Dysnatremia and the Endocrine Regulation of Fluid Balance during Exercise

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DEDICATION

This thesis is lovingly dedicated to my dearest husband, who believes in me
My family who supports me
My mentors who challenge me
My coaches who perfect me
My friends who ground me
And to everyone who loves to run.

You inspire me

TABLE OF CONTENTS

ABSTRACT

The aim of this thesis is to evaluate both abnormal and normal fluid balance during exercise. The central theme permeating all investigations is an underlying desire to understand exercise-associated hyponatremia. This thesis reflects a journey of scientific investigation primarily launched by outcomes from the 1st International Consensus Development Conference on Exercise-Associated Hyponatremia. Each individual investigation directly follows from the results of the previous investigation. Hence, the journey towards a greater understanding of exercise-associated hyponatremia went full circle and ultimately encompassed the spectrum of fluid regulation and dysregulation during exercise.

This thesis begins with a brief overview of the literature which serves to intertwine previous knowledge with new knowledge gained from each successive study. A review of the literature on exercise-associated hyponatremia opens the first chapter and lays the foundation for the following eight investigations. This thesis closes with a final summary of the literature which defends fluid balance physiology as the ultimate guide for developing more "physiologically appropriate" fluid replacement strategies. More specifically, the first two chapters document the abnormal regulation of serum sodium concentration during exercise (Chapter 1: The Hyponatremia of Exercise and Chapter 2: The Hypernatremia of Exercise) while the second two chapters investigate normal fluid balance physiology (Chapter 3: The Endocrine Regulation of Fluid Balance during Exercise and Chapter 4: Proposed Fluid Replacement Strategies).

A majority of the original data (75%) in this thesis is collected from "field" trials which encompass both intervention and observational studies. Both asymptomatic and symptomatic athletes are evaluated to delineate mechanisms underlying both fluid regulation and dysregulation. Data from laboratory studies are presented to compare the neuroendocrine response to high intensity, steady-state and prolonged endurance running in a subset of ten well-trained runners.

The main findings from these four chapters (eight investigations and two position statements) encompass the pathophysiology, physiology, prevention and treatment of dysnatremias associated with exercise. The conclusions from this thesis can be summarized as follows:

Pathophysiology of Dysnatremia:

1) Exercise-associated hyponatremia is dilutional and most likely results from a combination of non-suppressed arginine vasopressin (AVP) secretion with sustained fluid intake (exceeding output).
2) Exercise-associated hypernatremia primarily results from the inability to tolerate oral fluid ingestion, secondary to nausea and vomiting.

Endocrine Physiology of Normal Fluid Balance:

3) Non-osmotic AVP stimulation appears to be heightened during prolonged endurance exercise.
4) AVP, aldosterone, oxytocin and brain natriuretic peptide appear to participate in the maintenance of fluid homeostasis during exercise.
5) Sweat, urine and serum sodium concentrations appear to be homeostatically regulated and respond to small changes in AVP concentration.

Prevention of Dysnatremia:

6) Bodyweight loss is not an accurate surrogate of body fluid homeostasis during prolonged endurance exercise.
7) Sodium supplementation is not required to maintain plasma sodium concentrations during prolonged endurance exercise.

Treatment of Dysnatremia:

8) Administration of a small bolus of hypertonic saline solution rapidly reverses altered mental status changes associated with hyponatremic encephalopathy.
9) Dysnatremia predicts a delayed recovery in collapsed marathon runners.
10) A return to normonatremia is not required for dysnatremic athletes to recover and to be discharged from the medical tent.

OUTLINE OF THESIS

My chosen path of investigation was exercise-associated hyponatremia (EAH). I happened to be in the medical tent of the Houston Marathon in 1999, working as a podiatrist in charge of the podiatry/orthopedic unit, when four "collapsed" marathon runners were treated for presumed "dehydration" with intravenous fluids. One by one, the condition of each runner deteriorated until all four athletes experienced seizures and was transferred to hospital. These four runners were diagnosed with hyponatremia and critically ill for a week with cerebral and pulmonary edema [1]. The very next year, the medical team of the Houston Marathon purchased a portable electrolyte analyzer and twenty-one cases of symptomatic hyponatremia were documented in the medical tent of the 2000 Houston Marathon [2]. This growing "epidemic" of EAH in marathon runners culminated in the high profile deaths of two female runners from hyponatremic encephalopathy in the 2002 Boston and Marine Corp Marathons [3]. It was at that point where I personally realized that this "new" complication of endurance exercise warranted more urgent scientific attention.

Exercise-associated hyponatremia was first reported in four South African ultramarathon runners in 1985 and hypothesized to result from fluid overload [4]. Conversely, researchers investigating Ironman triathletes in Hawaii suggested that EAH resulted from excessive sodium loss [5-7]. Over time, cases of EAH quickly progressed from a singular phenomenon of ultraendurance activity [8;9] into the most common serious medical complication associated with endurance exercise [2;10;11]. Unfortunately, the scientific debate as to whether the pathophysiology of EAH was primarily dilutional [4;12-16] or depletional [17-20] continued; thereby widening an academic gap that threatened the well-being of all athletes participating in prolonged endurance activity.

My appeal for worldwide consensus in 2005 brought twelve "experts" from clinical medicine and exercise science to Cape Town, South Africa, to evaluate the existing evidence on EAH. The outcomes from this carefully deliberated review of the literature were published as the Position Statement from the First International Consensus

Development Conference on Exercise-Associated Hyponatremia [21]. The Consensus Panel concluded that EAH was primarily dilutional and secondary to fluid intake in excess of output. Evidence supporting a role for sodium supplementation during exercise and the use of hypertonic saline in the treatment of symptomatic EAH required further investigation, however.

Two trials were subsequently performed to help clarify the answer to these questions. The first investigation (Study 1A) evaluated the effect of ad libitum sodium supplementation on both performance and serum sodium concentration ($[Na^+]$) in Ironman triathletes [22]. Using a randomized-controlled prospective study design, either sodium or placebo capsules were dispensed to triathletes prior to race start; with instructions to consume the capsules ad libitum. It was concluded from these data that there were no performance benefits nor adverse medical consequences associated with supplemental sodium ingestion during prolonged endurance activity (~12 hours). More importantly, post-race serum $[Na^+]$ did not significantly differ between those athletes receiving sodium supplementation versus those triathletes consuming the placebo (corn starch). Therefore, it was concluded that sodium supplementation was not required to prevent the development of hyponatremia in athletes who lost weight during prolonged endurance exercise.

While sodium supplementation was not required to prevent the development of EAH during prolonged endurance activity, the administration of a small bolus of hypertonic saline solution was deemed by the Consensus Panel to be the most appropriate treatment strategy for symptomatic EAH [8;23-25]. Unfortunately, the safety and efficacy of such a practice had not yet been tested in the field. Study 1B documented the first case of an athlete who responded favorably to the administration of hypertonic saline within a field setting [26]. This case was atypical as the athlete's serum $[Na^+]$ was "borderline" throughout the entire period of observation (serum $[Na^+]$ between 132-135 mmol/L). Reversal of both the lethargy and confusion were dramatic and rapid in this triathlete following administration of a 50 mL bolus of 5% hypertonic saline. Therefore, the conclusions from this one case study suggested that: 1) hypertonic saline administration

could rapidly reverse altered mental status changes resulting from hyponatremic encephalopathy 2) hypertonic saline could be safely administered in the field and 3) the treatment of EAH should be guided by symptomatology and not by the serum [Na^+].

Further investigations detailing the exact pathophysiology and treatment of EAH were difficult to undertake because cases of hyponatremia were becoming increasingly rare in South African races [27-29]. Serendipitously, the incidence of *hyper*natremia in collapsed marathon runners seemed to surge, and the opportunity to investigate the opposite extreme of fluid imbalance was immediately seized.

Hypernatremia can cause morbidity and mortality secondary to cellular dehydration [30;31]. However, no known fatalities have been causally related to exercise-associated hypernatremia. This finding may be attributed to the existence of evolutionarily robust physiological mechanisms (arginine vasopressin secretion combined with the stimulation of thirst) which act to conserve body fluids [32].

Hypernatremia was biochemically defined as any [Na^+] above 145 mmol/L and CNS symptoms typically manifest when [Na^+] exceeds 160 mmol/L [31;33;34]. Hypertonic hyperosmolality caused an osmotic shift of water from the intracellular to the extracellular compartment which leads to cellular dehydration and cerebral shrinkage. Neurological symptoms often mimic those of hyponatremia and include: lethargy, tremor, weakness, irritability, delirium, mental confusion, seizures and coma [31;35]. Orthostatic hypotension and tachycardia have been shown to be common features of severe volume depletion, an expected adjunct to hypernatremia [31].

Study 2C of this thesis aimed to investigate the treatment of dysnatremia in collapsed marathon runners [36]. More specifically, the efficacy of oral versus intravenous (IV) fluid administration was targeted as the main outcome measure of this randomized-controlled prospective study. Although intravenous rehydration had been shown to facilitate a faster restoration of plasma volume - since this method of delivery bypasses

the delay associated with gastric absorption [37;38] - the clinical significance of this practice had yet to be fully evaluated in collapsed athletes.

The unexpected, but most interesting, finding from Study 2C was that nearly half (45%) of the collapsed cohort of ultramarathon runners were hypernatremic upon entrance into the medical tent [36]. This was the highest reported incidence of hypernatremia in collapsed runners to date [39] and hypernatremia predicted a delayed recovery in this cohort of collapsed athletes. Additionally, 45% of runners who were randomly assigned into the oral fluid replacement group were unable to tolerate oral fluid ingestion, secondary to nausea and vomiting. A significant majority of those runners who could not tolerate oral fluid ingestion were hypernatremic. Whether a return to normonatremia was required for hypernatremic athletes to recover and be discharged from the medical tent was not answered by that study.

Study 2D of this thesis was subsequently designed to evaluate if a return to normonatremia was a prerequisite to the resolution of symptomatology in dysnatremic runners. This observational study, performed at the same event the very next year, revealed that a return to normonatremia was *not* required for collapsed hypernatremic runners to recover (resolution of debilitating symptomatology) and be discharged from the medical area. A clear majority of these collapsed ultramarathon runners were hypernatremic (58%) upon presentation into the medical tent. Collapsed hypernatremic runners reported significantly more vomiting (79 vs. 34%; $p < 0.05$) than collapsed normonatremic runners during the race. This finding suggested that hypernatremia may have resulted from the inability to ingest and retain fluids during the race, rather than from a defect in the thirst mechanism during exercise. Curiously, the administration of hypotonic or isotonic fluids to hypernatremic runners facilitated the resolution of symptomatology and subsequent discharge from the medical area within 80 minutes of admission.

Now that the pathogenesis and treatment of exercise-associated hypernatremia was better identified, a greater understanding of the pathogenesis of exercise-associated

hyponatremia was necessary. The need to understand normal fluid regulation during exercise - particularly with regard to arginine vasopressin (AVP) secretion - was deemed a research priority by the EAH Consensus Panel [21]. Inappropriate secretion of AVP had been identified as the likely mediator in the pathophysiology of EAH [21;25;40]. However, evidence detailing AVP secretion during prolonged endurance exercise had been scarce and of questionable accuracy [41-44].

The syndrome of antidiuretic hormone secretion (SIADH) has been well-described as the most common cause of hyponatremia in the clinical setting [45]. AVP secretion has been shown to be linearly related to changes in plasma osmolality above ~ 280 mOsmol/kg H_20 [32]. At plasma osmolalities below 280 mOsmol/kg H_20, however, AVP secretion should be suppressed to maximize free water excretion and normalize low serum [Na^+]. It has been suggested that osmotic AVP secretion may be potentiated or superceded by non-osmotic stimuli during prolonged endurance exercise [21]. Therefore, if hypoosmolality develops during exercise, plasma AVP concentrations ($[AVP]_P$) may not be maximally suppressed; thereby facilitating the development of EAH. Plasma AVP concentrations do not have to be outside the "normal" range for EAH to develop during exercise: if $[AVP]_P$ is *inappropriate* for the degree of hypoosmolality present, than water retention and dilutional hyponatremia will likely occur [14;46]. Factors which may *potentiate* the secretion of AVP during exercise include hypovolemia [47;48] and increased core temperature [49] while other non-osmotic stimuli to AVP secretion during exercise include hypoglycemia [32], nausea and vomiting [50], and elevated plasma concentrations of IL-6 [51-53].

Study 3E of this thesis was undertaken to more critically assess the hormonal regulation of fluid balance in well-trained runners participating in a 56 km ultramarathon. Data from previous field investigations were limited by small sample sizes (N <10) [42;54;55] or delays in blood sampling which exceeded the half-lives of many fluid regulatory hormones being evaluated [41;42;44;56]. Therefore, 82 runners were recruited to participate in this observational study; whereas an onsite laboratory was fashioned at race start and finish

to ensure that all blood samples were appropriately handled for subsequent endocrine assay.

Plasma AVP concentrations were significantly elevated immediately post-race, despite non-significant decreases in serum [Na^+]. This main finding would confirm that non-osmotic AVP stimulation may either potentiate or predominate over osmotic AVP stimulation during prolonged endurance exercise. Therefore, it would be tempting to speculate that the non-osmotic secretion of AVP during exercise may prevent maximal free water excretion from occurring *if* hypoosmolality were to develop from exuberant fluid consumption during exercise. This would confirm the plausible role that AVP secretion would play in the pathophysiology of EAH.

Prolonged endurance exercise also stimulated significant elevations in the plasma concentrations of: oxytocin, interleukin-6, brain natriuretic peptide and the majority of adrenal steroid hormones measured (progesterone, corticosterone, aldosterone, 17-hydroxyprogesterone, 11-deoxycortisol, cortisol, DHEA, DHEAS and androstenedione). The increased concentrations of both oxytocin and brain natriuretic peptide were quite curious as oxytocin had been shown to participate in fluid and sodium balance predominantly in animals [57-59] while brain natriuretic peptide had been shown to be stimulated mainly by hypervolemia [60] (and not hypovolemia, as documented in this study). Therefore, further investigation was deemed necessary to delineate a potential role for both oxytocin and brain natriuretic hormone in fluid regulation during exercise.

Study 3F of this thesis recruited seven runners who had participated in Study 3E (prolonged endurance exercise). They completed a more detailed laboratory investigation of the endocrine regulation of fluid balance during high intensity and steady-state running. The same fluid balance and endocrine parameters that had been measured before and after the 56 km ultramarathon where re-measured in these same subjects after: 1) a maximal oxygen consumption test to exhaustion (VO_2 max test; high intensity run) and 2) a 60 minute run at 60% of peak treadmill speed (steady-state run).

Combined data from the high intensity, steady-state and prolonged endurance running trials confirmed that both oxytocin and brain natriuretic peptide seemed to be associated with the regulation of plasma volume and sodium concentration during exercise. These data also confirmed relationships between fluid regulatory variables with AVP and aldosterone secretion, which have been confirmed elsewhere [61-63]. Therefore, Study 3F may likely be the first study to document fluid regulatory roles for both oxytocin and brain natriuretic peptide in humans during exercise. Since oxytocin has known anti-diuretic effects [64], and has facilitated the development of hyponatremia in humans at rest [65-67], a pathophysiological role for elevated oxytocin secretion in the development of EAH during exercise would require further investigation.

Three well-trained female runners, who did not participate in the previous 56 km ultramarathon, were also similarly evaluated during high intensity and steady-state treadmill running. The addition of these three females into the laboratory trials "balanced" the previous inequality that precluded meaningful gender analyses from Study 3F. Therefore, Study 3G of this thesis evaluated ten runners (5 males and 5 females) during high intensity and steady-state running on a motorized treadmill.

The most significant finding from Study 3G was that both high intensity and steady state running elicited a linear increase in both urine and sweat [Na^+], both of which were correlated with a corresponding increase in serum [Na^+]. Post-run $[AVP]_P$ significantly correlated with post-run urine [Na^+] during both high intensity and steady state exercise, but with sweat [Na^+] only during steady-state running. These combined findings suggested that serum [Na^+] regulated corresponding changes in sweat and urine [Na^+] during exercise via changes in $[AVP]_P$. Therefore, the maintenance of fluid and thermoregulatory balance was shown to be intimately linked and most likely regulated by changes in AVP secretion.

Therefore, Study 3G would be the first to support the regulation of sweat gland secretion by changes in $[AVP]_P$ to conserve water and promote fluid homeostasis during exercise. Since 92% of all water [68] and 87% of all sodium [69] lost during exercise

is derived from sweat, the acute regulation of sweat secretion would assist in both fluid regulation and thermoregulation during exercise. Thus, non-osmotic AVP secretion during exercise would limit fluid loss in both urine and sweat. This combination may benefit water conservation during exercise but would hypothetically accelerate the development of EAH if hypoosmolality and high fluid intakes were to persist during prolonged endurance activity. Gender did not significantly predict any fluid regulatory variable documented in this study.

Evidence obtained from the above-mentioned endocrine studies (Studies 3E-G) suggested that the aim of fluid regulatory mechanisms activated during high intensity, steady state and prolonged endurance running was to preserve plasma osmolality and serum [Na^+]. These findings directly conflicted with the current fluid replacement guidelines which recommend full replacement of bodyweight lost during exercise [20;70;71]. Therefore, Study 4H of this thesis was designed to critically evaluate if changes in bodyweight could accurately reflect changes in serum [Na^+] and plasma volume during an Ironman triathlon.

The main conclusion from this observational field study was that bodyweight loss was not an accurate surrogate of fluid balance homeostasis during prolonged endurance exercise. Significant bodyweight loss (~4%) occurred from pre- to post-race while both serum [Na^+] and plasma volume increased (~1%) during ~12 hours of endurance activity [72]. None of the 181 male triathletes who participated in this trial reported any adverse symptoms following the race. This suggests that a 4% bodyweight loss following 12 hours of endurance exercise did not promote any adverse medical consequences.

The combined findings from the investigations outlined in this thesis thereby suggest that serum [Na^+] (as a surrogate for plasma osmolality [39]) may be the **primary** regulated variable during exercise. Therefore, it would seem logical that fluid recommendations for athletes participating in endurance exercise be based on the preservation of serum [Na^+] rather than on the maintenance of bodyweight. This physiological argument was

detailed in the closing review of fluid homeostasis of this thesis. In this Position Statement for marathon runners, it was argued that thirst be designated as the most physiologically sound fluid replacement guide during exercise [73] as it is during rest [32]. These *physiologically-based* fluid recommendations would aim to prevent future morbidity and mortality resulting from *non-physiologically-based* drinking guidelines that are currently being promoted for athletes participating in prolonged exercise activity.

Reference List

1. Ayus JC, Varon J, Arieff AI. Hyponatremia, cerebral edema, and noncardiogenic pulmonary edema in marathon runners. *Ann Intern.Med* 2000;**132**:711-4.

2. Hew TD, Chorley JN, Cianca JC, Divine JG. The incidence, risk factors, and clinical manifestations of hyponatremia in marathon runners. *Clin.J.Sport Med.* 2003;**13**:41-7.

3. Noakes TD,.Speedy DB. Case proven: exercise associated hyponatraemia is due to overdrinking. So why did it take 20 years before the original evidence was accepted? *Br.J.Sports Med.* 2006;**40**:567-72.

4. Noakes TD, Goodwin N, Rayner BL, Branken T, Taylor RK. Water intoxication: a possible complication during endurance exercise. *Med.Sci.Sports Exerc.* 1985;**17**:370-5.

5. Hiller DB, O'Toole ML, Fortress EE, Laird RH, Imbert PC, Sisk TD. Medical and physiological considerations in triathlons. *Am.J.Sports Med.* 1987;**15**:164-8.

6. Hiller WDB. Dehydration and hyponatremia during triathlons. *Med.Sci.Sports Exerc.* 1989;**21(Suppl)**:219-21.

7. Hiller WDB. Hyponatremia and ultramarathons. *JAMA* 1986;256-13.

8. Frizzell RT, Lang GH, Lowance DC, Lathan SR. Hyponatremia and ultramarathon running. *JAMA* 1986;**255**:772-4.

9. Surgenor S,.Uphold RE. Acute hyponatremia in ultra-endurance athletes. *Am.J Emerg Med* 1994;**12**:441-4.

10. Almond CS, Shin AY, Fortescue EB, Mannix R, Wypij D. Hyponatremia among Runners in the Boston Marathon. *N.Engl.J Med* 2005;**352**:1550-6.

11. Stuempfle KJ, Lehmann DR, Case HS, Bailey S, Hughes SL, McKenzie J *et al.* Hyponatremia in a cold weather ultraendurance race. *Alaska Med.* 2002;**44**:51-5.

12. Speedy DB, Noakes TD, Rogers IR, Thompson JM, Campbell RG, Kuttner JA *et al.* Hyponatremia in ultradistance triathletes. *Med.Sci.Sports Exerc.* 1999;**31**:809-15.

13. Speedy DB, Rogers IR, Noakes TD, Wright S, Thompson JM, Campbell R *et al.* Exercise-induced hyponatremia in ultradistance triathletes is caused by inappropriate fluid retention. *Clin.J.Sport Med.* 2000;**10**:272-8.

14. Speedy DB, Noakes TD, Rogers IR, Hellemans I, Kimber NE, Boswell DR *et al.* A prospective study of exercise-associated hyponatremia in two ultradistance triathletes. *Clin.J.Sport Med.* 2000;**10**:136-41.

15. Speedy DB, Rogers IR, Noakes TD, Thompson JM, Guirey J, Safih S *et al.* Diagnosis and prevention of hyponatremia at an ultradistance triathlon. *Clin.J.Sport Med.* 2000;**10**:52-8.

16. Speedy DB, Noakes TD, Schneider C. Exercise-associated hyponatremia: a review. *Emerg.Med.(Fremantle.)* 2001;**13**:17-27.

17. Vrijens DM,.Rehrer NJ. Sodium-free fluid ingestion decreases plasma sodium during exercise in the heat. *J Appl.Physiol* 1999;**86**:1847-51.

18. Twerenbold R, Knechtle B, Kakebeeke TH, Eser P, Muller G, von Arx P *et al.* Effects of different sodium concentrations in replacement fluids during prolonged exercise in women. *Br.J.Sports Med.* 2003;**37**:300-3.

19. Montain SJ, Cheuvront SN, Sawka MN. Exercise associated hyponatraemia: quantitative analysis to understand the aetiology. *Br.J.Sports Med.* 2006;**40**:98-105.

20. Convertino VA, Armstrong LE, Coyle EF, Mack GW, Sawka MN, Senay LC, Jr. *et al.* American College of Sports Medicine position stand. Exercise and fluid replacement. *Med Sci.Sports Exerc* 1996;**28**:i-vii.

21. Hew-Butler TD, Almond CS, Ayus JC, Dugas JP, Meeuwisse WH, Noakes TD *et al.* Consensus Statement of the 1st International Exercise-Associated Hyponatremia Consensus Development Conference, Cape Town, South Africa 2005. *Clin.J.Sport Med* 2005;**15**:208-13.

22. Hew-Butler TD, Sharwood K, Collins M, Speedy D, Noakes T. Sodium supplementation is not required to maintain serum sodium concentrations during an Ironman triathlon. *Br.J.Sports Med.* 2006;**40**:255-9.

23. Ayus JC, Arieff A, Moritz ML. Hyponatremia in marathon runners. *N.Engl.J.Med.* 2005;**353**:427-8.

24. Davis DP, Videen JS, Marino A, Vilke GM, Dunford JV, Van Camp SP *et al.* Exercise-associated hyponatremia in marathon runners: a two-year experience. *J.Emerg.Med.* 2001;**21**:47-57.

25. Siegel AJ, Verbalis JG, Clement S, Mendelson JH, Mello NK, Adner M *et al.* Hyponatremia in marathon runners due to inappropriate arginine vasopressin secretion. *Am.J.Med.* 2007;**120**:461-7.

26. Hew-Butler T, Anley C, Schwartz P, Noakes T. The treatment of symptomatic hyponatremia with hypertonic saline in an Ironman triathlete. *Clin.J.Sport Med.* 2007;**17**:68-9.

27. Sharwood K, Collins M, Goedecke J, Wilson G, Noakes T. Weight changes, medical complications and performance during an Ironman triathlon. *Br J Sports Med* 2004;**38**:718-24.

28. Sharwood K, Collins M, Goedecke J, Wilson G, Noakes T. Weight changes, sodium levels, and performance in the South African Ironman Triathlon. *Clin.J.Sport Med.* 2002;**12**:391-9.

29. Noakes TD, Sharwood K, Collins M, Perkins DR. The dipsomania of great distance: water intoxication in an Ironman triathlete. *Br.J.Sports Med.* 2004;**38**:E16.

30. van der Helm-van Mil AH, van Vugt JP, Lammers GJ, Harinck HI. Hypernatremia from a hunger strike as a cause of osmotic myelinolysis. *Neurology* 2005;**64**:574-5.

31. Adrogue HJ,.Madias NE. Hypernatremia. *N.Engl.J.Med.* 2000;**342**:1493-9.

32. Verbalis JG. Disorders of body water homeostasis. *Best.Pract.Res.Clin.Endocrinol.Metab* 2003;**17**:471-503.

33. Riggs JE. Neurologic manifestations of electrolyte disturbances. *Neurol.Clin.* 2002;**20**:227-39, vii.

34. Weiss-Guillet EM, Takala J, Jakob SM. Diagnosis and management of electrolyte emergencies. *Best.Pract.Res.Clin.Endocrinol.Metab* 2003;**17**:623-51.

35. Rocha-e-Silva. Hypertonic/hyperoncotic treatment for brain damage. *Crit Care Med.* 2003;**31**:2559-60.

36. Hew-Butler T, Sharwood K, Boulter J, Collins M, Tucker R, Dugas J *et al.* Dysnatremia predicts a delayed recovery in collapsed ultramarathon runners. *Clin.J.Sport Med* 2007;**17:** 289-296.

37. Nose H, Mack GW, Shi XR, Morimoto K, Nadel ER. Effect of saline infusion during exercise on thermal and circulatory regulations. *J.Appl.Physiol* 1990;**69**:609-16.

38. Casa DJ, Maresh CM, Armstrong LE, Kavouras SA, Herrera JA, Hacker FT, Jr. *et al.* Intravenous versus oral rehydration during a brief period: responses to subsequent exercise in the heat. *Med Sci.Sports Exerc.* 2000;**32**:124-33.

39. Kratz A, Siegel AJ, Verbalis JG, Adner MM, Shirey T, Lee-Lewandrowski E *et al.* Sodium status of collapsed marathon runners. *Arch.Pathol.Lab Med.* 2005;**129**:227-30.

40. Noakes TD, Sharwood K, Speedy D, Hew T, Reid S, Dugas J *et al.* Three independent biological mechanisms cause exercise-associated hyponatremia: evidence from 2,135 weighed competitive athletic performances. *Proc.Natl.Acad.Sci.U.S.A* 2005;**102**:18550-5.

41. Fellmann N, Bedu M, Giry J, Pharmakis-Amadieu M, Bezou MJ, Barlet JP *et al.* Hormonal, fluid, and electrolyte changes during a 72-h recovery from a 24-h endurance run. *Int.J.Sports Med.* 1989;**10**:406-12.

42. Dessypris A, Wagar G, Fyhrquist F, Makinen T, Welin MG, Lamberg BA. Marathon run: effects on blood cortisol -- ACTH, iodothyronines -- TSH and vasopressin. *Acta Endocrinol.(Copenh)* 1980;**95**:151-7.

43. Rocker L, Kirsch KA, Heyduck B, Altenkirch HU. Influence of prolonged physical exercise on plasma volume, plasma proteins, electrolytes, and fluid-regulating hormones. *Int.J.Sports Med.* 1989;**10**:270-4.

44. Nelson PB, Ellis D, Fu F, Bloom MD, O'Malley J. Fluid and electrolyte balance during a cool weather marathon. *Am.J.Sports Med.* 1989;**17**:770-2.

45. Schwartz WB, Bennett W, Curelop S, Bartter FC. A syndrome of renal sodium loss and hyponatremia probably resulting from inappropriate secretion of antidiuretic hormone. 1957. *J.Am.Soc.Nephrol.* 2001;**12**:2860-70.

46. Stuempfle KJ, Lehmann DR, Case HS, Hughes SL, Evans D. Change in serum sodium concentration during a cold weather ultradistance race. *Clin.J.Sport Med.* 2003;**13**:171-5.

47. Robertson GL. Posterior Pituitary. In Felig P, et.al., eds. *Endocrinology and Metabolism*, pp 385-432. New York: Mcgraw-Hill, 1995.

48. Convertino VA, Keil LC, Greenleaf JE. Plasma volume, renin, and vasopressin responses to graded exercise after training. *J.Appl.Physiol* 1983;**54**:508-14.

49. Takamata A, Mack GW, Stachenfeld NS, Nadel ER. Body temperature modification of osmotically induced vasopressin secretion and thirst in humans. *Am.J.Physiol* 1995;**269**:R874-R880.

50. Rowe JW, Shelton RL, Helderman JH, et.al. Influence of the emetic reflex on vasopressin release in man. *Kidney Int* 1979;**16**:729-35.

51. Gionis D, Ilias I, Moustaki M, Mantzos E, Papadatos I, Koutras DA *et al.* Hypothalamic-pituitary-adrenal axis and interleukin-6 activity in children with head trauma and syndrome of inappropriate secretion of antidiuretic hormone. *J.Pediatr.Endocrinol.Metab* 2003;**16**:49-54.

52. Mastorakos G, Weber JS, Magiakou MA, Gunn H, Chrousos GP. Hypothalamic-pituitary-adrenal axis activation and stimulation of systemic vasopressin secretion by recombinant interleukin-6 in humans: potential implications for the syndrome of inappropriate vasopressin secretion. *J.Clin.Endocrinol.Metab* 1994;**79**:934-9.

53. Siegel AJ. Exercise-associated hyponatremia: role of cytokines. *Am.J.Med.* 2006;**119**:S74-S78.

54. Wade CE, Dressendorfer RH, O'Brien JC, Claybaugh JR. Renal function, aldosterone, and vasopressin excretion following repeated long-distance running. *J.Appl.Physiol* 1981;**50**:709-12.

55. Gastmann U, Dimeo F, Huonker M, Bocker J, Steinacker JM, Petersen KG *et al.* Ultra-triathlon-related blood-chemical and endocrinological responses in nine athletes. *J.Sports Med.Phys.Fitness* 1998;**38**:18-23.

56. Freund BJ, Claybaugh JR, Hashiro GM, Buono M, Chrisney S. Exaggerated ANF response to exercise in middle-aged vs. young runners. *J.Appl.Physiol* 1990;**69**:1607-14.

57. Conrad KP, Gellai M, North WG, Valtin H. Influence of oxytocin on renal hemodynamics and sodium excretion. *Ann.N.Y.Acad.Sci.* 1993;**689**:346-62.

58. Verbalis JG, Mangione MP, Stricker EM. Oxytocin produces natriuresis in rats at physiological plasma concentrations. *Endocrinology* 1991;**128**:1317-22.

59. Stricker EM,.Verbalis JG. Interaction of osmotic and volume stimuli in regulation of neurohypophyseal secretion in rats. *Am.J.Physiol* 1986;**250**:R267-R275.

60. Huang WS, Lee MS, Perng HW, Yang SP, Kuo SW, Chang HD. Circulating brain natriuretic peptide values in healthy men before and after exercise. *Metabolism* 2002;**51**:1423-6.

61. Grant SM, Green HJ, Phillips SM, Enns DL, Sutton JR. Fluid and electrolyte hormonal responses to exercise and acute plasma volume expansion. *J.Appl.Physiol* 1996;**81**:2386-92.

62. Shoemaker JK, Green HJ, Ball-Burnett M, Grant S. Relationships between fluid and electrolyte hormones and plasma volume during exercise with training and detraining. *Med.Sci.Sports Exerc.* 1998;**30**:497-505.

63. Melin B, Koulmann N, Jimenez C, Savourey G, Launay JC, Cottet-Emard JM *et al.* Comparison of passive heat or exercise-induced dehydration on renal water and electrolyte excretion: the hormonal involvement. *Eur.J.Appl.Physiol* 2001;**85**:250-8.

64. Abdul-Karim R,.Assali NS. Renal function in human pregnancy. V. Effects of oxytocin on renal hemodynamics and water and electrolyte excretion. *J.Lab Clin.Med.* 1961;**57**:522-32.

65. Lauersen NH,.Birnbaum SJ. Water intoxication associated with oxytocin administration during saline-induced abortion. *Am.J.Obstet.Gynecol.* 1975;**121**:2-6.

66. Gupta DR,.Cohen NH. Oxytocin, "salting out," and water intoxication. *JAMA* 1972;**220**:681-3.

67. Seifer DB, Sandberg EC, Ueland K, Sladen RN. Water intoxication and hyponatremic encephalopathy from the use of an oxytocin nasal spray. A case report. *J.Reprod.Med.* 1985;**30**:225-8.

68. Pivarnik JM, Leeds EM, Wilkerson JE. Effects of endurance exercise on metabolic water production and plasma volume. *J.Appl.Physiol* 1984;**56**:613-8.

69. Nose H, Mack GW, Shi XR, Nadel ER. Shift in body fluid compartments after dehydration in humans. *J Appl.Physiol* 1988;**65**:318-24.

70. Cheuvront SN, Carter R, III, Sawka MN. Fluid balance and endurance exercise performance. *Curr.Sports Med.Rep.* 2003;**2**:202-8.

71. Coyle EF. Fluid and fuel intake during exercise. *J.Sports Sci.* 2004;**22**:39-55.

72. Hew-Butler T, Collins M, Bosch A, Sharwood K, Wilson G, Armstrong M *et al.* Maintenance of plasma volume and serum sodium concentration despite body weight loss in ironman triathletes. *Clin.J.Sport Med.* 2007;**17**:116-22.

73. Hew-Butler T, Verbalis JG, Noakes TD. Updated Fluid Recommendation: Position Statement from the International Marathon Medical Directors Association (IMMDA). *Clin.J.Sport Med* 2006;**16**.

CHAPTER 1

THE HYPONATREMIA OF EXERCISE

A Review of the Literature

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INTRODUCTION

Over the past decade, exercise-associated hyponatremia (EAH) has emerged as an important complication of prolonged endurance activity[1-7]. Prior to 1985, this condition was not reported, and runners generally finished marathons with weight loss but without serious medical complications. Abnormalities of serum sodium concentrations ($[Na^+]$), when measured, were confined to elevated levels consistent with varying degrees of volume depletion[8-15].

In March 2005, a panel of twelve international experts on exercise physiology, sport medicine, water metabolism and body fluid homeostasis convened in Cape Town, South Africa, for the 1st International Exercise-Associated Hyponatremia Consensus Development Conference. The primary goal of this panel was to review all of the existing data on EAH and formulate an evidence-based analysis that would define the current understanding of the pathophysiology of EAH. In particular, the panel was constituted to facilitate integration of existing medical and scientific knowledge of other forms of hyponatremia with the occurrence of this homeostatic imbalance during endurance exercise.

A secondary goal of the EAH Consensus Development Conference was to prepare a statement that would serve to curtail the growing problem of EAH by disseminating the most current information to both medical personnel and the greater public on the prevalence, nature and treatment of this disorder. The panel strived to clearly articulate what we agreed upon, debate issues that we did not agree upon, and describe in detail

what we did and did not know, including minority viewpoints that were supported by clinical and experimental data.

The following statement reflects a concise summary of the data deliberated and synthesized by the panel and provides a "snapshot in time" of the current state of knowledge on EAH. New knowledge will continue to advance regarding our understanding of EAH, and will mandate future updates to this consensus statement.

METHODS

The International Exercise-Associated Hyponatremia Consensus Development Conference (CDC) followed the guidelines set forth by the National Institutes of Health (NIH)[16]. The basic principles governing the conduct of a CDC are summarized below:

1. A broad based non-government, non-advocacy panel was assembled to give balanced, objective and knowledgeable attention to the topic. Panel members excluded anyone with scientific or commercial conflicts of interest and included researchers in clinical medicine (C.A., J.C.A., A.S. and J.V.), sports medicine (T.H., T.N., S.R., D.S.) and sports scientists (K.S., J.D.).
2. These experts presented data in a public session, followed by inquiry and discussion. The panel then met in an executive session to prepare the consensus statement.
3. A number of specific questions were prepared and posed in advance to define the scope and guide the direction of the conference. The principle task of the panel was to elucidate responses to these questions.
4. A systematic literature review was prepared and circulated in advance for use by the panel in addressing the conference questions (see reference list).
5. The consensus statement is intended to serve as the scientific record of the conference.
6. The consensus statement will be widely disseminated to achieve maximum impact on both current health care practice and future medical research.

The panel chairperson (W.M) did not identify with any advocacy position nor present data on EAH[16]. The chairperson was responsible for directing the plenary session and guiding the panel's deliberations.

There was a strict criterion for invitation to the EAH Consensus Panel: publication of original data on hyponatremia within the last five years. The U.S. Military, which has recently come to its own consensus, was excluded[17-19]. One delegate (L.W.) did not present original data, but has prepared a mathematical model of EAH and was therefore included as an invited participant[20].

The following focus questions formed the foundation for the EAH consensus statement:

1. What is the definition of exercise-associated hyponatremia (EAH)?
2. Can the severity of EAH be classified by clinical and laboratory criteria?
3. What is the etiology and pathophysiology of EAH?
4. What are the risk factors for the development of EAH?
5. How can EAH be prevented?
6. What are appropriate treatment protocols for EAH?
7. What advice should be disseminated for the prevention and treatment of EAH?
8. What studies should be performed to better understand EAH?

RESULTS AND DISCUSSION

1. What is the definition of exercise-associated hyponatremia (EAH)?

EAH is the occurrence of hyponatremia in individuals engaged in prolonged physical activity[1;3-7;21-46] and is defined by a serum or plasma sodium concentration ($[Na^+]$) below the normal reference range of the laboratory performing the test. For most laboratories, this is a $[Na^+]$ less than 135 mmol/L[47]. EAH can occur during or after physical activity,

and most commonly occurs in events lasting longer than four hours[1-6;21;23-26;29-35;38-45;48], although at least two cases have been reported in events of shorter duration[49;50]

2. Can the severity of EAH be classified by clinical and laboratory criteria?

EAH should be classified using the same clinical criteria as any acute or rapid onset hyponatremia[51]. As with any acute onset hyponatremia, the most important distinction is determining the presence or absence of clinical signs and symptoms, specifically neurological manifestations. In general, the lower the serum or plasma [Na^+], the more severe will be the neurological signs and symptoms[52] Individual variability is great, however, and the numerical value of [Na^+] is not a reliable index of the clinical severity of hyponatremia, including EAH [51].

In general, milder forms of hyponatremia ([Na^+] between 130-134 mmol/L) are relatively asymptomatic and are likely to resolve spontaneously, although exceptions to this rule have been reported[1;3;6;31;38;39;41;44]. Signs and symptoms of EAH tend to develop when the serum [Na^+] falls below 130 mmol/L[3;6;30;31;38;41]. Early signs and symptoms can include: bloating, "puffiness", nausea, vomiting, and headache. However, many of these signs and symptoms are non-specific and can be present following prolonged exercise in the absence of EAH[7;21;22;27;30;36;46;53]. As the severity of EAH progresses, more serious signs and symptoms can develop as a result of worsening cerebral edema (brain swelling), including altered mental status (confusion, disorientation, and agitation), seizures, respiratory distress (pulmonary edema), obtundation, coma and death[7;23;25-27;33;34;36;43;45;46;48;53-56]. The presence of any of these signs and symptoms represents an absolute indication to measure the serum or plasma [Na^+].

3. What is the etiology and pathophysiology of EAH?

Current evidence strongly indicates that EAH is a dilutional hyponatremia, caused by an increase in total body water relative to the amount of total body exchangeable Na^+ [1;4;21-23;26-29;29;30;33;35;36;39;46;48;53;55]. Although this increase can be relative in nature, in most

reported cases of symptomatic EAH, there is body weight gain suggestive of an absolute increase in total body water [1;4;5;21;23;29;35;39;41;48;53;57]. The primary etiologic factor in cases that have been adequately studied appears to be consumption of hypotonic fluids (water or sports drinks) in excess of insensible (respiratory and gastrointestinal), sweat and renal (urine) fluid losses[1;4;21-23;26-29;29;30;33;35;36;39;46;48;53].

Hyponatremia caused solely by the overconsumption of fluids has been demonstrated at rest in athletes with and without a history of EAH[57;58]. Weight gain in these athletes occurred despite an increase in free water excretion and what appeared to be maximally suppressed arginine vasopressin (AVP) levels (as reflected by mean urine osmolalities <100 mOsm/kg H_2O). This is consistent with known maximal urine excretory rates of 800-1,000 ml/h in normal adults[59].

During exercise, however, plasma AVP levels may not be maximally suppressed. This has been demonstrated in a 24-hour field march during which urinary osmolalities failed to reach the minimum concentrations expected in water loaded subjects[29] and in studies of hikers who developed hyponatremia in the Grand Canyon [22]. Plasma AVP levels within "normal ranges" are physiologically *inappropriate* in the presence of hyponatremia and/or hypervolemia[39-41]. Even small increases in circulating AVP levels markedly reduce the maximal kidney excretory capacity[51], thus increasing the propensity to retain ingested fluids even when rates of drinking do not exceed 800-1,000 ml/h. High urine osmolalities have been measured in athletes hospitalized with critical hyponatremia[4;22;23;28;46;60]. This further implicates inappropriate AVP secretion as an exacerbating factor in the development of dilutional hyponatremia during prolonged physical activity.

Accordingly, hyponatremic athletes can present with a spectrum of urine concentrations, ranging from the ability to excrete dilute urine freely[1;22] to an inability to void despite encouragement[28;35;53]. Thus, the risk of developing fluid overload with previously "normal" or excessive fluid intakes is enhanced when AVP is secreted inappropriately during prolonged exercise, as reflected by increased urine osmolality and decreased

urine volume. A lower rate of urine production correlates significantly with a higher rate of serum/plasma [Na^+] decrease in athletes drinking excessively during exercise[50]. Multiple potential stimuli to AVP secretion can exacerbate fluid retention at any time during prolonged exercise[21;61]. However, given the short half life (6-8 minutes)[61] of AVP, measurement of suppressed AVP levels at time points after the cessation of physical activity do not eliminate the possibility of inappropriate AVP secretion as a contributory factor to the development of EAH.

Excessive Na^+ loss has not been demonstrated to be a primary causative factor in the pathogenesis of EAH. Published data on Na^+ losses in EAH show that sodium loss is no greater in individuals who develop EAH than in individuals who do not[21;33;40;41]. Although symptomatic EAH is largely associated with weight gain, mild or asymptomatic EAH (generally [Na^+] in the range of 130-134 mmol/L) can be associated with a spectrum of weight change, from weight loss (-9%) to weight gain (+2%)[39]. The etiology of asymptomatic EAH with weight loss has not been clearly established [3;31;39;62]. However, in athletes with high sweat sodium concentrations (>100 mmol/L)[63] or high urinary sodium losses from inappropriate AVP secretion and water retention (>400 mOsm/L)[22;23;46] sodium losses may play a *secondary* role in the pathogenesis of EAH by either of two potential mechanisms: 1) hypovolemia produced by sodium losses can act as a stimulus to AVP secretion, producing a secondary retention of water, as is seen medically in some cases of diuretic-induced hyponatremia[64]; 2) sodium losses themselves can worsen the degree of hyponatremia, although in most cases not nearly as much as water retention[20] when summed up over time. Further studies are needed to fully investigate the role of sodium losses on this group of athletes who develop EAH, particularly those with a weight loss in excess of 3% or with a large volume of sweat over time, in warmer climates and in events lasting over 24 hours.

Ingestion of electrolyte-containing sports drinks does not prevent the development of EAH in athletes who drink to excess[1;22;32;62]. This is due to two factors: 1) all such drinks are hypotonic (< 135 mmol/L), and therefore will cause dilution of serum [Na^+] if water is retained in the body to excess; and 2) it is well known that even administration of

isotonic saline will not increase serum [Na^+] in hyponatremic patients with the syndrome of inappropriate antidiuretic hormone secretion (SIADH) because in a euvolemic or hypervolemic state, the infused sodium will be excreted in the urine rather than retained[65]. Sodium supplementation does not influence post-exercise [Na^+] in athletes who either lose[66] or maintain bodyweight[50;67]. However, in athletes who gain weight and develop EAH from excessive fluid intake, the serum [Na^+] is maintained somewhat better if the sodium concentration of the ingested beverage exceeds the amount present in currently available commercial sports drinks (i.e., >20 mmol/L)[44].

4. What are risk factors for EAH?

The presence of a risk factor implies a correlation with higher rates of EAH, but not necessarily causation. It is likely that these risk factors interact with each other and, in some cases, may not have an independent association with EAH. Recognized risk factors include:

- low body weight[1;6;40]
- female sex[1;6;7;22;30;39;54]
- 4 hours exercise duration[1-6;21;23-26;29-31;33-35;38-44;48]
- slow running or performance pace[1;4;7;30]
- race inexperience[30;45]
- excessive drinking behavior[1;4;7;26;30;37]
- high availability of drinking fluids[30]
- altered renal water excretory capacity (potentially impaired by drugs, such as nonsteroidal anti-inflammatory agents[7;23;43;54], intrinsic renal disease or SIADH)
- extreme hot[21;22;25;26;28;32;37] or cold[6] environmental conditions.

In a multivariate analysis, hyponatremia was associated with weight gain, a racing time > 4:00 hours and low body-mass-index extremes[1].

Low sodium ingestion, from the voluntary avoidance of sports drinks, sodium supplements or salty snacks, has not been shown to be a risk factor during events lasting <24 hours[12;67]. Published data on the cystic fibrosis genotype is inconclusive[68-71], with only one documented case of EAH reported in an infantryman whose fluid intake was high[63] . Further assessment will need to be made before excluding these variables as potential risk factors for EAH.

5. How can EAH be prevented?

EAH is caused primarily by the consumption of fluid in excess of urinary and sweat losses. Therefore, it follows that any individual participating in endurance exercise, and particularly those at increased risk for EAH, should avoid over consumption of fluids[42;72]. There is wide variability in sweat rates and renal water excretory capacity* during exercise, both among individuals and in the same individual depending on ambient environmental conditions during the time of exercise; thus, blanket universal drinking guidelines are not possible. The primary means of preventing EAH is to avoid excess fluid retention, as manifested by weight gain, during or after exercise. There are at least two ways to insure that weight gain does not occur during exercise: 1) drink only according to thirst (i.e., *ad libitum*)[73] or 2) utilize the USATF guidelines, or analogous methods, to estimate hourly sweat losses during exercise and avoid consuming amounts greater than this during endurance exercise events[74].

Published data show that an education program advising athletes on the risks of overdrinking, together with limiting fluid availability at a race, has been associated with a reduction in the incidence of EAH without deleterious effects[42;75]. Specifically for an Ironman distance triathlon, cycle aid station placement every 20 km, and run stations every 2.5 km are recommended[42]. In a standard marathon footrace, placement of aid stations every 5 km has been associated with an absence of EAH[72].

There is no currently available evidence to support the suggestion that Na^+ supplementation prevents the development of EAH[66;76] nor is there any evidence that

consumption of electrolyte-containing hypotonic fluids (i.e., sports drinks) can prevent the development of EAH in athletes who drink to excess[20;67]. Although some studies suggest that ingestion of electrolyte-containing drinks can decrease the severity of EAH[44], it is clear that this approach will not prevent the occurrence of this disorder in the presence of overdrinking.

* Sweat rates during sustained endurance exercise can vary markedly between individuals, ranging from as high as >2L/h[27] to as low as <250 mL/h[77]. Under resting conditions, maximum rates of renal water excretion in normal individual can reach levels as high as 1.4 l/h[21]. However, during sustained endurance exercise, rates of renal water excretion can be significantly decreased by antidiuresis due to inappropriate AVP secretion, potentially to as little as 0 to 60 mL/h (resting values)[67].

6. What are appropriate treatment protocols for laboratory confirmed EAH?

Medical facilities at endurance events should include onsite analysis of serum or plasma $[Na^+]$[78]. Any athlete exhibiting signs and symptoms of acute hyponatremia listed above should be screened for EAH by measuring plasma or serum $[Na^+]$. Based on this determination, the following treatment protocols are advised:

Asymptomatic EAH

Asymptomatic hyponatremia is not normally detected unless an athlete has blood or serum electrolyte concentrations tested for some other reason[1;3;6;29;31;38;39;41;44]. In athletes with this biochemical diagnosis, oral fluid intake should be restricted until the onset of urination. Athletes should also be advised to seek urgent medical attention if any signs or symptoms of EAH develop within 24 h. Asymptomatic EAH is a contraindication for the administration of intravenous normal saline, which can worsen the degree of hyponatremia and fluid overload in some cases.

Symptomatic – Onsite

Intravenous access must be established in athletes with symptomatic EAH, but care must be taken to avoid the administration of isotonic or hypotonic fluids to prevent worsening the degree of hyponatremia and fluid overload (with the exception of cases where there is evidence of circulatory insufficiency where standard Advanced Cardiac Life Support protocols apply)[55]. Oxygen should be administered and the athlete immediately transferred to a definitive medical care facility. The diagnosis of EAH must be communicated to the emergency room physician upon transfer of care.

If the medical staff is experienced in treating hyponatremia in the field, any athlete with EAH who exhibits signs of respiratory insufficiency, confusion, obtundaton, nausea and vomiting can be treated with 100 ml of 3% NaCl over ten minutes to acutely raise [Na^+] and decrease brain edema. This maneuver can raise the [Na^+] an average of 2-3 mmol/L and should not pose any substantial danger to the patient. This therapy aims to stabilize the athlete prior to hospital transfer without producing complications. The efficacy of hypertonic 3% NaCl infusion treatment has been documented in the hospital setting [54;79-83] and this approach has been recently proposed in the field [87].

Symptomatic – In hospital

Both clinical and laboratory reassessment must be performed upon admission, taking care to avoid treatment delays while awaiting diagnostic tests such as brain imaging[7;43;54;60]. Administration of hypotonic or isotonic intravenous fluids during this reevaluation is again contraindicated because of the potential to exacerbate hyponatremia and fluid overload.

If symptomatic EAH persists or worsens, current treatment guidelines for acute symptomatic hyponatremia should be followed. These should entail administration of hypertonic solutions of NaCl to immediately decrease brain edema. Several different protocols have been employed to accomplish this goal, including the following: 1) administer either a 100 ml or a 1 ml/kg bolus of 3% NaCl and repeat hourly at a rate of 100 ml/hour[7;43] or 2) infuse 3% NaCl at a rate of 1-2 ml/kg/hour[84]. Alternatively, if

hypertonic NaCl as not immediately available, hypertonic mannitol can be administered to accomplish this goal [85]. Regardless of which method is chosen, treatment should be continued until the patient regains consciousness. Subspecialty consultation is strongly advised (nephrology/endocrinology) with regard to further therapy (e.g. loop diuretics, fluid restriction, etc.). Plasma or serum [Na^+] should be monitored every hour until the symptoms subside and the patient is clinically stable with an appropriate urine output. Osmotic demyelination, or central pontine myelinolysis, in association with the rapid correction of an acute hyponatremia (i.e., <48 hour duration), has not been reported[86]and should never be an impediment to rapidly correcting hyponatremia in symptomatic EAH [7;21-23;26;43;46;54;60].

7. What advice should be disseminated for prevention and treatment of EAH?

Athletes and coaches

Strategies aimed at preventing the over consumption of hypotonic fluids (water or sports drinks), as outlined above, need to be communicated effectively to coaches and athletes. Furthermore, athletes and coaches must be better educated on the signs and symptoms of EAH regarding when to seek medical attention.

Medical directors and race directors

Aid stations should be placed at appropriately distanced intervals. Medical and race directors should strongly consider pre-race weight measurements as a routine part of race registration. The pre-race weights of collapsed athletes should be readily available to medical personnel either electronically or on the athletes' race bib number. Medical directors should ensure the availability of onsite [Na^+] analysis to screen for EAH before medical treatment is initiated. A self audit regarding the incidence and outcome of EAH cases is strongly advised after completion of each yearly event. Since the condition is preventable, appropriate action should be taken to avert future recurrences.

Medical tent staff

Medical personnel within all medical areas must be educated on the signs, symptoms and treatment strategies of EAH. Mandatory weighing of all participants presenting to the medical tent is strongly advised. Whether symptomatic EAH should be treated with 3% NaCl in the medical tent prior to transfer to a hospital emergency facility has not been critically evaluated at this time. Decisions regarding use of 3% NaCl for treatment of EAH in the field will therefore depend on the level of expertise of the medical staff on site.

Emergency medical services and hospitals

Prior to the race or event, the medical team should establish a relationship with the local emergency teams, medical facilities and emergency room physicians. The availability of 3% NaCl should be confirmed and the administration of 3% NaCl should be guided by the treatment protocol detailed above.

Local media

The media should disseminate the same information that is provided to athletes and coaches.

8. What studies should be performed to better understand EAH?

Prospective and controlled clinical trials on fluid replacement during exercise should be performed both in the laboratory and in the field. An international registry of all cases of EAH should be established. The following areas were identified by the Panel as priorities for further study:

- Which cases of symptomatic EAH can be safely monitored and managed on site rather than transferred to hospital emergency rooms?

- What are the appropriate indications for administering hypertonic NaCl in symptomatic EAH? Should initiation of hypertonic NaCl begin onsite in the medical tent when symptoms are severe?

- Are oral hypertonic NaCl solutions of use in mild to moderate EAH? Could they be utilized when hypertonic NaCl solutions are unavailable (i.e., onsite)?

- What is the etiology of EAH when it occurs in association with weight loss?

- What are the mechanisms (renal, cardiovascular, hormonal) responsible for fluid retention during prolonged physical activity?

- What is the etiology/mechanism for inappropriate (i.e., non-suppressed) plasma AVP concentrations during endurance exercise?

- What is the rate of endogenous water production or release during endurance exercise, in particular the water complexed to muscle and liver glycogen? How does this reduce the need to ingest fluids to replace sweat losses?

Reference List

1. Almond CS, Shin AY, Fortescue EB, Mannix R, Wypij D. Hyponatremia among Runners in the Boston Marathon. N.Engl.J Med 2005;**352**:1550-6.

2. Hiller WDB. Dehydration and hyponatremia during triathlons. Med.Sci.Sports Exerc. 1989;**21(Suppl)**:219-21.

3. O'Toole ML, Douglas PS, Laird RH, Hiller DB. Fluid and electrolyte status in athletes receiving medical care at an ultradistance triathlon. Clin.J.Sport Med 1995;**5**:116-22.

4. Noakes TD, Goodwin N, Rayner BL, Branken T, Taylor RK. Water intoxication: a possible complication during endurance exercise. Med.Sci.Sports Exerc. 1985;**17**:370-5.

5. Speedy DB, Faris JG, Hamlin M, Gallagher PG, Campbell RG. Hyponatremia and weight changes in an ultradistance triathlon. Clin.J Sport Med 1997;**7**:180-4.

6. Stuempfle KJ, Lehmann DR, Case HS, Bailey S, Hughes SL, McKenzie J et al. Hyponatremia in a cold weather ultraendurance race. Alaska Med. 2002;**44**:51-5.

7. Davis DP, Videen JS, Marino A, Vilke GM, Dunford JV, Van Camp SP et al. Exercise-associated hyponatremia in marathon runners: a two-year experience. J.Emerg.Med. 2001;**21**:47-57.

8. Maron MB, Horvath SM, Wilkerson JE. Acute blood biochemical alterations in response to marathon running. Eur.J.Appl.Physiol Occup.Physiol 1975;**34**:173-81.

9. McKechnie JK, Leary WP, Noakes TD. Metabolic responses to a 90 km running race. S.Afr.Med.J. 1982;**61**:482-4.

10. Cohen I,.Zimmerman AL. Changes in serum electrolyte levels during marathon running. S.Afr.Med J. 1978;**53**:449-53.

11. Rose LI, Carroll DR, Lowe SL, Peterson EW, Cooper KH. Serum electrolyte changes after marathon running. J.Appl.Physiol 1970;**29**:449-51.

12. Kavanagh T,.Shephard RJ. On the choice of fluid for the hydration of middle-aged marathon runners. Br.J.Sports Med. 1977;**11**:26-35.

13. Kavanagh T, Shephard RH, Pandit V. Marathon running after myocardial infarction. JAMA 1974;**229**:1602-5.

14. Shephard RJ,.Kavanagh T. Fluid and Mineral Needs and Postcoronary. Physician Sports Med 1978;**6**:90-102.

15. Whiting PH, Maughan RJ, Miller JB. Dehydration and serum biochemical changes in marathon runners. Eur.J Appl.Physiol 1984;**52**:183-7.

16. National Institutes of Health (NIH), Office of the Director Office of Medical Applications of Research. Guidelines for the Planning and Management of NIH Consensus Development Conference Online. Bethesda (MD). http://consensus.nih.gov/about/process.htm . 10-1-0001.

17. Montain SJ, Latzka WA, Sawka MN. Fluid replacement recommendations for training in hot weather. Mil.Med 1999;**164**:502-8.

18. Kolka MA, Latzka WA, Montain SJ, Corr WP, O'Brien KK, Sawka MN. Effectiveness of revised fluid replacement guidelines for military training in hot weather. Aviat.Space Environ.Med. 2003;**74**:242-6.

19. Department of the Army. Policy Guidance for Fluid Replacement during Training. Washington DC. Office of the Surgeon General 2005.

20. Weschler LB. Exercise-associated hyponatremia: A mathematical review. Sports Med 2005; 35:899-922.

21. Armstrong LE, Curtis WC, Hubbard RW, Francesconi RP, Moore R, Askew EW. Symptomatic hyponatremia during prolonged exercise in heat. Med Sci.Sports Exerc 1993;**25**:543-9.

22. Backer HD, Shopes E, Collins SL, Barkan H. Exertional heat illness and hyponatremia in hikers. Am.J Emerg Med 1999;**17**:532-9.

23. Clark JM,.Gennari FJ. Encephalopathy due to severe hyponatremia in an ultramarathon runner. West J Med 1993;**159**:188-9.

24. Dugas JP,.Noakes TD. Case Report: Hyponatraemic encephalopathy despite a modest rate of fluid intake during a 109 km cycle race. Br J Sports Med 2005; 39:e38.

25. Flinn SD,.Sherer RJ. Seizure after exercise in the heat. Physician Sports Med 2000;**28**:61-7.

26. Frizzell RT, Lang GH, Lowance DC, Lathan SR. Hyponatremia and ultramarathon running. JAMA 1986;**255**:772-4.

27. Gardner JW. Death by water intoxication. Mil.Med. 2002;**167**:432-4.

28. Garigan TP,.Ristedt DE. Death from hyponatremia as a result of acute water intoxication in an Army basic trainee. Mil.Med. 1999;**164**:234-8.

29. Galun E, Tur-Kaspa I, Assia E, Burstein R, Strauss N, Epstein Y et al. Hyponatremia induced by exercise: a 24-hour endurance march study. Miner.Electrolyte Metab 1991;**17**:315-20.

30. Hew TD, Chorley JN, Cianca JC, Divine JG. The incidence, risk factors, and clinical manifestations of hyponatremia in marathon runners. Clin.J.Sport Med. 2003;**13**:41-7.

31. Hiller DB, O'Toole ML, Fortress EE, Laird RH, Imbert PC, Sisk TD. Medical and physiological considerations in triathlons. Am.J.Sports Med. 1987;**15**:164-8.

32. Hsieh M, Roth R, Davis DL, Larrabee H, Callaway CW. Hyponatremia in runners requiring on-site medical treatment at a single marathon. Med.Sci.Sports Exerc. 2002;**34**:185-9.

33. Irving RA, Noakes TD, Buck R, van Zyl SR, Raine E, Godlonton J et al. Evaluation of renal function and fluid homeostasis during recovery from exercise-induced hyponatremia. J.Appl.Physiol 1991;**70**:342-8.

34. Nelson PB, Robinson AG, Kapoor W, Rinaldo J. Hyponatremia in a Marathoner. Physician Sports Med 1988;**16**:78-87.

35. Noakes TD, Sharwood K, Collins M, Perkins DR. The dipsomania of great distance: water intoxication in an Ironman triathlete. Br.J.Sports Med. 2004;**38**:E16.

36. O'Brien KK, Montain SJ, Corr WP, Sawka MN, Knapik JJ, Craig SC. Hyponatremia associated with overhydration in U.S. Army trainees. Mil.Med 2001;**166**:405-10.

37. Reynolds NC, Jr., Schumaker HD, Feighery S. Complications of fluid overload in heat casualty prevention during field training. Mil.Med 1998;**163**:789-91.

38. Speedy DB, Campbell R, Mulligan G, Robinson DJ, Walker C, Gallagher P et al. Weight changes and serum sodium concentrations after an ultradistance multisport triathlon. Clin.J Sport Med 1997;**7**:100-3.

39. Speedy DB, Noakes TD, Rogers IR, Thompson JM, Campbell RG, Kuttner JA et al. Hyponatremia in ultradistance triathletes. Med.Sci.Sports Exerc. 1999;**31**:809-15.

40. Speedy DB, Rogers IR, Noakes TD, Wright S, Thompson JM, Campbell R et al. Exercise-induced hyponatremia in ultradistance triathletes is caused by inappropriate fluid retention. Clin.J.Sport Med. 2000;**10**:272-8.

41. Speedy DB, Noakes TD, Rogers IR, Hellemans I, Kimber NE, Boswell DR et al. A prospective study of exercise-associated hyponatremia in two ultradistance triathletes. Clin.J.Sport Med. 2000;**10**:136-41.

42. Speedy DB, Rogers IR, Noakes TD, Thompson JM, Guirey J, Safih S et al. Diagnosis and prevention of hyponatremia at an ultradistance triathlon. Clin.J.Sport Med. 2000;**10**:52-8.

43. Surgenor S,.Uphold RE. Acute hyponatremia in ultra-endurance athletes. Am.J Emerg Med 1994;**12**:441-4.

44. Twerenbold R, Knechtle B, Kakebeeke TH, Eser P, Muller G, von Arx P et al. Effects of different sodium concentrations in replacement fluids during prolonged exercise in women. Br.J.Sports Med. 2003;**37**:300-3.

45. Young M, Sciurba F, Rinaldo J. Delirium and pulmonary edema after completing a marathon. Am.Rev.Respir.Dis. 1987;**136**:737-9.

46. Zelingher J, Putterman C, Ilan Y, Dann EJ, Zveibil F, Shvil Y et al. Case series: hyponatremia associated with moderate exercise. Am.J Med Sci. 1996;**311**:86-91.

47. Kratz A, Lewandrowski KB, Siegel AJ, Chun KY, Flood JG, Van Cott EM et al. Effect of marathon running on hematologic and biochemical laboratory parameters, including cardiac markers. Am.J.Clin.Pathol. 2002;**118**:856-63.

48. Speedy DB, Rogers IR, Safih S, Foley B. Profound hyponatremia and seizures in an Ironman triathlete. J Emerg Med 2000;**18**:41-4.

49. Glace, B. W. Hyponatremia and Rhabdomyolysis - Runner. Med Sci.Sports Exerc. 35(5), S261. 2003.

50. Vrijens DM,.Rehrer NJ. Sodium-free fluid ingestion decreases plasma sodium during exercise in the heat. J Appl.Physiol 1999;**86**:1847-51.

51. Verbalis JG. Disorders of body water homeostasis. Best.Pract.Res.Clin.Endocrinol.Metab 2003;**17**:471-503.

52. Arieff AI. Neurological manifestations and morbidity of hyponatremia: correlation with brain water and electrolytes. Medicine (Baltimore) 1976;**55**:121.

53. Gardner JW,.Gutmann FD. Fatal water intoxication of an Army trainee during urine drug testing. Mil.Med. 2002;**167**:435-7.

54. Ayus JC, Varon J, Arieff AI. Hyponatremia, cerebral edema, and noncardiogenic pulmonary edema in marathon runners. Ann Intern.Med 2000;**132**:711-4.

55. Herfel R, Stone CK, Koury SI, Blake JJ. Iatrogenic acute hyponatraemia in a college athlete. Br.J.Sports Med 1998;**32**:257-8.

56. Thompson J,.Wolff A.J. Hyponatremic encephalopathy in a marathon runner. Chest 2003;**124**:313S.

57. Noakes TD, Wilson G, Gray DA, Lambert MI, Dennis SC. Peak rates of diuresis in healthy humans during oral fluid overload. S.Afr.Med.J. 2001;**91**:852-7.

58. Speedy DB, Noakes TD, Boswell T, Thompson JM, Rehrer N, Boswell DR. Response to a fluid load in athletes with a history of exercise induced hyponatremia. Med.Sci.Sports Exerc. 2001;**33**:1434-42.

59. Knepper MA. Urinary Concentrating Mechanism. In Brenner B, ed. The Kidney, London: W.B. Saunders, 2003.

60. Speedy DB, Rogers I, Safih S, Foley B. Hyponatremia and seizures in an ultradistance triathlete. J.Emerg.Med. 2000;**18**:41-4.

61. Wade CE. Response, regulation, and actions of vasopressin during exercise: a review. Med Sci Sports Exerc 1984;**16**:506-11.

62. Speedy DB, Noakes TD, Kimber NE, Rogers IR, Thompson JM, Boswell DR et al. Fluid balance during and after an ironman triathlon. Clin.J.Sport Med. 2001;**11**:44-50.

63. Smith HR, Dhatt GS, Melia WM, Dickinson JG. Cystic fibrosis presenting as hyponatraemic heat exhaustion. BMJ 1995;**310**:579-80.

64. Spital,A. Diuretic-induced hyponatremia. Am J Nephrol 1999;**19**:447-452.

65. Schwartz WB, Bennett W, Curelop S, Bartter FC. A Syndrome of Renal Sodium Loss and Hyponatremia Probably Resulting from Inappropriate Secretion of Antidiuretic Hormone. Am J Med 1957;**23**:529-42.

66. Speedy DB, Thompson JM, Rodgers I, Collins M, Sharwood K, Noakes TD. Oral salt supplementation during ultradistance exercise. Clin.J.Sport Med. 2002;**12**:279-84.

67. Barr SI, Costill DL, Fink WJ. Fluid replacement during prolonged exercise: effects of water, saline, or no fluid. Med Sci.Sports Exerc 1991;**23**:811-7.

68. Orenstein DM, Henke KG, Costill DL, Doershuk CF, Lemon PJ, Stern RC. Exercise and heat stress in cystic fibrosis patients. Pediatr.Res. 1983;**17**:267-9.

69. Bar-Or O, Blimkie CJ, Hay JA, MacDougall JD, Ward DS, Wilson WM. Voluntary dehydration and heat intolerance in cystic fibrosis. Lancet 1992;**339**:696-9.

70. Stanghelle JK, Maehlum S, Skyberg D, et.al. Biochemical changes and endocrine responses in cystic fibrosis in relation to a marathon race. Int.J Sports Med 1988;**9 (suppl.)**:45-50.

71. Kriemler S, Wilk B, Schurer W, Wilson WM, Bar-Or O. Preventing dehydration in children with cystic fibrosis who exercise in the heat. Med Sci.Sports Exerc. 1999;**31**:774-9.

72. Reid SA, Speedy DB, Thompson JM, Noakes TD, Mulligan G, Page T et al. A study of haematological and biochemical parameters in runners completing a standard marathon. Clin.J.Sport Med. 2004;**14**:344-53.

73. Noakes TD. Fluid Replacement during Marathon Running. Clin.J.Sport Med 2003;**13**:309-18.

74. Casa, D. J. USATF Self-Testing Program for Optimal Hydration. http://www.org/groups/Coaches/library/hydration/USATFSelfTestingforOptimalHydration.pdf . 2003.

75. Sharwood K, Collins M, Goedecke J, Wilson G, Noakes T. Weight changes, medical complications and performance during an Ironman triathlon. Br J Sports Med 2004;**38**:718-24.

76. Noakes TD. Sodium ingestion and the prevention of hyponatraemia during exercise. Br J Sports Med 2004;**38**:790-3.

77. Sawka MN. Physiological consequences of hypohydration: exercise performance and thermoregulation. Med Sci Sports Exerc 1992;**24**:657-70.

78. Kratz A, Siegel AJ, Verbalis JG, Adner MM, Shirey T, Lee-Lewandrowski E et al. Sodium status of collapsed marathon runners. Arch.Pathol.Lab Med. 2005;**129**:227-30.

79. Ayus JC, Olivero JJ, Frommer JP. Rapid correction of severe hyponatremia with intravenous hypertonic saline solution. Am.J.Med 1982;**72**:43-8.

80. Ayus JC,.Arieff AI. Chronic hyponatremic encephalopathy in postmenopausal women: association of therapies with morbidity and mortality. JAMA 1999;**281**:2299-304.

81. Ayus JC, Wheeler JM, Arieff AI. Postoperative hyponatremic encephalopathy in menstruant women. Ann.Intern.Med 1992;**117**:891-7.

82. Ayus JC, Krothapalli RK, Arieff AI. Treatment of symptomatic hyponatremia and its relation to brain damage. A prospective study. N.Engl.J.Med. 1987;**317**:1190-5.

83. Ayus JC,.Arieff AI. Pulmonary complications of hyponatremic encephalopathy. Noncardiogenic pulmonary edema and hypercapnic respiratory failure. Chest 1995;**107**:517-21.

84. Verbalis JG. The Syndrome of Inappropriate Antidiuretic Hormone Secretion and Other Hypoosmolar Disorders. In Schrier RW, ed. Diseases of the Kidney, pp 2511-48. Philadelphia: Lippincott, 2001.

85. Berl T. Mannitol: a therapeutic alterntive for treatment of acute hyponatremia. Crit Care Med. 2000;**28**:2152-3.

86. Cheng JC, Zikos D, Skopicki HA, et.al. Long-term neurological outcome in psychogenic water drinkers with severe symptomatic hyponatremia: the effect of rapid correction. Am J Med 1990;**88**:561-6.

87. Ayus JC, Arieff A, Moritz M. Treatment of Marathon associated hyponatremia. N Eng J Med. 2005;353:427-8..

Study 1A: Sodium supplementation is not required to maintain serum sodium concentrations during an Ironman Triathlon

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INTRODUCTION

Current doctrine advises that athletes ingest between 460 – 920mg/L (20 -40 mmol L^{-1}) of sodium (Na^+) during exercise [1-4]. The prevailing theory argues that salt ingestion is the sole method by which a fall in serum Na^+ concentration ([Na^+]) can be prevented during prolonged exercise. This hypothesis is based on the model which predicts that Na^+ losses during exercise are large [2;4], especially in "salty sweaters" [5] including those with the gene for cystic fibrosis [4-6]. Failure to replace large Na^+ losses is then believed to cause a progressive fall in serum [Na^+] leading to the hyponatremia of exercise and, ultimately, hyponatremic encephalopathy. It is further argued that this mechanism becomes important during ultradistance events such as the 90km Comrades Marathon [4] and the 226km Ironman triathlon [5] where total sweat Na^+ losses might be as high as 400-650 mmol [4].

Guidelines promoting increased Na^+ intake during exercise conflict with those recently released by the Institute of Medicine (IOM) [7]. The IOM argues that the daily adequate intake of Na^+, which is 65 mmol or 1.5 g, is sufficient for physically active individuals. Since the median daily Na^+ intake in the United States is between 3.1 – 4.7 g (135 – 204 mmol) for men and 2.3 - 3.1 g (100 - 135 mmol) for women, additional supplementation seems unnecessary in athletes who ingest a Western diet. Furthermore, the body has appropriate defense mechanisms to protect against the development of a Na^+ deficit during prolonged exercise. Two immediate defense mechanisms are the release of Na^+ from internal body stores [8-11] or contraction of the extracellular fluid (ECF) volume. Contraction of the ECF volume by as little as 1 liter (~7%) would release 140mmol (~3 g) of Na^+, equivalent to the amount of Na^+ present in

7.8L of the popular sports drinks Gatorade® (USA) or Energade® (South Africa) or 28L of Powerade® (USA) [12].

Accordingly, to determine the potential role of Na^+ supplementation during very prolonged exercise, this study was designed to answer the following questions: (i) Do athletes who ingest additional Na^+ during a 226 km Ironman Triathlon maintain higher serum [Na^+] than those who do not ingest additional Na^+ ?; (ii) Are those athletes who fail to ingest additional Na^+ during the Ironman Triathlon able to maintain their serum [Na^+] within the normal range, or are they at increased risk for the development of hyponatremia ?; (iii) Is Na^+ supplementation associated with superior performance in the Ironman triathlon; and (iv) Does Na^+ supplementation reduce the probability that athletes will require medical care after finishing the race ?

METHODS

One hundred and forty-five triathletes competing in the 2001Cape Town Ironman Triathlon (3.8 km swim, 180 km cycle and 42.2 km run) volunteered to participate in this study and were randomly assigned into either a control (placebo) or experimental (Na^+ supplementation) group. Before participating in this trial, each athlete signed an informed consent form. The study was approved by the Research and Ethics Committee of the Faculty of Health Sciences of the University of Cape Town.

Three days before the race, subjects were weighed (Adamlab JPS electronic scales, South Africa) in racing attire and a baseline blood sample was obtained from an antecubital vein to measure serum [Na^+] (Easylyte PLUS Na/K/Cl analyzer, Bedford MA). Participants in the control group were provided with 40 "placebo" tablets, each filled with 596mg of starch (Cakes pride™ superfine corn flour). Subjects in the experimental group were given 40 identical looking tablets, each containing 620mg of table salt (244mg/10.6 mmoles of Na^+ with 376mg/ 10.6 mmoles of Cl^-). Both the Na^+ containing and placebo tablets were prepared by the researchers. All athletes were asked to ingest tablets "*ad libitum*" within a suggested range of 1-4 tablets every hour

and were discouraged from taking any other electrolyte-containing tablets during the race. After the race, all subjects returned their packets so that any remaining tablets could be counted. From this, each subject's intake of Na^+ or placebo tablets could be accurately quantified. Food and fluid intake were allowed *ad libitum* and were not considered in this analysis. All subjects confirmed that they had not ingested electrolyte-containing tablets other than those provided as part of the trial.

During the race, athletes were provided with access to water or the sports drink (Energade® [Na^+] = 18mmol/L) at stations placed every 20km in the cycling leg and every 2.5km in the running leg. Seconding stations placed this infrequently are associated with a reduced incidence of hyponatremia in the Ironman Triathlon [13 14].

Upon completion of the race, all subjects were immediately weighed and a venous blood sample obtained for measurement of post race [Na^+]. Blood pressure and rectal temperatures were then recorded according to the methods previously described [15]. Each athlete was then examined clinically for indicators of post-race fluid status including the presence or absence of sweating, the ability to form saliva (spit test), measurements of skin turgor (pinch test) and swelling of the hands (observation of the tightness of fit of rings and watches). Where indicated, an appropriate clinical diagnosis was made and recorded in any triathlete requiring medical care after the race.

Athletes in the experimental trial were asked specific questions regarding perceived exercise intensity during the race, post-race muscle soreness and mental wellness, all rated on a scale of 0 – 10 (Table 1A.1).

Data were analyzed using the STATISTICA 7.0 (Tulsa, OK, USA) software program. Using the one-way ANOVA technique, the experimental and control groups were compared alone and with a group consisting of all the other triathletes in the race in which these same measurements had been made (n = 299). The Kruskal-Wallis variation was used to evaluate non-parametric data. Statistical significance was set at $p < 0.05$.

RESULTS

Five hundred and ninety athletes successfully completed the Ironman triathlon. Air temperature during the race ranged from 15.6°C to 20.9°C with a midday value of 20°C and an average value of 17.2°C. Average humidity was 63% with a maximum value of 79% and a minimum value of 48%. Sea temperature was 15°C. Average wind speed was 6.4 m s^{-1} with maximum gusts of 22.3 m/s (81 km h^{-1}).

One hundred and fourteen of the 145 athletes ingested the supplemental tablets and had complete pre and post-race data collected. Of these 114 athletes, 53 were in the experimental group and 61 in the control group. Two hundred and ninety-nine triathletes, who voluntarily participated in other medical research, were included as a third comparison group. This comparison group had identical measurements obtained during this triathlon [15] but did not ingest either placebo or Na+ supplements during the race. Only 8-10% of the participants were female including four (8%) in the experimental, six (10%) in the placebo and 26 (9%) in the comparison group. The small number of female competitors precluded further evaluation of the effect of gender on serum [Na^+] and fluid balance during the race.

There were no significant differences between the three groups for age, finishing time, pre-race [Na^+], post-race [Na^+], pre-race weight, weight change during the race, percent dehydration during the race, post-race rectal temperature, post-race systolic or diastolic blood pressures or in the prevalence of medical diagnoses (Table 1A.2). Mean post-race serum [Na+] was unchanged from pre-race concentrations in all groups. Clinical measures of post-race fluid status were also not significantly different between groups.

The experimental group ingested an average of 14.7 ± 8.3 (mean ± SD) salt tablets (156 ± 88 mmol Na^+) while the average number of starch tablets consumed by the placebo group was 15.8 ± 10.1 ($p = 0.55$; NS). Measures of exercise intensity, muscle soreness and mental wellness were also not significantly different between the control and experimental groups (Table 1A.3).

A significant inverse correlation was noted between the post-race serum [Na^+] and the body weight change in all groups (Figure 1A.1) so that serum [Na^+] increased with increasing body weight loss during exercise. The slope of this relationship was not different between groups. The athlete who developed hyponatremic encephalopathy during the race is identified (arrow).

Table 1A.1: Measurement scales for exercise intensity, muscle soreness and mental wellness.

EXERCISE INTENSITY	
0	Rest
1	Very easy
2	Easy
3	Moderate
4	Somewhat hard
5	Hard
6	
7	Very hard
8	
9	Very, very hard
10	Maximal

MUSCLE SORENESS	
0	No soreness
1	Very little soreness
2	Little soreness
3	Moderate soreness
4	Somewhat intense soreness
5	Intense soreness
6	
7	Very intense soreness
8	
9	Very, very intense soreness
10	Maximal soreness

MENTAL WELLNESS	
0	Best I've every felt
1	Fell great
2	Fell good
3	Fell okay
4	Somewhat depressed
5	Depressed
6	
7	Very depressed
8	
9	Very, very depressed
10	Maximally depressed

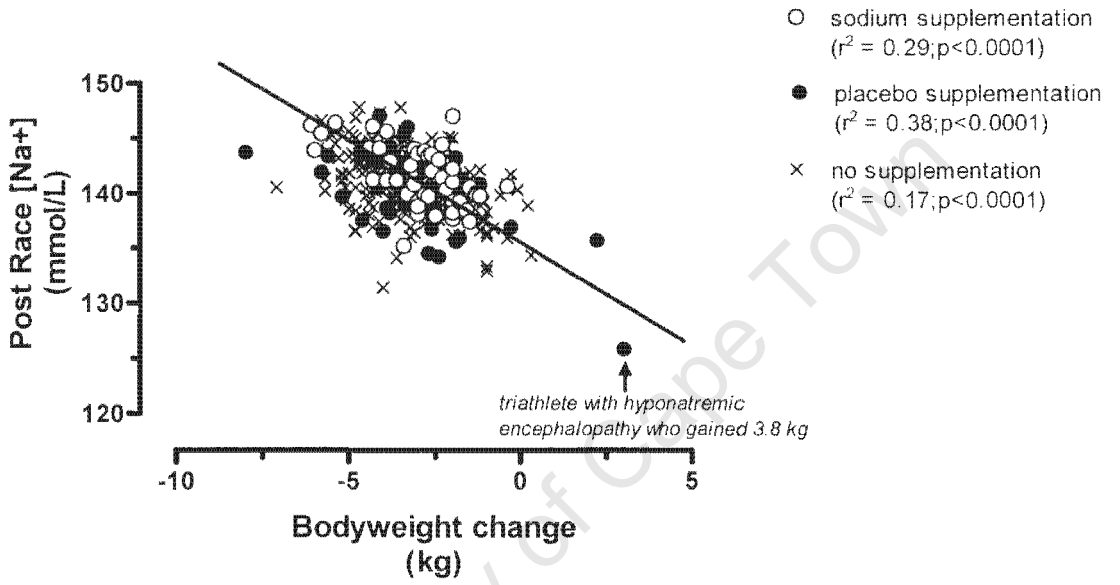
Table1A.2: Measured variables in three groups of triathletes completing the 2001 South African Ironman Triathlon (mean ± SD).

Variable	Experimental Group (N=53)	Placebo Group (N=61)	Comparison Group (N=299)	Average (N=413)	P-value
Age (years)	33.4 ± 7.4	33.9 ± 7.3	35.3 ± 8.1	35.0 ± 8.0	0.09
Finish time (minutes)	758.3 ± 87.6	762.0 ± 100.7	741.3 ± 96.7	745.4 ± 96.4	0.14
Pre-race [Na^+] (mmol/L)	140.6 ± 1.7	140.7 ± 1.7	140.5 ± 1.7	140.5 ± 1.7	0.67
Post-race [Na^+] (mmol/L)	141.5 ± 2.7	140.5 ± 3.5	140.9 ± 2.7	140.9 ± 2.8	0.15
Pre-race weight (kg)	78.2 ± 9.6	75.7 ± 10.2	76.4 ± 10.5	76.5 ± 10.4	0.36
Pre- to Post-race Weight change (kg)	-2.9 ± 1.3	-3.0 ± 1.7	-3.1 ± 1.3	-3.1 ± 1.4	0.36
Pre- to Post-race Dehydration (%)	-3.6 ± 1.4	-3.9 ± 2.1	-4.0 ± 1.6	-3.9 ± 1.7	0.16
Post Race Rectal Temperature (°C)	37.1 ± 0.6	37.4 ± 0.7	37.2 ± 0.9	37.2 ± 0.9	0.46
Post-race Systolic Blood Pressure (mmHg)	112 ± 13	109 ± 14	112 ± 17	112 ± 16	0.91
Post-race Diastolic Blood Pressure (mmHg)	72 ± 9	73 ± 10	73 ± 10	73 ± 10	0.59

Table 1A.3: Subjective assessment of physical condition at the end of the race in the experimental and control groups.

Variable	Experimental	Control	Average	P-value
Exercise Intensity	6.3 ± 2.1	6.5 ± 2.0	6.4 ± 2.1	0.59
Muscle Soreness	3.9 ± 2.1	4.3 ± 2.2	4.1 ± 2.1	0.40
Mental Wellness	1.8 ± 1.3	1.9 ± 1.2	1.9 ± 1.3	0.62
Tablets consumed	14.7 ± 8.3	15.8 ± 10.1	15.2 ± 9.1	0.55

Figure 1A.1: Post race [Na^+] versus bodyweight change in 413 athletes completing the 2001 South African Ironman Triathlon in the sodium, placebo and no supplement groups.



Legend to Figure 1:

**Note that the sole athlete in this trial to develop hyponatremic encephalopathy (arrow) was also the athlete who gained the most weight (3.8 kg) during the race.*

DISCUSSION

The first important finding of this study was that triathletes who ingested either placebo or salt tablets "*ad libitum*", or who ate and drank as they would normally, all maintained their serum [Na^+] within the normal range while exercising for a mean duration of ~12½ hours (Table 1A.2; Figure 1A.1). The sole exception (arrowed in Figure 1A.1) was one triathlete in the placebo group who gained the most weight during exercise because he drank to excess, ultimately developing hyponatremic encephalopathy, for which he required hospitalization for 12 hours [14].

In this study, subjects in the experimental group ingested an average of 156 mmoles Na^+ (3.6g) in addition to any Na^+ they might have imbibed either in sports drinks or foods consumed during the race. These 156 mmoles Na^+ is equivalent to the Na^+ present in nine liters of Gatorade® or Energade® or 32L of Powerade®. If, in addition, subjects in the experimental group had ingested another 7.5L of sports drinks (as seems probable since this equates to a fluid intake rate of 600 ml/hr for 12.75 hours), their total Na^+ intake would have been only ~297 mmoles during the Ironman race. This combined intake is substantially less than the calculated sweat Na+ loss of 400-650 mmol postulated to occur during even only nine hours of prolonged exercise [4]. Yet hyponatremia did not occur, even though it was highly probable that the majority of these athletes developed an acute whole body Na^+ deficit which may have been substantial in most of the athletes we studied.

Our finding that Na^+ supplementation did not alter the response of the serum [Na^+] during prolonged exercise supports the findings from our previous laboratory studies during which subjects drank sufficiently to replace either 50 or 100% of the weight they lost during 3 hours of laboratory exercise [16;17], as well as our earlier uncontrolled field study of competitors in the 2000 South African Ironman triathlon [18]. The absence of an adequate control group in our previous Ironman Triathlon study limited the scientific strength of that particular finding.

Based on these studies, we can reasonably conclude that it is unnecessary to ingest additional Na^+ supplementation during prolonged endurance exercise in order to maintain the serum [Na^+] within the normal range.

Historical evidence verifies that when athletes were advised ***not*** to drink during exercise [19] and hence did not ingest any Na^+ during prolonged exercise, they completed prolonged exercise with elevated serum [Na^+] [20-22]. For example, in 1990 we showed that only one of 101 competitors in the 1986 South African 186 km ultradistance triathlon developed asymptomatic hyponatremia (lowest serum [Na^+] = 131 mmol/L), whereas the mean post race serum [Na^+] was 142 mmol/L compared to the pre-race value of 143 mmol/L. This was despite the fact that the only fluids available during the race were water and Coca-Cola®, which has a very low [Na^+] (3.4 mmol/L) [23]. Opportunities for fluid replacement during that event were quite limited.

However, despite this evidence to the contrary, two studies are frequently quoted as evidence that Na^+ ingestion during exercise is essential if a progressive fall in serum [Na+] is to be prevented. Both studies contain important logical flaws, not least because they encouraged trial subjects to drink to excess during exercise.

The goal of the study of Vrijens and Rehrer was for athletes to drink sufficiently to insure that they did not lose weight during two hours of laboratory exercise [24]. Normal fluid balance during exercise requires that some weight must be lost due to (i) the release of stored water consequent to glycogenolysis, and (ii) irreversible loss of fuel through substrate oxidation [25-27]. As a result, athletes in any trial who do not lose weight during exercise must complete the trial in a mild state of over-hydration. That study therefore evaluated the effect of Na^+ supplementation on serum [Na^+] in subjects encouraged to over-drink during prolonged exercise. The data show that the response of the serum [Na^+] to overdrinking was determined by the renal response to exercise so that those athletes who passed the most urine during exercise were best able to maintain their serum [Na^+]. This is compatible with the conclusion that the serum [Na^+] is far more sensitive to changes in total body water [TBW] than to Na^+ balance during prolonged

exercise [28] and to the explanation that acute hyponatremia is always due to altered renal function in which the rate of free water clearance fails to match the rate of free water ingestion, whether at rest or during exercise [29]. Finally only 4 of 10 subjects completed all trials in that study, further limiting the validity of these findings.

Similarly Twerenbold et al studied athletes who drank to excess while running ~40km in 4 hours [1]. Since sweat rates were only ~500ml/hr whereas rates of fluid ingestion were ~1000ml/hr, subjects gained an average of 2kg weight during the run. In the presence of this large weight gain, the ingestion of additional [Na^+] predictably lessened the fall in serum [Na^+] by about 2-3 mmol/L. Yet, despite the ingestion of an additional 118 mmol Na^+, the group that ingested the most Na^+ still developed marked hyponatremia during exercise (mean post run serum [Na^+] = 134 mmol/L). The authors' suggestion that their data prove that all athletes should ingest additional Na^+ during exercise is incorrect, as fully argued elsewhere [12]. Rather the correct conclusion is that since the single best predictor of the post-exercise serum Na^+ is the change in body mass during exercise (Figure 1A.1), avoidance of overhydration is the most important intervention necessary to prevent the development of exercise associated hyponatremia [14;30-32].

Another line of argument used by the proponents of Na^+ supplementation during exercise is their claim that athletes carrying the gene for cystic fibrosis have much higher sweat [Na^+] than normal. As a result, these "salty sweaters" are at increased risk of developing hyponatremia during exercise [4;5]. However, the published evidence contradicts this conclusion. Four separate studies show that subjects with cystic fibrosis maintain their serum [Na^+] within the normal range during exercise [33-36]. These studies clearly establish that even patients with cystic fibrosis and who excrete a "salty sweat" during exercise, do not need to replace their excessive Na^+ losses during exercise in order to maintain their serum [Na^+] within the normal range.

The second important finding of our study was that Na^+ supplementation was not associated with any difference in triathlon racing performance. The intervention group finished the race 13 minutes slower than the mean time for all other triathletes (758.3 ±

87.6 versus 745.4 ± 96.4; p=0.14, NS) with no significant differences in finishing times between any of the groups (Table 1A.2). An absence of association in a cross sectional study does not, of course, exclude the possibility that a carefully controlled prospective trial might reveal such a relationship.

Subjective scores for exercise intensity and mental wellness were also not significantly different between the groups (Table 1A.3) suggesting that Na^+ supplementation did not alter the perception of fatigue during the race.

The third important finding was that Na^+ supplementation did not alter the probability of requiring medical care after the race. Weight change and percent weight loss during the race, clinical measures of fluid status, and post race rectal temperatures and blood pressures did not differ significantly between triathletes in the different groups (Table 1A.2). There were also no significant differences in the degree of muscle soreness experienced by triathletes in the different groups (Table 1A.3).

Finally, subjects in the experimental and control groups chose to ingest a similar number of supplemental tablets (14.7 ± 8.3 versus 15.8 ± 10.1 respectively; p=0.55, NS). This number was substantially less than the recommended intake of 1-4 tablets per hour. Our finding that athletes in the control group did not ingest more tablets than those in the experimental group (who would have been less Na+ deficient since they ingested an additional 156 mmol Na^+) suggests that neither group "craved" Na^+, as is the normal response in mammals who are Na^+ deficient [37-39]. Had a Na^+ deficiency been present, one would have assumed that (i) the control group would have ingested more tablets than the experimental group, in an attempt to correct their greater Na^+ deficit, whereas (ii) the experimental group would have ingested closer to the 400-600 mmol Na^+ that reasonable calculations suggest is lost during the Ironman triathlon.

CONCLUSION

Triathletes competing in the 2001 South African Ironman Triathlon maintained their serum [Na^+] within the normal range whether they drank the Na^+-poor drinks (water or a sports drink with [Na^+] = 18mmol/L) or whether they supplemented with ~156 mmol Na^+. The sole athlete to develop hyponatremic encephalopathy was also the only athlete to show a substantial (3.8kg) weight gain during the race [14].

Na^+ supplementation was not associated with a difference in triathlon performance. Athletes in the placebo, sodium supplementation and "no" supplementation groups did not differ in their finishing time nor in subjective measures of exercise intensity and mental wellness, nor in the prevalence of post-race medical diagnoses.

Clinical measures of fluid status, rectal temperature, blood pressure, absolute and percent weight loss were also not different between groups.

Therefore, predictions of the expected consequences of "large" Na^+ losses during prolonged exercise are inaccurate either because athletes sweat less or have lower sweat [Na^+] than are currently believed. Alternatively, during acute states of Na^+ loss, additional Na^+ may be released from either intracellular body stores (i.e. bone, skin) or by contraction of the ECF volume, in order to buffer acute Na^+ losses until these are replenished by Na+ ingestion during the next meal.

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Reference List

1. Twerenbold R, Knechtle B, Kakebeeke TH, Eser P, Muller G, von Arx P *et al.* Effects of different sodium concentrations in replacement fluids during prolonged exercise in women. *Br.J.Sports Med.* 2003;**37**:300-3.

2. Coyle EF. Fluid and fuel intake during exercise. *J.Sports Sci.* 2004;**22**:39-55.

3. Convertino VA, Armstrong LE, Coyle EF, Mack GW, Sawka MN, Senay LC, Jr. *et al.* American College of Sports Medicine position stand. Exercise and fluid replacement. *Med Sci.Sports Exerc* 1996;**28**:i-vii.

4. Montain SJ, Sawka MN, Wenger CB. Hyponatremia associated with exercise: risk factors and pathogenesis. *Exerc.Sport Sci.Rev.* 2001;**29**:113-7.

5. Murray B,.Eichner ER. Hyponatremia of Exercise. *Current Sports Medicine Reports* 2004;**3**:117-8.

6. Smith HR, Dhatt GS, Melia WM, Dickinson JG. Cystic fibrosis presenting as hyponatraemic heat exhaustion. *BMJ* 1995;**310**:579-80.

7. Institute of Medicine of the National Academies. Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate. Washington D.C.: The National Academies Press, 2004.

8. Heer M, Baisch F, Kropp J, Gerzer R, Drummer C. High dietary sodium chloride consumption may not induce body fluid retention in humans. *Am.J.Physiol Renal Physiol* 2000;**278**:F585-F595.

9. Titze J, Maillet A, Lang R, Gunga HC, Johannes B, Gauquelin-Koch G *et al.* Long-term sodium balance in humans in a terrestrial space station simulation study. *Am.J.Kidney Dis.* 2002;**40**:508-16.

10. Titze J, Krause H, Hecht H, Dietsch P, Rittweger J, Lang R *et al.* Reduced osmotically inactive Na storage capacity and hypertension in the Dahl model. *Am.J.Physiol Renal Physiol* 2002;**283**:F134-F141.

11. Titze J, Lang R, Ilies C, Schwind KH, Kirsch KA, Dietsch P *et al.* Osmotically inactive skin Na+ storage in rats. *Am.J.Physiol Renal Physiol* 2003;**285**:F1108-F1117.

12. Noakes TD. Sodium ingestion and the prevention of hyponatraemia during exercise. *Br J Sports Med* 2004;**38**:790-3.

13. Speedy DB, Rogers IR, Noakes TD, Thompson JM, Guirey J, Safih S *et al.* Diagnosis and prevention of hyponatremia at an ultradistance triathlon. *Clin.J.Sport Med.* 2000;**10**:52-8.

14. Noakes TD, Sharwood K, Collins M, Perkins DR. The dipsomania of great distance: water intoxication in an Ironman triathlete. *Br.J.Sports Med.* 2004;**38**:E16.

15. Sharwood K, Collins M, Goedecke J, Wilson G, Noakes T. Weight changes, medical complications and performance during an Ironman triathlon. *Br J Sports Med* 2004;**38**:718-24.

16. Sanders B, Noakes TD, Dennis SC. Sodium replacement and fluid shifts during prolonged exercise in humans. *Eur.J.Appl.Physiol* 2001;**84**:419-25.

17. Sanders B, Noakes TD, Dennis SC. Water and electrolyte shifts with partial fluid replacement during exercise. *Eur.J.Appl.Physiol Occup.Physiol* 1999;**80**:318-23.

18. Speedy DB, Thompson JM, Rodgers I, Collins M, Sharwood K, Noakes TD. Oral salt supplementation during ultradistance exercise. *Clin.J.Sport Med.* 2002;**12**:279-84.

19. Noakes TD. Fluid replacement during exercise. *Exerc.Sport Sci.Rev.* 1993;**21**:297-330.

20. Riley WJ, Pyke FS, Roberts AD, England JF. The effect of long-distance running on some biochemical variables. *Clin.Chim.Acta* 1975;**65**:83-9.

21. Noakes TD,.Carter JW. Biochemical parameters in athletes before and after having run 160 kilometres. *S.Afr.Med.J.* 1976;**50**:1562-6.

22. Muir AL, Percy-Robb IW, Davidson IA, Walsh EG, Passmore R. Physiological aspects of the Edinburgh commonwealth games. *Lancet* 1970;**2**:1125-8.

23. Noakes TD, Norman RJ, Buck RH, Godlonton J, Stevenson K, Pittaway D. The incidence of hyponatremia during prolonged ultraendurance exercise. *Med.Sci.Sports Exerc.* 1990;**22**:165-70.

24. Vrijens DM,.Rehrer NJ. Sodium-free fluid ingestion decreases plasma sodium during exercise in the heat. *J Appl.Physiol* 1999;**86**:1847-51.

25. Pastene J, Germain M, Allevard AM, Gharib C, Lacour JR. Water balance during and after marathon running. *Eur.J Appl.Physiol Occup.Physiol* 1996;**73**:49-55.

26. Kavanagh T,.Shephard RJ. On the choice of fluid for the hydration of middle-aged marathon runners. *Br.J.Sports Med.* 1977;**11**:26-35.

27. Rogers G, Goodman C, Rosen C. Water budget during ultra-endurance exercise. *Med Sci.Sports Exerc* 1997;**29**:1477-81.

28. Weschler LB. Exercise-associated hyponatremia: A mathematical review. *Sports Med* 2005; 35:899-922.

29. Wolfson AB. Acute hyponatremia in ultra-endurance athletes. *Am.J.Emerg.Med.* 1995;**13**:116-7.

30. Noakes TD. Overconsumption of fluids by athletes. *BMJ* 2003;**327**:113-4.

31. Noakes TD,.Martin DE. IMMDA-Aims Advisory statement on guidelines for fluid replacement during marathon running. *TBA* 2004;1-19.

32. Noakes TD, Sharwood KA, Speedy DB, Hew TD, Reid SA, Dugas JP. Dehydration prevents the development of hyponatremia during exercise: Evidence from 1423 weighed competitive athletic performances. *PNAS* 2005.

33. Orenstein DM, Henke KG, Costill DL, Doershuk CF, Lemon PJ, Stern RC. Exercise and heat stress in cystic fibrosis patients. *Pediatr.Res.* 1983;**17**:267-9.

34. Bar-Or O, Blimkie CJ, Hay JA, MacDougall JD, Ward DS, Wilson WM. Voluntary dehydration and heat intolerance in cystic fibrosis. *Lancet* 1992;**339**:696-9.

35. Stanghelle JK, Maehlum S, Skyberg D, et.al. Biochemical changes and endocrine responses in cystic fibrosis in relation to a marathon race. *Int.J Sports Med* 1988;**9 (suppl.)**:45-50.

36. Kriemler S, Wilk B, Schurer W, Wilson WM, Bar-Or O. Preventing dehydration in children with cystic fibrosis who exercise in the heat. *Med Sci.Sports Exerc.* 1999;**31**:774-9.

37. Denton DA, Eichberg JW, Shade R, Weisinger RS. Sodium appetite in response to sodium deficiency in baboons. *Am.J.Physiol* 1993;**264**:R539-R543.

38. Epstein AN. The dependence of the salt appetite of the rat on the hormonal consequences of sodium deficiency. *J.Physiol (Paris)* 1984;**79**:496-8.

39. Stellar E. Salt appetite: its neuroendocrine basis. *Acta Neurobiol.Exp.(Wars.)* 1993;**53**:475-84.

Study 1B: Case Report: The treatment of symptomatic hyponatremia with hypertonic saline in an Ironman triathlete

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INTRODUCTION

Exercise-associated hyponatremia (EAH) is as an important complication of endurance exercise [1]. The treatment of symptomatic cases in the field remains untested, although the benefits of hypertonic saline administration have been recently promoted [2]. We present a case of "mild" but symptomatic EAH in an Ironman triathlete who responded favorably to the administration of a 50 ml bolus of 5% hypertonic saline in the medical tent and was discharged without medical complication.

CASE REPORT

A 41 year old male triathlete finished his first Ironman Triathlon (3.8 km swim, 180 km cycle and 42.2 km run) in 10:49 (hours: minutes). Race conditions were cloudy and mild, with a starting temperature of 17°C, a maximum temperature of 21°C and a moderate 32 km/h wind present throughout most of the day. This athlete admitted to drinking > 10 L of fluid, mainly Cytomax™ (9 mmol/L sodium, 6 mmol/L potassium, 8% carbohydrate: maltodextrin, fructose and dextrose), on the bicycle portion of the race which he completed in 5:54 minutes (~1.7 L/hour). He stated that he was never thirsty, but drank to "stay ahead of thirst". He voluntarily fluid loaded two days prior to the race by ingesting "four to five" liters of Cytomax™ per day.

During the running portion of the race, he reported having three bouts of diarrhea and feeling "delusional" towards the end of his 3:51 run. He related urinating twice at the beginning of the run, although his urine was "sparse" and "appeared green". He ingested two pain killers before and during the triathlon (an over-the-counter mix of

acetaminophen and ibuprofen). His medical history was unremarkable, except for mild asthma for which he did not take regular medication.

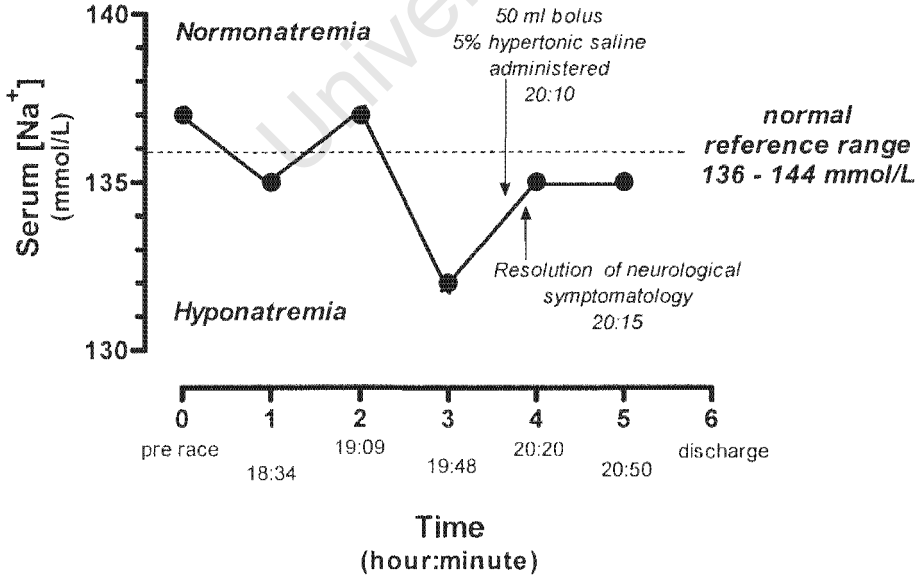
This triathlete consented to participate in a large research trial where pre race laboratory values and a pre race weight of 68.0 kg were recorded. Upon completion of the Ironman event, the subject presented to the research team at the finish area complaining of nausea. His post race weight was 68.2 kg: a bodyweight gain of 0.2 kg. Blood pressure on admission was 104/73 mmHg, pulse was 63 bpm and axillary temperature was 35°C (Measurement of axillary temperature was part of the existing protocol for the Ironman Medical Team and because this patient did not present with any signs or symptoms of heat illness, rectal temperature was not obtained).

The initial serum sodium concentration ($[Na^+]$) was 135 mmol/L (normal range: 136 – 144 mmol/L), serum potassium was 3.25 mmol/L (normal range: 3.4 – 4.5 mmol/L) and glucose was 4.0 mmol/L (normal range: 4 – 8 mmol/L). He was placed in the Trendelenburg position and intravenous access was established without fluid administration: this was the protocol for a larger prospective study, formally approved by the ethical committee at the University of Cape Town, on collapsed athletes to which the subject had given written informed consent to participate prior to race start. The subject was offered a choice of oral fluids, from which he chose Coke™. He drank one to two sips of Coke™ before relating that he felt "worse". He was sleepy, lethargic, disinterested and did not interact voluntarily with other participants or study volunteers. He kept his eyes closed during most of our questioning, but could answer four out of five questions correctly in the initial assessment of consciousness (he could not recall the date). He did not report any vomiting during the race, and never became stuporous, comatose or obtunded within the medical tent. An intramuscular injection of 10 mg metoclopramide, an antiemetic, was given 14 minutes after admission for persistent nausea. Sequential serum $[Na^+]$ determinations were obtained every ~ 30 minutes (Figure 1B.1).

The patient's condition did not change for 100 minutes following admission. When the third serum sodium determination decreased to 132 mmol/L, this provided clinical and biochemical justification to administer a 50 ml bolus of 5% hypertonic saline over a twenty minute period through an intravenous line (3% hypertonic saline is not readily available in South Africa). Within five minutes of the start of the infusion of hypertonic saline, the patient's mental state improved dramatically: he became awake and alert and conversed with the medical staff. After completion of the 20 minute infusion, the triathlete wished to stand and urinate.

The patient was discharged from the medical area 30 minutes after the hypertonic saline infusion was completed with instructions to present to the nearest emergency room if his symptoms reappeared and to consume fluids only according to thirst. Follow up communication with this athlete, nine days after completion of the Ironman Triathlon, confirmed that he recovered uneventfully, was without unusual or residual symptoms and did not seek medical care after discharge from the medical tent.

Figure 1B.1: Sequential serum sodium determinations for the Ironman triathlete. The triathlon commenced at 07:00 and the time noted on the X-axis refers to "real-time" on the 24 hour clock.



DISCUSSION

This is the first published report supporting the efficacy of hypertonic saline administration in the treatment of symptomatic EAH in the field. Although the serum sodium concentration was only mildly lowered compared to other reported cases of EAH encephalopathy, the rapid improvement in mental status following the administration of hypertonic saline suggests that this triathlete's symptoms were due to mild hyponatremic encephalopathy which was likely reversed by the administration of hypertonic saline.

These neurological symptoms associated with "mild" EAH are not well described, as symptomatic hyponatremia with mental status changes generally occur when serum [Na^+] falls below 130 mmol/L [3] or when serum [Na^+] declines precipitously over a short time period [4]. This athlete's pre race electrolyte values were within the lowest 5% found in this cohort; perhaps indicating that this subject was maximally hydrated before the triathlon, secondary to his "fluid loading" practices.

Although the numerical values for both serum [Na^+] and bodyweight gain were not alarming, the history and symptomatology were sufficient to suspect mild hyponatremic encephalopathy. Non-steroidal anti-inflammatory (NSAID) use has recently been shown to be an independent risk factor for the development of EAH [5]. Thus, this athlete was at greater risk for water retention and dilutional hyponatremia because he ingested two NSAID tablets before and during the triathlon. Furthermore, it was noted that the maintenance of bodyweight during an Ironman triathlon represented an approximately 2.5 kg weight gain, secondary to metabolic water production and substrate utilisation[6]. Thus, this athlete was at least 2.7 kg overhydrated at the end of the race despite the apparent "marginal" increase in bodyweight. Lastly, it has recently been verified that beverages containing fructose (i.e. Cytomax™) retard the absorption of water from the intestinal tract [7]. This delayed fluid absorption may have contributed to the sudden drop in serum [Na^+] to 132 mmol/L during the third sequential electrolyte evaluation; roughly one hour after admission into the medical tent. Since the serum [Na^+] in venous blood

may be 4 mmol/L higher than that measured in arterial blood for 30 - 40 minutes after ingesting a fluid load, the initial serum [Na^+] may have been "falsely" elevated in the initial determinations because all electrolyte evaluations were obtained through venipuncture[7].

Therefore, the combination of: 1) a **history** of high fluid intake, low urine output and anti-inflammatory use with 2) **clinical findings** of weight gain, lethargy and confusion, combined with 3) **laboratory findings** of electrolyte values below the normal range with normal blood glucose, axillary temperature, blood pressure and pulse rates along with 4) positive treatment outcomes with the administration of a bolus of hypertonic saline all support a diagnosis of EAH with emerging, but mild, encephalopathy. Although the weight gain was minimal and the sequential serum sodium concentrations were mildly reduced, neurological symptoms were present and reversed rapidly by the infusion of a concentrated dose of 5% saline. The total amount of sodium infused was 43 mmol: enough to raise the serum [Na^+] by roughly 1 mmol/L in a 68 kg male. However, the rapid reversal of cerebral hypoosmolality combined with the initiation of diuresis following the intravenous administration of hypertonic saline probably most influenced this patient's recovery from EAH; irrespective of any change in serum [Na^+] as measured in venous blood. Administration of this small concentrated amount of sodium was unlikely to have precipitated untoward effects, as the infusion of 50 ml of 29.2% hypertonic saline (250 mmol/L Na^+) over 10 minutes has been documented in hyponatremic patients with clinical success[8].

CONCLUSION

Mild neurological symptoms resulting from mild EAH in a well trained triathlete reversed rapidly following a bolus of intravenous hypertonic saline. Treatment of clinical symptomatology should always take precedence over laboratory values. Hypertonic saline can be used judiciously, and without complication, in the treatment of symptomatic exercise-associated hyponatremia when other potential causes of collapse have been excluded.

From this case study, it appears that a 50 ml bolus of 5% hypertonic saline can be safely administered in the field. Therefore, the authors' would not hesitate to administer such a bolus to stabilize any athlete with symptomatic hyponatremia. Waiting until presentation to a hospital to administer a bolus of hypertonic saline, in a hyponatremic patient with clear mental status changes/seizures, may be far more dangerous than administration of a bolus in the field prior to transfer.

Reference List

1. Hew-Butler TD, Almond CS, Ayus JC, Dugas JP, Meeuwisse WH, Noakes TD *et al.* Consensus Statement of the 1st International Exercise-Associated Hyponatremia Consensus Development Conference, Cape Town, South Africa 2005. *Clin.J.Sport Med* 2005;**15**:208-13.

2. Ayus JC, Arieff A, Moritz ML. Hyponatremia in marathon runners. *N.Engl.J.Med.* 2005;**353**:427-8.

3. Speedy DB, Noakes TD, Rogers IR, Thompson JM, Campbell RG, Kuttner JA *et al.* Hyponatremia in ultradistance triathletes. *Med.Sci.Sports Exerc.* 1999;**31**:809-15.

4. Verbalis JG. Disorders of body water homeostasis. *Best.Pract.Res.Clin.Endocrinol.Metab* 2003;**17**:471-503.

5. Wharam PC, Speedy DB, Noakes TD et al. NSAID use increases the risk of developing hyponatremia during an Ironman triathlon. Med Sci Sports Exerc. 2006;38:618-22.

6. Speedy DB, Noakes TD, Kimber NE et al. Fluid balance during and after an Ironman triathlon. Clin J Sport Med 2001;11;44-50.

7. Shafiee MA, Charest AF, Cheema-Dhadli S, Glick DN, Napolova O, Roozbeh J *et al.* Defining conditions that lead to the retention of water: the importance of the arterial sodium concentration. *Kidney Int.* 2005;**67**:613-21.

8. Worthley LIG, Thomas PD. Treatment of hyponatremic seizures with intravenous 29.2% saline. BMJ 1986;292:168-170.

CHAPTER 2

THE HYPERNATREMIA OF EXERCISE

Study 2C: Dysnatremia predicts a delayed recovery in collapsed ultramarathon runners

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INTRODUCTION

Exercise-induced abnormalities in serum sodium concentrations ($[Na^+]$) are uncommon [1] with the incidence of dysnatremia in collapsed athletes generally low. The highest reported incidence of *hyper*natremia in collapsed marathon runners is 25% [1] while the highest reported incidence of *hypo*natremia in collapsed runners is 8% [2]. Yet, when present, both exercise induced hyponatremia and hypernatremia may lead to severe central nervous system (CNS) dysfunction and death [3-6]. The brain has a limited capacity to adapt to rapid changes in osmolality, which produce acute changes in brain volume [7]. The rapidity with which hyponatremia or hypernatremia develops often dictates the severity of the CNS disturbance [8]. Prompt reversal of the abnormal $[Na^+]$ can ameliorate CNS dysfunction by restoring brain osmolality while normalizing cerebral cell size.

The treatment of hypo-, normo- and hypernatremia with intravenous versus oral fluids and the effect of initial $[Na^+]$ on recovery time has yet to be properly evaluated in collapsed athletes. Intravenous rehydration may lead to a faster restoration of plasma volume, since this method of delivery bypasses the delay associated with gastric absorption, [9;10]. Overzealous intravenous rehydration, however, may lead to fluid overload hyponatremia [11]. Oral rehydration has been shown to ameliorate thirst [12] with the ingestion of sodium containing beverages facilitating the restoration of fluid balance in the volume depleted state [13-15]

This study was designed to address the following questions: 1) What is the incidence of hyponatremia, hypernatremia and normonatremia in collapsed runners presenting to the

medical tent of the Comrades Marathon and did initial [Na^+] status influence time to discharge; 2) Do intravenous fluids restore [Na^+] to 140 mmol/L faster than the administration of oral fluids in normonatremic and hypernatremic collapsed runners and thus influence time to discharge. It is hypothesized that exercise-induced dysnatremias are uncommon in collapsed athletes and that the administration of intravenous fluids will produce a more rapid correction of the dysnatremias leading to shorter discharge times from the medical facility.

METHODS

This study was approved by the Ethics Committee of the University of Cape Town and the Georgetown University Institutional Review Board. Informed written consent and pre-race bodyweights were obtained from 7,299 runners during registration for the 2005 Comrades Marathon.

Runners who "collapsed" during or following the race were triaged in the medical area. Blood pressure, heart rate, serum sodium concentration ([Na^+]) and mental status were immediately evaluated and the runners were placed in the Trendelenburg position.

Runners were randomized by race number into two separate groups with treatment dictated by [Na^+]. All collapsed runners whose last digit of the race number was "even" received intravenous rehydration; conversely, all runners whose last digit of their race number was "odd" received an intravenous line to maintain venous access (placebo IV) with oral rehydration. The appropriate hypertonic (3% NaCl), isotonic (0.9% NaCl/ normal saline) or hypotonic (0.45% NaCl/ half normal saline) fluid administered was dependant on the post race [Na^+]. Appropriate fluid therapy aimed to correct [Na^+] to140 mmol/L and any athlete who could not tolerate oral fluids was immediately reassigned to the oral - intravenous rehydration group.

Subjects were treated according to the following protocol:

1) If [Na^+] < 135 mmol/L: placebo IV with oral hypertonic rehydration

Or IV hypertonic rehydration

2) If [Na^+] between 135 – 140 mmol/L: placebo IV with oral isotonic rehydration

Or IV isotonic rehydration

3) If [Na^+] >140 mmol/L: placebo IV with oral hypotonic rehydration

Or IV hypotonic rehydration

The volume of fluid used was calculated from the following equation [5]:

$$\text{Change in [Na+]} = \frac{\text{infusate [Na}^+\text{] - serum [Na}^+\text{]}}{\text{*(Total Body Water + 1)}}$$

$$\text{Volume of infusate Required} = \frac{\text{Change in [Na}^+\text{] targeted}}{\text{Change in [Na}^+\text{] calculated above}}$$

*Total body water in liters (TBW) for females was calculated as: bodyweight (kg) x 0.5, and for males: bodyweight (kg) x 0.6

Oral fluid intake was administered ad libitum, up to the calculated amount necessary to return serum [Na^+] to140 mmol/L. The identical hypotonic, isotonic and hypertonic fluids used for IV resuscitation were used for oral rehydration, and were flavored with a non-caloric powdered drink mix (Crystal Light™) to enhance palatability. Although fluid intake was encouraged, it was not forced upon the athlete. All intravenous fluids were delivered at the maximal rate: stopcock wide open through an 18 bore catheter. Oral fluid intake was not permitted by those runners receiving intravenous fluid resuscitation.

Clinical symptoms were documented upon admission. Each athlete was asked to recall the frequency of fluid ingestion and urination throughout the 89 km course. Blood pressure and heart rate were measured every 30 minutes. Mental status was assessed using the Paris Island scale for nonfocal encephalopathy [16]. Sodium ([Na^+]), potassium ([K^+]), chloride ([Cl^-]), bicarbonate ([HCO_3^-]), and hematocrit ([Hct]) were measured

onsite with a portable electrolyte analyzer (Bayer-Chiron 348 ph/Blood Gas Analyser 2000, England) and repeated 30 minutes after treatment was initiated.

Any runner assigned to the oral resuscitation group but could not tolerate fluids by mouth and was switched to intravenous fluid resuscitation had sequential serum [Na^+] measurements obtained 30 minutes after the *oral* fluid was initiated. The within-run standard deviation (WRSD) of the portable analyzer was 0.09 while the coefficient of variation was 0.07% in regards to the precision and accuracy of serial serum [Na^+] measurements obtained from whole blood (Operator's Manual 1996, Chiron Diagnostics Ltd). Bodyweights were measured pre race and upon *discharge* from the medical tent, as it was logistically difficult to measure collapsed runners as they entered the medical tent. Discharge from the medical tent was authorized by the head physician (J.B.) when: 1) the runner was alert and oriented to person, place and time 2) the athlete could ambulate without assistance. Administration of the entire calculated dose of fluid was *not* a requirement for discharge. Time to discharge was the main outcome measure with the rate of recovery of [Na^+] a secondary outcome.

A cohort of 31 athletes was recruited at registration to serve as a control group. A venous blood sample, bodyweight and blood pressure were obtained at registration and immediately following completion of the race.

Descriptive analyses were compiled for the frequency of hyponatremic, normonatremic and hypernatremic runners who presented to the medical tent of the Comrades Marathon. The number of subjects in each subgroup (n) as well as in total (N) for each variable is listed in the tables, as full data could not be obtained in every runner who required an initial serum [Na^+]. Independent t-tests were performed between hypernatremic and normonatremic groups in Table 1, as the hyponatremic group was too small and [Na^+] values too close to the "normal" range (134 mmol/L) to define statistically meaningful relationships. All analyses were performed using the STATISTICA 7™ software program with significance set at $p < 0.05$. All data were presented as mean ± SD.

RESULTS

11,728 athletes completed the 2005 Comrades marathon within the 12 hour time limit, with an average finishing time of 602 minutes. The temperature in Pietermaritzburg at the start of the race was 5°C. The peak temperature in Durban (race finish) was 24°C. Coke™, Energade™ and water were freely available every 1.5 km. One hundred and thirty-three runners, who had given informed consent prior to the race and had their serum [Na^+] measured on entrance to the trial, collapsed during (12%) or after (88%) the marathon. There were no significant differences between any biochemical, cardiovascular and symptom parameter between those athletes who collapsed during or after the marathon. The mean finishing time for the collapsed athletes was 573 ± 92 minutes (range: 372-718 minutes).

A small majority (53%; n = 71) of collapsed athletes were normonatremic with [Na^+] between 135-145 mmol/L (Table 2C.1). Fewer than 2% (n = 2) of athletes were hyponatremic ([Na^+] < 135 mmol/L) with the remaining 45% (n = 60) hypernatremic ([Na^+] >145 mmol/L). Runners with a higher initial serum [Na^+] spent a significantly longer time in the medical tent compared with runners presenting to the medical tent with a lower initial serum [Na^+], with the exception of the two hyponatremic runners (Table 2C.1; Figure 2C.1).

There were no significant differences between the finishing times or split time difference between normonatremic and hypernatremic runners (Table 2C.1). Diastolic blood pressure was significantly lower in normonatremic runners compared with hypernatremic runners. All other biochemical, cardiovascular, symptom and fluid intake/output parameters were not significantly different between normonatremic and hypernatremic runners (Table 2C.1). The weight difference *at discharge* from the medical area was identical between normonatremic and hypernatremic runners. The hyponatremic group was too small to make any meaningful statistical conclusions, but is included in Table 2C.1 for descriptive comparisons.

Normonatremic runners had lower initial [Na^+] and [Cl^-] and required a lower volume of calculated hypotonic saline correction to return the initial [Na^+] to 140 mmol/L, compared with hypernatremic runners (Table 2C.1). Fluid administration lowered [Na^+] by only 0.9 ± 2.3 mmol/L in normonatremic runners (Figure 2C.2). This represents an error of 2.0 mmol/L in the expected response of [Na^+] to the volume of fluid calculated to return the serum [Na^+] to 140 mmol/L. Fluid administration lowered [Na^+] in hypernatremic runners by 2.6 ± 2.9 mmol/L representing an average error of 5.7 mmol/L compared to the expected response. There were no significant differences between intravenous and oral rehydration groups with regards to the extent of change in [Na^+] from the initial value to the first follow-up value (~30 minutes) after rehydration commenced (Figure 2C.3). The change in [Na^+] for the intravenous group was -2.1 ± 3.1 mmol/L while the change in [Na^+] in runners able to tolerate oral fluids was -0.7 ± 1.8 mmol/L.

Tolerance to oral rehydration proved to be problematic as 45% (n = 24) of collapsed runners assigned into the oral resuscitation group could not tolerate fluids by mouth and were immediately switched to intravenous therapy. This specific sub-group of collapsed athletes had a higher *initial* [Na^+], spent a significantly longer time in the medical tent, required larger volumes of hypotonic fluid correction, had higher hematocrits and experienced more nausea and vomiting than those runners assigned to the oral treatment group and were able to tolerate fluids by mouth (Table 2C.2). Collapsed runners that were assigned to the oral group, but later switched to intravenous fluids, decreased the initial [Na^+] by -0.7 ± 1.9 mmol/L.

There was wide variation in the response of [Na^+] to hypotonic fluid administration in all runners whose initial [Na^+] was > 140 mmol/L. (Figure 2C.4). Serum sodium concentrations were expected to decline in response to hypotonic fluid administration, but in a few cases, [Na^+] actually increased. The line of unity represented no change in serum [Na^+] from the initial [Na^+] to the follow-up [Na^+] ~30 minutes later. If the administration of hypotonic fluids caused [Na^+] to increase, then the follow up [Na^+] would lie above the line of unity. If the administration of hypotonic fluids caused [Na^+] to

decrease (as would be expected), than the follow up [Na^+] would lie below the line of unity.

The 31 runners in the control group finished the race in a mean time of 608.9 ± 84.5. The control group lost 2% bodyweight from pre to post race while serum [Na^+] increased significantly by 2.7 ± 6.0 mmol/L ($p < 0.05$; Table 2C.3). Systolic and diastolic blood pressure were within normal limits pre and post race, with the degree of post race hypotension similar to that observed in collapsed runners (Tables 2C.1 and 2C.3). None of the control group runners collapsed or reported any adverse symptomatology. The mean finishing time was not significantly different from that of the collapsed runners.

Table 2C.1: Analysis of collapsed ultramarathon runners classified by initial post-race [Na^+].

VARIABLE	HYPONATRAEMIA (<135 mmol/L) $^+$n = 2	NORMONATRAEMIA (135 – 145 mmol/L) $^+$n = 71	HYPERNATRAEMIA (>145 mmol/L) $^+$n = 60	AVERAGE $^+$N=133
Finish time (min)	596.0 ± 0.0 n = 1	576.0 ± 96.0 n = 63	568.2 ± 88.9 n = 53	572.6 ± 92.1 N = 117
Split time difference (min)	89.0 ± 0.0 n = 1	48.2 ± 31.8 n = 52	50.0 ± 36.4 n = 47	49.4 ±34.0 N = 100
Time in tent (min)	146.0 ± 121.6 n = 2	80.0 ± 30.7** n = 68	102.2 ± 36.0 n = 56	90.0 ± 36.9 N = 126
Pre-race weight (kg)	65.0 ± 0.0 n = 1	70.5 ± 10.8 n = 48	68.9 ± 11.1 n = 44	69.7 ± 10.0 N = 93
Weight difference at discharge (kg)	No data n = 0	-1.8 ± 2.2 n = 33	-1.8 ± 3.3 n = 32	-1.8 ± 2.8 N = 65
Initial [Na+] (mmol/L)	134.0 ± 0.0 n = 2	142.9 ± 1.9** n =71	148.3 ± 2.1 n = 60	145.2 ± 3.6 n = 133
Calculated hypotonic saline correction (L)	0.2 ± 0.1 (hypertonic: n = 2)	0.8 ± 0.4** n = 64	2.0 ± 0.9 n = 59	1.4 ± 0.9 N = 123
Initial [Cl-] (mmol/L)	97.0 ± 2.8 n = 2	103.1 ± 4.1** n = 47	106.2 ± 3.6 n = 44	104.4 ± 4.3 N = 93
Initial [HCO_3-] (mmol/L)	26.3 ± 2.9 n = 2	23.7 ± 2.5 n = 71	23.4 ± 3.0 n = 60	23.6 ± 2.8 N = 133
Initial [K+] (mmol/L)	4.5 ± 0.6 n = 2	5.1 ± 2.0 n = 70	4.7 ± 0.8 n = 60	4.9 ± 1.5 N = 132
Initial Hct (%)	48.5 ± 6.4 n = 2	51.9 ± 4.7 n = 71	51.6 ± 4.9 n = 60	51.7 ± 4.8 N = 133
Glucose (mmol)	5.3 ± 0.0 n =1	5.6 ± 1.5 n =17	5.7 ± 1.5 n =16	5.7 ± 1.4 N = 34
Initial Systolic BP (mmHg)	113 ± 4 n =2	104 ± 15 n = 63	108 ± 18 n = 59	106 ± 17 N = 124
Initial Diastolic BP (mmHg)	75 ± 7 n =2	64 ± 14* n = 63	69 ± 14 n = 59	67 ± 14 N = 124
Pulse (beats/min)	85 ± 7 n =2	83 ± 11 n = 61	83 ± 13 n = 58	83 ± 12 N = 121
Initial [Na+] – follow-up [Na+] Difference (mmol/L)	1.0 ± 4.2 n =2	-0.9 ± 2.3** n = 38	-2.6 ± 2.9 n = 43	-1.7 ± 2.8 N = 83
^Nausea	0.5 ± 0.7 n = 2	0.7 ± 0.5 n = 68	0.8 ± 0.4 n = 60	0.7 ± 0.4 n = 130
^Vomiting	0.5 ± 0.7 n = 2	0.4 ± 0.7 n = 68	0.6 ± 0.5 n = 60	0.5 ± 0.6 N = 130
#Fluid Intake	1.5 ± 0.7 n = 2	2.2 ± 1.2 n = 66	2.0 ± 1.0 n = 60	2.1 ± 1.1 N = 128
@Urination	3.0 ± 0.0 n = 2	1.7 ± 1.1 n = 66	1.7 ± 1.2 n = 59	1.7 ± 1.1 N = 129

*** p<0.05 and **p<0.01 between normonatremia and hypernatremia groups only**

$^+$ designates the total number of subjects with an initial [Na^+] value

LEGEND **(for non-continuous variables)**

^ **Nausea and vomiting**: 0 = no and 1 = yes

\# **Fluid intake**: 0 = drank at every table, 1 = drank at every 2nd table, 2 = drank at every 3rd table, and 4 = drank at > every third table

@ **Urination**: 0 = none, 1 = once, 2 = twice, 3 = 3 or more times

Table 2C.2: Analysis of runners on the basis of tolerance for oral fluid rehydration during recovery.

VARIABLE	ORAL GROUP $^{+}$n = 29	$^{++}$ORAL-IV GROUP $^{+}$n = 24
Finish time (min)	545.4 ± 93.6 n = 26	595.3 ± 85.7 n = 21
Split time difference (min)	47.3 ± 36.1 n = 24	53.4 ± 29.3 n = 17
Time in tent (min)	73.5 ± 30.5** n = 26	101.9 ± 35.5 n = 24
Pre-race weight (kg)	66.0 ± 7.5 n = 18	69.7 ± 11.1 n = 20
Weight difference at discharge (kg)	-1.3 ± 3.4 n = 9	-1.0 ± 3.4 n = 16
Initial [Na+] (mmol/L)	143.8 ± 3.7** n = 29	147.1 ± 3.5 n = 24
Calculated hypotonic saline correction (L)	1.0 ± 0.8** n = 29	1.7 ± 1.0 n = 24
Initial [Cl-] (mmol/L)	103.9 ± 5.2 n = 23	105.5 ± 3.0 n = 15
Initial [HCO_3-] (mmol/L)	23.1 ± 2.8 n = 29	23.0 ± 2.6 n = 24
Initial [K+] (mmol/L)	4.9 ± 1.1 n = 29	4.9 ± 0.8 n = 24
Initial Hct (%)	50.3 ± 4.8* n = 29	53.3 ± 5.3 n = 24
Glucose (mmol)	5.1 ± 1.8 n = 6	5.7 ± 0.8 n = 7
Initial Systolic BP (mmHg)	103 ± 14 n = 27	103 ± 19 = 23
Initial Diastolic BP (mmHg)	65 ± 14 n = 27	65 ± 15 n = 23
Pulse (beats/min)	82 ± 12 n = 27	89 ± 10 n = 21
Initial [Na+] – follow-up [Na+] Difference (mmol/L)	-0.7 ± 1.8 n = 15	-1.5 ± 1.9 n = 15
^Nausea	0.7 ± 0.5* n = 29	1.0 ± 0.2 n = 24
^Vomiting	0.4 ± 0.5* n = 29	0.7 ± 0.5 n = 24
#Fluid Intake	2.0 ± 1.2 n = 28	1.9 ± 0.9 n = 24
@Urination	1.5 ± 1.1 n = 28	2.1 ± 0.9 n = 24

*p < 0.05, **p < 0.01

$^{+}$ designates the total number of subjects with an initial [Na^{+}] value

$^{++}$group originally randomized to oral therapy but were unable to tolerate such therapy

LEGEND (for non-continuous variables)

^ Nausea and vomiting: 0 = no and 1 = yes

Fluid intake: 0 = drank at every table, 1 = drank at every 2nd table, 2 = drank at every 3rd table, and 4 = drank at > every third table

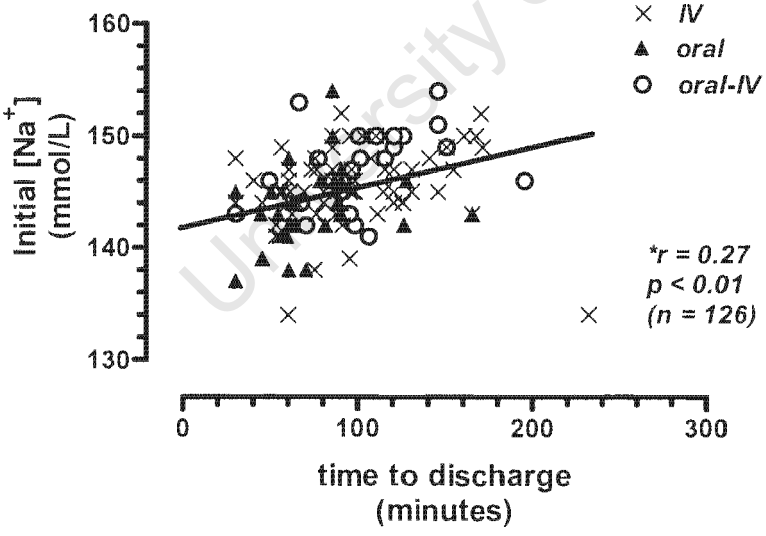
@ Urination: 0 = none, 1 = once, 2 = twice, 3 = 3 or more times

Table 2C.3: Pre and post race values for control group (asymptomatic) runners (N=31)

VARIABLE	PRE RACE	POST RACE
Bodyweight (kg)	71.3 ± 10.8	70.0 ± 10.6
$[Na^+]$ (mmol/L)	137.2 ± 4.6*	139.9 ± 4.1
Systolic Blood Pressure (mmHg)	116 ± 10	104 ± 6
Diastolic Blood Pressure (mmHg)	66 ± 12	68 ± 8

*$p < 0.05$

Figure 2C.1: Initial serum $[Na^+]$ at presentation versus time to discharge from the medical tent for all subjects with different treatments (N = 126).



*represents total group regardless of treatment.
No *single* treatment group showed significant correlation between variables.

Figure 2C.2: Correction of serum [Na^+] in normonatremic and hypernatremic collapsed ultramarathon runners, with a calculated dose of hypotonic fluid administration designed to return the initial serum [Na^+] to 140 mmol/L.

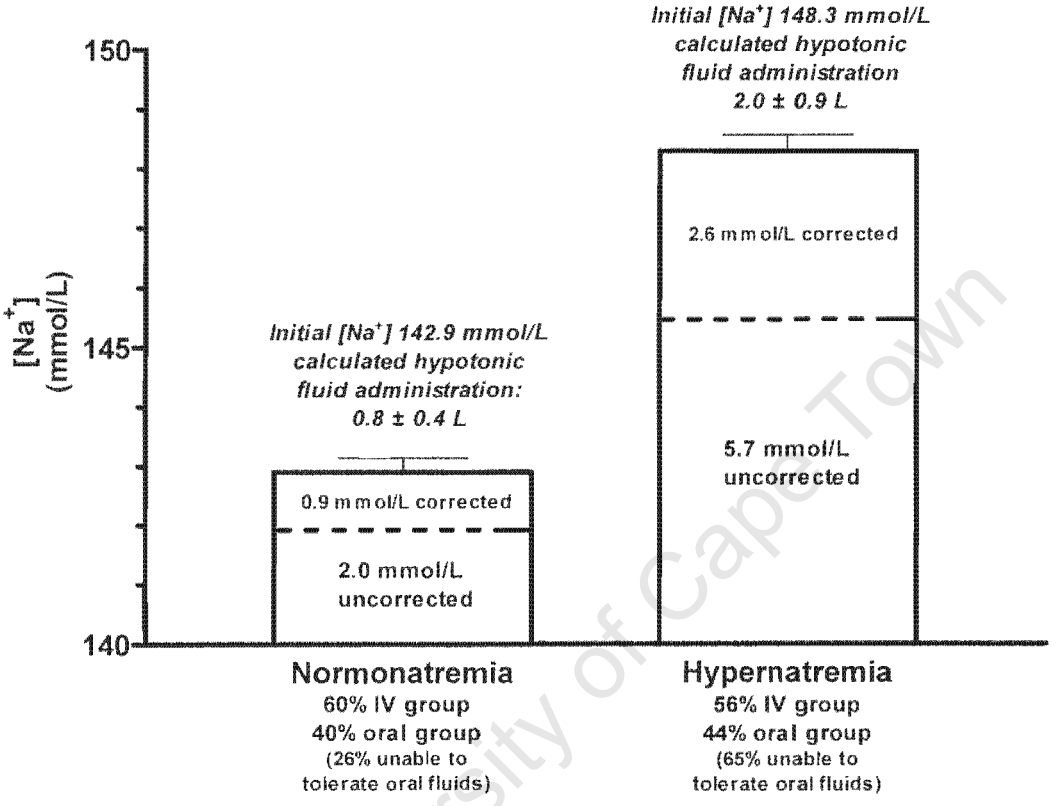


Figure 2C.3: Change in [Na^+] with oral and intravenous fluid resuscitation.

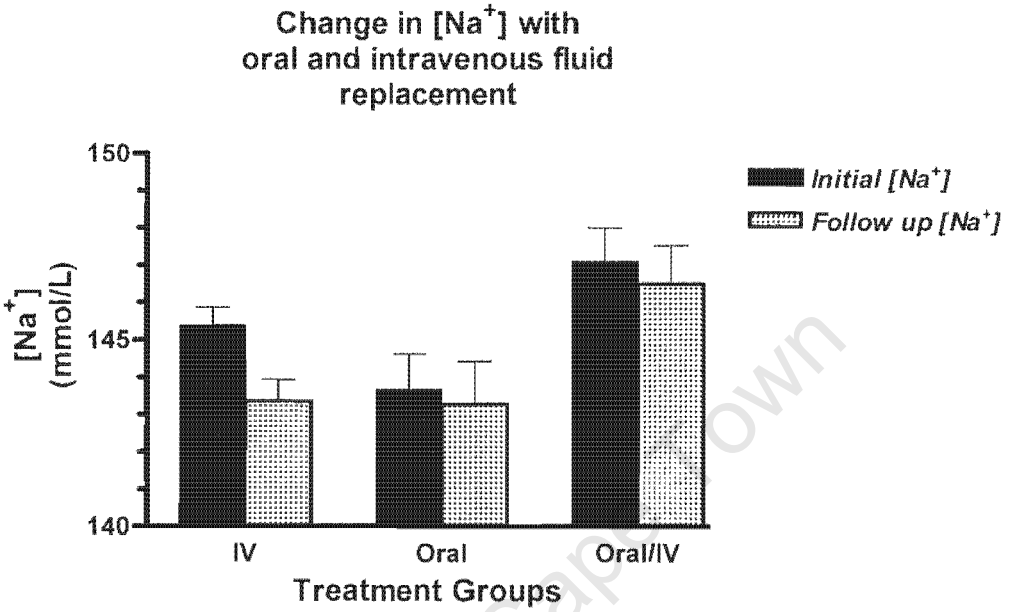


Figure 2C.4: Difference between initial [Na^+] and follow-up [Na^+] in athletes receiving hypotonic fluid administration (n = 84).

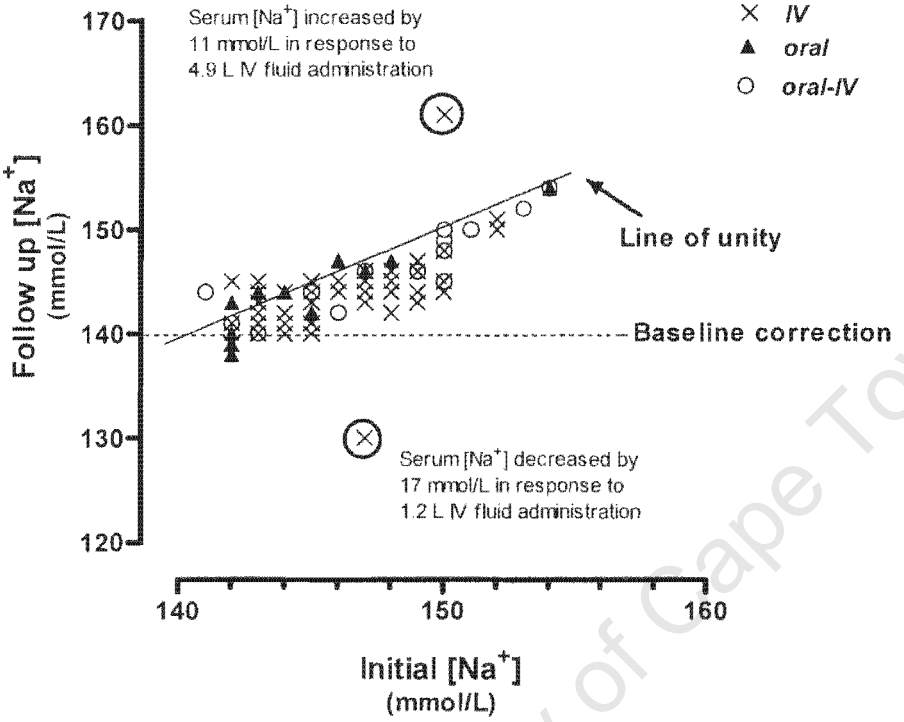
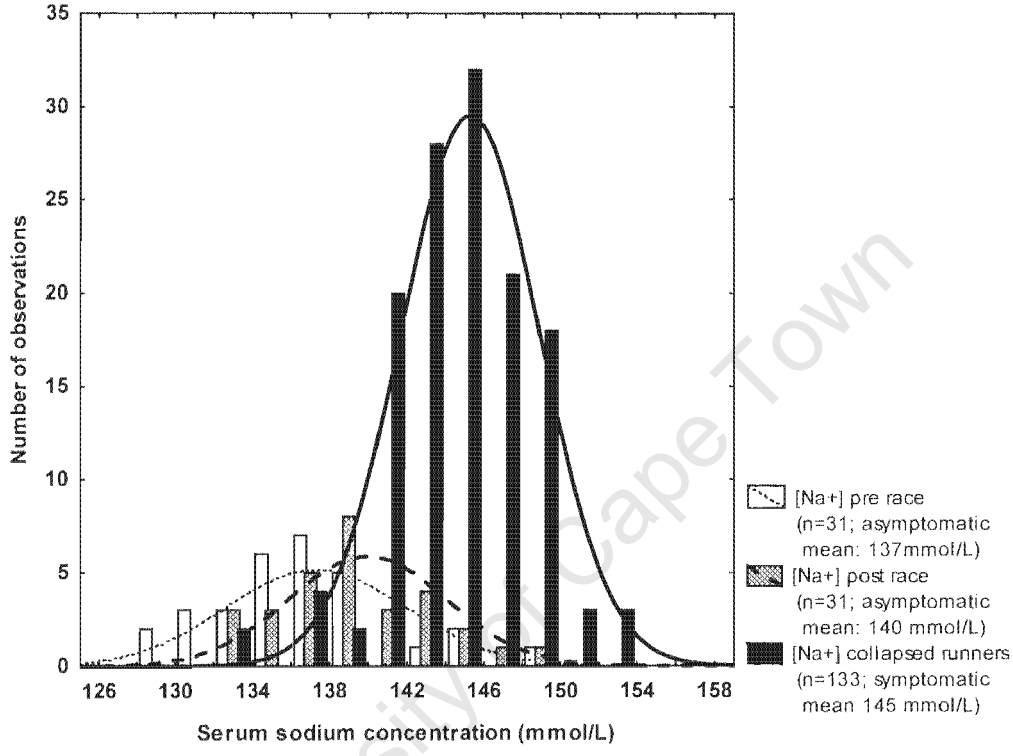


Figure 2C.5: Serum sodium concentrations in three cohorts of runners: pre-race, asymptomatic runners post race, and symptomatic collapsed runners post race.



DISCUSSION

The current study was unique in providing field-based data on the prevalence of dysnatremias in collapsed runners after an ultra-endurance event and investigating the efficacy of oral vs. intravenous therapy to correct serum [Na^+]. In summary, a narrow majority (53%) of collapsed runners had a normal serum [Na^+], with more hypernatremia (45%) and less hyponatremia (2%) documented in the current cohort than has previously been reported [1;2]. Normonatremic runners required less fluid, recovered and were discharged from the medical tent significantly faster than runners who presented with either hyponatremia or hypernatremia. Furthermore, there was a significant linear relationship between serum [Na^+] and time to discharge. The impact of oral vs. intravenous therapy for restoration of normal [Na^+] was not significant.

The positive correlation between initial serum [Na^+] and time to discharge highlights the potential deleterious effects of serum sodium derangements on the delayed recovery of collapsed athletes. Although the strength of this significant relationship was statistically weak ($r = 0.27$; $p<0.05$) this relationship confirmed our clinical suspicions that dysnatremia significantly contributes to the ability of collapsed runners to recover and re-establish physiological homeostasis.

The two documented cases of hyponatremia within the medical tent were both mild with an initial [Na^+] of 134 mmol/L. Both hyponatremic patients were alert and oriented upon admission. One hyponatremic runner received 266 ml of 3% hypertonic saline correction, in three boluses, without any apparent change in [Na^+] over a 232 minute period. Each bolus prompted a copious diuresis (2.3 L total) of electrolyte-poor water (urine osmolality ranged from 51-396 mOsmol/kg H_2O in four samples) which, curiously, did not change the serum [Na^+] in sequential analysis. Arginine vasopressin levels were maximally suppressed (< 1 pg/ml) only at the last blood sampling, with values between 2-3 pg/ml at initial sampling. Although collective data on these two hyponatremic runners were insufficient to derive any statistically meaningful conclusions, the descriptive trends suggest that these hyponatremic athletes were the slowest runners,

spent the longest time in the medical tent, slowed down considerably during the second half of the race, were lighter, ingested fluid and urinated more frequently on the course, had higher HCO_3-, lower hematocrit and hemoglobin concentrations and higher blood pressure and heart rates compared with normonatremic and hypernatremic runners.

There was a shift to the right (towards hypernatremia) in the [Na^+] distribution curve of the three cohorts of athletes tested at the 2005 Comrades Marathon (Figure 2C.5) suggesting that this is the most likely dysnatremia to occur with prolonged exercise. The mean serum [Na^+] of asymptomatic athletes tested pre-race was significantly lower than post race, with the mean serum [Na^+] of symptomatic athletes significantly higher than that of asymptomatic athletes. This shift to the right further supports the potential for deleterious effects from hypernatremia and possible contribution to the delayed recovery of collapsed athletes. Since post-race bodyweights were obtained only at discharge from the medical area, we cannot comment on the precise mechanism of the high prevalence of hypernatremia, although volume depletion [17] or the increased activation/inappropriate inactivation of sodium stores have been postulated [18]. Weight loss has been shown to be linearly related to increases in serum [Na^+] [18], however, suggesting that volume depletion seems the more likely cause of the hypernatremia in this cohort of collapsed athletes.

Hypernatremic runners assigned into the oral fluid resuscitation group were less likely to tolerate fluid intake by mouth (Table 2C.2). This subgroup of oral fluid intolerant hypernatremic athletes (45%) spent a significantly longer time in the medical tent, required greater amounts of fluid to return [Na^+] to the desired value of 140 mmol/L and had higher initial hematocrits compared with athletes who were able to tolerate oral fluids. These data suggest that hypernatremic athletes lost a greater proportion of their extracellular fluid volume during the race, even though the frequency of fluid intake and urination were similar for athletes who could and could not tolerate oral fluids at race finish. Since hypernatremic athletes reported significantly more nausea and vomiting during the race, it is difficult to ascertain whether the high incidence of gastrointestinal distress contributed to the development of hypernatremia (by the inability to ingest and

absorb fluids along the race course), or if the development of hypernatremia contributed to the development of gastrointestinal and other symptoms commonly associated with post exercise collapse.

Although the degree of hypernatremia in the present cohort of collapsed athletes was "mild", perhaps it was the rapidity at which hypernatremia developed or the duration of acute hypertonicity that facilitated the development of disabling symptoms and a delayed recovery. These findings suggest that the hypernatremia of exercise could contribute to the development of symptoms and delayed recovery in endurance athletes via a mechanism linked to cellular shrinkage [19] which could significantly alter plasma volume [20] and cellular function [15].

Another key finding of this study was that the calculated amount of hypotonic fluid necessary to restore initial (elevated) serum sodium concentrations back to 140 mmol/L and the actual decline in serum [Na^+] after the initiation of hypotonic fluid resuscitation was roughly one third of the "expected" decrease (Figure 2C.2). This error in correction between the actual and calculated decline in serum [Na^+] was irrespective of the route of fluid administration, as ~60% of each group received intravenous fluids. It should be noted that hypernatremic runners were less likely to tolerate oral fluid ingestion compared to normonatremic runners (65% versus 26% respectively); thus, hypernatremic athletes received a greater proportion of fluid resuscitation via the intravenous route although the ratio of correction was similar to that of the normonatremic group. This suggests that the route of administration did not significantly impact the change in serum [Na^+].

It is of interest, however, that calculated hypotonic fluid rehydration did not cause a decline in [Na^+] within ~30 minutes of administration in all hypertonic athletes (Figure 2C.4). Two athletes, in particular, displayed highly aberrant responses that were not expected from mathematical calculations. These findings suggest that fluid replacement calculations, which were designed for steady state conditions, may be less accurate

when used to correct dysnatremias in athletes recovering from prolonged endurance exercise.

Finally, we acknowledge the limitations to data collection in the field, particularly the variability in subject numbers for each measured parameter as noted in the tables. Nevertheless, the strength of this field study lies in its applicability to real life scenarios. Field medicine is a dynamic situation in which flexibility in the optimum management of patients takes precedence over adherence to a strict protocol. Although time to discharge may be influenced by the capacity of the medical team to accommodate the temporal variation in patient influx, there were no significant correlations noted between the time of admission into the medical tent with the time to discharge. This suggests that the main outcome variable of time to discharge was not biased by the ability of the medical research team to assess and treat collapsed runners when the medical tent was at maximal capacity.

CONCLUSION

The prevalence of hyponatremia (2%) and hypernatremia (45%) are reported for a cohort of collapsed runners presenting to the medical tent at the Comrades marathon. Dysnatremia did predict a delayed recovery after an 89 km footrace as normonatremic athletes spent significantly less time in the medical tent compared with hypernatremic and hyponatremic athletes. There were no significant differences between the effects of intravenous or oral fluid rehydration on the initial rate of decline of the serum sodium concentrations to "baseline" levels (140 mmol/L).

These data suggest that intravenous fluid resuscitation may best benefit hypernatremic collapsed runners who are intolerant to oral fluid ingestion. In all other cases of normo and hypernatremia, the efficacy of ad libitum oral fluid ingestion appears to be equivalent to intravenous fluid resuscitation; although dysnatremic runners will take a longer time to recover than normonatremic athletes.

Reference List

1. Kratz A, Siegel AJ, Verbalis JG, Adner MM, Shirey T, Lee-Lewandrowski E *et al.* Sodium status of collapsed marathon runners. *Arch.Pathol.Lab Med.* 2005;**129**:227-30.

2. Holtzhausen LM, Noakes TD, Kroning B, de Klerk M, Roberts M, Emsley R. Clinical and biochemical characteristics of collapsed ultra-marathon runners. *Med.Sci.Sports Exerc.* 1994;**26**:1095-101.

3. Hew TD, Chorley JN, Cianca JC, Divine JG. The incidence, risk factors, and clinical manifestations of hyponatremia in marathon runners. *Clin.J.Sport Med.* 2003;**13**:41-7.

4. Van der Helm-van Mil AH, van Vugt JP, Lammers GJ, Harinck HI. Hypernatremia from a hunger strike as a cause of osmotic myelinolysis. *Neurology* 2005;**64**:574-5.

5. Adrogue HJ,.Madias NE. Hypernatremia. *N.Engl.J.Med.* 2000;**342**:1493-9.

6. Adrogué HJ,.Madias NE. Hyponatremia. *N.Engl.J.Med.* 2000;**342**:1581-9.

7. Verbalis JG. Disorders of body water homeostasis. *Best.Pract.Res.Clin.Endocrinol.Metab* 2003;**17**:471-503.

8. Riggs JE. Neurologic manifestations of electrolyte disturbances. *Neurol.Clin.* 2002;**20**:227-39, vii.

9. Nose H, Mack GW, Shi XR, Morimoto K, Nadel ER. Effect of saline infusion during exercise on thermal and circulatory regulations. *J.Appl.Physiol* 1990;**69**:609-16.

10. Casa DJ, Maresh CM, Armstrong LE, Kavouras SA, Herrera JA, Hacker FT, Jr. *et al.* Intravenous versus oral rehydration during a brief period: responses to subsequent exercise in the heat. *Med Sci.Sports Exerc.* 2000;**32**:124-33.

11. Noakes TD, Berlinski N, Solomon E, Weight LM. Collapsed Runners: Blood Biochemical Changes After IV Fluid Therapy. *Physician Sports Med* 1991;**19**:70-81.

12. Maresh CM, Herrera-Soto JA, Armstrong LE, Casa DJ, Kavouras SA, Hacker FT, Jr. *et al.* Perceptual responses in the heat after brief intravenous versus oral rehydration. *Med Sci.Sports Exerc.* 2001;**33**:1039-45.

13. Nose H, Mack GW, Shi XR, Nadel ER. Role of osmolality and plasma volume during rehydration in humans. *J Appl.Physiol* 1988;**65**:325-31.

14. Shirreffs SM, Taylor AJ, Leiper JB, Maughan RJ. Post-exercise rehydration in man: effects of volume consumed and drink sodium content. *Med Sci.Sports Exerc.* 1996;**28**:1260-71.

15. Shirreffs SM,.Maughan RJ. Rehydration and recovery of fluid balance after exercise. *Exerc.Sport Sci.Rev.* 2000;**28**:27-32.

16. Gardner JW, Kark JA. Clinical diagnosis, management and surveillance of exertional heat illness. In Pandolf KB, Burr RE, eds. *Meidcal Aspects of Harsh Environments*, p 238. Washington DC: Borden Institute, 2001.

17. Coyle EF. Fluid and fuel intake during exercise. *J.Sports Sci.* 2004;**22**:39-55.

18. Noakes TD, Sharwood K, Speedy D, Hew T, Reid S, Dugas J *et al.* Three independent biological mechanisms cause exercise-associated hyponatremia: evidence from 2,135 weighed competitive athletic performances. *Proc.Natl.Acad.Sci.U.S.A* 2005;**102**:18550-5.

19. Astrand PO,.Saltin B. Plasma and cell volume alterations after prolonged severe exercise. *J Appl.Physiol* 1964;**19**:829-32.

20. Greenleaf JE, Convertino VA, Mangseth GR. Plasma volume during stress in man: osmolality and red cell volume. *J.Appl.Physiol* 1979;**47**:1031-8.

Study 2D: Hypernatremia and intravenous fluid resuscitation in collapsed ultramrathon runners

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INTRODUCTION

Symptomatic hyponatremia and hypernatremia are both medical emergencies which can present with vague complaints such as lightheadedness, nausea, headache, fatigue and confusion [1]. The consequence of inappropriate fluid administration in an individual with a serum sodium imbalance can be potentially devastating [2]. Therefore, determination of serum sodium concentration ($[Na^+]$) *before* treatment is initiated is highly recommended in athletes who collapse during or after an endurance event.

The administration of intravenous (IV) fluids to collapsed athletes has been discouraged as exuberant administration of IV fluids has been shown to cause or worsen hyponatremia [3]. Recent studies document no physiological advantage using IV fluid administration over oral fluid ingestion with regards to: the hormonal response and restoration of fluid volume following dehydration [4], performance after a prior exercise bout [5;6] or in the recovery of collapsed athletes [7]. Hypernatremic athletes unable to tolerate oral fluid ingestion, however, may benefit by the IV administration of hypotonic fluids to facilitate a return to normonatremia [7;8].

This study was a direct sequel to the 2005 Comrades Marathon Collapsed Runner Trial [7], where the previous results were unclear as to whether a return to normonatremia was physiologically necessary for symptomatology to resolve in collapsed hypernatremic runners. We hypothesized that a return to normonatremia, through the administration of IV hypotonic fluid, would ameliorate adverse symptomatology and facilitate the recovery and discharge of collapsed runners from the medical tent. Secondly, we wished to

verify that the administration of IV isotonic solution to normonatremic collapsed runners would not adversely affect serum [Na^+].

METHODS

This observational study was performed in the medical tent of the 2006 Comrades 90 km Marathon and was approved by the Ethics Committee of the University of Cape Town. The treatment of all collapsed runners was carried out at the discretion of the Comrades Medical Team, according to accepted standard of care protocols. All runners gave written consent to be treated in the medical tent and all identities were withheld from the researchers during data analysis.

A "collapsed" runner was defined in this study as any athlete who presented to the medical tent, either carried in via stretcher or ambulatory, with (non-orthopedic) symptomatology unresolved by Trendelenburg positioning and ad libitum oral fluid intake. All "collapsed" runners required a venous blood sample withdrawn for serum electrolyte determination. If the athlete was normonatremic (serum [Na^+] between 135 and 145 mmol/L) an isotonic solution (Ringers Lactate; RL), was infused intravenously. If the athlete was hypernatremic (serum [Na^+] >145 mmol/L) a hypotonic solution, (0.45% Normal Saline; HNS) was infused intravenously. If the athlete was hyponatremic (< 135 mmol/L) a 50 mL bolus of hypertonic solution (5% Saline) was administered and the runner immediately transferred to the nearest hospital. After administration of one liter (1L) of IV fluid, another venous blood sample was obtained for sequential serum [Na^+] analysis. If the runner still felt unwell, additional intravenous fluid was administered until the athlete felt well enough to be discharged from the medical tent. A venous blood sample was obtained after each additional liter of IV fluid was administered until the athlete was discharged.

Other biochemical variables that were measured from the venous blood sample included: potassium ([K^+]; normal range: 3.7 – 5.0 mmol/L), hemoglobin (Hb; normal range for males: 13 – 18 gm/dL), hematocrit (Hct; normal range for males: 40 – 54%),

calcium ([Ca^{++}]; normal range: 1.0 – 1.4 mmol/L) and pH (normal range: 7.36 – 7.42) (Bayer-Chiron 348 pH/Blood Gas analyzer 2000, England). The within-run standard deviation of the portable analyzer was 0.09 while the coefficient of variation was 0.07% in regards to the precision and accuracy of serum [Na^{+}] measurements obtained from whole blood (Operator's manual 1996, Chiron Diagnostics Ltd).

Blood pressure and pulse rate were recorded upon initial presentation into the medical tent and re-evaluated every 30 minutes. The change (Δ) in systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse rate were presented as the "final minus the initial" value. All athletes were queried if they experienced nausea or vomiting during the race. The percent change in blood volume (BV), cell volume (CV) and plasma volume (PV) were calculated from Hb and Hct using the equations of Dill and Costill [9].

All analyses were performed using the STATISTICA 7™ software program (StatSoft, Tulsa, OK) with significance set at $p < 0.05$. All data were presented as mean ± SD. The number of subjects in each subgroup (n) as well as in total (N) for each variable was listed in the tables, as full data could not be obtained in every runner who required serum [Na^{+}] determination.

.RESULTS

9,864 runners (82% of starters; 17% female) finished the Comrades 90 km Marathon with an average finishing time of 10:02 (hours: minutes). One-hundred and three "collapsed" runners (1%) were treated in the medical tent and had serial electrolyte determinations performed. The average finishing time of the collapsed cohort was 9:58. The minimum temperature was 12°C while the maximum temperature was 22°C.

Sixteen percent of the collapsed runners were female. The only variables that were significantly different between collapsed male and female runners were expected sex-derived differences in: initial Hb (18.0 ± 1.6 vs. 15.2 ± 1.3 gm/dL; male vs. female respectively) and initial Hct (53 ± 5 vs. 46 ± 4%), follow-up Hct (50 ± 5 vs. 43 ± 5%).

Eighteen percent (N = 18) of the collapsed cohort did not finish the Comrades Marathon under the cut-off time of 12:00. The only variables that were significantly different between those who did and did not finish the race were: initial systolic blood pressure (100 ± 15 vs. 112 ± 20 mmHg; finishers vs. non-finishers) and diastolic blood pressure (63 ± 12 vs. 72 ± 18 mmHg).

Fifty-eight percent of the collapsed runners were hypernatremic with the remaining 40% normonatremic and 2% hyponatremic. Nausea was reported in 77% while vomiting was reported in 50% of the total cohort. The hypernatremic runners experienced more nausea (82 vs. 72% respectively; NS) and significantly more vomiting than normonatremic runners (79 vs. 34%; $p<0.001$).

Runners who reported nausea had a similar initial serum [Na^+] compared with runners who did *not* report nausea (145.9 ± 5.6 vs. 144.9 ± 4.8 mmol/L respectively; NS). Runners who reported vomiting during the Comrades Marathon had significantly higher initial (146.9 ± 5.9 vs. 144.5 ± 4.7 mmol/L; $p<0.05$) and follow-up (145.6 ± 5.8 vs. 143.5 ± 4.1 mmol/L; $p<0.05$) serum [Na^+] compared with runners who did not report vomiting.

Although 58% of the total cohort was hypernatremic, our supply of 0.45% Normal Saline (HNS) was exhausted earlier than anticipated. Therefore, only 55% (N = 28) of collapsed hypernatremic runners received HNS (Table 2D.1) while the remaining 45% (N = 23) received Ringers Lactate (Table 2D.2). Collapsed hypernatremic runners receiving HNS had significantly higher initial serum [Na^+] (150.5 vs. 148 mmol/L; $p<0.01$), lower initial Hb (18 vs. 19 gm/dL; $p<0.05$) and reported more vomiting (79 vs. 30%; $p<0.001$) than those hypernatremic runners receiving RL. Otherwise, no significant difference in any biochemical or cardiovascular parameter was found in hypernatremic runners given either 1L of RL or HNS.

A significant decrease (Δ) in serum [Na^+] in hypernatremic collapsed runners occurred following the administration of both 1L of HNS (Table 2D.1; 150.5 ± 3.5 vs. 148.0 ± 4.6;

$p<0.05$) and RL (Table 2D.2; 147.7 ± 2.2 vs. 146.2 ± 2.1; $p<0.05$). The initial and final serum Hb and Hct were significantly higher in hypernatremic compared with normonatremic collapsed runners when both natremic groups received 1L RL (Table 2D.2).

When all serum [Na^+] data were analyzed sequentially, the initial serum [Na^+] for the entire cohort was in the "hypernatremic" (145.9 ± 5.5 mmol/L) range while all follow-up serum [Na^+] values were within the "normonatremic" (<145 mmol/L) range (Figure 2D.1). With respect to only the hypernatremic group, all serial serum [Na^+] remained above 145 mmol/L.

When all data were combined, there was a weak but significant inverse correlation between plasma volume Δ versus serum [Na^+] Δ (Figure 2D.2A) which was not present when the natremic groups were separated by the type of fluid administered (Table 2D.2B and 2D.2C). A weak inverse correlation was also noted between initial pH versus initial serum [Na^+] (Figure 2D.4), with a "clustering" of data points by natremic status. This "clustering" was also present in the positive correlation between initial serum [Ca^{++}] versus initial serum [Na^+] (Figure 2D.3A). When data were separated by the type of fluid administered and natremic group, there was a significant negative correlation between serum [Ca^{++}] Δ versus plasma volume Δ only in those hypernatremic runners receiving HNS (Figure 2D.3B). This relationship was not significant when collapsed hypernatremic runners were given 1L RL (Figure 2D.3C).

Table 2D.1: Biochemical and cardiovascular parameters of hypernatremic collapsed runners given 1L of 0.45% Normal Saline IV and normonatremic collapsed runners given 1L Ringers Lactate IV.

VARIABLE	HYPERNATREMIC (Mean ± SD)	NORMONATREMIC (Mean ± SD)
Initial [Na^+] (mmol/L)	150.5 ± 3.5** (n = 28)	141.7 ± 2.6 (n = 38)
Initial [K^+] (mmol/L)	4.8 ± 0.6 (n = 28)	4.7 ± 0.8 (n = 38)
Initial Hb (gm/dL)	17.7 ± 1.7 (n = 23)	16.9 ± 2.0 (n = 32)
Initial Hct (%)	52.4 ± 5.1 (n = 25)	49.7 ± 5.8 (n = 35)
Follow-up [Na^+] (mmol/L)	148.0 ± 4.6** (n = 28)	141.8 ± 3.8 (n = 38)
Follow-up [K^+] (mmol/L)	4.9 ± 0.7 (n = 28)	4.9 ± 0.7 (n = 38)
Follow-up Hb (gm/dL)	20.9 ± 1.9 (n = 25)	16.0 ± 1.9 (n = 34)
Follow-up Hct (%)	49.9 ± 5.1 (n = 27)	46.7 ± 5.5 (n = 37)
Blood Glucose (mmol/L)	5.7 ± 1.4 (n = 18)	5.7 ± 1.7 (n = 37)
Time in Tent (minutes)	79.3 ± 33.0 (n = 25)	77.7 ± 33.9 (n = 53)
Δ [Na^+] (mmol/L)	-2.5 ± 5.2* (n = 28)	-0.2 ± 2.6 (n = 38)
Δ [K^+] (mmol/L)	0.1 ± 0.8 (n = 28)	0.1 ± 0.6 (n = 38)
Δ SBP (mmHg)	9.1 ± 18.4 (n = 18)	1.3 ± 18.3 (n = 30)
Δ DBP (mmHg)	2.5 ± 17.1 (n = 18)	2.1 ± 15.3 (n = 30)
Δ Pulse (bpm)	-3.5 ± 13.0 (n = 17)	-4.4 ± 9.0 (n = 48)
% Δ Blood Volume 1 - 2	6.1 ± 5.8 (n = 19)	7.5 ± 5.5 (n = 28)
% Δ Cell Volume 1 - 2	-0.3 ± 1.2 (n = 19)	-0.2 ± 1.1 (n = 28)
% Δ Plasma Volume 1 - 2	13.5 ± 12.7 (n = 19)	15.6 ± 11.3 (n = 28)

** $p < 0.001$ between hypernatremic and normonatremic groups
* $p < 0.05$ between hypernatremic and normonatremic groups

Table 2D.2: Biochemical and cardiovascular parameters of hypernatremic and normonatremic collapsed runners given 1L of Ringers Lactate solution IV.

VARIABLE	HYPERNATREMIC (Mean ± SD)	NORMONATREMIC (Mean ± SD)
Initial [Na^+] (mmol/L)	147.7 ± 2.2** (n = 23)	141.7 ± 2.6 (n = 38)
Initial [K^+] (mmol/L)	4.9 ± 0.6 (n = 23)	4.7 ± 0.8 (n = 38)
Initial Hb (gm/dL)	18.8 ± 1.5** (n = 23)	16.9 ± 2.0 (n = 32)
Initial Hct (%)	54.8 ± 4.5** (n = 20)	49.7 ± 5.8 (n = 35)
Follow-up [Na^+] (mmol/L)	146.2 ± 2.1** (n = 23)	141.8 ± 3.8 (n = 38)
Follow-up [K^+] (mmol/L)	5.2 ± 1.0 (n = 23)	4.9 ± 0.7 (n = 38)
Follow-up Hb (gm/dL)	17.2 ± 1.9* (n = 19)	16.0 ± 1.9 (n = 34)
Follow-up Hct (%)	50.9 ± 5.5** (n = 22)	46.7 ± 5.5 (n = 37)
Blood Glucose (mmol/L)	5.8 ± 1.6 (n = 16)	5.7 ± 1.7 (n = 37)
Time in Tent (minutes)	84.7 ± 37.1 (n = 22)	77.7 ± 33.9 (n = 53)
Δ [Na^+] (mmol/L)	-1.7 ± 3.2* (n = 23)	-0.2 ± 2.6 (n = 38)
Δ [K^+] (mmol/L)	0.4 ± 0.9 (n = 23)	0.1 ± 0.6 (n = 38)
Δ SBP (mmHg)	4.5 ± 13.8 (n = 19)	1.3 ± 18.3 (n = 30)
Δ DBP (mmHg)	4.4 ± 18.5 (n = 18)	2.1 ± 15.3 (n = 30)
Δ Pulse (bpm)	1.1 ± 11.5 (n = 18)	-4.4 ± 9.0 (n = 48)
% Δ Blood Volume 1 - 2	7.3 ± 4.0 (n = 13)	7.5 ± 5.5 (n = 28)
% Δ Cell Volume 1 - 2	0.2 ± 0.6 (n = 14)	-0.2 ± 1.1 (n = 28)
% Δ Plasma Volume 1 - 2	16.7 ± 9.5 (n = 13)	15.6 ± 11.3 (n = 28)

** p< 0.001 between hypernatremic and normonatremic groups
* p< 0.05 between hypernatremic and normonatremic groups

Figure 2D.1: Sequential changes in serum sodium concentration from entrance into the medical tent (sample 1) until discharge (samples 2 – 4 in sequence over time).

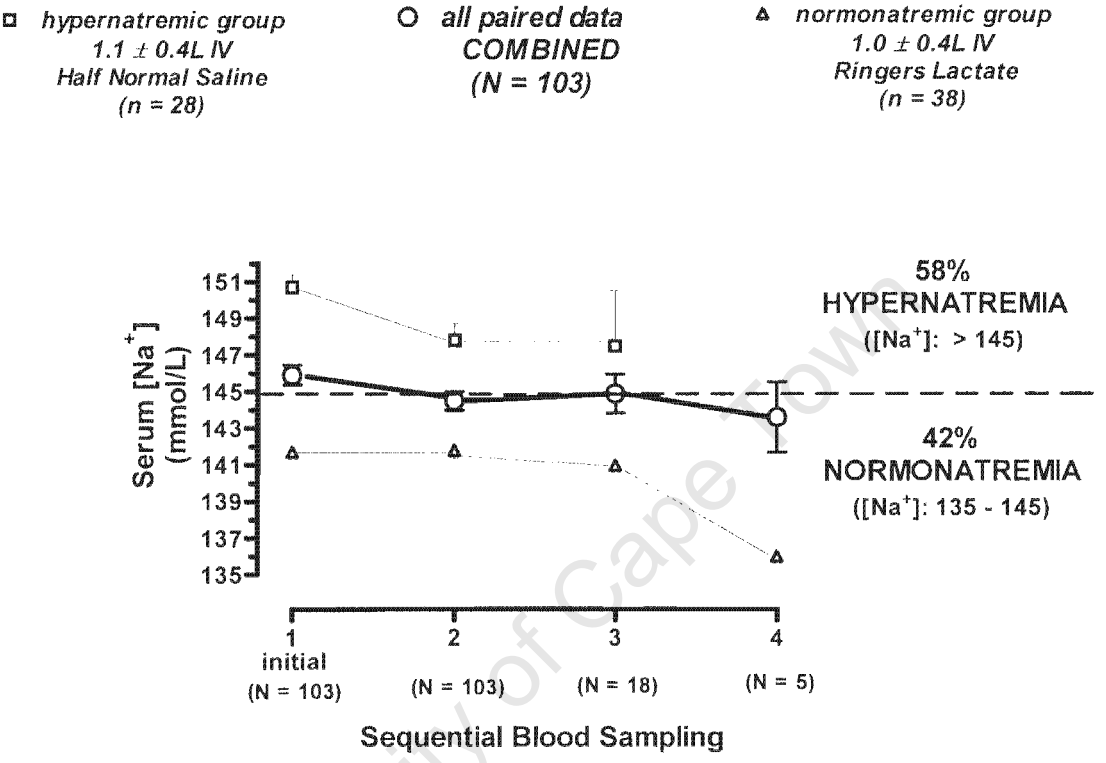


Figure 2D.2A: Changes in plasma volume versus changes in serum sodium concentration from first to second blood sampling in all collapsed runners (N = 68).

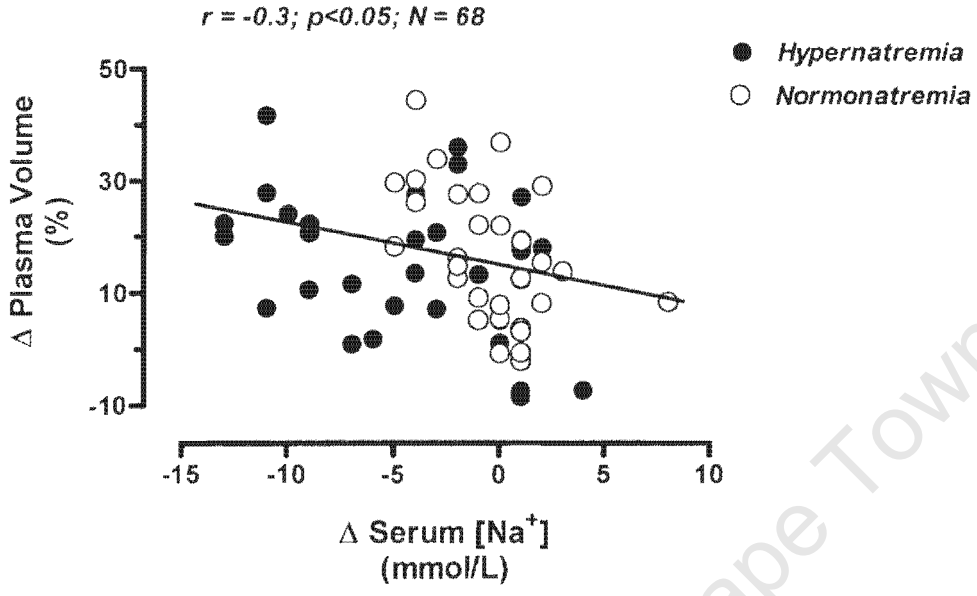


Figure 2D.2B: Changes in plasma volume versus changes in serum sodium concentration from first to second blood sampling in hypernatremic collapsed runners given 1L of 0.45% Normal Saline IV and normonatremic collapsed runners given 1L Ringers Lactate IV (N = 47).

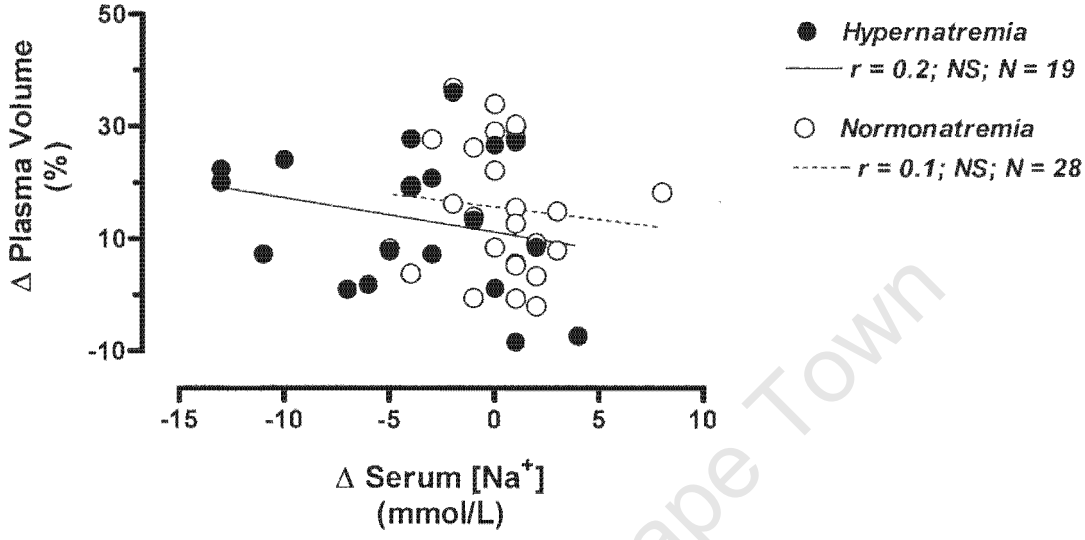


Figure 2D.2C: Changes in plasma volume versus changes in serum sodium concentration from first to second blood sampling in hypernatremic and normonatremic collapsed runners given 1L of Ringers Lactate solution IV (N = 41).

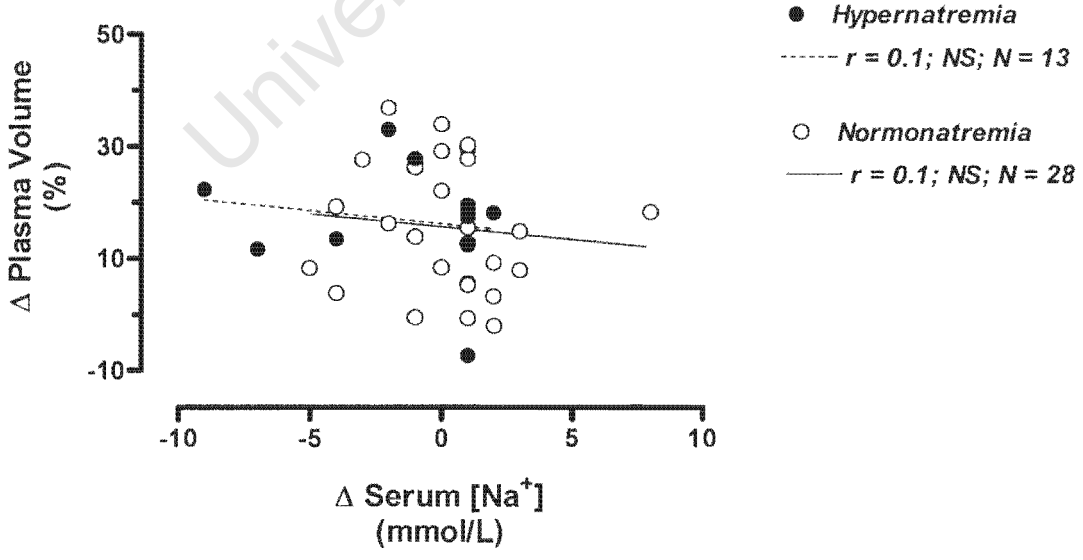


Figure 2D.3A: Initial serum calcium concentration versus initial serum sodium concentration in all collapsed runners (N = 103).

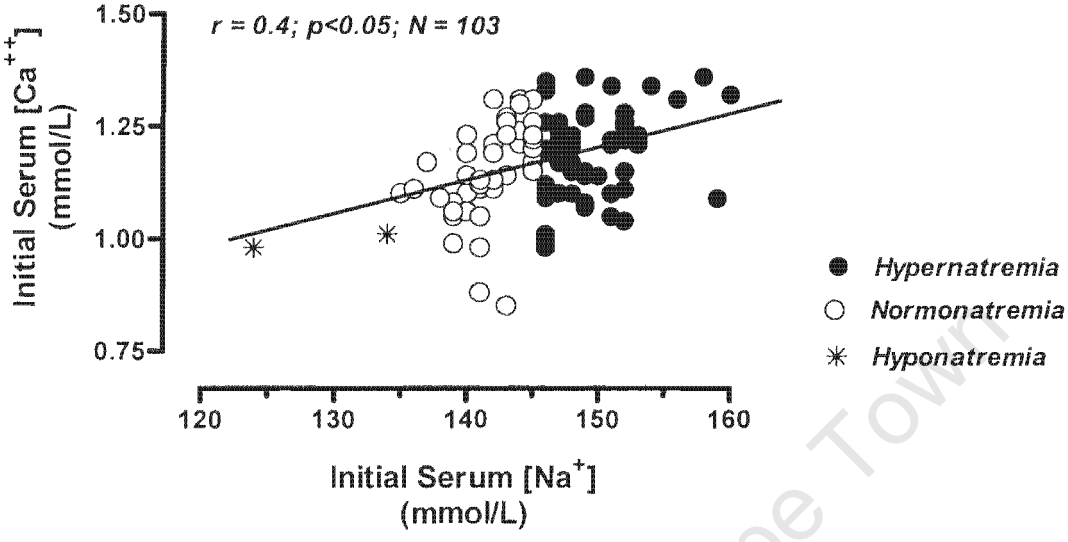


Figure 2D.3B: Initial serum calcium concentration versus initial serum sodium concentration in hypernatremic collapsed runners given 1L of 0.45% Normal Saline IV and normonatremic collapsed runners given 1L Ringers Lactate IV (N = 47).

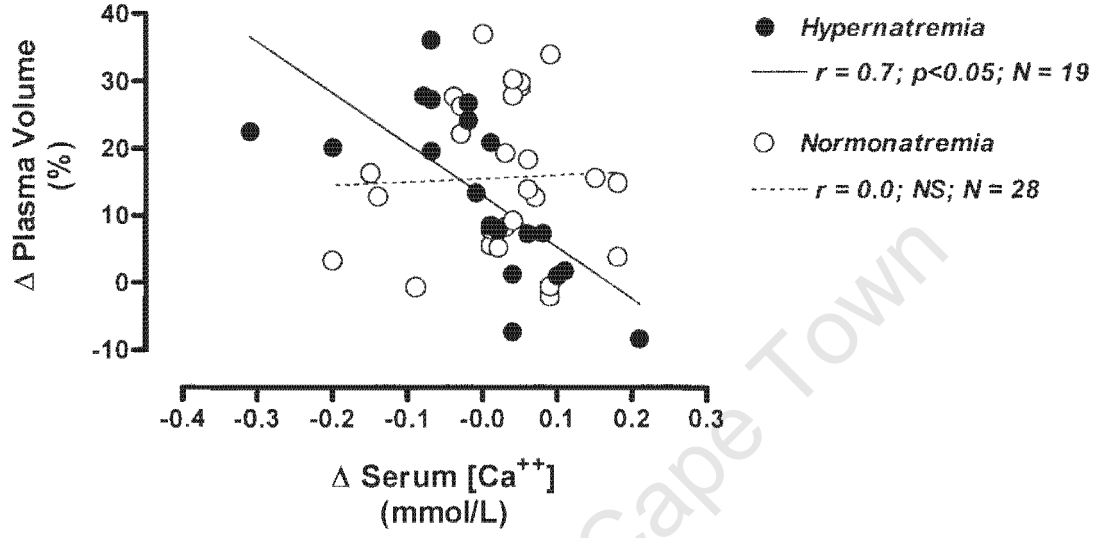


Figure 2D.3C: Initial serum calcium concentration versus initial serum sodium concentration sampling in hypernatremic and normonatremic collapsed runners given 1L of Ringers Lactate solution IV (N = 41).

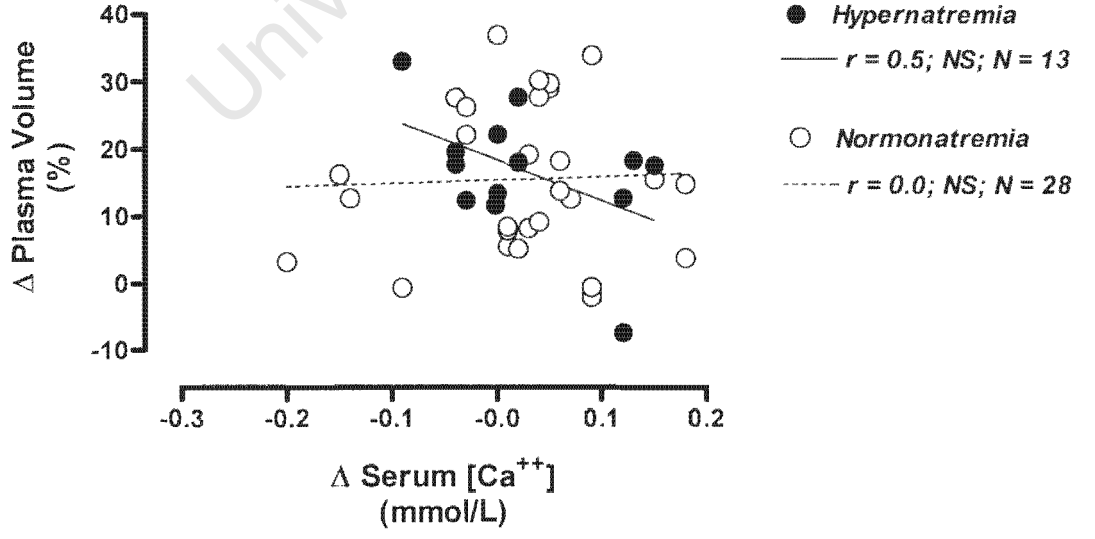
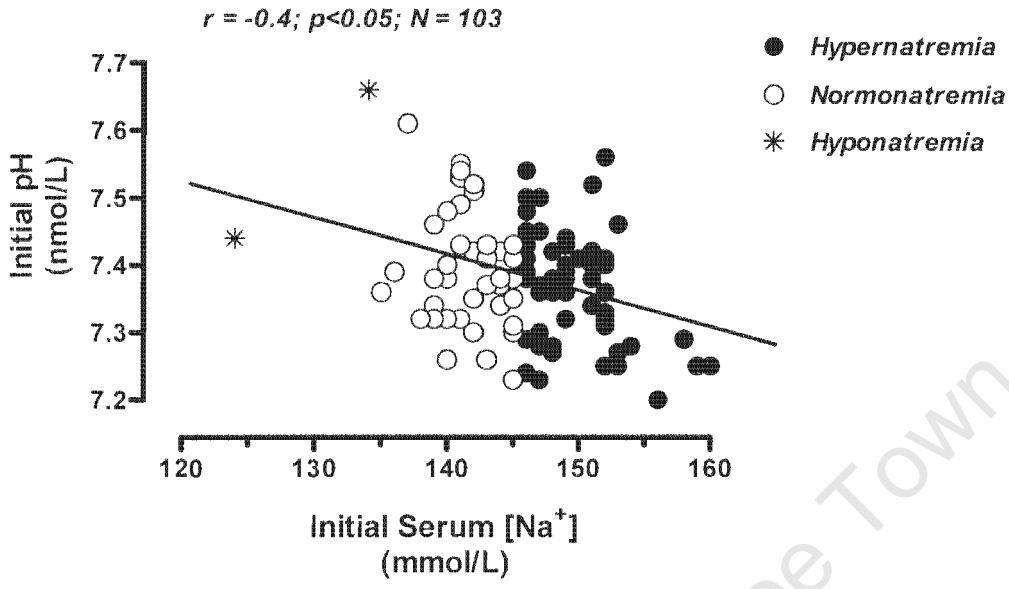


Figure 2D.4: Initial venous blood pH versus initial serum sodium concentration in all collapsed runners (N = 103).



DISCUSSION

Over half (58%) of the "collapsed" runners that were treated in the medical tent of the 2006 Comrades 90 km Marathon were hypernatremic. This was the highest reported incidence of hypernatremia ever recorded in the medical tent after a running race; higher than the 45% incidence of hypernatremia previously reported after the 2005 Comrades Marathon [7]. With fluid stations spaced every 1.5 km apart along the route, this finding was quite curious. Fluid stations spaced ≤ 1.6 km apart have been associated with an increased incidence of hyponatremia in marathon runners [10] while lengthening the distance between fluid stations has been shown to decrease the incidence of hyponatremia in Ironman Triathletes [11]. Therefore, the unexpectedly high incidence of hypernatremia and low incidence of hyponatremia - despite the generous availability of fluid along the course - was perhaps more reflective of a conservative approach to hydration by South African runners.

No deaths have been directly attributed to hypernatremia associated with exercise, although three fatal cases of hyperpyrexia in military recruits were associated with elevated serum [Na^+] and [K^+] [12]. Significant morbidity and mortality have been reported from hypernatremia following both extreme water deprivation [13] and excessive salt intake [14]. Conversely, asymptomatic hypernatremia has been documented following marathon foot races [15,16] and a long-distance ski race [17] while 40% of a small cohort of hypernatremic runners voluntarily withdrew from a "test" marathon from "heat stress" (rectal temperature reaching 40ºC) [18]. Hence, hypernatremia *per se* may not induce collapse in runners but may stimulate other pathophysiological processes which collectively impair performance [19], the capacity to continue exercise [18] or the ability to recover quickly [7].

The principle cause of the hypernatremia seen in this cohort of collapsed athletes remains unclear. Although excessive sodium intake was an unlikely mechanism, it cannot be determined if the abnormally high serum [Na^+] was secondary to significant body water loss or from an abnormal liberation of internal sodium stores [20]. The higher

Hb and Hct levels detected in hypernatremic compared with normonatremic runners (Tables 2D.1 and 2D.2), combined with the negative correlation between serum [Na^+] and plasma volume Δ (Figure 2D.2A) suggests that volume depletion accompanied the hypernatremia. The greater increase in SBP following IV fluid administration also supports the presence of concomitant hypovolemia in hypernatremic athletes [21].

Hypernatremic runners reported significantly more vomiting during the race than normonatremic runners (79 vs. 34%; $p<0.001$). Vomiting aggravates overall fluid loss and compromises fluid replacement and absorption. Whether the hypernatremia caused the high incidence of vomiting or whether vomiting caused the hypernatremia cannot be determined by these data. However, it does seem clear that vomiting amplifies the degree of hypernatremia. A previous report confirmed that 80% of a cohort of marathon runners who lost >4% of their body mass experienced (nonspecific) GI distress [22]. While this finding does not clarify whether hypernatremia induced the GI distress or whether the opposite response occurred, these data also suggest that hypernatremia was associated with fluid loss.

Ringers Lactate solution ([Na^+] = 131 mmol/L) was used by the medical team to both restore plasma volume [21] and "preferentially" rehydrate the intracellular space of collapsed normonatremic runners [23]. The half-life of RL is expected to be between 2-3 hours [24], with the retention of fluid within the vascular space proportional to the degree of hypovolemia present [21]. The administration of RL to runners in the normonatremic group caused an insignificant decrease in serum [Na^+] and pulse rate as well as an insignificant increase in serum [K^+], SBP and DBP (Tables 2D.1 and 2D.2). These minor biochemical and cardiovascular changes reflect an appropriate physiological response to RL infusion in subjects with hypovolemic normonatremia [21].

The administration of one liter of RL caused a 16% increase in plasma volume (Tables 1 and 2); an identical increase to that reported after 15 minutes of IV isotonic saline infusion in athletes previously dehydrated to 4-5% [4]. Interestingly enough, plasma volume was preferentially expanded at the expense of the intracellular space. This

"unexpected" decrease in the intracellular space has been previously documented and may have resulted from either 1) low sodium excretion by the kidney [23] or 2) preferential "binding" of lactic acid ([Lac-]) to [Na^+] in the vascular space [25].

Half Normal Saline ([Na^+] = 77 mmol/L) was used by the medical team to decrease serum [Na^+] in collapsed hypernatremic runners. The high incidence of hypernatremia in this cohort was unexpected; thus we underestimated our supply of HNS and were unable to treat all hypernatremic runners (N = 51) with hypotonic IV fluid. The administration of both HNS (Table 2D.1) and RL (Table 2D.2) to hypernatremic runners caused a significant decrease in serum [Na^+]. However, a return to normonatremia was *not* required for hypernatremic runners to successfully "recover" (resolution of adverse symptomatology) and be discharged from the medical tent (Figure 2D.1). The lack of statistical significance between both the time to discharge and the change (Δ) in any biochemical or cardiovascular parameter further suggests that the clinical and physiological response to both solutions was similar. Therefore, both HNS and RL seem equally effective in the treatment of collapsed runners with hypernatremia.

Hypotonic solutions have a minimal half-life and should leave the intravascular space quickly [24]. Only 8% of the infused HNS would be expected to remain in the intravascular space while the remaining 92% would be expected to distribute evenly between the interstitial and intracellular space [24]. The infusion of HNS in our cohort of collapsed hypernatremic runners elicited a plasma volume expansion that was greater than expected (14%; Table 2D.1). More surprising, however, was the 0.3% *loss* in intracellular volume that was largely unexpected given the hypotonicity of the infused solution. The degree of blood and plasma volume expansion, with concomitant cell volume contraction, was similar between the normonatremic and hypernatremic groups. This was despite wide disparities in the tonicity of both plasma and the two rehydration solutions. Therefore, in non-steady-state conditions following exercise, expansion of the intravascular compartment took precedence over expansion of the intracellular compartment following IV fluid resuscitation,

It cannot be concluded by these data that IV administration of either 1) an isotonic solution to normonatremic and hypernatremic runners or 2) a hypotonic solution to hypernatremic runners enhanced the "recovery" of collapsed athletes. It **can** be concluded, however, that the appropriate administration of one liter of IV fluid, based on the maintenance or return of serum [Na^+] back into the normal range, did not promote adverse physiological effects. Therefore, this practice should not be viewed as harmful if utilized in this context. Speculation that plasma volume expansion could facilitate a reduction in the sympatho-vagal ratio [26] or assist in the recovery of collapsed athletes by alternative mechanisms warrants further investigation.

The positive linear relationship between initial serum [Na^+] and initial serum [Ca^{++}] was an interesting ancillary finding (Figure 2D.3A). The apparent "clustering" of data according to natremic status might suggest that the concentration of the two cations increased with concomitant contraction of the plasma volume. However, the disparate response of the Δ in serum [Na^+] (Figure 2D.2B and 2D.2C) and [Ca^{++}] (Figure 2D.3B and 2D.3C) with the Δ plasma volume would suggest that the changes in these ions are not simply a result of hemodilution. Furthermore, hypercalcemia may induce nausea and vomiting because calcium stimulates gastric secretions [1]. Therefore, the higher incidence of vomiting in hypernatremic runner with highest serum [Na^+]'s may have been secondary to a concomitant increase in serum [Ca^{++}]. Significant correlations between calcium excretion with both daily sodium intake and sodium excretion have been verified in two Japanese studies [27;28]; further supporting the potential for an inter-relationship between calcium and sodium in plasma and bone.

The inverse linear relationship between initial [Na^+] and initial pH was more difficult to rationalize (Figure 2D.4). The presence of a Na^+, H^+ exchange mechanism in muscle cells has been hypothesized to explain a simultaneous disappearance of sodium ions, in proportion to the appearance of hydrogen ions in blood, after two minutes of maximal intensity exercise [29]. If this phenomenon were correct, then a *positive* relationship between serum [Na^+] and pH would be expected. However, because it is generally agreed that the skeleton acts as a "buffer reservoir" during chronic metabolic acidosis [30],

liberation of sodium from bone stores would be an expected response to chronically elevated hydrogen ion concentration (low pH). This case scenario would indeed validate the inverse relationship between serum [Na^+] and pH. However, whether prolonged endurance activity would create a state of chronic metabolic acidosis is unknown.

In summary, 58% of our cohort of collapsed runners was hypernatremic with vomiting either aggravating or facilitating the development of hypernatremia. A return to normonatremia was not required for hypernatremic runners to successfully "recover" and be discharged from the medical tent. The appropriate administration of 1L of IV RL or HNS fluid to collapsed runners, based on the maintenance or return of serum [Na^+] back into the normal range, did not promote any adverse physiological effects. Therefore, this practice should not be viewed as harmful if utilized in this context.

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Reference List

1. Weiss-Guillet EM, Takala J, Jakob SM. Diagnosis and management of electrolyte emergencies. *Best.Pract.Res.Clin.Endocrinol.Metab* 2003;**17**:623-51.

2. Lin M, Liu SJ, Lim IT. Disorders of water imbalance. *Emerg.Med.Clin.North Am.* 2005;**23**:749-70, ix.

3. Noakes TD, Berlinski N, Solomon E, Weight LM. Collapsed Runners: Blood Biochemical Changes After IV Fluid Therapy. *Physician Sports Med* 1991;**19**:70-81.

4. Kenefick RW, Maresh CM, Armstrong LE, Castellani JW, Riebe D, Echegaray ME *et al.* Plasma vasopressin and aldosterone responses to oral and intravenous saline rehydration. *J.Appl.Physiol* 2000;**89**:2117-22.

5. Casa DJ, Maresh CM, Armstrong LE, Kavouras SA, Herrera JA, Hacker FT, Jr. *et al.* Intravenous versus oral rehydration during a brief period: responses to subsequent exercise in the heat. *Med Sci.Sports Exerc.* 2000;**32**:124-33.

6. Castellani JW, Maresh CM, Armstrong LE, Kenefick RW, Riebe D, Echegaray M *et al.* Intravenous vs. oral rehydration: effects on subsequent exercise-heat stress. *J.Appl.Physiol* 1997;**82**:799-806.

7. Hew-Butler T, Sharwood K, Boulter J, Collins M, Tucker R, Dugas J *et al.* Dysnatremia predicts a delayed recovery in collapsed ultramarathon runners. *Clin.J.Sport Med* 2007;**17:** 289-296.

8. Speedy DB, Noakes TD, Holtzhausen LM. Exercise-Associate Collapse. *Phys Sportsmed* 2003;**31**:23-9.

9. Dill DB,.Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl.Physiol* 1974;**37**:247-8.

10. Hew TD, Chorley JN, Cianca JC, Divine JG. The incidence, risk factors, and clinical manifestations of hyponatremia in marathon runners. *Clin.J.Sport Med.* 2003;**13**:41-7.

11. Speedy DB, Rogers IR, Noakes TD, Thompson JM, Guirey J, Safih S *et al.* Diagnosis and prevention of hyponatremia at an ultradistance triathlon. *Clin.J.Sport Med.* 2000;**10**:52-8.

12. Baxter CR,.Teschan PE. Atypical heat stroke, with hypernatremia, acute renal failure, and fulminating potassium intoxication. *AMA.Arch.Intern.Med.* 1958;**101**:1040-50.

13. van der Helm-van Mil AH, van Vugt JP, Lammers GJ, Harinck HI. Hypernatremia from a hunger strike as a cause of osmotic myelinolysis. *Neurology* 2005;**64**:574-5.

14. Ofran Y, Lavi D, Opher D, Weiss TA, Elinav E. Fatal voluntary salt intake resulting in the highest ever documented sodium plasma level in adults (255 mmol L-1): a disorder linked to female gender and psychiatric disorders. *J.Intern.Med.* 2004;**256**:525-8.

15. Kavanagh T,.Shephard RJ. On the choice of fluid for the hydration of middle-aged marathon runners. *Br.J.Sports Med.* 1977;**11**:26-35.

16. Rocker L, Kirsch KA, Heyduck B, Altenkirch HU. Influence of prolonged physical exercise on plasma volume, plasma proteins, electrolytes, and fluid-regulating hormones. *Int.J.Sports Med.* 1989;**10**:270-4.

17. Astrand PO,.Saltin B. Plasma and cell volume alterations after prolonged severe exercise. *J Appl.Physiol* 1964;**19**:829-32.

18. Riley WJ, Pyke FS, Roberts AD, England JF. The effect of long-distance running on some biochemical variables. *Clin.Chim.Acta* 1975;**65**:83-9.

19. Coyle EF. Fluid and fuel intake during exercise. *J.Sports Sci.* 2004;**22**:39-55.

20. Noakes TD, Sharwood K, Speedy D, Hew T, Reid S, Dugas J *et al.* Three independent biological mechanisms cause exercise-associated hyponatremia: evidence from 2,135 weighed competitive athletic performances. *Proc.Natl.Acad.Sci.U.S.A* 2005;**102**:18550-5.

21. Drobin D,.Hahn RG. Volume kinetics of Ringer's solution in hypovolemic volunteers. *Anesthesiology* 1999;**90**:81-91.

22. Rehrer NJ, Janssen GM, Brouns F, Saris WH. Fluid intake and gastrointestinal problems in runners competing in a 25-km race and a marathon. *Int.J.Sports Med.* 1989;**10 Suppl 1**:S22-S25.

23. Hahn RG,.Drobin D. Rapid water and slow sodium excretion of acetated Ringer's solution dehydrates cells. *Anesth.Analg.* 2003;**97**:1590-4.

24. Cooper A,.Moore M. I.V. fluid therapy. Part 2. I.V. fluid selection. *Aust.Nurs.J.* 1999;**7**:suppl-4.

25. Nose H, Takamata A, Mack GW, Oda Y, Okuno T, Kang DH *et al.* Water and electrolyte balance in the vascular space during graded exercise in humans. *J.Appl.Physiol* 1991;**70**:2757-62.

26. Yun AJ, Lee PY, Bazar KA. Clinical benefits of hydration and volume expansion in a wide range of illnesses may be attributable to reduction of sympatho-vagal ratio. *Med.Hypotheses* 2005;**64**:646-50.

27. Kodama N, Nishimuta M, Suzuki K. Negative balance of calcium and magnesium under relatively low sodium intake in humans. *J.Nutr.Sci.Vitaminol.(Tokyo)* 2003;**49**:201-9.

28. Itoh R,.Suyama Y. Sodium excretion in relation to calcium and hydroxyproline excretion in a healthy Japanese population. *Am.J.Clin.Nutr.* 1996;**63**:735-40.

29. Medbo JI,.Sejersted OM. Acid-base and electrolyte balance after exhausting exercise in endurance-trained and sprint-trained subjects. *Acta Physiol Scand.* 1985;**125**:97-109.

30. Bettice JA,.Gamble JL, Jr. Skeletal buffering of acute metabolic acidosis. *Am.J.Physiol* 1975;**229**:1618-24.

CHAPTER 3

THE ENDOCRINE REGULATION OF FLUID BALANCE DURING EXERCISE

Study 3E: Hormonal changes during endurance exercise

INTRODUCTION

Marathon running has experienced robust growth and popularity over the last two decades, with the number of marathon finishers in the U.S. increasing from 120,000 in 1980, to 260,000 in 1995 and 450,000 in 2002. This large increase in the number of recreational athletes, who view the marathon as their ultimate fitness goal, has altered the physiological profile of marathon runners and uncovered an entirely new set of medical problems not previously seen in more experienced, better trained and faster athletes. The growing popularity of marathon running underscores the importance of understanding the physiological mechanisms that underlie both normal and abnormal fluid and electrolyte regulation during endurance exercise.

Understanding the normal hormonal regulation of fluid and sodium balance during prolonged endurance activity will allow better definition of disorders of fluid homeostasis related to neuroendocrine and endocrine mechanisms. Exercise-associated hyponatremia (EAH) represents the major life-threatening manifestation of dysregulation of fluid homeostasis during prolonged exercise. EAH has recently emerged as the most common serious medical complication of endurance exercise [1]. Four otherwise healthy female marathon runners have died from EAH since 1993 [2;3], and 23-27% of Ironman triathletes have finished triathlons with documented hyponatremia (serum sodium concentrations <135 mmol/L) [4;5]. Although the primary cause of EAH is a relative over-consumption of fluids beyond the ability of the kidneys to excrete excess fluid, the mechanisms that limit maximum renal excretory ability, as well as the question of why a smaller percentage of athletes develop hyponatremia despite a loss of body weight during endurance exercise, remain to be elucidated.

To date there is a paucity of data describing the endocrine regulation of fluid and sodium balance during prolonged endurance exercise. Most of the available field-based data has been evaluated in small (n<10) groups [6-8] under varying states of hydration [9], and with considerable time delays in blood sampling that often exceed the half-lives of

many of the regulatory hormones being evaluated [8;10-12]. Each of these factors markedly limits the strength of the scientific knowledge obtained. The aim of this study was to perform a comprehensive evaluation of the endocrine secretion of pituitary, natriuretic and adrenal steroid hormones, as well as cytokines, in well-trained endurance athletes immediately before and after running a 56 km ultramarathon.

METHODS

Subjects and sample collection

Informed written consent was obtained in 82 runners competing in the Two Oceans 56 km ultramarathon, held in Cape Town, South Africa on March 26, 2005. This study was approved by both the Ethics Committee of the University of Cape Town and the Georgetown University Institutional Review Board.

Baseline body weight, blood and urine samples were obtained within 60 min of the start of the race. Post-race body weight, blood and urine samples were obtained immediately upon race completion; the average time interval between race finish to post-race blood draw was 6.9 ± 0.5 min. All pre- and post-race blood samples were immediately placed on ice and centrifuged within 10 min at 3,000 rpm; separated plasma was stored on dry ice until the samples were frozen to -80° C. All samples remained frozen until further analysis was performed. Body weight was measured with athletes in racing attire without shoes on calibrated Adamlab JPS electronic scales placed on a hard, flat surface (Scales, Brackenfell, South Africa). A post-race questionnaire was administered to each runner immediately after blood and urine collection, which detailed the type and estimated amounts of fluid ingested along the course, the frequency of urination, and any medications taken during the race. Food and fluid intake were allowed *ad libitum* during the race.

Analytical measurements

Changes in plasma volume were estimated by comparing pre- and post-race measurements of plasma protein using a clinical refractometer (Schuco Clinical

Refractometer 5711-2020, Japan). Plasma and urine sodium ($[Na^+]$) and potassium ($[K^+]$) concentrations were measured using ion-selective electrodes (Beckman Synchron EI-ISE, Fullerton, CA).

Hormone Measurements

Plasma levels of arginine vasopressin (AVP) and oxytocin (OT) were measured by specific radioimmunoassay following acetone-ether extraction as described previously [13]. The standard curve for AVP is linear between 0.5 and 10.0 pg/tube with the use of a synthetic AVP standard (PerkinElmer Life Sciences Inc, Boston MA). The minimum detectable concentration of AVP in extracted plasma was 0.5 pg/ml. The AVP antiserum (R-4) displayed <1% cross-reactivity with OT. The standard curve of the OT assay was linear between 0.25 and 5.0 pg/tube with the use of a synthetic OT standard (PerkinElmer Life Sciences Inc, Boston MA). The minimum detectable concentration of OT in extracted plasma was 0.25 pg/ml. The OT antiserum (Pitt-Ab2) displayed <1% cross-reactivity with AVP.

Eleven adrenal steroid hormones (cortisol, 11-deoxycortisol, aldosterone, corticosterone, DHEA, DHEAS, testosterone, androstenedione, 17-hydroxyprogesterone, progesterone and 25-hydroxyvitamin D3) were measured using a liquid chromatography-tandem mass spectrometer, in conjunction with an atmospheric pressure photoionization source, using methodology described previously [14].
Brain natriuretic peptide was assessed via measurement of the more stable cleaved inactive fragment, N-terminal pro-brain natriuretic peptide (NT-pro-BNP), using the automated Dade RxL Dimension, as described previously [15]. Interleukin-6 (IL-6) was measured using a chemiluminescence method with a commercial kit and an automatic chemiluminassay analyzer (Immulite 1000 System: Diagnostic Products Corporation, Los Angeles, California). The minimal detectable limit of the assay was 5 pg/ml.

Statistical analysis

All data were analyzed using STATISTICA 7™ software (StatSoft Tulsa, OK). Where applicable, data were presented as means ± SEM, together with the range of values. Paired t-tests were utilized to assess significant differences between pre- and post-race.

Differences between male and female runners were assessed using paired t-tests. Statistical significance was accepted when $p < 0.05$.

RESULTS

Overall, a total of 5,472 men completed the Two Oceans race with a mean finishing time of 5:35 (hour: minutes), and 1,502 women (22%) completed the event with a mean finishing time of 5:55. The maximum temperature on race day was 23° C and the minimum temperature at race start was 15° C. Coke™ and sachets containing 100 ml of water and Powerade™ ($[Na^+]$ =10 mmol/L, 8% carbohydrate) were available at fluid stations located every 3 km along the 56 km course.

All eighty-two runners who consented to participate in the research trial completed the Two Oceans Ultramarathon, including 58 men (71%) and 24 women (29%), with a combined mean finishing time of 5:56. The mean age of the cohort was 43.7 ± 1.1 years. The mean pre-race body weight of the subjects was 74.3 ± 1.4 kg with the mean body mass index of 23.5 ± 0.3 kg/cm^2. The subjects' mean estimated total fluid intake during the race was 3.2 ± 1.7 L.

Physiological markers of fluid balance homeostasis were assessed by changes (Δ = post-race minus pre-race) in body weight, plasma $[Na^+]$, plasma volume, urine $[Na^+]$, and urine osmolality (Table 3E.1). The changes in body weight, plasma volume, urine $[Na^+]$ and urine osmolality were statistically significant from pre- to post-race, while the changes in plasma $[Na^+]$ were not.

Significant increases from pre- to post-race were observed for AVP, OT, NT-proBNP and IL-6. Several of the changes were particularly marked, with a five-fold increase in brain natriuretic peptide and a ten-fold increase in IL-6 (Table 3E.2).

Plasma concentrations of nine of the eleven adrenal steroid hormones measured increased significantly from pre- to post-race (Figure 3E.1). Cortisol, the major glucocorticoid hormone, and its two precursors, 17-hydroxyprogesterone and 11-deoxycortisol, increased significantly by 1.7-fold (cortisol and 17-hydroxyprogesterone)

and 3.1-fold (11-dexoxycortisol). Plasma levels of the major mineralocorticoid hormone, aldosterone, increased 5.4-fold and its immediate glucocorticoid precursor, corticosterone, increased 3.3-fold. The adrenal gonadal steroid precursors (DHEA, DHEAS, and androstenedione) all increased significantly, while the gonadal steroid end-products (testosterone and progesterone) did not change significantly from pre- to post-race in either sex. Plasma concentrations of 25-hydroxyvitamin D3 increased significantly from pre- to post-race.

Significant differences were noted between male versus female runners in the pre- and post-race plasma concentrations of: NT-proBNP (29.1 versus 45.7 pg/ml pre-race and 140.5 versus 216.6 pg/ml post-race; male versus female respectively), DHEAS (126.7 versus 91.8 µg/ml pre-race and 170.5 versus 127.5 µg/ml post-race, respectively), testosterone (502.7 versus 78.2 pre-race ng/ml and 453.8 versus 83.1 ng/ml post-race, respectively), and progesterone (1.8 versus 129.5 ng/ml pre-race and 18.0 versus 135.2 ng/ml post-race, respectively). Although the absolute pre- and post-race values of these four hormones were different between males and females, the *change* in the concentration from pre to post-race was not significantly different between sexes.

Simple regression analysis revealed significant positive correlations between post-race AVP versus post-race OT, post-race IL-6 and plasma $[Na^+]$ Δ. significant negative correlations were noted between post-race aldosterone and aldosterone Δ versus plasma volume Δ.

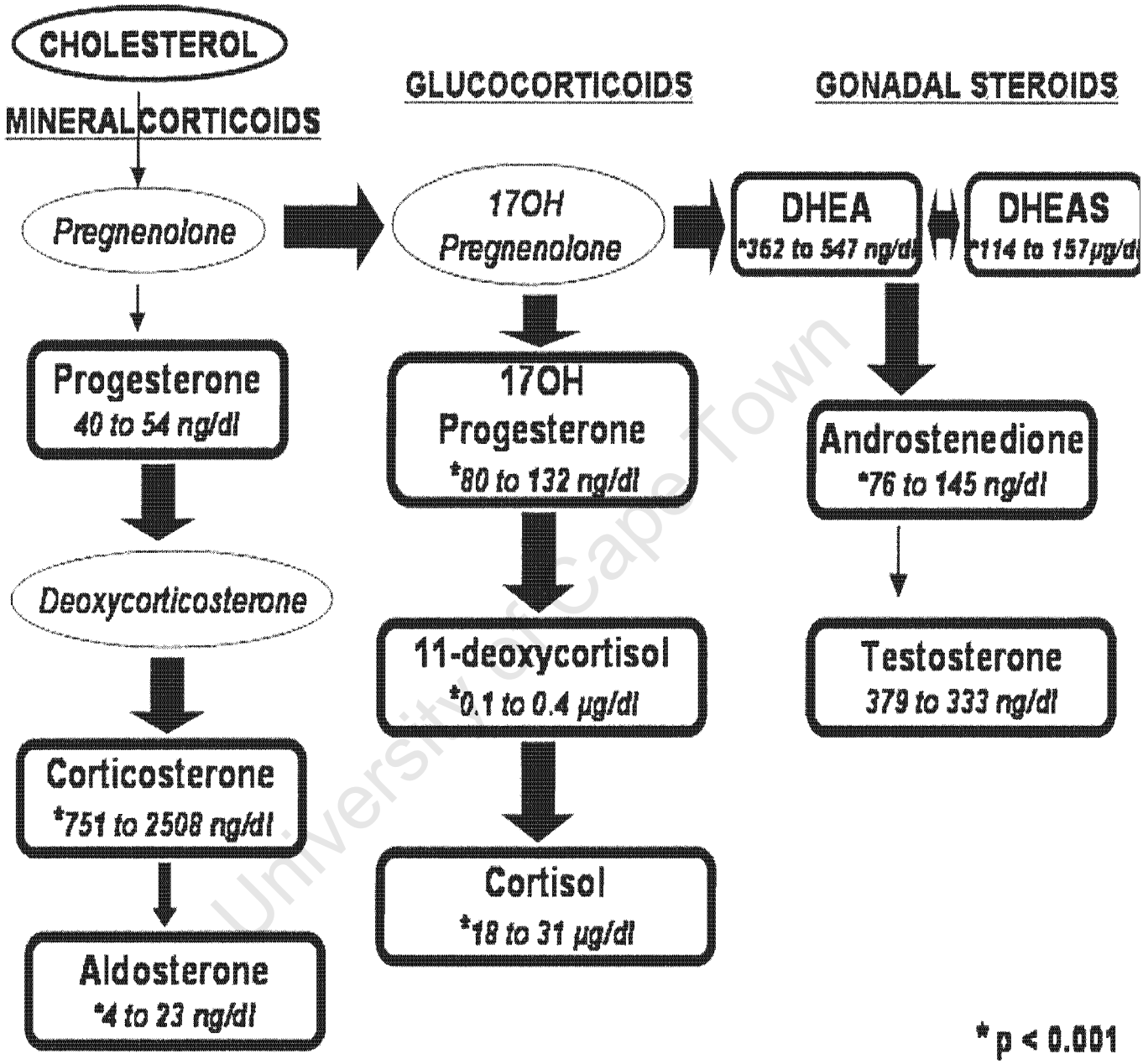
Table 3E.1: Physiological markers of fluid balance in runners completing a 56 km running race.

VARIABLE	Mean ± SEM	Minimum	Maximum
Bodyweight Δ (%) (N = 79)	-3.6 ± 0.1	-6.4	-0.4
Plasma $[Na^+]$ Δ (mmol/L) (N = 78)	-1.3 ± 0.5	-9.9	15.4
Plasma Volume Δ (%) (N = 79)	-8.5 ± 0.1	-21	9
Urine $[Na^+]$ Δ (mmol/L) (N = 64)	-25.5 ± 6.4	-163	116.5
Urine Osmolality Δ ($mOsmol/kgH_2O$) (N = 72)	122.3 ± 33.3	-557.0	665

Table 3E.2: Peptide hormone and cytokine changes from pre to post race.

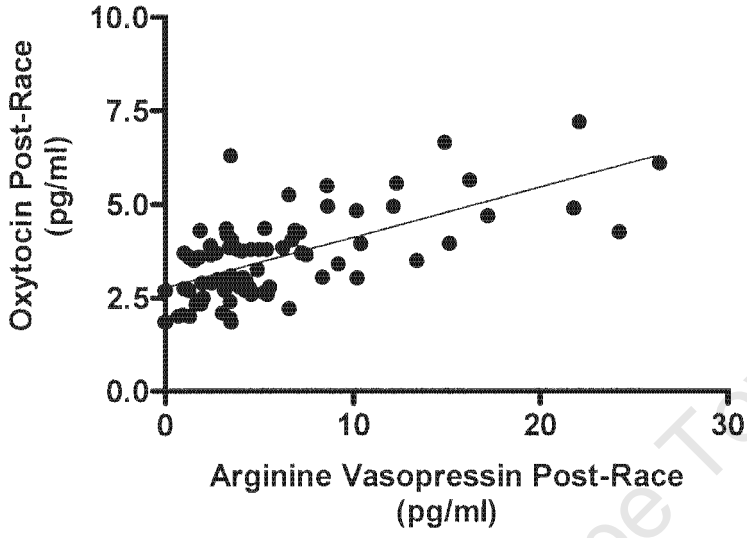
VARIABLE	PRE-RACE (Mean ± SEM)	POST-RACE (Mean ± SEM)	P - value
Arginine Vasopressin (pg/ml) (N = 78)	1.3 ± 0.1	7.1 ± 0.9	< 0.0001
Oxytocin (pg/ml) (N = 79)	1.9 ± 0.1	3.7 ± 0.1	< 0.0001
NT-proBNP (pg/ml) (N = 76)	34.1 ± 3.8	165.0 ± 16.2	< 0.0001
Interleukin-6 (pg/ml) (N = 61)	4.2 ± 0.1	52.7 ± 3.8	< 0.0001

Figure 3E.1: Adrenal steroid results from pre to post-race within the pathway of steroid biosynthesis from cholesterol.



Legend: The adrenal steroids measured in this study are noted inside each rectangular box. Pre and post-race concentrations are noted directly underneath each plasma steroid, respectively. The asterisks denote statistical significance from pre to post-race.

Figure 3E.2: Relationship between arginine vasopressin and oxytocin.



DISCUSSION

Body fluid homeostasis during prolonged endurance exercise (356 ± 4 minutes in this ultramarathon) was well maintained in this cohort of experienced athletes with *ad libitum* food and fluid intakes. Plasma sodium levels were maintained from pre-race (139.3 ± 0.3 mmol/L) to post-race (138.1 ± 0.4 mmol/L) despite significant decreases in body weight (-3.6 ± 0.1%, $p<0.01$) and significant reductions in estimated plasma volume (-8.5 ± 0.1%, $p<0.01$). Urine sodium excretion decreased while urine osmolality increased pre- to post-race, also contributing to the successful regulation of serum [Na^+] during this period of heightened physical stress. These findings suggest that the physiological mechanisms responsible for body fluid homeostasis primarily function to preserve plasma osmolality.

The endocrine hormone most responsible for the regulation of plasma osmolality and sodium concentration is AVP [16]. Plasma levels of AVP were significantly elevated 3.9-fold at the end of the ultramarathon despite unchanged plasma sodium concentrations. This suggests the presence of non-osmotic stimuli to AVP secretion during prolonged endurance exercise. For runners competing at sustained exercise intensities over long distances, these potentially include plasma volume contraction [17], nausea and/or vomiting [18], hypoglycemia [16], elevated body temperature [19] and elevated IL-6 concentrations [20]. However, this should not be interpreted to mean that osmotic stimulation of AVP secretion does not occur under these conditions, which is demonstrated by a positive correlation between post-race AVP levels and pre- to post-race changes in plasma [Na^+]. Osmotic stimulation of AVP secretion has also been shown in other field studies, such as a study involving 16 elite runners completing a 42.2 km marathon in 164 minutes. In this study, a 4-fold increase in post-race AVP was documented in conjunction with a serum [Na^+] increase of 6 mmol/L, a body weight loss of 5% and a decline in plasma volume of 12% [21]. The differences between these findings and our results likely reflect greater evaporative fluid losses with less fluid replacement in the previous marathon study, but also could indicate that non-osmotic stimulation may have had a lesser impact on AVP secretion in marathon runners who compete at higher exercise intensities.

The estimated 8.5 ± 0.1% plasma volume contraction from pre- to post-race is sufficient to both stimulate and increase the gain for osmotic AVP release from the neurohypophysis [17]. The decrease in plasma volume during exercise generally results from increased hydrostatic forces [22], the magnitude of which is linearly related to exercise intensity [23]. These exercise-induced reductions in plasma volume are apparent within the first 6 km of running and generally remain stable throughout the remainder of the exercise [24]. The initial development and maintenance of a reduced plasma volume could serve as a non-osmotic stimulus to AVP secretion, which would in turn produce an antidiuresis during exercise as a result of its actions at AVP V_2 receptors in the kidney. This stimulated antidiuresis could potentiate the development of a dilutional hyponatremia by causing retention of excessively consumed fluids. EAH may therefore represent an exercise-induced variant of the syndrome of inappropriate antidiuretic hormone secretion (SIADH), in which nonosmotic AVP secretion is inappropriate for the degree of hypoosmolality that develops during prolonged exercise as a result of drinking in excess of sweat losses.

Plasma concentrations of oxytocin were also significantly elevated in runners immediately following completion of the Two Oceans 56 km race, although the increase was only approximately half (1.9-fold) of the relative increase in AVP secretion. The secretion of both posterior pituitary hormones was positively correlated with one another (Figure 3E.2), which suggests that a complementary relationship may exist in humans between the two neurohypophysial hormones during periods of prolonged exertional stress. Osmotic stimulation of oxytocin and its role in stimulating natriuresis and inhibiting sodium appetite has been well described in rats [25;26], but equivocal in human studies [27;28]. The significant positive correlation between urine sodium excretion and post-race oxytocin levels, as found in this study, supports a possible physiological role for oxytocin in the maintenance of fluid homeostasis in exercising humans. The potential stimulatory effect of oxytocin on glucose production and metabolism [29], and on the restraint of exercise-associated tachycardia [30], may also stimulate oxytocin secretion unrelated to fluid homeostasis during prolonged endurance exercise.

Circulating levels of interleukin-6 were elevated 12.5-fold post-race, but these levels were much lower than the 8,000-fold increase seen after longer duration races (i.e., 245 km; [31]) or the 100-fold increase documented after a competitive, higher intensity marathon [32]. It was initially hypothesized that circulating monocytes were primarily responsible for the increased IL-6 production in response to inflammation and muscle damage associated with prolonged endurance exercise. However, these theories have not been supported in field studies [32;33]. Alternatively, it has been confirmed that active muscles produce IL-6 during exercise [34;35]. The physiological function of these marked increases in IL-6 production is unclear, but possible metabolic roles in the stimulation of lipolysis [36;37], and in the augmentation of endogenous glucose production and clearance [38], have been recently postulated. Mice deficient in IL-6 have a reduced energy expenditure and exercise capacity, further suggesting that IL-6 is necessary for normal exercise capacity [39].

Interleukin-6 also stimulates AVP production in non-exercising humans [20;40], and could play a role in the development of exercise-induced SIADH [41]. A possible pathophysiological role for IL-6 on fluid balance, via the documented stimulatory effects of IL-6 secretion on non-osmotic AVP secretion, is supported by the results of this study that show a significant, but weak, linear correlation between post-race IL-6 levels and post-race AVP secretion.

Circulating levels of BNP have been shown to increase in relation to both the distance and the time taken to complete an ultraendurance event [42;43], with a similar 4.5-fold increase in NT-proBNP documented in 10 healthy men immediately following completion of a 100 km running race [44]. Interest in the role of increased circulating levels of BNP after prolonged endurance exercise has focused on whether this might represent a marker of myocardial strain and dysfunction [42;43;45-47]. Although significantly higher levels of BNP have been documented in endurance athletes immediately post-race [42;43;45;47], 24 hours post-marathon [46], and at baseline control levels in marathon runners [48], a correlation between BNP elevations and other markers of myocardial damage (e.g., cardiac troponins) has not been substantiated using second generation assays [42;43;47]. One study did report a positive correlation between the change in BNP

levels and the change in cardiac troponin T, but this study utilized a less sensitive first generation assay with a 1-2% cross reactivity with skeletal troponin T [44].

The role of elevated plasma BNP concentrations in the absence of any clear evidence of cardiac wall stress or significant blood volume expansion during endurance exercise was intriguing. We chose to assess circulating levels of BNP rather than atrial natriuretic peptide (ANP) in our study design because high BNP concentrations have been observed in disease states characterized by fluid overload [49]. BNP is currently the only FDA-approved marker for the diagnosis of congestive heart failure, because of its superior sensitivity to blood volume expansion. Because body weight and plasma volume decreased in our cohort of runners, and blood pressure is known to remain stable or even decrease in athletes during prolonged endurance exercise [50-52], the known natriuretic and diuretic effects of elevated natriuretic peptides seem paradoxical in the context of prolonged exercise.

Strong evidence has linked natriuretic peptide secretion, stimulation and expression to lipid mobilization [53-58]. Prolonged exercise duration, at concomitantly lower exercise intensities, increase the dependency of the body on lipid utilization as a primary energy source [59]. Interestingly, female runners in our cohort had greater pre- and post-race concentrations of BNP than male runners. Because females have increased fat stores [60] and a heightened capacity to utilize lipids as an energy source during endurance exercise [61], it is tempting to speculate that the increased BNP levels found in our cohort resulted from the increased metabolic demand to increase fat metabolism during an ultradistance race, with female runners more physiologically efficient at mobilizing fat as an energy substrate than male runners.

The 1.7-fold increase in plasma cortisol from pre- to post-race was an expected response as muscular exercise represents a reproducible stressor that activates the hypothalamic-pituitary axis to induce gluconeogenesis and counteract the inflammatory response to exercise [62;63]. The magnitude of the cortisol increase was similar to that documented in ultradistance triathletes competing for 23 to 27 hours [7], runners participating in a 24-hour endurance race [12], and marathon runners completing 42.2 km

between 157 and 334 minutes [64;65]. A larger 3 to 5-fold increase in the magnitude of circulating cortisol has been documented in more elite athletes such as those competing in the Boston Marathon [66] and in a cohort of triathletes completing a world championship [67]. Therefore, it appears that the intensity of exercise, rather than the duration, is a stronger stimulus to cortisol secretion, presumably as a result of greater ACTH stimulation [67].

The precursor adrenal steroid for cortisol, 11-deoxycortisol, increased 3.1- fold, which was almost double the increase seen in the glucocorticoid end product, cortisol. A limitation in the rate of 11β-hydroxylase activity, increased binding of cortisol to elevated plasma concentrations of circulating albumin, increased binding or production of corticosteroid binding globulin (CBG), increased translocation of cortisol into active tissues and/or an increase in the degradation of free cortisol in the liver may contribute singularly or in combination to the disparity noted between the circulating amounts of cortisol and 11-deoxycortisol documented after prolonged endurance exercise. The 1.7-fold significant increase in plasma 17-hydroxyprogesterone, the precursor adrenal steroid to 11-deoxycortisol (Figure 3E.1), suggests that the rate of 21-hydroxylase activity was greater than the activity of 11β-hydroxylase during prolonged exercise stress.

Plasma concentrations of the major mineralocorticoid hormone, aldosterone, increased significantly from pre- to post-race. The 5.4-fold increase in aldosterone levels were consistent with the 3-8 fold range of increase documented in other endurance races lasting between 2 to 27 hours [11;12;21;66;68]. The variation in the magnitude of increase between these field studies was likely dependant on the degree of thermal exposure [69], the level of dehydration before and during the race [70], the tonicity of the rehydration beverage [71], and exercise intensity [72], with higher aldosterone levels found in faster runners competing in shorter distances [21] and lower aldosterone levels found in slower runners competing in longer distances [12]. Aldosterone concentrations have been shown to increase 2-fold 10 km into a 42 km run [11] and remain elevated for up to two days following a 24 hour run [12]. The initial increase in aldosterone levels are most likely due to ACTH stimulation [73] combined with the coupling between sympathetic nerve activity

and the renin-angiotensin system that has been described elsewhere during exercise [74;75]. The sustained elevation in aldosterone levels 1-2 days post-race may have facilitated the isotonic return, or even expansion, of plasma volume over pre-race levels in response to prolonged endurance exercise [76]. Both the pre- to post-race change and post-race concentrations of aldosterone were negatively correlated with the change in plasma volume, which is an expected physiological response since plasma volume contraction is a strong stimulus of the renin-angiotensin-aldosterone system [77].

There was a significant 3.3-fold increase in the concentration of the weak glucocorticoid and mineralocorticoid precursor, corticosterone, from pre- to post-race. In humans, corticosterone is mainly an intermediary in the production of aldosterone, with no specific physiological role [78]. Although corticosterone, cortisol and aldosterone have equal affinity for the mineralocorticoid receptor, aldosterone accounts for 90% of the total mineralocorticoid activity expressed [79]. Perhaps the larger circulating concentrations of cortisol and corticosterone, produced during prolonged endurance exercise, stimulate more rapid nongenomic actions at mineralocorticoid receptors in cardiomyocytes and vascular smooth muscle cells before the slower genomic actions of increased aldosterone concentrations facilitate increased sodium reabsorption across epithelial cells [80;81].

Dihydroepiandrosterone (DHEA) and its readily conjugated sulfated ester, DHEAS, increased 1.5 and 1.4-fold, respectively, from pre- to post-race in our cohort of ultramarathon runners. These values were similar to the 1.7-fold increase in DHEAS seen in 18 runners completing a 42.2 km marathon [65] and 2-fold increase seen in 35 male triathletes after a world championship [67]. The magnitude of increase was similar between male and female runners in our cohort of experienced ultramarathon runners, but the *absolute* pre- and post-race plasma concentrations of DHEAS were significantly higher in males than in females. This finding was in direct contrast with the results of another study involving previously *untrained* males and females participating in a 15 km, 25 km and 42 km road race where both sexes had similar DHEAS levels throughout [82]. The disparity between these results and the physiological rationale between the gender differences in DHEAS levels is unclear.

In agreement with the literature, however, is our confirmation of the significant negative correlation found between both pre and post-race DHEA and DHEAS levels with age, which has been previously documented in both athletic [67] and non-athletic adults [83]. In both genders, DHEAS has been shown to remain elevated for 1-2 days following a marathon. This suggests that the adrenal production of this androgen and estrogen precursor may be sustained after prolonged endurance races [82], in direct contrast to other endocrine secretions which for the most part return to baseline within 24 hours post-race [21;33;67]. Although DHEAS is the most abundant circulating hormone in the body, the physiological role of both DHEA and DHEAS remains uncertain [84]. The documented participation of DHEA and DHEAS in the regulation of interleukin synthesis, modulation of insulin output and anti-glucocorticoid (anti-catabolic) effect may support a physiological role for the increased secretion of these hormones during and after a prolonged endurance exercise [84].

There was a non-significant decrease in testosterone in males and females from pre- to post-race, despite similar significant increases in androstenedione in both males (1.9-fold) and females (1.8-fold). The decrease in plasma testosterone levels in males has been well documented in other endurance races; with a systematic decline noted in male subjects running 15 km, 25 km and 42 km [82], and a 50% decline in pre-race testosterone levels noted 8 hours into a 24 hour race [85]. The magnitude of the testosterone decline was similar in a cohort of younger versus older males completing a championship triathlon, with further decline in testosterone levels noted 16-18 hours after race completion [67]. These cumulative results support the existence of the "exercise-hypogonadal male condition" that has been fully described elsewhere [86].

The marginal increase in plasma testosterone concentrations found in female runners has been documented previously, where testosterone increased almost linearly in five women completing a 42.2 km marathon [87]. Androstenedione was also elevated in this cohort, with peak concentrations noted between 20 and 30 km [87]. In contrast to the male athletes, there was a sharp decline in testosterone and androstenedione immediately following the race in the female athletes, with a return to baseline levels noted for both hormones within 60-120 minutes. Unlike in male athletes where a majority of

testosterone is produced by the testis, the majority of testosterone formation in females is derived from the adrenal gland from the peripheral conversion of androstenedione to testosterone. The disparity in the magnitude and kinetics of testosterone levels between males and females reflects the separate origin of these androgen hormones, with plasma estradiol perhaps a better marker for exercise-induced gonadal dysfunction in female athletes.

Plasma concentrations of progesterone increased 10-fold in male and remained constant in female runners. The reason for the large increase in progesterone levels in male runners remains unclear, and elevated progesterone levels have not been reported previously in male athletes completing a prolonged endurance event. Marginal increases in progesterone concentration in female runners, however, has been previously documented in one study involving only two female marathon runners [87]. The influence of progesterone in the regulation of fluid balance during endurance exercise is not supported by any data found in this study, although progesterone may play a minor role in sodium retention as an intermediary in aldosterone formation (Figure 3E.1).

The 1.1-fold increase in plasma 25-hydroxyvitamin D3 concentrations in runners completing 56 km in a mean time of 356 minutes was an expected physiological response. 25-hydroxyvitamin D3 is a precursor to the most active vitamin D metabolite (1, 25-dihydroxyvitamin D), which is produced from sun exposure [88]. Since the 2005 Two Oceans Ultramarathon was held on a cloudless day and elevated serum levels of 25-hydroxyvitamin D3 have been documented in physically active versus non-active men, it seems likely that the increased sun exposure during six hours of running would stimulate an increase in vitamin D synthesis. The physiological significance of increased circulating levels of 25-hydroxyvitamin D3 during prolonged endurance exercise is uncertain; however it's contribution to bone mineralization [89] and glucose tolerance [90] have been documented.

In conclusion, the increase in AVP during endurance exercise is consistent with the need to conserve water, but inappropriate in the presence of decreases in serum [Na^+],

suggesting a non-osmotic stimulus underlying the AVP secretion. The smaller increases in oxytocin levels are significant, but of uncertain physiological significance. The large increase in NT-proBNP was not due to blood volume expansion and also is of unknown origin. The increase in IL-6 was consistent with rhabdomyolysis following prolonged endurance exercise. The increase in cortisol was expected as a result of HPA-axis stimulation by stress. The increase in aldosterone was consistent with the need to conserve sodium and protect against further decreases in plasma volume in response to sweat sodium losses during prolonged endurance exercise. Plasma sodium concentration was maintained in well-trained runners participating in a 56 km race by stimulating hormonal mechanisms that protect fluid and sodium balance. Plasma volume contraction, from the increased hydrostatic forces that occur during long distance running, facilitated the retention of total body water and sodium mainly through the direct stimulation of AVP, OT and aldosterone.

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Reference List

1. Hew-Butler TD, Almond CS, Ayus JC, Dugas JP, Meeuwisse WH, Noakes TD *et al.* Consensus Statement of the 1st International Exercise-Associated Hyponatremia Consensus Development Conference, Cape Town, South Africa 2005. *Clin.J.Sport Med* 2005;**15**:208-13.

2. Hew TD, Chorley JN, Cianca JC, Divine JG. The incidence, risk factors, and clinical manifestations of hyponatremia in marathon runners. *Clin.J.Sport Med.* 2003;**13**:41-7.

3. Ayus JC, Varon J, Arieff AI. Hyponatremia, cerebral edema, and noncardiogenic pulmonary edema in marathon runners. *Ann Intern.Med* 2000;**132**:711-4.

4. Speedy DB, Campbell R, Mulligan G, Robinson DJ, Walker C, Gallagher P *et al.* Weight changes and serum sodium concentrations after an ultradistance multisport triathlon. *Clin.J Sport Med* 1997;**7**:100-3.

5. Hiller WDB. Dehydration and hyponatremia during triathlons. *Med.Sci.Sports Exerc.* 1989;**21(Suppl)**:219-21.

6. Wade CE, Dressendorfer RH, O'Brien JC, Claybaugh JR. Renal function, aldosterone, and vasopressin excretion following repeated long-distance running. *J.Appl.Physiol* 1981;**50**:709-12.

7. Gastmann U, Dimeo F, Huonker M, Bocker J, Steinacker JM, Petersen KG *et al.* Ultra-triathlon-related blood-chemical and endocrinological responses in nine athletes. *J.Sports Med.Phys.Fitness* 1998;**38**:18-23.

8. Dessypris A, Wagar G, Fyhrquist F, Makinen T, Welin MG, Lamberg BA. Marathon run: effects on blood cortisol -- ACTH, iodothyronines -- TSH and vasopressin. *Acta Endocrinol.(Copenh)* 1980;**95**:151-7.

9. Mudambo KS, Coutie W, Rennie MJ. Plasma arginine vasopressin, atrial natriuretic peptide and brain natriuretic peptide responses to long-term field training in the heat: effects of fluid ingestion and acclimatization. *Eur.J.Appl.Physiol Occup.Physiol* 1997;**75**:219-25.

10. Nelson PB, Ellis D, Fu F, Bloom MD, O'Malley J. Fluid and electrolyte balance during a cool weather marathon. *Am.J.Sports Med.* 1989;**17**:770-2.

11. Freund BJ, Claybaugh JR, Hashiro GM, Buono M, Chrisney S. Exaggerated ANF response to exercise in middle-aged vs. young runners. *J.Appl.Physiol* 1990;**69**:1607-14.

12. Fellmann N, Bedu M, Giry J, Pharmakis-Amadieu M, Bezou MJ, Barlet JP *et al.* Hormonal, fluid, and electrolyte changes during a 72-h recovery from a 24-h endurance run. *Int.J.Sports Med.* 1989;**10**:406-12.

13. Verbalis JG, McHale CM, Gardiner TW, Stricker EM. Oxytocin and vasopressin secretion in Response to Stimuli Producing Learned Taste Aversion in Rats. *Behav.Neurosci.* 1986;**100**:466-75.

14. Guo T, Chan M, Soldin SJ. Steroid profiles using liquid chromatography-tandem mass spectrometry with atmospheric pressure photoionization source. *Arch.Pathol.Lab Med.* 2004;**128**:469-75.

15. Soldin SJ, Soldin OP, Boyajian AJ, Taskier MS. Pediatric brain natriuretic peptide and N-terminal pro-brain natriuretic peptide reference intervals. *Clin.Chim.Acta* 2006;**366**:304-8.

16. Verbalis JG. Disorders of body water homeostasis. *Best.Pract.Res.Clin.Endocrinol.Metab* 2003;**17**:471-503.

17. Robertson GL. Posterior Pituitary. In Felig P, et.al., eds. *Endocrinology and Metabolism*, pp 385-432. New York: Mcgraw-Hill, 1995.

18. Rowe JW, Shelton RL, Helderman JH, et.al. Influence of the emetic reflex on vasopressin release in man. *Kidney Int* 1979;**16**:729-35.

19. Takamata A, Mack GW, Stachenfeld NS, Nadel ER. Body temperature modification of osmotically induced vasopressin secretion and thirst in humans. *Am.J.Physiol* 1995;**269**:R874-R880.

20. Gionis D, Ilias I, Moustaki M, Mantzos E, Papadatos I, Koutras DA *et al.* Hypothalamic-pituitary-adrenal axis and interleukin-6 activity in children with head trauma and syndrome of inappropriate secretion of antidiuretic hormone. *J.Pediatr.Endocrinol.Metab* 2003;**16**:49-54.

21. Rocker L, Kirsch KA, Heyduck B, Altenkirch HU. Influence of prolonged physical exercise on plasma volume, plasma proteins, electrolytes, and fluid-regulating hormones. *Int.J.Sports Med.* 1989;**10**:270-4.

22. Maw GJ, Mackenzie IL, Taylor NA. Human body-fluid distribution during exercise in hot, temperate and cool environments. *Acta Physiol Scand.* 1998;**163**:297-304.

23. Wilkerson JE, Gutin B, Horvath SM. Exercise-induced changes in blood, red cell, and plasma volumes in man. *Med.Sci.Sports* 1977;**9**:155-8.

24. Myhre LG, Hartung GH, Nunneley SA, Tucker DM. Plasma volume changes in middle-aged male and female subjects during marathon running. *J.Appl.Physiol* 1985;**59**:559-63.

25. Verbalis JG, Mangione MP, Stricker EM. Oxytocin produces natriuresis in rats at physiological plasma concentrations. *Endocrinology* 1991;**128**:1317-22.

26. Stricker EM,.Verbalis JG. Central inhibition of salt appetite by oxytocin in rats. *Regul.Pept.* 1996;**66**:83-5.

27. Williams TD, Abel DC, King CM, Jelley RY, Lightman SL. Vasopressin and oxytocin responses to acute and chronic osmotic stimuli in man. *J.Endocrinol.* 1986;**108**:163-8.

28. Kostoglou-Athanassiou I, Treacher DF, Forsling ML. Is Oxytocin Natriuretic in Man? *J.Endocrinol* 1994;**143(suppl.O)**:39.

29. Paolisso G, Sgambato S, Passariello N, Torella R, Giugliano D, Mignano S *et al.* Pharmacological doses of oxytocin affect plasma hormone levels modulating glucose homeostasis in normal man. *Horm.Res.* 1988;**30**:10-6.

30. Braga DC, Mori E, Higa KT, Morris M, Michelini LC. Central oxytocin modulates exercise-induced tachycardia. *Am.J.Physiol Regul.Integr.Comp Physiol* 2000;**278**:R1474-R1482.

31. Margeli A, Skenderi K, Tsironi M, Hantzi E, Matalas AL, Vrettou C *et al.* Dramatic elevations of interleukin-6 and acute-phase reactants in athletes participating in the ultradistance foot race spartathlon: severe systemic inflammation and lipid and lipoprotein changes in protracted exercise. *J.Clin.Endocrinol.Metab* 2005;**90**:3914-8.

32. Ostrowski K, Schjerling P, Pedersen BK. Physical activity and plasma interleukin-6 in humans--effect of intensity of exercise. *Eur.J.Appl.Physiol* 2000;**83**:512-5.

33. Starkie RL, Rolland J, Angus DJ, Anderson MJ, Febbraio MA. Circulating monocytes are not the source of elevations in plasma IL-6 and TNF-alpha levels after prolonged running. *Am.J.Physiol Cell Physiol* 2001;**280**:C769-C774.

34. Steensberg A, van Hall G, Osada T, Sacchetti M, Saltin B, Klarlund PB. Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *J.Physiol* 2000;**529 Pt 1**:237-42.

35. Ostrowski K, Rohde T, Zacho M, Asp S, Pedersen BK. Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. *J.Physiol* 1998;**508 (Pt 3)**:949-53.

36. Holmes AG, Watt MJ, Febbraio MA. Suppressing lipolysis increases interleukin-6 at rest and during prolonged moderate-intensity exercise in humans. *J.Appl.Physiol* 2004;**97**:689-96.

37. van Hall G, Steensberg A, Sacchetti M, Fischer C, Keller C, Schjerling P *et al.* Interleukin-6 stimulates lipolysis and fat oxidation in humans. *J.Clin.Endocrinol.Metab* 2003;**88**:3005-10.

38. Febbraio MA,.Pedersen BK. Contraction-induced myokine production and release: is skeletal muscle an endocrine organ? *Exerc.Sport Sci.Rev.* 2005;**33**:114-9.

39. Faldt J, Wernstedt I, Fitzgerald SM, Wallenius K, Bergstrom G, Jansson JO. Reduced exercise endurance in interleukin-6-deficient mice. *Endocrinology* 2004;**145**:2680-6.

40. Mastorakos G, Weber JS, Magiakou MA, Gunn H, Chrousos GP. Hypothalamic-pituitary-adrenal axis activation and stimulation of systemic vasopressin secretion by recombinant interleukin-6 in humans: potential implications for the syndrome of inappropriate vasopressin secretion. *J.Clin.Endocrinol.Metab* 1994;**79**:934-9.

41. Siegel AJ. Exercise-associated hyponatremia: role of cytokines. *Am.J.Med.* 2006;**119**:S74-S78.

42. Scharhag J, Herrmann M, Urhausen A, Haschke M, Herrmann W, Kindermann W. Independent elevations of N-terminal pro-brain natriuretic peptide and cardiac troponins in endurance athletes after prolonged strenuous exercise. *Am.Heart J.* 2005;**150**:1128-34.

43. Scharhag J, Urhausen A, Schneider G, Herrmann M, Schumacher K, Haschke M *et al.* Reproducibility and clinical significance of exercise-induced increases in cardiac troponins and N-terminal pro brain natriuretic peptide in endurance athletes. *Eur.J.Cardiovasc.Prev.Rehabil.* 2006;**13**:388-97.

44. Ohba H, Takada H, Musha H, Nagashima J, Mori N, Awaya T *et al.* Effects of prolonged strenuous exercise on plasma levels of atrial natriuretic peptide and brain natriuretic peptide in healthy men. *Am.Heart J.* 2001;**141**:751-8.

45. Niessner A, Ziegler S, Slany J, Billensteiner E, Woloszczuk W, Geyer G. Increases in plasma levels of atrial and brain natriuretic peptides after running a marathon: are their effects partly counterbalanced by adrenocortical steroids? *Eur.J.Endocrinol.* 2003;**149**:555-9.

46. Siegel AJ, Lewandrowski EL, Chun KY, Sholar MB, Fischman AJ, Lewandrowski KB. Changes in cardiac markers including B-natriuretic peptide in runners after the Boston marathon. *Am.J.Cardiol.* 2001;**88**:920-3.

47. Neumayr G, Pfister R, Mitterbauer G, Eibl G, Hoertnagl H. Effect of competitive marathon cycling on plasma N-terminal pro-brain natriuretic peptide and cardiac troponin T in healthy recreational cyclists. *Am.J.Cardiol.* 2005;**96**:732-5.

48. Date H, Imamura T, Onitsuka H, Maeno M, Watanabe R, Nishihira K *et al.* Differential increase in natriuretic peptides in elite dynamic and static athletes. *Circ.J.* 2003;**67**:691-6.

49. Huang WS, Lee MS, Perng HW, Yang SP, Kuo SW, Chang HD. Circulating brain natriuretic peptide values in healthy men before and after exercise. *Metabolism* 2002;**51**:1423-6.

50. Ketelhut R, Losem CJ, Messerli FH. Depressed systolic and diastolic cardiac function after prolonged aerobic exercise in healthy subjects. *Int.J.Sports Med.* 1992;**13**:293-7.

51. Seals DR, Rogers MA, Hagberg JM, Yamamoto C, Cryer PE, Ehsani AA. Left ventricular dysfunction after prolonged strenuous exercise in healthy subjects. *Am.J.Cardiol.* 1988;**61**:875-9.

52. Vanoverschelde JL, Younis LT, Melin JA, Vanbutsele R, Leclercq B, Robert AR *et al.* Prolonged exercise induces left ventricular dysfunction in healthy subjects. *J.Appl.Physiol* 1991;**70**:1356-63.

53. Moro C, Polak J, Hejnova J, Klimcakova E, Crampes F, Stich V *et al.* Atrial natriuretic peptide stimulates lipid mobilization during repeated bouts of endurance exercise. *Am.J.Physiol Endocrinol.Metab* 2006;**290**:E864-E869.

54. Sengenes C, Zakaroff-Girard A, Moulin A, Berlan M, Bouloumie A, Lafontan M *et al.* Natriuretic peptide-dependent lipolysis in fat cells is a primate specificity. *Am.J.Physiol Regul.Integr.Comp Physiol* 2002;**283**:R257-R265.

55. Moro C, Galitzky J, Sengenes C, Crampes F, Lafontan M, Berlan M. Functional and pharmacological characterization of the natriuretic peptide-dependent lipolytic pathway in human fat cells. *J.Pharmacol.Exp.Ther.* 2004;**308**:984-92.

56. Birkenfeld AL, Boschmann M, Moro C, Adams F, Heusser K, Franke G *et al.* Lipid mobilization with physiological atrial natriuretic peptide concentrations in humans. *J.Clin.Endocrinol.Metab* 2005;**90**:3622-8.

57. Sengenes C, Stich V, Berlan M, Hejnova J, Lafontan M, Pariskova Z *et al.* Increased lipolysis in adipose tissue and lipid mobilization to natriuretic peptides during low-calorie diet in obese women. *Int.J.Obes.Relat Metab Disord.* 2002;**26**:24-32.

58. Sarzani R, Dessi-Fulgheri P, Paci VM, Espinosa E, Rappelli A. Expression of natriuretic peptide receptors in human adipose and other tissues. *J.Endocrinol.Invest* 1996;**19**:581-5.

59. Romijn JA, Coyle EF, Sidossis LS, Gastaldelli A, Horowitz JF, Endert E *et al.* Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am.J.Physiol* 1993;**265**:E380-E391.

60. Hellstrom L, Blaak E, Hagstrom-Toft E. Gender differences in adrenergic regulation of lipid mobilization during exercise. *Int.J.Sports Med.* 1996;**17**:439-47.

61. Kiens B, Roepstorff C, Glatz JF, Bonen A, Schjerling P, Knudsen J *et al.* Lipid-binding proteins and lipoprotein lipase activity in human skeletal muscle: influence of physical activity and gender. *J.Appl.Physiol* 2004;**97**:1209-18.

62. Duclos M, Corcuff JB, Pehourcq F, Tabarin A. Decreased pituitary sensitivity to glucocorticoids in endurance-trained men. *Eur.J.Endocrinol.* 2001;**144**:363-8.

63. de Vries WR, Bernards NT, de Rooij MH, Koppeschaar HP. Dynamic exercise discloses different time-related responses in stress hormones. *Psychosom.Med.* 2000;**62**:866-72.

64. Maron MB, Horvath SM, Wilkerson JE. Acute blood biochemical alterations in response to marathon running. *Eur.J.Appl.Physiol Occup.Physiol* 1975;**34**:173-81.

65. Ponjee GA, De Rooy HA, Vader HL. Androgen turnover during marathon running. *Med.Sci.Sports Exerc.* 1994;**26**:1274-7.

66. Newmark ST, Himathongkam T, Martin RP, Cooper KH, Rose LI. Adrenocortical response to marathon running. *J.Clin.Endocrinol.Metab* 1976;**42**:393-4.

67. Malarkey WB, Hall JC, Rice RR, Jr., O'Toole ML, Douglas PS, Demers LM *et al.* The influence of age on endocrine responses to ultraendurance stress. *J.Gerontol.* 1993;**48**:M134-M139.

68. Altenkirch HU, Gerzer R, Kirsch KA, Weil J, Heyduck B, Schultes I *et al.* Effect of prolonged physical exercise on fluid regulating hormones. *Eur.J.Appl.Physiol Occup.Physiol* 1990;**61**:209-13.

69. Kosunen KJ, Pakarinen AJ, Kuoppasalmi K, Adlercreutz H. Plasma renin activity, angiotensin II, and aldosterone during intense heat stress. *J.Appl.Physiol* 1976;**41**:323-7.

70. Maresh CM, Gabaree-Boulant CL, Armstrong LE, Judelson DA, Hoffman JR, Castellani JW *et al.* Effect of hydration status on thirst, drinking, and related hormonal responses during low-intensity exercise in the heat. *J.Appl.Physiol* 2004;**97**:39-44.

71. Brandenberger G, Candas V, Follenius M, Libert JP, Kahn JM. Vascular fluid shifts and endocrine responses to exercise in the heat. Effect of rehydration. *Eur.J.Appl.Physiol Occup.Physiol* 1986;**55**:123-9.

72. Montain SJ, Laird JE, Latzka WA, Sawka MN. Aldosterone and vasopressin responses in the heat: hydration level and exercise intensity effects. *Med.Sci.Sports Exerc.* 1997;**29**:661-8.

73. Yamauchi T, Harada T, Kurono M, Matsui N. Effect of exercise-induced acidosis on aldosterone secretion in men. *Eur.J.Appl.Physiol Occup.Physiol* 1998;**77**:409-12.

74. Melin B, Koulmann N, Jimenez C, Savourey G, Launay JC, Cottet-Emard JM *et al.* Comparison of passive heat or exercise-induced dehydration on renal water and electrolyte excretion: the hormonal involvement. *Eur.J.Appl.Physiol* 2001;**85**:250-8.

75. Melin B, Jimenez C, Savourey G, Bittel J, Cottet-Emard JM, Pequignot JM *et al.* Effects of hydration state on hormonal and renal responses during moderate exercise in the heat. *Eur.J.Appl.Physiol Occup.Physiol* 1997;**76**:320-7.

76. Takamata A, Mack GW, Gillen CM, Nadel ER. Sodium appetite, thirst, and body fluid regulation in humans during rehydration without sodium replacement. *Am.J.Physiol* 1994;**266**:R1493-R1502.

77. Fitzsimons JT. Angiotensin, thirst, and sodium appetite. *Physiol Rev.* 1998;**78**:583-686.

78. Raubenheimer PJ, Young EA, Andrew R, Seckl JR. The role of corticosterone in human hypothalamic-pituitary-adrenal axis feedback. *Clin.Endocrinol.(Oxf)* 2006;**65**:22-6.

79. Holst JP, Soldin OP, Guo T, Soldin SJ. Steroid hormones: relevance and measurement in the clinical laboratory. *Clin.Lab Med.* 2004;**24**:105-18.

80. Boldyreff B,.Wehling M. Aldosterone: refreshing a slow hormone by swift action. *News Physiol Sci.* 2004;**19**:97-100.

81. Mihailidou AS. Nongenomic actions of aldosterone: physiological or pathophysiological role? *Steroids* 2006;**71**:277-80.

82. Keizer H, Janssen GM, Menheere P, Kranenburg G. Changes in basal plasma testosterone, cortisol, and dehydroepiandrosterone sulfate in previously untrained males and females preparing for a marathon. *Int.J.Sports Med.* 1989;**10 Suppl 3**:S139-S145.

83. Orentreich N, Brind JL, Rizer RL, Vogelman JH. Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. *J.Clin.Endocrinol.Metab* 1984;**59**:551-5.

84. Corrigan B. DHEA and sport. *Clin.J.Sport Med.* 2002;**12**:236-41.

85. Nagel D, Seiler D, Franz H. Biochemical, hematological and endocrinological parameters during repeated intense short-term running in comparison to ultra-long-distance running. *Int.J.Sports Med.* 1992;**13**:337-43.

86. Hackney AC, Moore AW, Brownlee KK. Testosterone and endurance exercise: development of the "exercise-hypogonadal male condition". *Acta Physiol Hung.* 2005;**92**:121-37.

87. Bonen A,.Keizer HA. Pituitary, ovarian, and adrenal hormone responses to marathon running. *Int.J.Sports Med.* 1987;**8 Suppl 3**:161-7.

88. Rockell JE, Green TJ, Skeaff CM, Whiting SJ, Taylor RW, Williams SM *et al.* Season and Ethnicity Are Determinants of Serum 25-hydroxyvitamin D Concentrations in New Zealand Children Aged 5-14 y. *J Nutr* 2005;**135**:2602-8.

89. Ruohola JP, Laaksi I, Ylikorni T, Haataja R, Mattila VM, Sahi T *et al.* Association Between Serum 25(OH)D Concentrations and Bone Stress Fractures in Finnish Young Men. *J Bone Miner Res* 2006;**21**:1483-8.

90. Scragg R, Holdaway I, Singh V, Metcalf P, Baker J, Dryson E. Serum 25-hydroxyvitamin D3 levels decreased in impaired glucose tolerance and diabetes mellitus. *Diabetes research and Clinical Practice* 1995;**27**:181-8.

Study 3F: Fluid balance and acute changes in Oxytocin and Brain Natriuretic Peptide durino high intensity, steady-state and prolonged endurance running

INTRODUCTION

Arginine vasopressin (AVP), aldosterone and atrial natriuretic peptide (ANP) are commonly identified as the principle hormones regulating overall fluid balance in humans [1]. During exercise, however, the relationships between these hormones with common markers of fluid balance [2] become less predictable. The dissociations that are evident between the established steady-state relationships of: AVP with plasma osmolality [3;4], aldosterone with plasma volume [4;5] and ANP with volume overload [5] suggest that other related endocrine factors - such as oxytocin (OT) and brain natriuretic peptide (BNP) - may assist in the regulation of fluid homeostasis during periods of heightened physical stress. Alternatively, the primary fluid regulatory hormones may be stimulated by perturbations from other regulatory systems when homeostasis is acutely disrupted [6-8].

Running stimulates both the sympathetic-adrenal medullary system and the hypothalamic-pituitary-adrenal (HPA) axis [9]. Treadmill running is considered a reproducible and quantifiable stressor; capable of provoking a neuroendocrine response that is proportional to relative exercise intensity [9;10] and duration [11]. This neuroendocrine stress response is similar regardless of baseline training [9], disease [12] and hydration status [13]. However, the response of fluid regulatory hormones to these variations in exercise intensity and duration are not well established or understood.

The aim of this study is to investigate the endocrine response to high intensity, steady-state and prolonged endurance running in seven well-trained runners. More specifically, we wish to evaluate the relationships between common markers of fluid balance [2] (plasma and urine [Na^+], plasma volume and bodyweight) with those endocrine factors which are known (AVP [14], aldosterone [15;16]) or **not** known (oxytocin [17], brain natriuretic

peptide [18;19], interleukin-6 [20], cortisol [21;22]) to effect the acute regulation of fluid homeostasis in humans during exercise.

METHODS

Subjects: Seven well-trained endurance runners (five male and two female) were recruited and signed written informed consent for participation in this study, which was approved by both the Ethics Committee of the University of Cape Town and the Georgetown University Institutional Review Board.

Testing protocol: All seven runners participated in the Two Oceans 56 km ultramarathon (prolonged endurance running), held in Cape Town, South Africa on March 26, 2005. Baseline body weight, blood and urine samples were obtained within 60 min of the start of the race. Post-race body weight, blood and urine samples were obtained immediately upon race completion. Food and fluid intake were allowed *ad libitum* during the race.

Approximately six months after completion of the prolonged endurance running race, all seven runners presented to the laboratory twice, one week apart, during the same part of the day to prevent individual diurnal variation between exercise tests. Each subject was asked to refrain from vigorous exercise 24 hours prior to each test and consume a similar diet. No food or fluid was allowed during either laboratory treadmill running test.

A high intensity (VO_2 max) exercise test was performed on the first visit to the laboratory. This test was of a maximal running test to volitional exhaustion on a motorized treadmill (Quinton type BA-1 treadmill, USA). Each athlete was allowed to warm-up on the treadmill at a self-selected speed for five minutes. After completion of the warm-up, a heart-rate monitor (Polar Heart Rate Monitor, USA) was fitted around each runner's chest and a mask tightly secured over the nose and mouth; in order to prevent air from escaping through the boundary of the mask. Oxygen uptake was measured continuously using an Oxycon Alpha Analyzer (Jaeger, Netherlands) with maximal oxygen consumption (VO_2 maximum) defined as the highest value obtained in

each subject before volitional exhaustion. The exercise protocol utilized was a peak treadmill running test, where each runner ran for one minute at a speed of 7.5 km/hr. Thereafter, the speed was increased by 0.5 km/h every 30 seconds until the subject could no longer keep pace with the treadmill [23]. The last 30 second stage that was completed was designated as each athlete's peak treadmill running speed.

A steady-state treadmill test was performed in the laboratory one week later. Each subject ran for 60 minutes on the treadmill at a speed that corresponded to 60% of the peak treadmill running speed reached during the high intensity test.

Sample collection and measurements: Immediately prior to each exercise test, bodyweight was obtained on a calibrated Adamlab JPS electronic scale (Scales, Brackenfell, South Africa) in running attire and without shoes after the subject voided. A urine sample was obtained from this pre-race void. Venous blood was obtained with each subject in a seated position. Ten milliliters of venous blood were collected into chilled lithium heparin tubes and immediately centrifuged for 10 minutes at 3000 rpm. The separated plasma was immediately frozen at -80°C until further analysis could be performed.

Within five minutes of completing each exercise test, all subjects returned to the seated position and 10 ml of venous blood was collected into chilled lithium heparin tubes, centrifuged, separated and frozen. Each subject voided and the urine was collected. Urine samples were immediately frozen and stored at -80°C until further analysis could be performed. A post-exercise bodyweight was then obtained.

Analytical measurements. Changes in plasma volume were estimated by comparing pre- and post-race measurements of plasma protein using a clinical refractometer (Schuco Clinical Refractometer 5711-2020, Japan). Plasma and urine sodium concentrations ($[Na^+]$) were measured using ion-selective electrodes (Beckman Synchron EI-ISE, Fullerton, CA).

Hormone Measurements. Plasma levels of arginine vasopressin (AVP) and oxytocin (OT) were measured by specific radioimmunoassay following acetone-ether extraction as described previously [24]. The standard curve for AVP is linear between 0.5 and 10.0 pg/tube with the use of a synthetic AVP standard (PerkinElmer Life Sciences Inc, Boston MA). The minimum detectable concentration of AVP in extracted plasma was 0.5 pg/ml. The AVP antiserum (R-4) displayed <1% cross-reactivity with OT. The standard curve of the OT assay was linear between 0.25 and 5.0 pg/tube with the use of a synthetic OT standard (PerkinElmer Life Sciences Inc, Boston MA). The minimum detectable concentration of OT in extracted plasma was 0.25 pg/ml. The OT antiserum (Pitt-Ab2) displayed <1% cross-reactivity with AVP.

Four adrenal steroid hormones (cortisol, 11-deoxycortisol, aldosterone, corticosterone) were measured using a liquid chromatography-tandem mass spectrometer, in conjunction with an atmospheric pressure photoionization source, using methodology described previously [25].

Brain natriuretic peptide concentrations were assessed with measurement of the more stable cleaved inactive fragment, N-terminal pro-brain natriuretic peptide (NT-proBNP), using the automated Dade RxL Dimension as previously described [26]. Interleukin-6 (IL-6) was measured by chemiluminescence (Immulite 1000 Diagnostic Product Corporation, Los Angeles, California).

Statistical analysis. All data were analyzed using the STATISTICA 7.0™ software (StatSoft Inc., Tulsa, OK). Where applicable, data were presented as means ± SEM Statistical significance was accepted when $p < 0.05$. A univariate regression model was utilized to assess the relationships between fluid markers with the endocrine secretions that were most likely to affect fluid balance during exercise. The regression model was performed when data from all the three running tests were combined. Each fluid marker (as a dependant variable) was evaluated against a group of independent variables that included: AVP, OT, NT-proBNP, aldosterone, IL-6 and cortisol. The "change" (Δ) in each designated variable denoted post- minus pre-run measurements.

RESULTS

The mean age of our seven subjects was 44 ± 4 years, training distance 56 ± 6 km/week, running experience 11± 3 years with an average VO_2 max of 55 ± 5 ml/kg/min. The mean finishing time for the ultramarathon was 359 ± 13 minutes while the mean time to exhaustion for the VO_2 max test was 10 ± 1 minute.

Comparisons between the three different running conditions (high intensity, steady-state and prolonged endurance) for the posterior pituitary, natriuretic peptide and cytokine endocrine secretions are documented in Table 3F.1. AVP was significantly suppressed before the ultramarathon while post-run OT and the change in both AVP and OT were significantly elevated after the ultramarathon, compared to steady-state running. Significant elevations in NT-proBNP and IL-6 were noted following the ultramarathon compared to both high intensity and steady-state running.

Comparisons between high intensity, steady-state and prolonged endurance running for the mineralocorticoid and glucocorticoid steroid hormones are documented in Table 3F.2. Significant elevations in corticosterone and 11-deoxycortisol were noted before and after the ultramarathon, when compared to both high intensity and steady-state running. Cortisol was significantly elevated only after the ultramarathon compared to both treadmill runs.

Both plasma volume and body weight decreased immediately following all three running conditions (Figure 3F.1a and 3F.1b). The plasma volume contraction was largest following the VO_2 max run (-11 ± 1%) and smallest after the ultramarathon (-8 ± 0%). The plasma volume change after the ultramarathon was significantly different from the plasma volume change following both high intensity and steady-state running (-9 ± 2%). Conversely, body weight loss was largest following the ultramarathon (-4 ± 0%) compared with high intensity (-0.3 ± 0%) and steady-state running (-2 ± 0%). The change in body weight between all three running conditions was statistically significant.

Plasma [Na^+] Δ increased after both the high intensity (3 ± 1 mmol/L) and steady-state (2 ± 1 mmol/L) runs but decreased following the ultramarathon (-4 ± 2 mmol/L) (Figure 2a). Conversely, urine osmolality Δ decreased following the high intensity (-43 ± 45 mOsmol/kgH_20) and steady-state (-12 ± 31 mOsmol/kgH_20) runs but increased markedly after the ultramarathon (224 ± 97 mOsmol/kgH_20; Figure 2b). The differences between the ultramarathon with both laboratory treadmill tests were statistically significant with regard to both of these fluid balance markers. Urine [Na^+] Δ decreased following all three running tests, with the decrease after the steady-state run (-41 ± 13 mmol/L) significantly lower than that seen after the VO_2 max test (-7 ± 6 mmol/L) (Figure 3F.3).

The plasma concentrations of both AVP (Figure 3F.4) and OT (Figure 3F.5) were increased immediately following all three running conditions. However, only after the ultramarathon was the increase in both posterior pituitary hormones statistically significant from pre- to post-run. The response of these two posterior pituitary hormones did not parallel one another - with regard to the degree of elevation between exercise conditions - although AVP and OT were positively correlated with each other when data from all three exercise tests were combined (Figure 3F.6).

The increase in plasma aldosterone concentrations from pre- to post-run were significant after all three running conditions (Figure 3F.7). However, the plasma concentrations of cortisol, corticosterone, IL-6 and NT-proBNP were significantly elevated only after prolonged endurance running.

When data from all three running conditions were combined, a significant inverse correlation was noted between NT-proBNP Δ versus plasma [Na^+] Δ (Figure 3F.8a) while a significant positive correlation was noted between NT-proBNP versus urine osmolality Δ (Figure 3F.8b). In addition, univariate tests of significance suggested that aldosterone, AVP, OT and NT-proBNP were the primary hormones significantly associated with changes in plasma volume (aldosterone, NT-proBNP and OT), body

weight (OT and NT-proBNP), plasma [Na^+] (NT-proBNP) and urine [Na^+] (AVP) (Table 3F.3).

Table 3F.1: Comparison between high intensity, steady state and prolonged endurance running for posterior pituitary, natriuretic peptide and cytokine secretions.

VARIABLE	VO_2 MAX (Mean ± SEM)	STEADY STATE (Mean ± SEM)	ULTRAMARATHON (Mean ± SEM)
Vasopressin pre-run (pg/mL)	3.6 ± 0.7	3.1 ± 0.3	*1.9 ± 0.5
Vasopressin post-run (pg/mL)	14.4 ± 5.2	3.9 ± 0.4	6.7 ± 1.4
Vasopressin Δ (pg/mL)	10.9 ± 5.0	0.7 ± 0.3	*4.9 ± 1.6
Oxytocin pre-run (pg/mL)	1.3 ± 0.2	1.1 ± 0.3	1.5 ± 0.2
Oxytocin post-run (pg/mL)	2.6 ± 0.7	1.5 ± 0.1	**3.5 ± 0.5
Oxytocin Δ (pg/mL)	1.3 ± 0.7	0.4 ± 0.3	*2.0 ± 1.0
NT-proBNP pre-run (pg/mL)	26.9 ± 8.1	18.4 ± 4.6	23.6 ± 7.6
NT-proBNP post-run (pg/mL)	#30.6 ± 14.4	26.6 ± 6.2	**117.9 ± 29.0
NT-proBNP Δ (pg/mL)	##3.7 ± 6.6	8.1 ± 3.1	**94.3 ± 22.5
IL-6 pre-run (pg/mL)	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0
IL-6 post-run (pg/mL)	##4.3 ± 0.3	4.2 ± 0.2	**59.6 ± 9.8
IL-6 Δ (pg/mL)	##0.3 ± 0.3	0.1 ± 0.1	**55.6 ± 9.8

*p <0.05, * *p <0.01 between steady state and ultramarathon

#p <0.05, ##p < 0.01 between VO_2Max and ultramarathon

@p <0.05, @@ p < 0.01 between VO_2Max and steady state

Table 3F.2: Comparison between high intensity, steady state and prolonged endurance running for mineralocorticoid and glucocorticoid steroid hormones.

VARIABLE	VO_2 MAX (Mean ± SEM)	STEADY STATE (Mean ± SEM)	ULTRAMARATHON (Mean ± SEM)
Aldosterone pre-run (ng/mL)	4.9 ± 0.5	6.1 ± 1.9	2.6 ± 1.2
Aldosterone post-run (ng/mL)	12.5 ± 1.9	16.9 ± 2.7	19.7 ± 6.2
Aldosterone Δ (ng/mL)	7.6 ± 2.3	10.8 ± 2.5	17.1 ± 6.1
Cortisol pre-run (ug/mL)	11.6 ± 2.4	11.3 ± 2.3	14.6 ± 1.7
Cortisol post-run (ug/mL)	##15.9 ± 3.0	10.4 ± 1.0	**32.6 ± 4.3
Cortisol Δ (ug/mL)	#4.4 ± 1.5	-0.9 ± 2.3	**17.6 ± 4.5
Corticosterone pre-run (ng/mL)	#170.7 ± 68.6	139.6 ± 61.0	*652.8 ± 183.3
Corticosterone post-run (ng/mL)	#518.9 ± 187.5	226.2 ± 60.0	**3491.4 ± 978.8
Corticosterone Δ (ng/mL)	#348.2 ± 153.6	86.6 ± 74.0	**2838.6 ± 872.8
11-deoxycortisol pre-run (ug/mL)	#0.03 ± 0.01	0.03 ± 0.01	*0.10 ± 0.03
11-deoxycortisol post-run (ug/mL)	#0.10 ± 0.03	0.05 ± 0.01	*0.54 ± 0.17
11-deoxycortisol Δ (ug/mL)	#0.06 ± 0.03	0.02 ± 0.01	*0.44 ± 0.16

*p <0.05, **p <0.01 between steady state and ultramarathon
#p <0.05, ##p < 0.01 between VO_2Max and ultramarathon
@p <0.05, @@ p < 0.01 between VO_2Max and steady state

Table 3F.3: Univariate tests of significance when data from all three running conditions are combined (N = 21).

FLUID BALANCE MARKER	HORMONE	P-Value	F-value
% Plasma volume loss	Aldo post	0.02	7.6
	Aldo Δ	0.03	6.1
	BNP post	0.04	5.3
	OT post	0.05	5.0
% Body weight loss	OT post	0.01	8.7
	BNP post	0.02	7.3
Plasma $[Na^+]$ Δ	BNP Δ	0.01	7.2
Plasma $[Na^+]$ post	BNP Δ	0.01	8.3
	Aldo Δ	0.04	5.3
Urine $[Na^+]$ post	AVP post	0.04	5.6

Figure 3F.1a: Plasma volume changes between high intensity, steady state and prolonged endurance running.

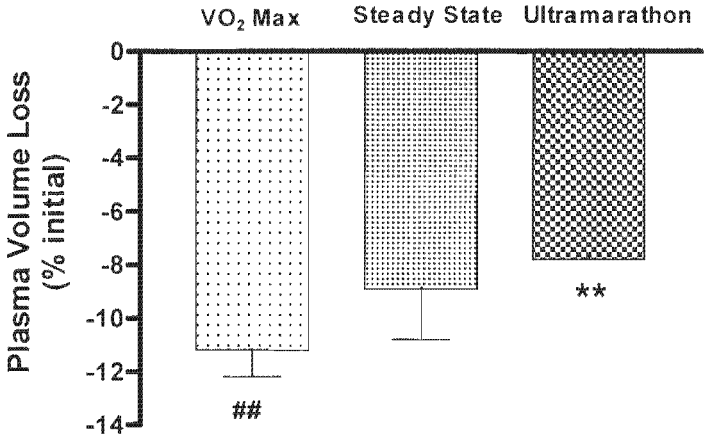
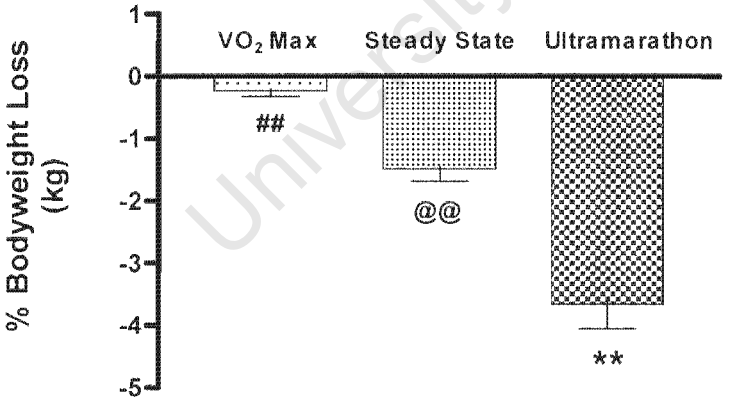


Figure 3F.1b: Bodyweight changes between high intensity, steady state and prolonged endurance running.



*p <0.05, * *p <0.01 between steady state and ultramarathon
#p <0.05, # #p < 0.01 between VO_2Max and ultramarathon
@p <0.05, @@ p < 0.01 between VO_2Max and steady state

Figure 3F.2a: Plasma [Na^+] changes between high intensity, steady state and prolonged endurance running.

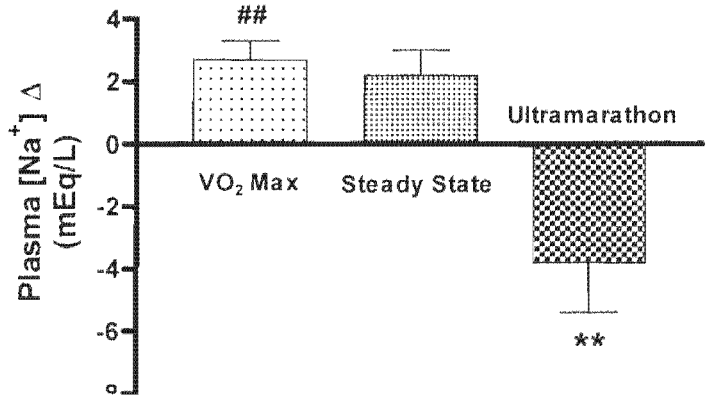
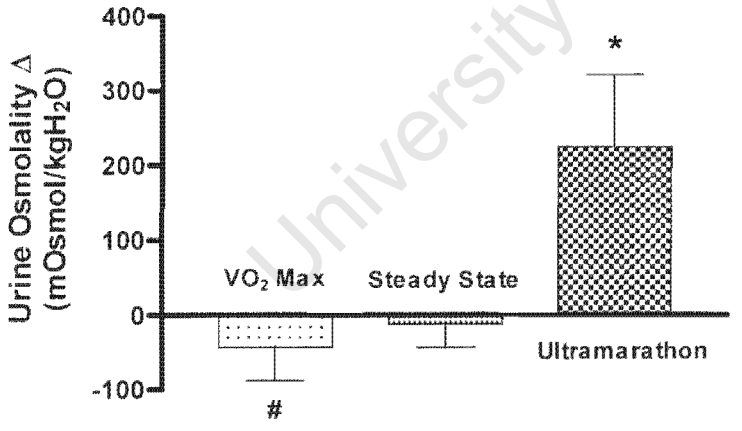
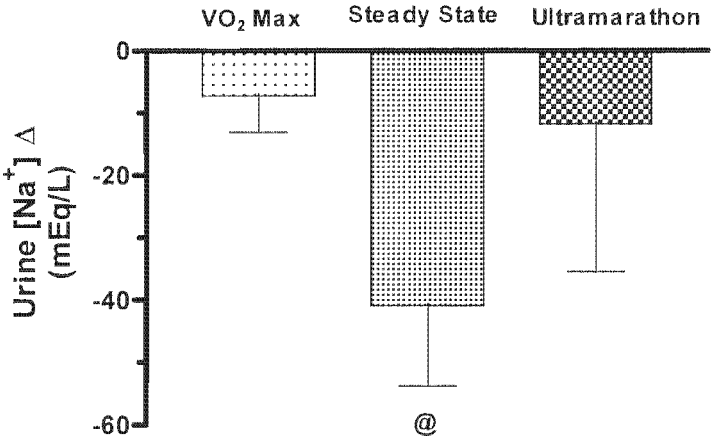


Figure 3F.2b: Changes in urine osmolality between high intensity, steady state and prolonged endurance running.



*p <0.05, * *p <0.01 between steady state and ultramarathon
#p <0.05, # #p < 0.01 between VO_2Max and ultramarathon
@p <0.05, @@ p < 0.01 between VO_2Max and steady state

Figure 3F.3: Changes in urine [Na^+] between high intensity, steady state and prolonged endurance running.



*p <0.05, * *p <0.01 between steady state and ultramarathon
#p <0.05, # #p < 0.01 between VO_2Max and ultramarathon
@p <0.05, @@ p < 0.01 between VO_2Max and steady state

Figure 3F.4: Changes in AVP between high intensity, steady state and prolonged endurance running.

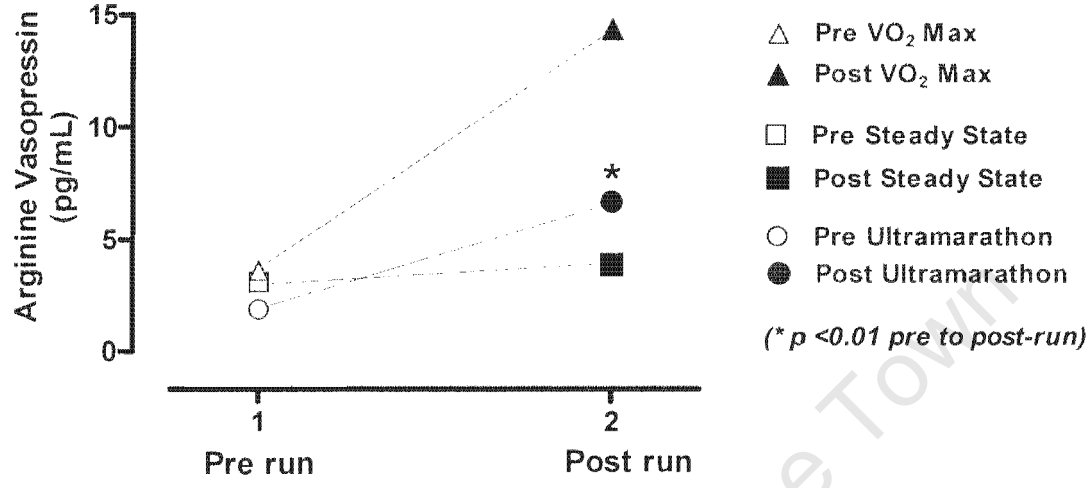


Figure 3F.5: Changes in OT between high intensity, steady state and prolonged endurance running.

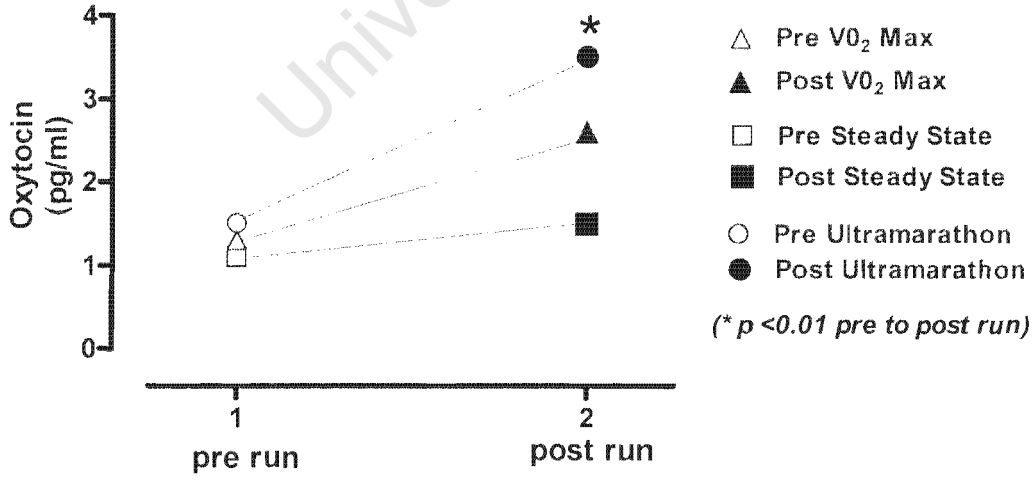


Figure 3F.6: Correlation between oxytocin post-exercise versus the change in AVP when all three exercise conditions are combined (N = 21).

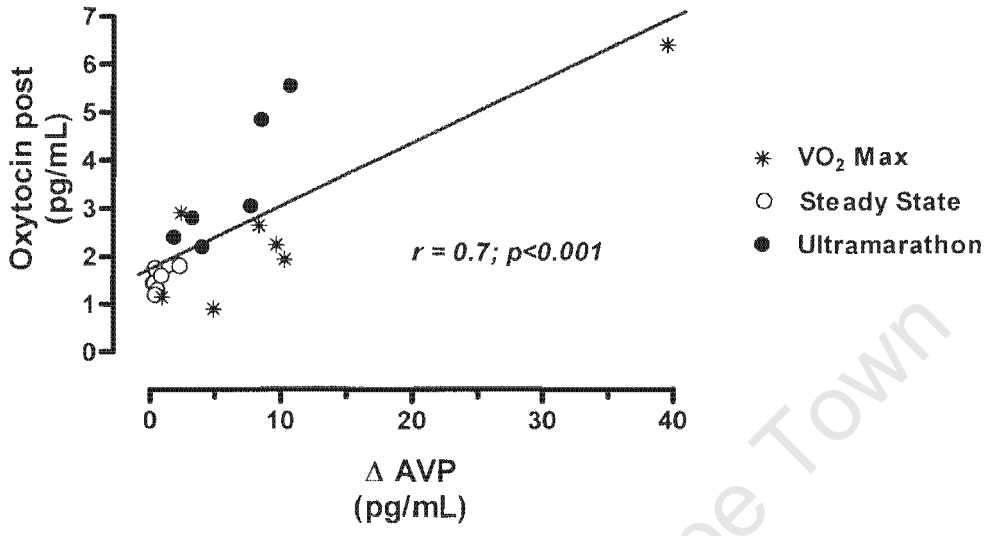


Figure 3F.7: Changes in aldosterone between high intensity, steady state and prolonged endurance running.

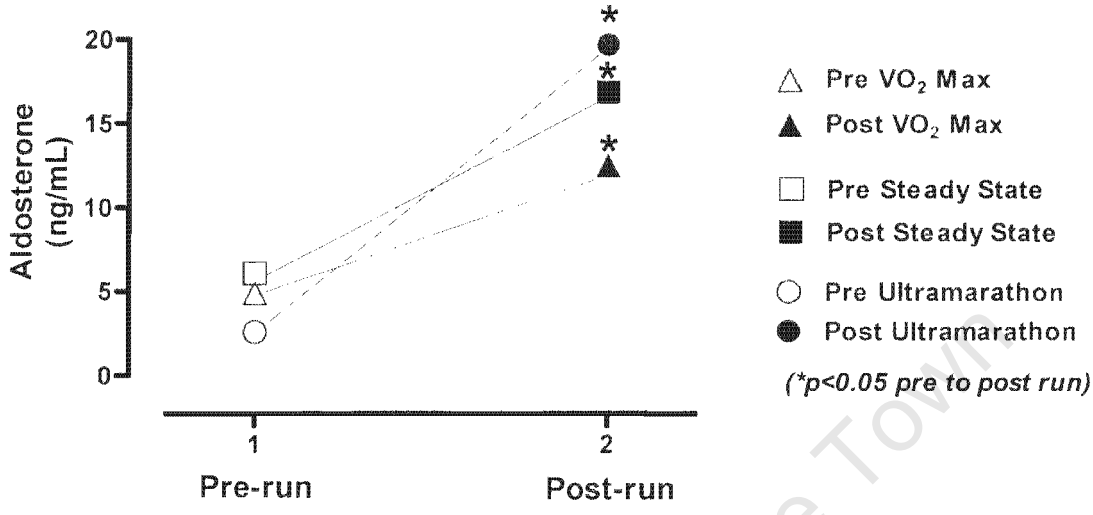


Figure 3F.8a: Correlation between the change in serum sodium concentration versus the change in NT-proBNP (post minus pre-race) when all three exercise conditions are combined (N=21).

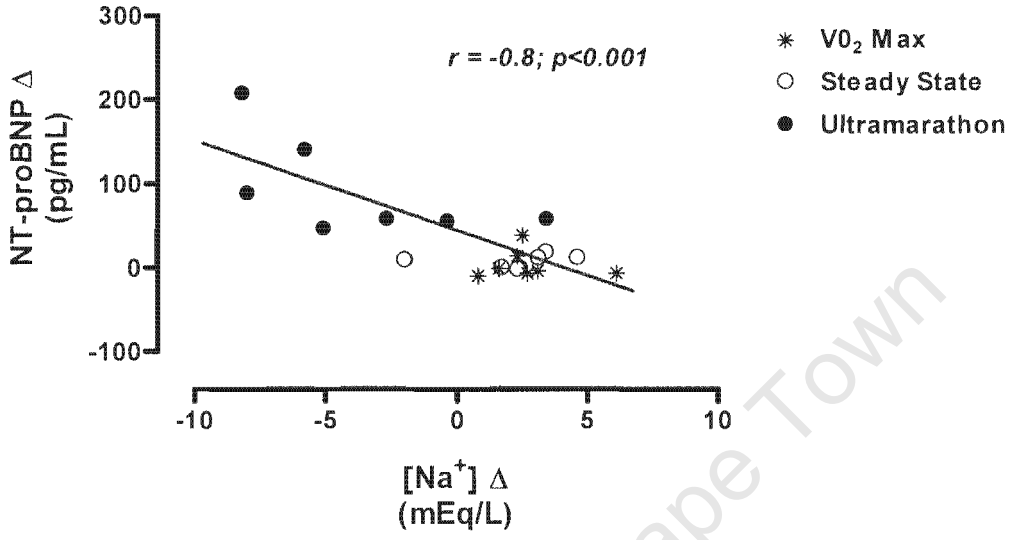
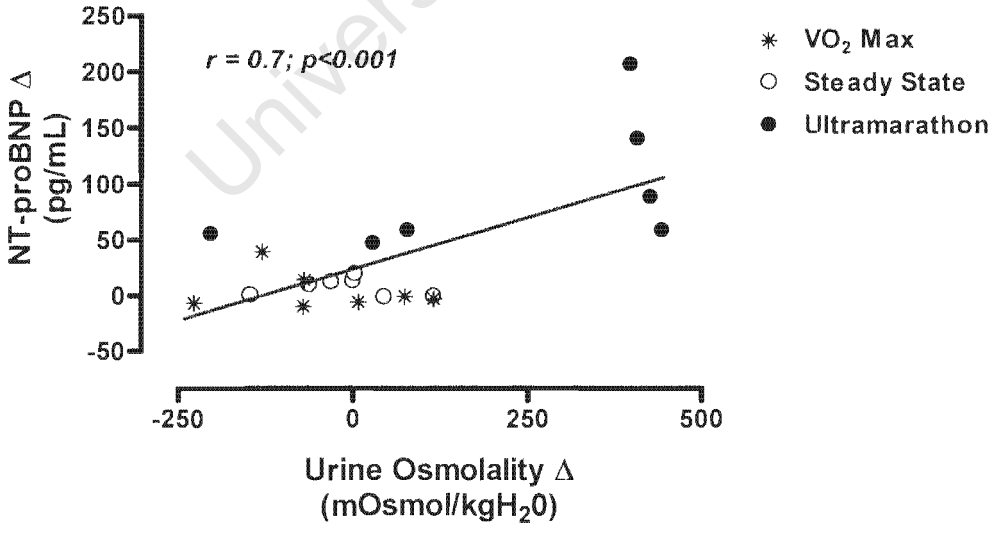


Figure 3F.8b: Correlation between the change in urine osmolality versus the change in NT-proBNP when all three exercise conditions are combined (N=21).



DISCUSSION

The established relationships between plasma AVP and aldosterone with perturbations in fluid balance during exercise are expected and confirmed by the results of this study. However, the potential contributions of oxytocin and brain natriuretic hormone to changes in fluid balance are unexpected and not well clarified.

Of the two posterior pituitary hormones, AVP is widely regarded as the principle regulator of body water homeostasis [27]. In univariate regression analyses, post-run urine [Na^+] is positively correlated with AVP (Table 3F.3). This is an expected physiological response, as AVP secretion generally promotes free water clearance [27]. The increase in AVP per unit rise in plasma osmolality during exercise, however, tends to be higher than the per unit rise in AVP seen after infusion of hypertonic saline; suggesting that other factors may be involved in the stimulation of AVP secretion during exercise [3].

During high intensity exercise, there appears to be dissociation between plasma AVP concentrations with plasma osmolality; where AVP secretion may exceed predicted levels [28]. This highly variable and substantial increase in AVP suggests the inclusion of "non" [29] or "high" [30] responders in our well-trained cohort of runners (Table 3F.1; Figure 3F.4). "High-responders" are characterized by: 1) a resistance to glucocorticoid suppression by the administration of dexamethasone, 2) a higher corticotropin (ACTH) response following exercise 3) an enhancement of AVP secretion with administration of dexamethasone and 4) a higher plasma ACTH response after AVP infusion [30]. It is hypothesized that "high responders" have an enhanced hypothalamic drive for AVP [30], with the wide variability in the response of the HPA-axis primarily related to the magnitude of AVP release [28]. Administration of GABA agonists (sodium valproate [31] or alprazolam [32]) abolishes the AVP response to high intensity exercise in humans while administration of a GABA antagonist (DHEA) [32] increases the AVP–ACTH-cortisol response. These findings suggest that an osmotic/hypovolemic stimulus primarily

controls AVP release during exercise [31;32], despite the apparent dissociation that occurs in some individuals at maximal exercise intensities.

The disparity between AVP and plasma [Na^+] seen after high intensity exercise appears to be exaggerated following prolonged endurance exercise; where plasma AVP concentrations are significantly elevated despite a significant *decrease* in plasma [Na^+] from pre- to post-run (Table 3F.1; Figure 3F.2a and 3F.4). This finding would suggest that factors in addition to plasma osmolality strongly influence AVP secretion during six hours of prolonged endurance running. These non-osmotic factors might include plasma volume contraction [1;4], elevated body temperature [6] fluid ingestion prior to exercise [33], composition of the fluid consumed [21], nausea [8] or other unknown factors [34]. Conversely, pre-race plasma AVP concentrations are suppressed prior to the ultramarathon compared with the pre-run VO_2 max and steady-state laboratory runs. This finding would infer that subjects are optimally hydrated prior to the start of the "competitive" ultramarathon, as opposed to before the "non-competitive" treadmill runs, perhaps as a pre-race ritual guided by psychological rather than physiological stimuli.

In univariate regression analyses, post-run urine [Na^+] is positively correlated with AVP (Table 3F.4). This is an expected physiological response, as AVP secretion generally promotes free water clearance [27]. In contrast to these findings from combined data, when AVP secretion and urine [Na^+] are separately assessed by running condition only, the increase in AVP is smallest after the SS run (Table 3F.1; Figure 3F.4) while urine [Na^+] is highest after the SS run (Figure 3F.3). The apparent disparity between AVP secretion and free water clearance is reported elsewhere following exercise [5;35;36] and remains a somewhat curious phenomenon. Alternatively, plasma aldosterone concentrations are also stimulated by exercise [5 35;36]; suggesting that perhaps it is not a paradoxical "increase" in free water clearance that accompanies AVP secretion but rather a reabsorption of sodium ions from enhanced aldosterone secretion that jointly alters urine [Na^+].

Oxytocin is the "other" posterior pituitary hormone which participates in the regulation of fluid balance in animals [37] but seemingly not in humans [38]. Accordingly, very few studies have measured OT in humans during exercise. The only study documenting an increase in plasma OT concentrations following exercise involves five well-trained males running on a treadmill for ~60 minutes at an intensity of 80% of $V0_2$ max [17]. Peak levels of OT ranged between 4.5 – 23.9 pg/ml and were elevated in three out of the five runners. In contrast, OT did not change following high intensity exercise in male cyclists exercising for 20-25 minutes until exhaustion [39] or in healthy females during 20 minutes of graded exercise up to 90% $V0_2$ max [40;41]. These cumulative findings suggest that OT may not be stimulated by short duration high intensity exercise but may be stimulated by continuous exercise activity lasting ≥ 60 minutes. This suggestion is partially supported by our results, where plasma OT concentrations are significantly elevated after prolonged endurance, but not steady state running (Table 3F.1; Figure 3F.5).

The magnitude and pattern of change in OT between high intensity, steady-state and prolonged endurance exercise did not parallel the changes noted for AVP (Figure 3F.4 and 3F.5). These findings support an independent secretion of the two posterior pituitary hormones in response to different exercise conditions, and could suggest that the relationship between AVP and ACTH is independent of OT. The independent secretion of AVP and OT has been previously recorded in humans after chronic dehydration and sodium loading [42] and in assessments of diurnal variation [17]. Despite the apparent dissociation that is seen in our subjects from pre- to post-race, a positive correlation is noted between post-run concentrations of AVP and OT when data from all three exercise conditions are combined (Figure 3F.6). A significant positive correlation between the two hormones is documented in both rats [43;44] and dogs [45] and shown to remain robust under dissimilar stimuli such as hypovolemia, hyperosmolality, hypotension, uremia and nausea [43].

The physiological stimulus and role for OT secretion in humans during prolonged endurance running remain unclear. Although it is well-documented that OT causes a natriuresis under physiological concentrations in male rats [37], evidence supporting a

natriuretic effect of OT in humans is conflicting [38;46-48]. OT, like AVP, can cause an antidiuresis in humans [49]. However, the dosage of OT required to stimulate an equivalent antidiuresis is 100-fold the amount of AVP [46]. Curiously, in univariate analyses, OT was correlated with changes in both plasma volume and bodyweight when data from all three trials were combined (Table 3F.3).These associations need further evaluation during prolonged endurance running to clarify if OT does, in fact, participate in the maintenance of fluid balance in humans during exercise.

The significant increase in brain natriuretic peptide (BNP) following prolonged endurance exercise is also unexpected (Table 3F.1). While the role of ANP in the regulation of fluid balance during exercise has been widely assessed [1;4;5;35;36;50], the role of BNP in fluid homeostasis has not [18]. BNP is considered a superior marker of fluid overload [51] and has been measured after exercise primarily as an indicator of cardiac dysfunction [52;53]. The significant increase in NT-proBNP that is seen in our subjects following prolonged endurance exercise, but not high intensity or steady-state exercise, has been verified elsewhere [52;53]. Because NT-proBNP is *not* significantly elevated after the VO_2 max test (where blood pressure, heart rate and cardiac output would be highest [54]) but is increased four-fold after the ultramarathon (where mean arterial pressure and left ventricular diameter are significantly reduced [55-57]) it appears that other stimuli, apart from the cardiovascular system, are responsible for the increase in BNP seen after prolonged endurance exercise. Whether BNP secretion is alternatively stimulated by inflammation [58], decreased renal blood flow [59], lipolysis [60], or from a yet to be determined factor remain unclear.

When data from all three running tests are combined, a significant positive correlation between NT-proBNP Δ versus urine osmolality Δ (Figure 3F.8b) and a negative associated between NT-proBNP Δ versus plasma $[Na^+]$ Δ (Figure 3F.8a) become apparent. These relationships would suggest that BNP participates in the maintenance of fluid balance via the promotion of solute excretion in response to an increase in plasma $[Na^+]$ *if* the inverse relationship between NT-proBNP Δ versus plasma $[Na^+]$ Δ represents an *immediate* response of plasma $[Na^+]$ against a *delay* in the

disappearance of the more stable inactive fragment of BNP (NT-proBNP). Elevations in NT-proBNP are also significantly associated with decreases in plasma volume and bodyweight (Table 3F.4; Figures 3F.1a and 3F.1b). These paradoxical relationships further support the suggestion that BNP is not stimulated by volume expansion during exercise or alternatively may reflect a decrease in both plasma volume and bodyweight from BNP stimulation from other physiological sources.

The significant increase in the secretion of the principle mineralocorticoid hormone, aldosterone, following all three running conditions is a well-documented physiological response [16;61] (Figure 3F.7). Additionally, since aldosterone promotes sodium conservation and blood pressure maintenance, the significant inverse correlations between plasma aldosterone with both plasma volume and plasma [Na^+] are not surprising (Table 3F.3). These collective data suggest that aldosterone secretion is rapid, predictable and consistent following exercise and confirms that aldosterone participates in the regulation of fluid balance during periods of heightened physical stress [5;13;16;61;62].

Elevated post-run plasma aldosterone concentrations are not significantly different between high intensity, steady-state and prolonged endurance running, although the trend in the magnitude of secretion appears to be temporally related. The increase in aldosterone is greatest following the ultramarathon (six hours) and least following the VO_2 max test (10 minutes) (Table 3F.2). The increase in plasma aldosterone concentration over time may represent the slow "genomic" response facilitating the sustained increase in the magnitude of aldosterone production, as seen after steady-state and prolonged endurance running [63]. Conversely, the actions of the fast "non-genomic" response may contribute to the small and rapid increase in plasma aldosterone concentrations seen after high intensity running [63].

The smallest increase in plasma aldosterone concentration (8 ± 2 ng/ml; high intensity running) paradoxically correlates with the largest decrease in plasma volume (-11 ± 1%; Figure 3F.1a) and smallest loss in urine [Na^+] (-7 ± 6 mmol/L; Figure 3F.3).

Furthermore, if ACTH stimulation elicits a proportional increase in aldosterone secretion [64;65], than plasma aldosterone concentrations are expected to be higher after high intensity, compared with steady-state, running. These discrepancies between the plasma concentrations of aldosterone with corresponding changes in fluid balance markers allude to the complexity of the stimulus-response relationship. Potential stimuli to aldosterone secretion during exercise include changes in: plasma volume [1], plasma osmolality [4], sympathetic nerve activity [5], renin and angiotension secretion [34], hydration status [13], ambient temperature [66], sodium status [67], and potassium levels [68]. Alternatively, since the smallest increase in plasma aldosterone concentration corresponds to the largest increase in plasma [Na^+] (3 ± 1 mmol/L; Figure 3F.2a; high intensity running) while the largest increase in aldosterone concentration corresponds to the largest decrease in plasma [Na^+] (-4 ± 2 mmol/L; Figure 3F.2a; ultramarathon running); it is tempting to speculate that the maintenance of plasma [Na^+] during each exercise condition takes precedence over all other competing stimuli with regard to plasma aldosterone secretion.

The plasma concentrations of the precursor hormone to aldosterone, corticosterone, do not reflect concomitant changes in the magnitude of aldosterone secretion (Table 3F.2). Although corticosterone is not known to play a significant role in any physiological process in humans [69] the significant elevations documented before and after prolonged endurance running, compared to high intensity and steady-state running (Table 3F.2), are notable. Furthermore, the five-fold elevations in corticosterone before and after the ultramarathon infer heightened stimulation of the adrenal cortex by ACTH. The corresponding seven-fold increase in plasma aldosterone concentrations, however, may suggest the potentiation of aldosterone synthase activity to accelerate aldosterone formation during periods of heightened physical stress.

Significant elevations in both IL-6 (Table 3F.1) and cortisol (Table 3F.2) following the ultramarathon exemplify the significant inflammatory and stress imposed by prolonged endurance running. A direct role for cortisol and IL-6 secretion in overall fluid balance however, is not apparent from these data.

In conclusion, AVP and aldosterone appear to influence changes in urine [Na^+] and plasma volume, respectively, when all three running conditions are combined. The expected linear relationships between AVP with plasma [Na^+] and between aldosterone with plasma volume, however, are not supported at the extremes of exercise intensity and duration. These apparent "disassociations" suggest that other endocrine factors may influence the maintenance of fluid balance during high intensity, steady-state and prolonged endurance running. The significant increases in both OT and BNP secretion following prolonged endurance running as well as the linear relationships between OT with plasma volume and NT-proBNP with plasma [Na^+] suggest that these endocrine secretions participate in the regulation of fluid homeostasis during periods of heightened physical stress.

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Reference List

1. Grant SM, Green HJ, Phillips SM, Enns DL, Sutton JR. Fluid and electrolyte hormonal responses to exercise and acute plasma volume expansion. *J.Appl.Physiol* 1996;**81**:2386-92.

2. Hew-Butler T, Collins M, Bosch A, Sharwood K, Wilson G, Armstrong M *et al.* Maintenance of plasma volume and serum sodium concentration despite body weight loss in ironman triathletes. *Clin.J.Sport Med.* 2007;**17**:116-22.

3. Takamata A, Nose H, Kinoshita T, Hirose M, Itoh T, Morimoto T. Effect of acute hypoxia on vasopressin release and intravascular fluid during dynamic exercise in humans. *Am.J.Physiol Regul.Integr.Comp Physiol* 2000;**279**:R161-R168.

4. Shoemaker JK, Green HJ, Ball-Burnett M, Grant S. Relationships between fluid and electrolyte hormones and plasma volume during exercise with training and detraining. *Med.Sci.Sports Exerc.* 1998;**30**:497-505.

5. Melin B, Koulmann N, Jimenez C, Savourey G, Launay JC, Cottet-Emard JM *et al.* Comparison of passive heat or exercise-induced dehydration on renal water and electrolyte excretion: the hormonal involvement. *Eur.J.Appl.Physiol* 2001;**85**:250-8.

6. Takamata A, Mack GW, Stachenfeld NS, Nadel ER. Body temperature modification of osmotically induced vasopressin secretion and thirst in humans. *Am.J.Physiol* 1995;**269**:R874-R880.

7. Chikanza IC, Petrou P, Chrousos G. Perturbations of arginine vasopressin secretion during inflammatory stress. Pathophysiologic implications. *Ann.N.Y.Acad.Sci.* 2000;**917**:825-34.

8. Rowe JW, Shelton RL, Helderman JH, et.al. Influence of the emetic reflex on vasopressin release in man. *Kidney Int* 1979;**16**:729-35.

9. Deuster PA, Chrousos GP, Luger A, Debolt JE, Bernier LL, Trostmann UH *et al.* Hormonal and metabolic responses of untrained, moderately trained, and highly trained men to three exercise intensities. *Metabolism* 1989;**38**:141-8.

10. Luger A, Deuster PA, Kyle SB, Gallucci WT, Montgomery LC, Gold PW *et al.* Acute hypothalamic-pituitary-adrenal responses to the stress of treadmill exercise. Physiologic adaptations to physical training. *N.Engl.J.Med.* 1987;**316**:1309-15.

11. Viru A. Plasma hormones and physical exercise. *Int.J.Sports Med.* 1992;**13**:201-9.

12. Ferrari R, Anand IS, Ceconi C, De Giuli F, Poole-Wilson PA, Harris P. Neuroendocrine response to standing and mild exercise in patients with

untreated severe congestive heart failure and chronic constrictive pericarditis. *Heart* 1996;**76**:50-5.

13. Montain SJ, Laird JE, Latzka WA, Sawka MN. Aldosterone and vasopressin responses in the heat: hydration level and exercise intensity effects. *Med.Sci.Sports Exerc.* 1997;**29**:661-8.

14. Wade CE. Response, regulation, and actions of vasopressin during exercise: a review. *Med Sci Sports Exerc* 1984;**16**:506-11.

15. Costill DL, Branam G, Fink W, Nelson R. Exercise induced sodium conservation: changes in plasma renin and aldosterone. *Med.Sci.Sports* 1976;**8**:209-13.

16. Luger A, Deuster PA, Debolt JE, Loriaux DL, Chrousos GP. Acute exercise stimulates the renin-angiotensin-aldosterone axis: adaptive changes in runners. *Horm.Res.* 1988;**30**:5-9.

17. Landgraf R, Hacker R, Buhl H. Plasma vasopressin and oxytocin in response to exercise and during a day-night cycle in man. *Endokrinologie.* 1982;**79**:281-91.

18. Mudambo KS, Coutie W, Rennie MJ. Plasma arginine vasopressin, atrial natriuretic peptide and brain natriuretic peptide responses to long-term field training in the heat: effects of fluid ingestion and acclimatization. *Eur.J.Appl.Physiol Occup.Physiol* 1997;**75**:219-25.

19. Niessner A, Ziegler S, Slany J, Billensteiner E, Woloszczuk W, Geyer G. Increases in plasma levels of atrial and brain natriuretic peptides after running a marathon: are their effects partly counterbalanced by adrenocortical steroids? *Eur.J.Endocrinol.* 2003;**149**:555-9.

20. Siegel AJ. Exercise-associated hyponatremia: role of cytokines. *Am.J.Med.* 2006;**119**:S74-S78.

21. Deuster PA, Singh A, Hofmann A, Moses FM, Chrousos GC. Hormonal responses to ingesting water or a carbohydrate beverage during a 2 h run. *Med.Sci.Sports Exerc.* 1992;**24**:72-9.

22. Brandenberger G, Candas V, Follenius M, Libert JP, Kahn JM. Vascular fluid shifts and endocrine responses to exercise in the heat. Effect of rehydration. *Eur.J.Appl.Physiol Occup.Physiol* 1986;**55**:123-9.

23. Scrimgeour AG, Noakes TD, Adams B, Myburgh K. The influence of weekly training distance on fractional utilization of maximum aerobic capacity in marathon and ultramarathon runners. *Eur.J.Appl.Physiol Occup.Physiol* 1986;**55**:202-9.

24. Verbalis JG, McHale CM, Gardiner TW, Stricker EM. Oxytocin and vasopressin secretion in Response to Stimuli Producing Learned Taste Aversion in Rats. *Behav.Neurosci.* 1986;**100**:466-75.

25. Guo T, Taylor RL, Singh RJ, Soldin SJ. Simultaneous determination of 12 steroids by isotope dilution liquid chromatography-photospray ionization tandem mass spectrometry. *Clin.Chim.Acta* 2006;**372**:76-82.

26. Soldin SJ, Soldin OP, Boyajian AJ, Taskier MS. Pediatric brain natriuretic peptide and N-terminal pro-brain natriuretic peptide reference intervals. *Clin.Chim.Acta* 2006;**366**:304-8.

27. Verbalis JG. Disorders of body water homeostasis. *Best.Pract.Res.Clin.Endocrinol.Metab* 2003;**17**:471-503.

28. Inder WJ, Hellemans J, Swanney MP, Prickett TC, Donald RA. Prolonged exercise increases peripheral plasma ACTH, CRH, and AVP in male athletes. *J.Appl.Physiol* 1998;**85**:835-41.

29. Duclos M, Corcuff JB, Pehourcq F, Tabarin A. Decreased pituitary sensitivity to glucocorticoids in endurance-trained men. *Eur.J.Endocrinol.* 2001;**144**:363-8.

30. Petrides JS, Gold PW, Mueller GP, Singh A, Stratakis C, Chrousos GP *et al.* Marked differences in functioning of the hypothalamic-pituitary-adrenal axis between groups of men. *J.Appl.Physiol* 1997;**82**:1979-88.

31. Chiodera P, Volpi R, Maffei ML, Caiazza A, Caffarri G, Papadia C *et al.* Role of GABA and opioids in the regulation of the vasopressin response to physical exercise in normal men. *Regul.Pept.* 1993;**49**:57-63.

32. Deuster PA, Faraday MM, Chrousos GP, Poth MA. Effects of dehydroepiandrosterone and alprazolam on hypothalamic-pituitary responses to exercise. *J.Clin.Endocrinol.Metab* 2005;**90**:4777-83.

33. Wade CE,.Claybaugh JR. Plasma renin activity, vasopressin concentration, and urinary excretory responses to exercise in men. *J.Appl.Physiol* 1980;**49**:930-6.

34. Maresh CM, Gabaree-Boulant CL, Armstrong LE, Judelson DA, Hoffman JR, Castellani JW *et al.* Effect of hydration status on thirst, drinking, and related hormonal responses during low-intensity exercise in the heat. *J.Appl.Physiol* 2004;**97**:39-44.

35. Freund BJ, Shizuru EM, Hashiro GM, Claybaugh JR. Hormonal, electrolyte, and renal responses to exercise are intensity dependent. *J.Appl.Physiol* 1991;**70**:900-6.

36. Melin B, Jimenez C, Savourey G, Bittel J, Cottet-Emard JM, Pequignot JM *et al.* Effects of hydration state on hormonal and renal responses during moderate exercise in the heat. *Eur.J.Appl.Physiol Occup.Physiol* 1997;**76**:320-7.

37. Conrad KP, Gellai M, North WG, Valtin H. Influence of oxytocin on renal hemodynamics and sodium excretion. *Ann.N.Y.Acad.Sci.* 1993;**689**:346-62.

38. Cross RB, Dicker SE, Kitchen AH, Lloyd S, Pickford M. The effect of oxytocin on the urinary excretion of water and electrolytes in man. *J.Physiol* 1960;**153**:553-61.

39. Chicharro JL, Hoyos J, Bandres F, Gomez GF, Perez M, Lucia A. Plasma oxytocin during intense exercise in professional cyclists. *Horm.Res.* 2001;**55**:155-9.

40. Altemus M, Deuster PA, Galliven E, Carter CS, Gold PW. Suppression of hypothalmic-pituitary-adrenal axis responses to stress in lactating women. *J.Clin.Endocrinol.Metab* 1995;**80**:2954-9.

41. Altemus M, Roca C, Galliven E, Romanos C, Deuster P. Increased vasopressin and adrenocorticotropin responses to stress in the midluteal phase of the menstrual cycle. *J.Clin.Endocrinol.Metab* 2001;**86**:2525-30.

42. Williams TD, Abel DC, King CM, Jelley RY, Lightman SL. Vasopressin and oxytocin responses to acute and chronic osmotic stimuli in man. *J.Endocrinol.* 1986;**108**:163-8.

43. Stricker EM, Hosutt JA, Verbalis JG. Neurohypophyseal secretion in hypovolemic rats: inverse relation to sodium appetite. *Am.J.Physiol* 1987;**252**:R889-R896.

44. Stricker EM,.Verbalis JG. Interaction of osmotic and volume stimuli in regulation of neurohypophyseal secretion in rats. *Am.J.Physiol* 1986;**250**:R267-R275.

45. Weitzman RE, Glatz TH, Fisher DA. The effect of hemorrhage and hypertonic saline upon plasma oxytocin and arginine vasopressin in conscious dogs. *Endocrinology* 1978;**103**:2154-60.

46. Thomson WB. The effect of oxytocin and vasopressin and of phenylalanyl 3-oxytocin on the urinary excretion of water and electrovtes. *J.Physiol* 1960;**150**:284-94.

47. Rasmussen MS, Simonsen JA, Sandgaard NC, Hoilund-Carlsen PF, Bie P. Effects of oxytocin in normal man during low and high sodium diets. *Acta Physiol Scand.* 2004;**181**:247-57.

48. Kostoglou-Athanassiou I, Treacher DF, Forsling ML. Is Oxytocin Natriuretic in Man? *J.Endocrinol* 1994;**143(suppl.O)**:39.

49. Abdul-Karim R,.Assali NS. Renal function in human pregnancy. V. Effects of oxytocin on renal hemodynamics and water and electrolyte excretion. *J.Lab Clin.Med.* 1961;**57**:522-32.

50. Roy BD, Green HJ, Burnett M. Prolonged exercise following diuretic-induced hypohydration effects on fluid and electrolyte hormones. *Horm.Metab Res.* 2001;**33**:540-7.

51. Huang WS, Lee MS, Perng HW, Yang SP, Kuo SW, Chang HD. Circulating brain natriuretic peptide values in healthy men before and after exercise. *Metabolism* 2002;**51**:1423-6.

52. Scharhag J, Urhausen A, Schneider G, Herrmann M, Schumacher K, Haschke M *et al.* Reproducibility and clinical significance of exercise-induced increases in cardiac troponins and N-terminal pro brain natriuretic peptide in endurance athletes. *Eur.J.Cardiovasc.Prev.Rehabil.* 2006;**13**:388-97.

53. Scharhag J, Herrmann M, Urhausen A, Haschke M, Herrmann W, Kindermann W. Independent elevations of N-terminal pro-brain natriuretic peptide and cardiac troponins in endurance athletes after prolonged strenuous exercise. *Am.Heart J.* 2005;**150**:1128-34.

54. Staessen J, Fagard R, Hespel P, Lijnen P, Vanhees L, Amery A. Plasma renin system during exercise in normal men. *J.Appl.Physiol* 1987;**63**:188-94.

55. Vanoverschelde JL, Younis LT, Melin JA, Vanbutsele R, Leclercq B, Robert AR *et al.* Prolonged exercise induces left ventricular dysfunction in healthy subjects. *J.Appl.Physiol* 1991;**70**:1356-63.

56. Seals DR, Rogers MA, Hagberg JM, Yamamoto C, Cryer PE, Ehsani AA. Left ventricular dysfunction after prolonged strenuous exercise in healthy subjects. *Am.J.Cardiol.* 1988;**61**:875-9.

57. Douglas PS, O'Toole ML, Hiller WD, Reichek N. Different effects of prolonged exercise on the right and left ventricles. *J.Am.Coll.Cardiol.* 1990;**15**:64-9.

58. Shor R, Rozenman Y, Bolshinsky A, Harpaz D, Tilis Y, Matas Z *et al.* BNP in septic patients without systolic myocardial dysfunction. *Eur.J.Intern.Med.* 2006;**17**:536-40.

59. Hutchens MP,.Weinmann M. Renal protection with recombinant b-type natriuretic peptide in a burn patient with rhabdomyolysis. *Burns* 2006;**32**:128-31.

60. Moro C, Galitzky J, Sengenes C, Crampes F, Lafontan M, Berlan M. Functional and pharmacological characterization of the natriuretic peptide-dependent lipolytic pathway in human fat cells. *J.Pharmacol.Exp.Ther.* 2004;**308**:984-92.

61. Viru A, Karelson K, Smirnova T. Stability and variability in hormonal responses to prolonged exercise. *Int.J.Sports Med.* 1992;**13**:230-5.

62. Brandenberger G, Candas V, Follenius M, Kahn JM. The influence of the initial state of hydration on endocrine responses to exercise in the heat. *Eur.J.Appl.Physiol Occup.Physiol* 1989;**58**:674-9.

63. Boldyreff B,.Wehling M. Aldosterone: refreshing a slow hormone by swift action. *News Physiol Sci.* 2004;**19**:97-100.

64. Holst JP, Soldin SJ, Tractenberg RE, Guo T, Kundra P, Verbalis JG *et al.* Use of steroid profiles in determining the cause of adrenal insufficiency. *Steroids* 2007;**72**:71-84.

65. Yamauchi T, Harada T, Kurono M, Matsui N. Effect of exercise-induced acidosis on aldosterone secretion in men. *Eur.J.Appl.Physiol Occup.Physiol* 1998;**77**:409-12.

66. Kosunen KJ, Pakarinen AJ, Kuoppasalmi K, Adlercreutz H. Plasma renin activity, angiotensin II, and aldosterone during intense heat stress. *J.Appl.Physiol* 1976;**41**:323-7.

67. Takamata A, Mack GW, Gillen CM, Nadel ER. Sodium appetite, thirst, and body fluid regulation in humans during rehydration without sodium replacement. *Am.J.Physiol* 1994;**266**:R1493-R1502.

68. Holst JP, Soldin OP, Guo T, Soldin SJ. Steroid hormones: relevance and measurement in the clinical laboratory. *Clin.Lab Med.* 2004;**24**:105-18.

69. Raubenheimer PJ, Young EA, Andrew R, Seckl JR. The role of corticosterone in human hypothalamic-pituitary-adrenal axis feedback. *Clinical Endocrinology* 2006;**65**:22-6.

Study 3G: Arginine Vasopressin and acute changes in serum, sweat and urinary sodium concentrations during exercise in humans

INTRODUCTION

Plasma tonicity changes are known to account for 72% of the variance noted in the onset threshold for thermoregulatory sweating [1]. The integration between tonicity and thermoregulation is centrally mediated [2], with fluid conservation taking precedence over the maintenance of core temperature during both passive heat stress [3] and exercise [4]. Exercise training [5] can induce an expansion of blood volume, which attenuates the hyperosmotic suppression of cutaneous vasodilation. Therefore, both osmoreceptors and volume receptors appear to play important regulatory roles in fluid and thermoregulatory balance during exercise. The co-existence of osmosensitive and thermosensitive neurons in the pre-optic anterior hypothalamic area [6] combined with widespread pre-optic and hypothalamic networks of vasopressinergic neurons [2] identifies arginine vasopressin (AVP) as a putative endocrine regulator of sweat and urine sodium and water excretion during intense or prolonged exercise.

During exercise, osmotic and non-osmotic stimuli to AVP secretion limit renal water excretion for as long as exercise continues above a threshold intensity of 50% of maximal oxygen uptake (VO_2 Max) [7]. An exercise intensity of >50% VO_2 Max is required to contract the plasma volume by >7%, which is sufficient to stimulate both AVP and renin release during exercise [7].

Normally, sweat glands play an active role in thermoregulation while the kidneys act to maintain fluid and solute homeostasis [8]. However, since antidiuresis and sodium retention are increased during physical activity performed above 50% VO_2 Max, sweat production predominates as the primary source of water and electrolyte loss during exercise. Ninety-two percent of all water [9] and 87% of all sodium lost [10] during exercise is derived from sweat, which establishes the large impact that sweating has on fluid and electrolyte balance under these circumstances. Despite these functional and structural differences, regulatory mechanisms that govern the concentration of sweat may serve

important physiological roles in water and sodium balance when renal excretion is suppressed. The parallel changes of sweat rate and urine production in response to changes in plasma osmolality and volume at rest further support AVP as the main endocrine regulator of both excretions [11].

The aim of this study was to evaluate the endocrine regulation of fluid balance during high intensity and steady state running. Fluid regulatory parameters such as plasma, sweat and urine sodium concentration and osmolality, plasma volume, and bodyweight were evaluated in association with plasma concentrations of cytokines, neurohypophyseal and natriuretic peptides, and adrenal steroid hormones. Specifically, we sought to identify which endocrine factors were most associated with serum, urine and sweat sodium concentrations during exercise.

METHODS

Subjects: Ten trained endurance runners (five male and five female) were recruited and signed written informed consent for participation in this study, which was approved by both the Ethics Committee of the University of Cape Town and the Georgetown University Institutional Review Board.

Testing protocol: Each runner presented to the laboratory twice, one week apart, during the same part of the day to prevent individual diurnal variation between exercise tests. Each subject was asked to refrain from vigorous exercise 24 hours prior to each test and to consume a similar diet. No food or fluid was allowed during either exercise test.

A high intensity (HI) exercise test was performed during the first experimental session. This consisted of a maximal running test to volitional exhaustion on a motorized treadmill (Quinton type BA-1 treadmill, USA). Each athlete was allowed to warm-up on the treadmill at a self-selected speed for five minutes. After completion of the warm-up, a heart-rate monitor (Polar Heart Rate Monitor, USA) was fitted around each runner's chest and a mask tightly secured over the nose and mouth in order to prevent air from escaping through the boundary of the mask. Oxygen uptake was measured

continuously using an Oxycon Alpha Analyzer (Jaeger, Netherlands) with maximal oxygen consumption (VO_2 max) defined as the highest value obtained in each subject before volitional exhaustion. The exercise protocol utilized was a peak treadmill running test, during which each runner ran for one minute at a speed of 7.5 km/hr. Thereafter, the speed was increased by 0.5 km/h every 30 seconds until the subject could no longer keep pace with the treadmill [12]. The last 30 second stage that was completed was designated as each athlete's peak treadmill running speed. A steady-state treadmill test (SS) was performed one week later. Each subject ran for 60 minutes on the treadmill at a speed that corresponded to 60% of the peak speed reached during the HI test.

Sample collection and measurements: Immediately prior to each exercise test, bodyweight was obtained on a calibrated Adamlab JPS electronic scale (Scales, Brackenfell, South Africa) in running attire and without shoes after the subject voided. A urine sample was obtained from this pre-race void. Venous blood was obtained with each subject in a seated position. Ten milliliters of venous blood were collected into chilled lithium heparin tubes and immediately centrifuged for 10 minutes at 3000 rpm. The separated plasma was immediately frozen at -80°C until further analysis could be performed. A central area on the back, between the shoulder blades, was cleansed with distilled water and one 5 cm x 5 cm piece of sterile gauze was secured with a sheet of plastic wrap and tape for sweat collection, similar to the method described by Ikai [13].

Within one minute of completing each exercise test, all subjects returned to the seated position and 10 ml of venous blood was collected into chilled lithium heparin tubes, centrifuged, separated and frozen. Sweat was collected from the sterile gauze and transferred into an eppendorf tube. The subject voided and the urine was collected. The sweat and urine samples were immediately frozen and stored at -80°C until further analysis could be performed. A post-exercise bodyweight was then obtained.

Analytical measurements. Changes in plasma volume were estimated by comparing pre- and post-race measurements of plasma protein using a clinical refractometer (Schuco Clinical Refractometer 5711-2020, Japan). Serum, urine and sweat sodium ($[Na^+]$) and potassium ($[K^+]$) concentrations were measured using ion-selective

electrodes (Beckman Synchron EI-ISE, Fullerton, CA). Osmolality was measured using a vapor pressure osmometer (VAPRO 5520, WESCOR, Logan, UT)

Hormone Measurements. Plasma concentrations of arginine vasopressin ($[AVP]_P$) and oxytocin ($[OT]_P$) were measured by specific radioimmunoassay following acetone-ether extraction as described previously [14]. The standard curve for AVP is linear between 0.5 and 10.0 pg/tube with the use of a synthetic AVP standard (PerkinElmer Life Sciences Inc, Boston MA). The minimum detectable concentration of AVP in extracted plasma was 0.5 pg/ml. The AVP antiserum (R-4) displays <1% cross-reactivity with OT. The standard curve of the OT assay is linear between 0.25 and 5.0 pg/tube with the use of a synthetic OT standard (PerkinElmer Life Sciences Inc, Boston MA). The minimum detectable concentration of OT in extracted plasma is 0.25 pg/ml. The OT antiserum (Pitt-Ab2) displays <1% cross-reactivity with AVP.

Eleven adrenal steroid hormones (cortisol, 11-deoxycortisol, aldosterone, corticosterone, DHEA, DHEAS, testosterone, androstenedione, 17-hydroxyprogesterone, progesterone and 25-hydroxyvitamin D3) were measured using a liquid chromatography-tandem mass spectrometer, in conjunction with an atmospheric pressure photoionization source, using methodology described recently [15].

Brain natriuretic peptide concentrations were assessed with measurement of the more stable cleaved inactive fragment, N-terminal pro-brain natriuretic peptide (NT-proBNP), using the automated Dade RxL Dimension as previously described [16]. Interleukin-6 (IL-6) was measured by chemiluminescence (Immulite 1000 Diagnostic Product Corporation, Los Angeles, CA)

Statistical analysis. All data were analyzed using STATISTICA 7.0™ software (StatSoft Inc., Tulsa, OK). Data are presented as means ± SEM, together with the range of values. Statistical significance was accepted when $p<0.05$.

RESULTS

Descriptive fitness, demographic and training characteristics of the subjects are presented in Table 3G.1. There were no significant differences between male and female runners with regard to any of these characteristics.

The change (Δ = post- minus pre-exercise measurement) in the physiological markers of fluid balance for both the high intensity and steady state runs are summarized in Tables 3G.2A and 3G.2B, respectively. For HI exercise, the pre- to post-test increase in serum [Na^+] (137.6 ± 0.6 mmol/L to 140.4 ± 0.7 mmol/L; $p<0.01$) and plasma osmolality (294.1 ± 2.2 mOsm/kg H_2O to 306.7 ± 2.2 mOsm/kg H_2O; $p<0.001$) were significantly different, while for SS exercise, only the pre- to post-test increase in serum [Na^+] was significant (138.1 ± 0.6 mmol/L to 140.2 ± 0.5 mmol/L; $p<0.05$).

Sweat samples were obtained in only six runners following the HI test and in all ten runners following the SS run. After the HI run, the sweat [K^+] = 7.7 ± 0.5 mmol/L, [Na^+] = 69.7 ± 8.5 mmol/L, and osmolality = 170.7 ± 14.2 mOsm/kg H_2O. After the SS run, the sweat [K^+] = 4.8 ± 0.4 mmol/L, [Na^+] = 76.9 ± 7.7 mmol/L and osmolality = 172.2 ± 13.6 mOsm/kg H_2O.

Measurements of plasma hormone concentrations from before and after exercise are summarized separately for the HI test (Table 3G.3) and for the SS run (Table 3G.4). Only the changes in oxytocin and aldosterone concentrations were significantly different before and after exercise in both exercise tests, while AVP concentrations were significantly increased only after the HI run.

There was a significant difference between % bodyweight Δ, plasma osmolality Δ, AVP Δ and post-test, cortisol Δ and post-test, oxytocin post-test and DHEA Δ between HI and SS exercise (Table 3G.5).

Significant gender differences (female vs. male) were noted in NT-proBNP Δ after the high intensity run (54.6 vs. 12.4 pg/mL; $p<0.01$) and in testosterone concentrations ($p<0.001$) after both the high intensity (pre: 18.2 vs. 413 ng/mL; post: 25.6 vs. 586.2

ng/mL; Δ: 7.4 vs. 172.4 ng/mL), and steady state runs (pre: 17.6 vs. 423.2 ng/mL; post: 23.6 vs. 528.0 ng/mL; Δ: 6.0 vs. 104.8 ng/mL).

When data from both the high intensity and steady state runs were combined, significant positive linear correlations were noted between the following parameters: sweat [Na^+] versus post urine [Na^+] (Figure 3G.1a), sweat osmolality versus post urine osmolality (Figure 3G.1b), post serum [Na^+] versus both sweat [Na^+] (Figure 3G.2a) and post urine [Na^+] (Figure 3G.2b), post urine osmolality versus post urine [Na^+] (Figure 3G.3a) and sweat osmolality versus sweat [Na^+] (Figure 3G.3b). There were no significant relations noted between post plasma osmolality versus post urine and sweat osmolality or between plasma osmolality versus serum [Na^+] when data from the HI and SS runs were analyzed in combination or separately.

Post urine [Na^+] (Figure 3G.4b), but not sweat [Na^+] (Figure 3G.4a), was significantly correlated with post $[AVP]_P$ when the HI and SS trials were combined. Conversely, no significant relation existed between $[AVP]_P$ versus post urine and sweat osmolality when data were combined. When data were separated by trial, however, there were significant positive correlations noted between $[AVP]_P$ versus sweat osmolality following both HI and SS running (Figure 3G.5a) and between post $[AVP]_P$ versus post urine osmolality following the SS run only (Figure 3G.5b). When data from only the steady state running condition were considered, there were significant relations between post $[AVP]_P$ and both post urine and sweat [Na^+] (Figure 3G.5a) and between post $[AVP]_P$ versus both post urine and sweat osmolality (Figure 3G.5b). Conversely, only after the high intensity condition was post serum [Na^+] (Figure 3G.7a) and post plasma osmolality (Figure 3G.7b) significantly correlated with aldosterone Δ.

Table 3G.1: Physiological and training parameters of subjects participating in High Intensity and Steady State laboratory trials (N = 10: 5 females and 5 males)

VARIABLE	Mean ± SEM	Minimum	Maximum
VO_2 Maximum ($ml/kg/O_2$)	56.1 ± 3.2	34.0	70.8
Time to VO_2 Max (minutes)	10.4 ± 0.9	5.5	15.0
Peak Running Speed (km/hr)	16.7 ± 0.8	12.5	21.5
Maximum Heart Rate (beats per minute)	175.7 ± 4.9	141	194
Training Distance (km/week)	57.3 ± 6.8	36	100
Years Running	10.9 ± 2.7	1	30
Age (years)	40.4 ± 3.4	25	53
Body Mass Index (kg/cm^2)	22.3 ± 0.7	17.9	25.4

Table 3G.2A: Measures of fluid balance: High Intensity run (VO_2 Max Test) (N=10)

VARIABLE	Mean ± SEM	Minimum	Maximum
Urine Osmolality Δ (mOsmol/kgH_2O)	**-44.0 ± 31.2**	**-227.0**	**115.0**
Urine [Na^+] Δ (mmol/L)	**-5.6 ± 4.1**	**-37.0**	**12.0**
Plasma Volume Δ (%)	**-9.5 ± 1.3**	**-14.6**	**0.0**
Bodyweight Δ (%)	**-0.3 ± 0.1**	**-0.1**	**-0.8**
Serum [Na^+] Δ (mmol/L)	**2.8 ± 0.5**	**-0.8**	**6.1**
Plasma Osmolality Δ (mOsmol/kgH_2O)	**12.6 ± 1.9**	**5.0**	**23.0**

Table 3G.2B: Measures of fluid balance: Steady State run (60% peak treadmill running speed) (N=10)

VARIABLE	Mean ± SEM	Minimum	Maximum
Urine Osmolality Δ ($mOsmol kgH_2O$)	-6.3 ± 23.6	-147.0	114.0
Urine [Na^+] Δ (mmol/L)	-28.9 ± 10.8	-98.0	3.0
Plasma Volume Δ (%)	-8.2 ± 1.4	-19.1	-4.3
Bodyweight Δ (%)	-1.4 ± 0.1	-0.7	-2.3
Serum [Na^+] Δ (mmol/L)	2.1 ± 0.6	-2.0	4.6
Plasma Osmolality Δ ($mOsmol/kgH_2O$)	3.2 ± 2.0	-5.0	15.0

Table 3G.3: Plasma hormone concentrations before and after exercise: High Intensity run (VO_2 Max Test) (N=10)

VARIABLE	PRE-TEST (Mean ± SEM)	POST-TEST (Mean ± SEM)	P-value
Arginine Vasopressin (pg/mL)	**3.3 ± 0.5**	**15.8 ± 4.6**	***<0.01***
Oxytocin (pg/mL)	**1.3 ± 0.1**	**2.9 ± 0.5**	***<0.01***
NT-proBNP (pg/mL)	**27.9 ± 5.6**	**33.5 ± 10.0**	**0.6**
IL-6 (pg/mL)	**<4.0 ± 0.0**	**4.3 ± 0.3**	**0.3**
DHEAS (ug/dL)	**105.8 ± 13.0**	**117.2 ± 15.9**	**0.6**
Aldosterone (ng/dL)	**5.4 ± 0.7**	**12.6 ± 1.3**	***<0.01***
Cortisol (ug/dL)	**12.1 ± 1.7**	**15.1 ± 2.2**	**0.3**
Corticosterone (ng/dL)	**175.9 ± 49.3**	**466.0 ± 133.3**	**0.06**
11-deoxycortisol (ug/dL)	**0.03 ± 0.00**	**0.09 ± 0.02**	**0.06**
Androstenedione (ng/dL)	**54.0 ± 6.3**	**73.3 ± 9.2**	**0.1**
Testosterone (ng/dL)	**216.0 ± 69.6**	**305.9 ± 95.9**	**0.5**
17-Hydroxyprogesterone (ng/dL)	**56.5 ± 25.5**	**86.5 ± 34.1**	**0.5**
DHEA (ng/dL)	**185.9 ± 37.4**	**306.0 ± 62.3**	**0.1**
Progesterone (ng/dL)	**147.4 ± 145.8**	**184.9 ± 180.6**	**0.9**
25-Hydroxyvitamin D3 (ug/L)	**58.7 ± 6.8**	**73.6 ± 10.0**	**0.2**

Table 3G.4: Plasma hormone concentrations before and after exercise: Steady State run (60% peak treadmill running speed) (N=10)

VARIABLE	PRE-TEST (Mean ± SEM)	POST-TEST (Mean ± SEM)	P-value
Arginine Vasopressin (pg/mL)	3.1 ± 0.2	4.3 ± 0.7	0.2
Oxytocin (pg/mL)	1.0 ± 0.2	1.5 ± 0.1	*<0.05*
NT-proBNP (pg/mL)	21.4 ± 5.5	33.2 ± 0.7	0.2
IL-6 (pg/mL)	4.0 ± 0.0	4.2 ± 0.2	0.2
DHEAS (ug/dL)	156.3 ± 52.3	110.9 ± 13.8	0.4
Aldosterone (ng/dL)	6.0 ± 1.3	20.3 ± 3.4	*<0.01*
Cortisol (ug/dL)	12.2 ± 1.7	9.4 ± 0.8	0.2
Corticosterone (ng/dL)	139.9 ± 44.7	177.9 ± 47.9	0.6
11-deoxycortisol (ug/dL)	0.03 ± 0.01	0.04 ± 0.01	0.2
Androstenedione (ng/dL)	47.0 ± 6.8	65.2 ± 7.6	0.09
Testosterone (ng/dL)	220.4 ± 71.4	275.8 ± 86.3	0.6
17-Hydroxyprogesterone (ng/dL)	35.1 ± 6.6	54.9 ± 7.4	0.06
DHEA (ng/dL)	168.3 ± 38.9	179.1 ± 24.6	0.8
Progesterone (ng/dL)	36.0 ± 25.4	36.0 ± 34.6	1.0
25-Hydroxyvitamin D3 (ug/L)	58.0 ± 6.5	67.5 ± 5.5	0.3

Table 3G.5: Measurements that were significantly different between High Intensity and Steady State exercise (N=10)

VARIABLE	High Intensity (Mean ± SEM)	Steady State (Mean ± SEM)	p-value
% Bodyweight Δ (kg)	-0.3 ± 0.1	-1.4 ± 0.1	0.000001
Plasma Osmolality Δ ($mOsmol/kgH_2O$)	12.6 ± 1.9	3.2 ± 2.0	0.003
Oxytocin post (pg/mL)	2.9 ± 0.5	1.5 ± 0.1	0.02
Vasopressin post (pg/mL)	15.8 ± 4.6	4.3 ± 0.7	0.02
Vasopressin Δ (pg/mL)	12.6 ± 4.5	1.1 ± 0.7	0.02
Cortisol post (ug/mL)	15.1 ± 2.2	9.4 ± 0.8	0.02
Cortisol Δ (ug/mL)	3.0 ± 1.5	-2.8 ± 1.9	0.03
DHEA Δ (ug/mL)	120.0 ± 29.0	10.9 ± 27.3	0.01

Figure 3G.1a: Sweat sodium concentration versus urine sodium concentration immediately following both High Intensity and Steady State running.

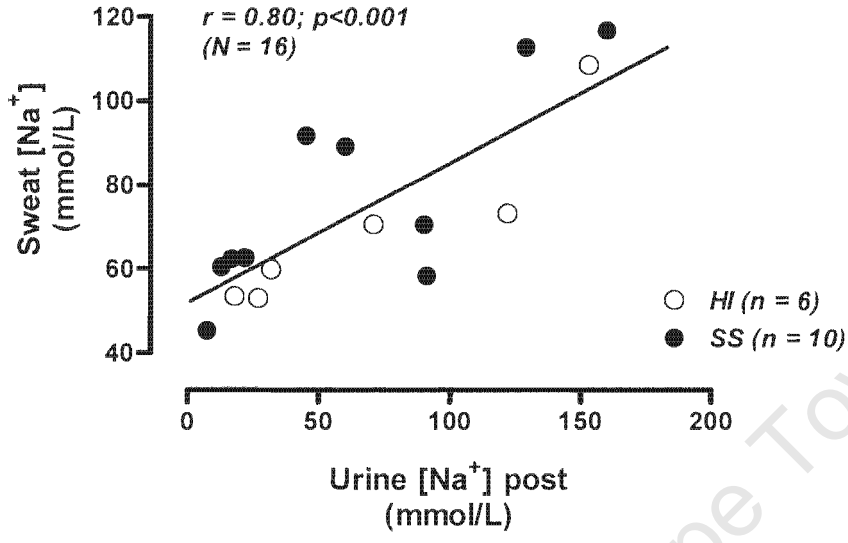


Figure 3G.1b: Sweat osmolality versus urine osmolality immediately following both High Intensity and Steady State running.

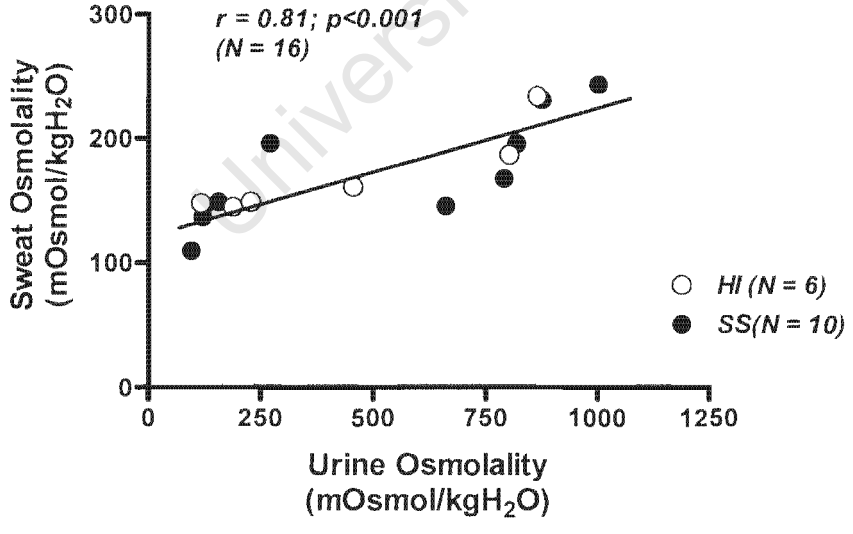


Figure 3G.2a: Sweat sodium concentration versus serum sodium concentration immediately following both High Intensity and Steady State running.

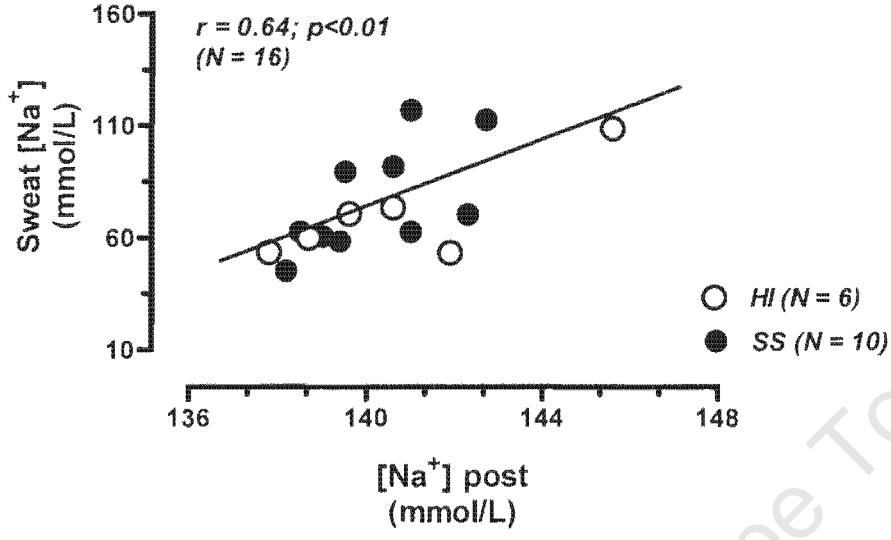


Figure 3G.2b: Urine sodium concentration versus serum sodium concentration immediately following both High Intensity and Steady State running.

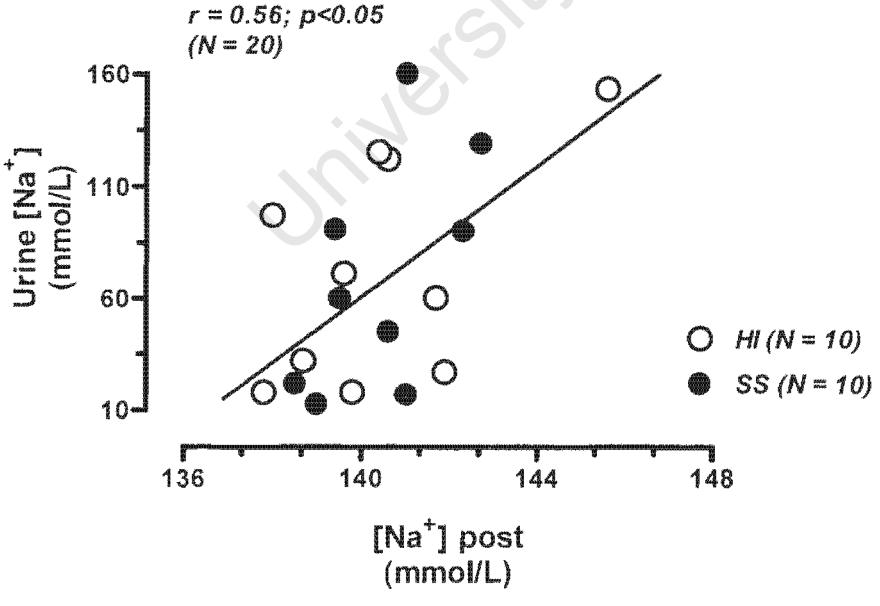


Figure 3G.3a: Urine sodium concentration versus urine osmolality immediately following both High Intensity and Steady State running.

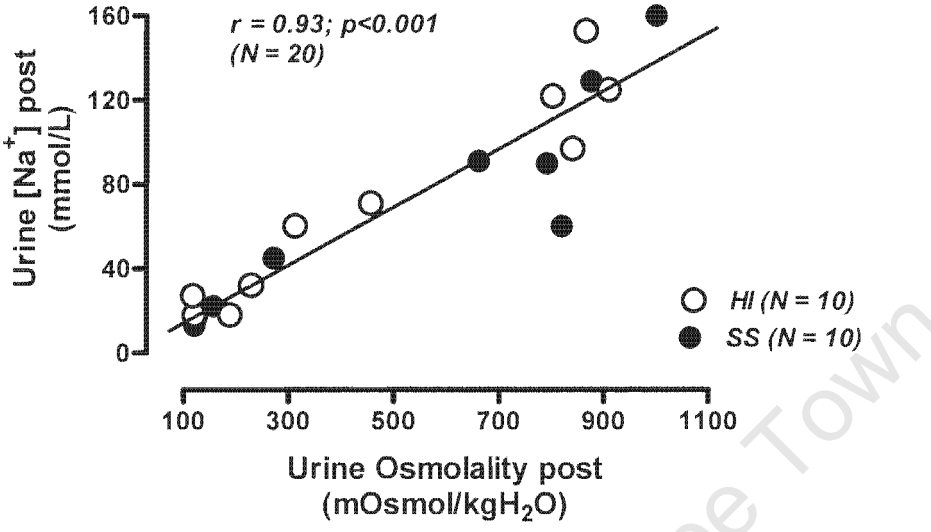


Figure 3G.3b: Sweat sodium concentration versus sweat osmolality immediately following both High Intensity and Steady State running.

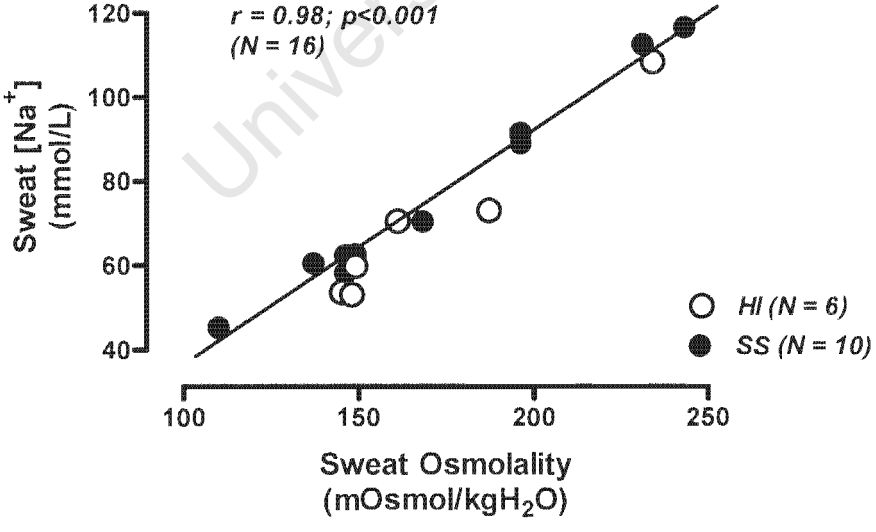


Figure 3G.4a: Sweat sodium concentrations versus $[AVP]_P$ immediately following both High Intensity and Steady State running.

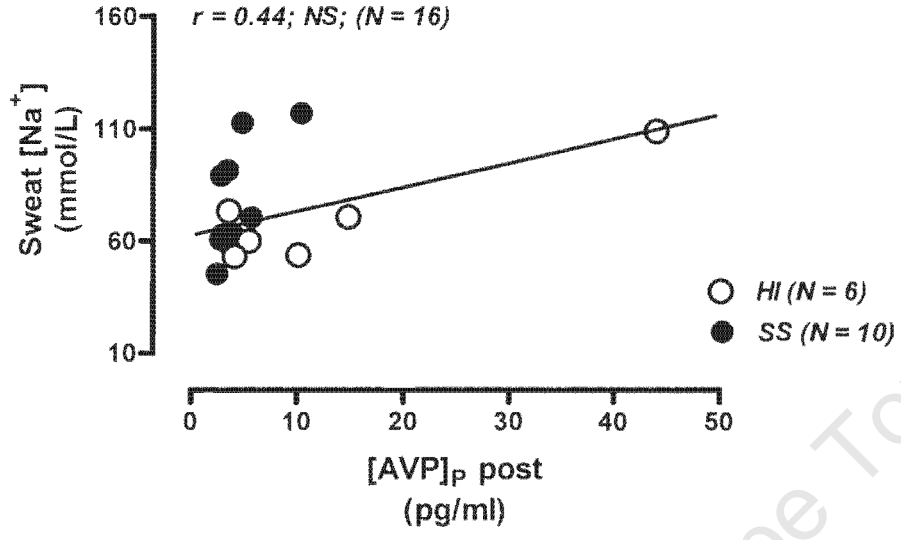


Figure 3G.4b: Urine sodium concentrations versus $[AVP]_P$ immediately following both High Intensity and Steady State running.

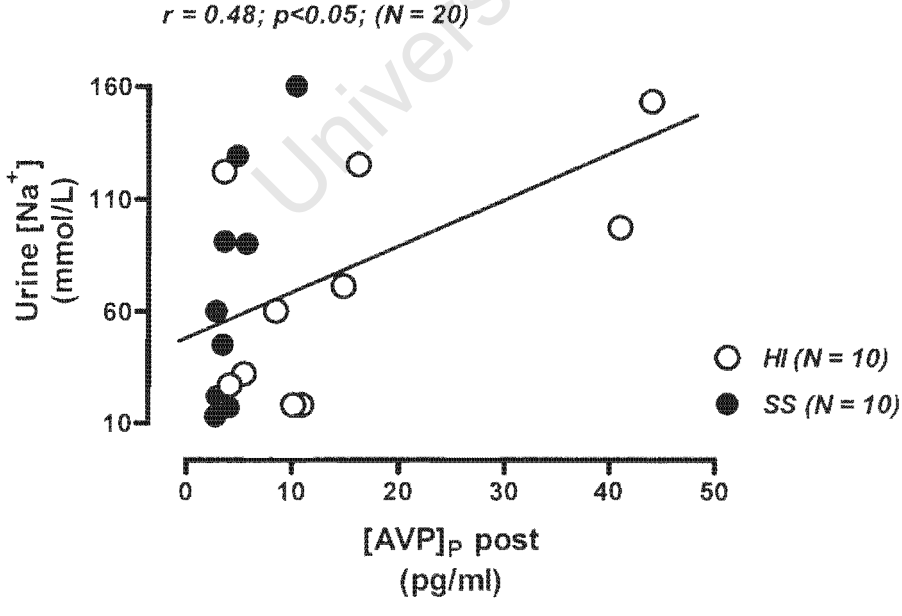


Figure 3G.5a: Sweat osmolality versus $[AVP]_P$ immediately following both High Intensity and Steady State running.

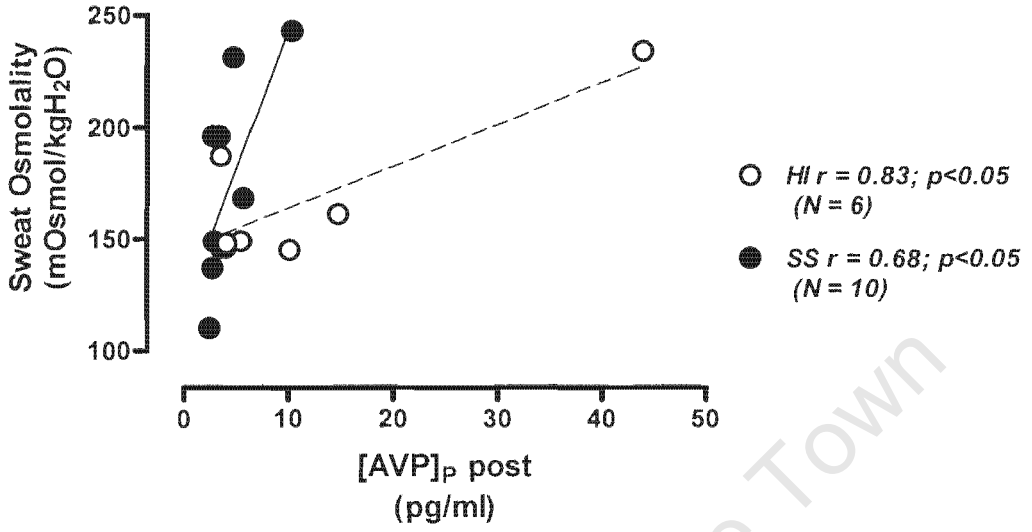


Figure 3G.5b: Urine osmolality versus $[AVP]_P$ immediately following both High Intensity and Steady State running.

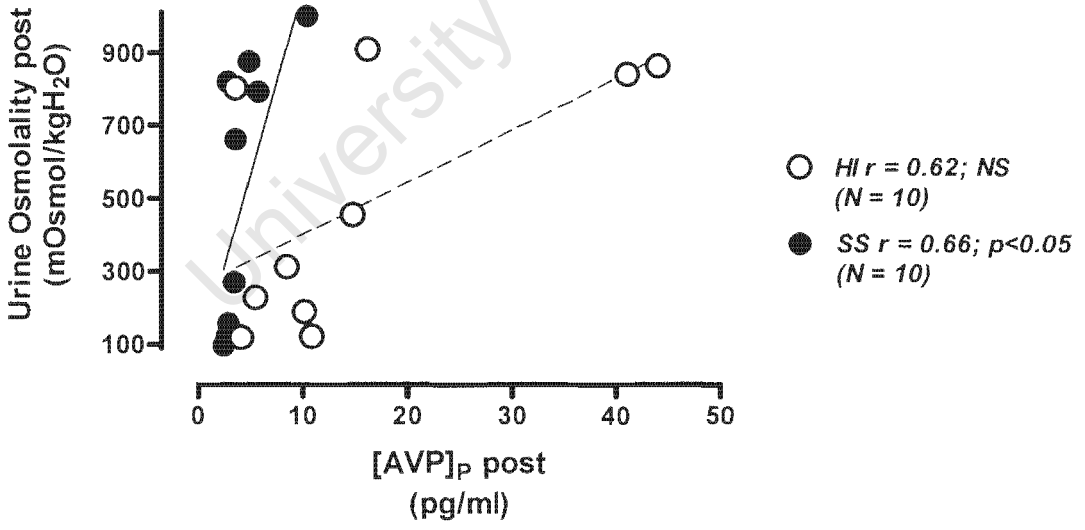


Figure 3G.6a: Both urine and sweat sodium concentration versus [AVP] $_P$ immediately following Steady State running only.

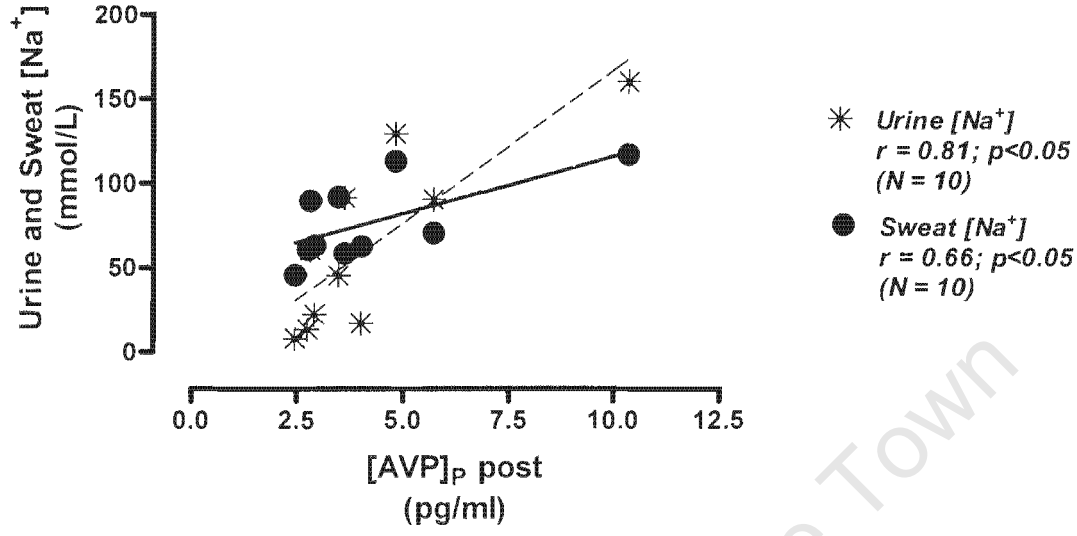


Figure 3G.6b: Both urine and sweat osmolality versus [AVP] $_P$ immediately following Steady State running only.

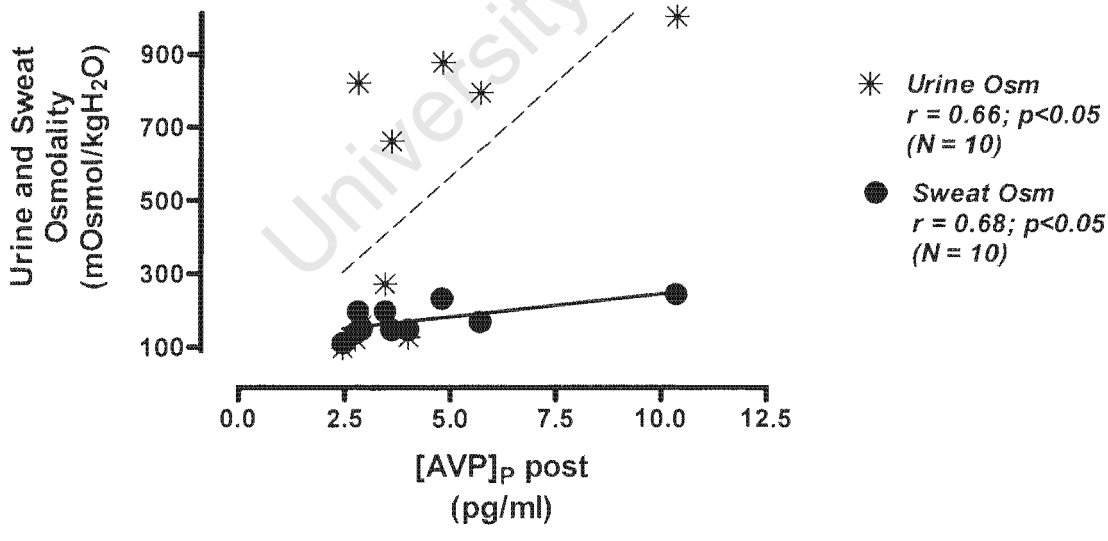


Figure 3G.7a: Serum sodium concentration versus the change in aldosterone concentration (post- minus pre-exercise) immediately following the High Intensity run.

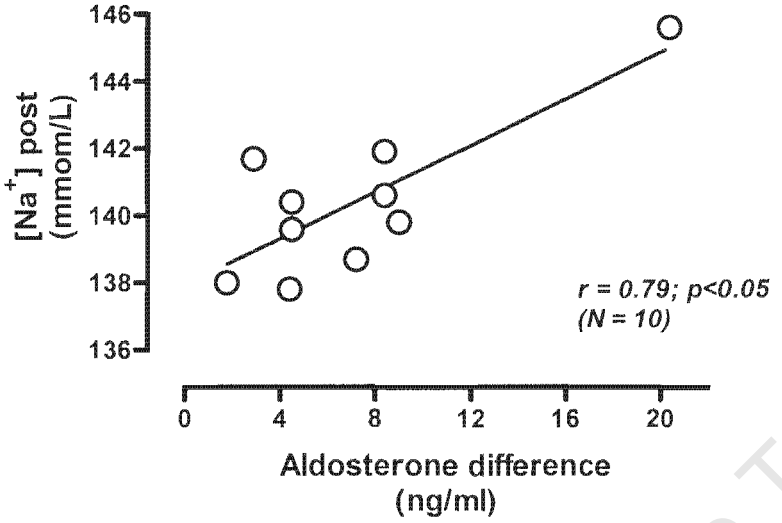
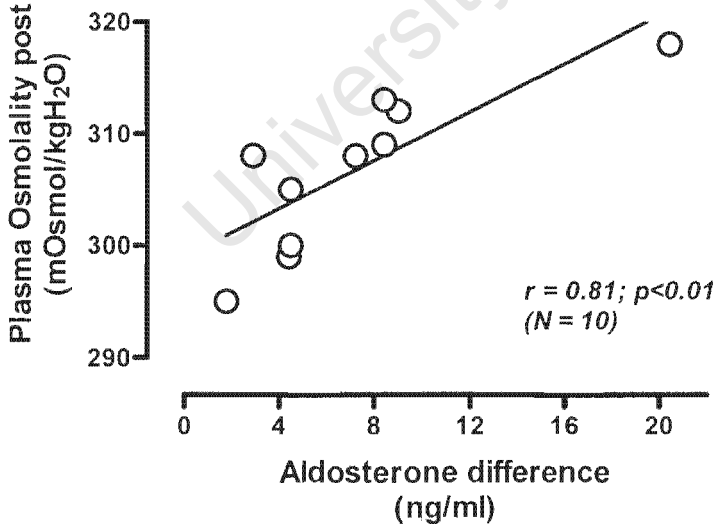


Figure 3G.7b: Plasma osmolality versus the change in aldosterone concentration (post-minus pre-exercise) immediately following the High Intensity run.



DISCUSSION

This is the first study to document significant linear relationships between both sweat and urine [Na^+] versus serum [Na^+] during exercise in humans. The urine and sweat sodium concentrations represent aggregate samples that were collected immediately following HI and SS running. Therefore, whether or not the observed changes in urine and sweat [Na^+] are primarily due to an increase in sodium excretion, a decrease in sweat water excretion, or both, cannot be ascertained from these data.

AVP is the hormone that appears best situated to mediate the changes in sweat and urine [Na^+] (Figure 3G.4b and 3G.6a) and osmolality (Figure 3G.5a, 3G.5b and 3G.6b). The capacity for water reabsorption in the kidneys via AVP activation of V_2 receptors, appears to be greater than that present in the sweat glands (Figure 3G.6a and 3G.6b), which are not known to possess AVP V_2 receptors. The tight correlations between urine osmolality versus urine [Na^+] (Figure 3G.3a) and between sweat osmolality versus sweat [Na^+] (Figure 3G.3b) strongly suggest that the changes in [Na^+] are mainly due to a concentrating mechanism of water reabsorption rather than from a hormonally-mediated alteration in sodium excretion, since no other endocrine variable correlated significantly with urine or sweat [Na^+]. Thus, AVP stimulation appears to promote water conservation in both urine and sweat production to actively defend fluid homeostasis during exercise.

The positive association between serum and urine [Na^+] is largely expected, as osmotically-induced AVP secretion generally induces a linear increase in urine [Na^+] and osmolality as a result of water reabsorption through aquaporin 2 (AQP2) water channels [17]. The positive relationships between sweat [Na^+] versus both urine [Na^+] (Figure 1a) and serum [Na^+] (Figure 3G.2a) are largely unexpected, however. A similar increase in sweat [Na^+] in response to dehydration-induced hypernatremia has been previously documented in one study involving eight cyclists riding for two hours in the heat [18]. Since these collections also represent aggregated samples, the underlying mechanism(s) for the observed changes in sweat [Na^+] remain uncertain.

Sweat glands possess all of the essential elements required for acute fluid and sodium regulation including aquaporins (AQP5) [19], Na^+/H^+ exchangers [20], adrenergic nerve terminals, β-adrenergic receptors, and cAMP regulatory components that activate or inactivate CFTR-Cl channels within the sweat duct [21]. Sweat sodium concentration is neither constant over time nor identical over different regions of the body [22], with significant inter- and intra-subject variability [8] supporting a plausible role for sweat glands in fluid and electrolyte balance. Although the activation [8;13;23], anatomy [8;20] and function [8;24] of the sweat glands are morphologically and functionally distinct from those of the renal tubules, similar responses to perturbations in both salt intake [24-26] and plasma chloride concentration (as a surrogate for NaCl) [27] suggest that they may perform similar and complementary functions in the acute regulation of serum $[Na^+]$ and plasma osmolality.

AVP may affect sweat rate and composition via two possible mechanisms: 1) vasoconstriction of cutaneous blood flow (via AVP V_{1A} receptors) or 2) water reabsorption (via AVP V_2 receptors). Injections of either Pitressin™ or Octapressin™ provide evidence against vasoconstriction (V_{1A}) as the principle mechanism affecting sweat output. Fifty mU of Octapressin™ contains 2.5-fold the amount of pressor activity as 20 mU of Pitressin™; yet sweat rates remain indistinguishable when the two analogues are compared [28]. Furthermore, local injections of bradykinin have similar effects on sweating rate as local injections of AVP, even though bradykinin stimulates vasodilation, not vasoconstriction, of skin blood flow [29]. Dissociations between $[AVP]_P$ and skin blood flow [30] plus documentation that sweat production continues after arterial occlusion [31] also suggest that changes in cutaneous blood flow contribute minimally to changes in sweat rate or composition.

Plasma AVP concentrations are not positively correlated with either sweat $[Na^+]$ or osmolality when data from both the HI and SS trials are combined (Figure 3G.4a). When the data are separated, however, significant correlations are evident between $[AVP]_P$ versus both sweat $[Na^+]$ and sweat osmolality after SS running (Figure 3G.6a and 3G.6b) and between $[AVP]_P$ versus sweat osmolality after HI running (Figure 3G.5a). This disparity may be due to both the duration and the intensity of the running

trials, which differentially affects the gain of each linear relationship (Figure 3G.5a). Since the number of activated sweat glands increase rapidly during the first eight minutes following the onset of sweating [32], sweat gland output would be most limited following HI running (10 minutes), as evidenced by our inability to obtain sweat samples in 40% of subjects following the VO_2 max test. Furthermore, because exercise delays the onset thermoregulatory sweating due to an increase in plasma osmolality [33], it is also expected that the onset of sweating would be most delayed following HI running because changes in plasma osmolality are greatest following maximal intensity exercise [34] (Table 3G.5). Thus, the large variability in post-exercise $[AVP]_P$ following HI running further supports the possibility that a relation between sweat $[Na^+]$ and $[AVP]_P$ lacked sufficient time to materialize.

Previous studies have provided conflicting results regarding the influence of exogenous AVP administration on sweat rate and sweat $[Na^+]$. Investigations that have supported a positive relationship between AVP administration and sweat $[Na^+]$ primarily have utilized *local* injections of Pitressin™ [28;29]. In contrast, investigations that document either an increase [11;35;36] or no change [37;38] in sweat rate and composition have utilized subcutaneous or intramuscular injections of Pitressin™.

Increases in sweat rate are noted in temperatures exceeding 29°C [35;36]. The rationale for the increase in sweat rate following Pitressin™ administration evolves from the concomitant finding that skin blanching occurs 1-3 minutes following injection [11]. Skin blanching and the sensation of "warmth" [37] suggests that Pitressin™ induces a powerful cutaneous vasoconstriction that overrides the vasodilator influences activated by exercise in the heat [11;39]. Diminished skin blood flow causes skin temperatures to fall and body temperatures to rise. This combination would stimulate a transient increase in sweat rate that is not seen in thermoneutral conditions [36]. A similar increase in sweating rate is also documented when Pitressin™ is introduced locally into the cerebral ventricles [35], thereby supporting a central control mechanism.

Studies that have not documented changes in sweat rate or composition following Pitressin™ administration show clear methodological differences pertaining to the:

timing of collection (>60 minutes) [31], inadequate dosing (no change in urinary indices) [38], or hyperhydration in extreme heat (maximal sweating rates achieved) [37]. Furthermore, the only study to evaluate the relation between endogenous $[AVP]_P$ and sweat $[Na^+]$ failed to collect sweat and blood samples on the same testing day [40].

From these collective data, it can be concluded that exogenous Pitressin™ administration would likely cause a decrease in sweat rate combined with an increase in sweat $[Na^+]$ when local concentrations of AVP are above a certain threshold [29]. Conversely, systemic administration of 5-10 units of Pitressin™ may cause a transient (< 60 minutes) increase in sweat rate in hot (>29°C) environments [11;35;36]. Therefore, it is tempting to speculate that steady-state running (in a thermoneutral environment) may stimulate a constant and sufficient "local" accumulation of AVP that in turn effectively alters sweat $[Na^+]$. These findings also suggest the possibility of a more intimate relation between AVP and the thermoregulatory system, in which suppressed AVP levels would facilitate both maximum sweat rates and maximal thermoregulatory responses.

Only during the high intensity run were the changes in plasma aldosterone concentrations significantly correlated with post-run serum $[Na^+]$ (Figure 3G.7a) and plasma osmolality (Figure 3G.7b). These positive linear relationships might appear paradoxical, since aldosterone facilitates sodium conservation rather than sodium excretion. However, in this case the primary stimulus to aldosterone secretion would be activation of the renin-angiotensin system by induced intravascular volume depletion, as indicated by the significant increases in plasma protein concentration (Tables 3G.2A and 3G.2B), rather than by hypernatremia or hyperosmolality. The degree of plasma volume contraction was marginally higher following HI compared with SS running (9.5 vs. 8.2% respectively) despite the contradictory differences in bodyweight loss (-0.3 vs. 1.4% respectively; $p<0.0001$). The apparent contradiction between plasma volume and bodyweight changes can be best explained by the higher hydrostatic forces associated with the higher workloads, however [7]. Thus, despite the absence of a significant correlation between plasma volume and aldosterone, plasma volume contraction may have been the primary stimulus for aldosterone secretion during high intensity exercise;

with the linear increase in serum [Na^+] resulting from the fast "nongenomic" actions of aldosterone which can influence sodium channels within two minutes [41].

The physiological role of OT in fluid balance in humans is widely debated. Although some studies show that OT produces natriuresis in man [42] other studies have failed to demonstrate any association between plasma OT concentrations and natriuresis [43]. Electrophysiological studies performed on human immortalized sweat gland lines confirm that OT may regulate the sodium content of sweat [44]. However, even though the plasma OT concentrations after both high intensity (Table 3G.3) and steady state (Table 3G.4) running are significantly elevated, OT concentrations did not correlate with either urine or sweat [Na^+] in these subjects. This lack of an association may be misleading, however, since OT has been shown to play a more central role in fluid balance in rats [45] than plasma levels would indicate.

High intensity exercise provokes a significantly greater increase in plasma osmolality than steady-state running (Table 3G.5), due to the increased hydrostatic forces at higher workloads [7]. The greater increase in plasma osmolality accounts for the greater stimulation of AVP [7] and OT secretion (Table 3G.5). The increase in cortisol and DHEA concentration after high intensity exercise most likely reflects greater stimulation of ACTH from maximal physical effort [46]. The increase in these two steroid hormones, however, was not significantly associated with any changes in measured fluid balance parameters.

Although NT-proBNP concentrations were higher in females following the high intensity run and testosterone concentrations were higher in males before and after both exercise trials, the influence of gender on fluid regulatory variables appears insignificant, as changes in neither of these variables correlated with changes in any of the fluid regulatory parameters.

Limitations: We acknowledge that our method of sweat sodium collection may not be reflective of whole body sweat [Na^+]. However, our procedure was standardized between individuals and across trials. The mean sweat [Na^+] of our subjects was 69.9 mmol/L and 76.9 mmol/L following the high intensity and steady state runs, respectively.

These concentrations are comparable to the mean values obtained in exercising subjects reported by Ikai (75.8 mmol/L), whose procedure we replicated [13].

CONCLUSION

Changes in serum [Na^+] and osmolality during exercise are strongly correlated with corresponding changes in sweat and urine [Na^+]. Associations of both of these variables with $[AVP]_P$ suggest that they reflect attempts by the body to preserve fluid homeostasis via AVP-mediated decreases in water losses, both in the urine and in the sweat. Therefore, the maintenance of fluid and thermoregulatory balance appear to be intimately linked, and are most likely regulated coordinately by changes in pituitary AVP secretion.

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Reference List

1. Sawka MN, Gonzalez RR, Young AJ, Dennis RC, Valeri CR, Pandolf KB. Control of thermoregulatory sweating during exercise in the heat. *Am.J.Physiol* 1989;**257**:R311-R316.

2. Horowitz M, Kaspler P, Simon E, Gerstberger R. Heat acclimation and hypohydration: involvement of central angiotensin II receptors in thermoregulation. *Am.J.Physiol* 1999;**277**:R47-R55.

3. Takamata A, Nagashima K, Nose H, Morimoto T. Osmoregulatory inhibition of thermally induced cutaneous vasodilation in passively heated humans. *Am.J.Physiol* 1997;**273**:R197-R204.

4. Kamijo Y, Okumoto T, Takeno Y, Okazaki K, Inaki M, Masuki S *et al.* Transient cutaneous vasodilatation and hypotension after drinking in dehydrated and exercising men. *J.Physiol* 2005;**568**:689-98.

5. Ichinose T, Okazaki K, Masuki S, Mitono H, Chen M, Endoh H *et al.* Ten-day endurance training attenuates the hyperosmotic suppression of cutaneous vasodilation during exercise but not sweating. *J.Appl.Physiol* 2005;**99**:237-43.

6. Silva NL,.Boulant JA. Effects of osmotic pressure, glucose, and temperature on neurons in preoptic tissue slices. *Am.J.Physiol* 1984;**247**:R335-R345.

7. Convertino VA, Keil LC, Greenleaf JE. Plasma volume, renin, and vasopressin responses to graded exercise after training. *J.Appl.Physiol* 1983;**54**:508-14.

8. Gibinski K. Some controversial problems in sweat gland function. *Environ.Res.* 1971;**4**:365-89.

9. Pivarnik JM, Leeds EM, Wilkerson JE. Effects of endurance exercise on metabolic water production and plasma volume. *J.Appl.Physiol* 1984;**56**:613-8.

10. Nose H, Mack GW, Shi XR, Nadel ER. Shift in body fluid compartments after dehydration in humans. *J Appl.Physiol* 1988;**65**:318-24.

11. Pearcy M, Robinson S, Miller DI, Thomas JT, Jr., Debrota J. Effects of dehydration, salt depletion and pitressin on sweat rate and urine flow. *J.Appl.Physiol* 1956;**8**:621-6.

12. Scrimgeour AG, Noakes TD, Adams B, Myburgh K. The influence of weekly training distance on fractional utilization of maximum aerobic capacity in marathon and ultramarathon runners. *Eur.J.Appl.Physiol Occup.Physiol* 1986;**55**:202-9.

13. Ikai K, Sato K, Sugiyama K, Otsuka Y, Nitta H. Comparison of human sweat electrolyte concentration in mental, thermal and exercise perspiration. *Nagoya Med.J.* 1969;**15**:47-66.

14. Verbalis JG, McHale CM, Gardiner TW, Stricker EM. Oxytocin and vasopressin secretion in Response to Stimuli Producing Learned Taste Aversion in Rats. *Behav.Neurosci.* 1986;**100**:466-75.

15. Guo T, Taylor RL, Singh RJ, Soldin SJ. Simultaneous determination of 12 steroids by isotope dilution liquid chromatography-photospray ionization tandem mass spectrometry. *Clin.Chim.Acta* 2006;**372**:76-82.

16. Soldin SJ, Soldin OP, Boyajian AJ, Taskier MS. Pediatric brain natriuretic peptide and N-terminal pro-brain natriuretic peptide reference intervals. *Clin.Chim.Acta* 2006;**366**:304-8.

17. Verbalis JG. Disorders of body water homeostasis. *Best.Pract.Res.Clin.Endocrinol.Metab* 2003;**17**:471-503.

18. Morgan RM, Patterson MJ, Nimmo MA. Acute effects of dehydration on sweat composition in men during prolonged exercise in the heat. *Acta Physiol Scand.* 2004;**182**:37-43.

19. Nejsum LN, Kwon TH, Jensen UB, Fumagalli O, Frokiaer J, Krane CM *et al.* Functional requirement of aquaporin-5 in plasma membranes of sweat glands. *Proc.Natl.Acad.Sci.U.S.A* 2002;**99**:511-6.

20. Granger D, Marsolais M, Burry J, Laprade R. Na+/H+ exchangers in the human eccrine sweat duct. *Am.J.Physiol Cell Physiol* 2003;**285**:C1047-C1058.

21. Reddy MM,.Quinton PM. Rapid regulation of electrolyte absorption in sweat duct. *J.Membr.Biol.* 1994;**140**:57-67.

22. Kondo N, Takano S, Aoki K, Shibasaki M, Tominaga H, Inoue Y. Regional differences in the effect of exercise intensity on thermoregulatory sweating and cutaneous vasodilation. *Acta Physiol Scand.* 1998;**164**:71-8.

23. Shibasaki M, Kondo N, Crandall CG. Non-thermoregulatory modulation of sweating in humans. *Exerc.Sport Sci.Rev.* 2003;**31**:34-9.

24. Sigal CB,.Dobson RL. The effect of salt intake on sweat gland function. *J.Invest Dermatol.* 1968;**50**:451-5.

25. Robinson S, Nicholas JR, Smith JH, Daly WJ, Pearcy M. Time relation of renal and sweat gland adjustments to salt deficiency in men. *J.Appl.Physiol* 1955;**8**:159-65.

26. Allsopp AJ, Sutherland R, Wood P, Wootton SA. The effect of sodium balance on sweat sodium secretion and plasma aldosterone concentration. *Eur.J.Appl.Physiol Occup.Physiol* 1998;**78**:516-21.

27. Robinson S, Maletich RT, Robinson WS, Rohrer BB, Kunz AL. Output of NaCl by sweat glands and kidneys in relation to dehydration and to salt depletion. *J.Appl.Physiol* 1956;**8**:615-20.

28. Quatrale RP,.Speir EH. The effect of ADH on eccrine sweating in the rat. *J.Invest Dermatol.* 1970;**55**:344-9.

29. Fasciolo JC, Totel GL, Johnson RE. Antidiuretic hormone and human eccrine sweating. *J.Appl.Physiol* 1969;**27**:303-7.

30. Takamata A, Mack GW, Gillen CM, Jozsi AC, Nadel ER. Osmoregulatory modulation of thermal sweating in humans: reflex effects of drinking. *Am.J.Physiol* 1995;**268**:R414-R422.

31. Ratner AC,.Dobson RL. The effect of antidiuretic hormone on sweating. *J.Invest Dermatol.* 1964;**43**:379-81.

32. Kondo N, Shibasaki M, Aoki K, Koga S, Inoue Y, Crandall CG. Function of human eccrine sweat glands during dynamic exercise and passive heat stress. *J.Appl.Physiol* 2001;**90**:1877-81.

33. Takamata A, Nagashima K, Nose H, Morimoto T. Role of plasma osmolality in the delayed onset of thermal cutaneous vasodilation during exercise in humans. *Am.J.Physiol* 1998;**275**:R286-R290.

34. Wilkerson JE, Horvath SM, Gutin B, Molnar S, Diaz FJ. Plasma electrolyte content and concentration during treadmill exercise in humans. *J.Appl.Physiol* 1982;**53**:1529-39.

35. Ladell WSS. The effect of pituitrin upon performance in moderate heat. *S.Afr.J.Med Sci* 1948;**13**:145-50.

36. Allen JA,.Roddie IC. The effect of antidiuretic hormone on the rate of sweat production in man. *J Physiol* 1971;**212**:37P-8P.

37. Senay LC, Jr.,.Van Beaumont W. Antidiuretic hormone and evaporative weight loss during heat stress. *Pflugers Arch.* 1969;**312**:82-90.

38. Gibinski K, Kozlowski S, Chwalbinska-Moneta J, Giec L, Zmudzinski J, Markiewicz A. ADH and thermal sweating. *Eur.J.Appl.Physiol Occup.Physiol* 1979;**42**:1-13.

39. Kellogg DL, Jr., Johnson JM, Kosiba WA. Control of internal temperature threshold for active cutaneous vasodilation by dynamic exercise. *J.Appl.Physiol* 1991;**71**:2476-82.

40. Takamata A, Yoshida T, Nishida N, Morimoto T. Relationship of osmotic inhibition in thermoregulatory responses and sweat sodium concentration in humans. *Am.J.Physiol Regul.Integr.Comp Physiol* 2001;**280**:R623-R629.

41. Boldyreff B,.Wehling M. Aldosterone: refreshing a slow hormone by swift action. *News Physiol Sci.* 2004;**19**:97-100.

42. Kostoglou-Athanassiou I, Treacher DF, Forsling ML. Is Oxytocin Natriuretic in Man? *J.Endocrinol* 1994;**143(suppl.O)**:39.

43. Rasmussen MS, Simonsen JA, Sandgaard NC, Hoilund-Carlsen PF, Bie P. Effects of oxytocin in normal man during low and high sodium diets. *Acta Physiol Scand.* 2004;**181**:247-57.

44. Ring A,.Mork AC. Electrophysiological responses to oxytocin and ATP in monolayers of a human sweat gland cell line. *Biochem.Biophys.Res.Commun.* 1997;**234**:30-4.

45. Stricker EM,.Verbalis JG. Central inhibitory control of sodium appetite in rats: correlation with pituitary oxytocin secretion. *Behav.Neurosci.* 1987;**101**:560-7.

46. de Vries WR, Bernards NT, de Rooij MH, Koppeschaar HP. Dynamic exercise discloses different time-related responses in stress hormones. *Psychosom.Med.* 2000;**62**:866-72.

CHAPTER 4

PROPOSED FLUID REPLACEMENT STRATEGIES

Study 4H: The maintenance of plasma volume and serum sodium concentration despite bodyweight loss in Ironman triathletes

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INTRODUCTION

The maintenance of bodyweight during physical activity is promoted in order to maintain circulatory volume, minimise cardiovascular strain and reduce thermoregulatory compromise [1-3]. The recommendation that individual athletes calculate and fully replace bodyweight losses is promoted to protect individuals from medical complications resulting from both under and over hydration [4-6]. Dill (1933) first hypothesized that man does not defend body mass during exercise, but rather protects plasma osmolality [7]. Since some bodyweight loss can occur without any loss of total body water, the maintenance of body mass may not be necessary to maintain fluid homeostasis during prolonged endurance exercise [8].

The body defends plasma osmolality to preserve cell size and optimise cellular function [9-11]. This internal homeostatic regulation occurs mainly through the secretion of the antidiuretic hormone, arginine vasopressin (AVP), and subsequently through the stimulation of thirst when osmolality rises 5 – 10 mOsmol/kg H_2O above the threshold for AVP secretion (280 – 285 mOsmol/kg H_2O) [12]. Conversely, when plasma osmolality falls below 280 mOsmol/kg H_2O, AVP is maximally suppressed thereby promoting renal free water excretion and maintaining tonicity within the normal physiological range (280 – 295 mOsmol/ kg H_2O) [10]. The tonicity of the body fluids is driven by osmotic gradients from solutes that are impermeable to the cell membrane. Water moves freely between the extracellular and intracellular fluid compartments from areas of lower solute concentration to areas of higher solute concentration until osmotic equilibrium is achieved. Since sodium is the main extracellular cation and not readily diffusible across

the cell membrane, serum sodium concentration largely dictates plasma osmolality – and therefore cell size and function - during prolonged endurance activity.

The circulating plasma volume consists of all the fluid located within the vascular space, except that contained within the red blood cells. Although the maintenance of plasma volume is considered important [2], this volume constitutes less than 10% of the total body water [8;10]. Therefore, the capacity for fluid shifts from other body fluid compartments to maintain circulating plasma volume is great, despite significant (> 2%) bodyweight loss [13-19].

This study examines the relationship between bodyweight, plasma volume and serum [Na^+] in triathletes completing a 226 km Ironman Triathlon. We predict that a one-to-one linear relationship will not exist between bodyweight, plasma volume and serum [Na^+] loss.

METHODS

Subjects

All entrants of the 2000 South African Ironman Triathlon, (3.8 km swim, 180 km cycle and a 42.2 km run) were invited to participate in this study [20]. Approval for this study was obtained from the Research and Ethics Committee of the Faculty of Health Sciences, University of Cape Town. During race registration, 181 male triathletes agreed to participate in the research project and gave written informed consent. Fluid and food intake were allowed ad libitum during the race.

Measurement of body weights

The triathletes were weighed at registration (registration weight), prior to the start of the race (start weight) and immediately after completion of the race (finish weight) as previously described [20]. All bodyweight measurements were adjusted to the "standardised" body mass of that obtained only in swimming attire without shoes. The registration weight was measured in standardised clothing (250 g) and the net body

weight was corrected accordingly. For measurement of start weight, subjects were weighed in swimming attire within 2 hours before the commencement of the race. Immediately on cornpletion of the race, each athlete removed his or her shoes, was weighed in running clothes (approximately 200 g) from which a corrected finish weight was calculated and recorded. If athletes were too exhausted to remove his or her shoes, their finish weight was corrected for the additional weight of their shoes. A sample of 20 pairs of running shoes were weighed and the average weight of a pair of running shoes was found to be 750 g [20].

Hematological analysis

Blood was sampled at race registration and within 10 minutes of finishing the race for the analysis of pre- and post-race haematocrit (Hct) and blood haemoglobin (Hb) concentrations. A 4.5 ml venous blood sample was drawn by venipuncture with the subjects in either a supine or sitting position into lithium heparin vacutainer tubes. Haematocrit was immediately measured in triplicate using heparinised microcapillary tubes and corrected for trapped plasma [21]. The microcapillary tubes were centrifuged in a Hawksley micro-haematocrit centrifuge for 5 minutes and read with a Hawksley micro-haematocrit reader. Haemoglobin concentration was measured in duplicate using a standard cyanmethaemoglobin procedure[22]. Serum sodium analysis was performed using an EasyLyte PLUS Na/K/Cl analyser (Medica Corp., Bedford MA) after onsite centrifugation of the remaining blood sample at 3000 x g for ten minutes at 4°.

Calculations

Percent of body weight lost or gained during the race (% Δ Weight (Start)) was calculated as the difference between the start and finish weights divided by the start weight and expressed as a percentage. Percent of body weight lost or gained from registration until the end of the triathlon (% Δ Weight (Registration)) was calculated as the difference between the registration and finish weights divided by the registration weight and expressed as a percentage. Both pre-race and registration weights were used as the athletes' weights changed during the three days prior to the race if they

were carbohydrate loading. To correct for the fuel utilisation during the race, 1.1 kg (see estimations from Table 4H.2 in the results section) was added to the difference between the start and finish weights and expressed as a percentage (% Δ Weight (Fuel)).

Changes in blood (BV), cell (CV) and plasma (PV) volumes were determined from the pre- and post-race Hb and Hct values using the equations of Dill and Costill [21]. The equations are:

(1) $BV_{post} = BV_{pre} \times (Hb_{pre}/Hb_{post})$, where $BV_{pre} = 100$ ml
(2) $CV_{post} = BV_{post} \times Hct_{post}$ and $CV_{pre} = BV_{pre} \times Hct_{pre}$
(3) $PV_{post} = BV_{post} - CV_{post}$ and $PV_{pre} = BV_{pre} - CV_{pre}$
(4) $\Delta BV\ (\%) = 100 \times (BV_{post} - BV_{pre}) / BV_{pre}$
(5) $\Delta CV\ (\%) = 100 \times (CV_{post} - CV_{pre}) / CV_{pre}$
(6) $\Delta PV\ (\%) = 100 \times (PV_{post} - PV_{pre}) / PV_{pre}$

Statistical analysis

Data were analyzed using the STATISTICA 7.0™ (Stat Soft Inc., Tulsa, OK) statistical programme. Where applicable, data were presented as the mean ± standard deviation, together with the range of values. Statistical significance was accepted when $p < 0.05$.

RESULTS

All 181 male triathletes who consented to participate in this study completed the 2000 South African Ironman triathlon. The physiological characteristics and overall race and split times are summarised in Table 4H.1.

The average temperature during the race was 20.5° C (ranging from 17.0 at the start of the race to 23.9° C), while the average humidity was 68%, (ranging from 46 to 87%). The sea water temperature was 16° C while the average wind speed was 4.6 $m \cdot sec^{-1}$.

Fuel substrate utilisation was estimated from the average age, bodyweight, and finishing times of the athletes for each component (swim, bike and run) of the triathlon (Table 4H.2). The average total endogenous substrate utilised was calculated to be 1.1 kg during the 226 km event.

There was a slight decrease in blood and cell volume from pre to post race, with the 2.6 ± 5.5% decline in red blood cell volume statistically significant (Table 4H.3). Plasma volume and serum [Na^+] were both elevated post race, with the increase in the average serum [Na^+] statistically significant. The average bodyweight of the study group at race start was 0.9 kg higher than the average bodyweight obtained at registration. Bodyweight loss at race completion was significant, with athletes losing an average of 4% of their body mass from race registration to finish and 5% from race start to race finish. None of the athletes in this cohort reported any adverse clinical symptoms after the race.

There was a strong correlation (r = 0.9) between the change in blood volume and plasma volume (Figure 4H.1). However, when viewed as a percentage (Figure 4H.1B) instead of as an absolute change in fluid volume (Figure 4H.1A), the slope of the relationship deviated from the line of identity. Greater changes in plasma volume were necessary to elicit smaller changes in corresponding blood volume when the relationship was viewed as a percent change. The line of identity refers to the hypothetical line of correlation if blood and plasma volume changed equally.

Significant correlations were noted between bodyweight change and both plasma and blood volume change (Figure 4H.2). The slope of the correlation lines deviated furthest from the hypothesised line of identity (dotted line) when bodyweight measurements were taken just prior to race start (Figure 4H.2A and 4H.B). The correlation coefficients became slightly more robust and the slope of these correlation lines moved closer toward the line of identity if the "lower" registration bodyweights were employed (Figure 4H.2C and 4H.D). If these registration bodyweights – which may better represent "true

bodyweight - were further modified to accommodate fuel utilisation during the race (registration bodyweight minus 1.1 kg of total endogenous substrate utilised as calculated in Table 4H.2) than the correlation coefficients stabilised but the slope of the correlation lines angled closer to the line of identity (Figure 4H.2E and 4H.F).

There were no significant correlations between either plasma or blood volume change and post race serum [Na^+] or serum [Na^+] change (Figure 4H.3) despite significant correlations between % bodyweight change versus % plasma volume change (Figure 4H.2), post race serum [Na^+] versus % bodyweight change [20], and between serum [Na^+] change versus % bodyweight change under all bodyweight conditions measured (Figure 4H.4).

There was a significant inverse correlation between the percent change in red blood cell volume versus serum [Na^+] change as well as between % bodyweight change versus serum [Na^+] change (Figure 4H.4). When these two significant relationships were superimposed with respect to serum [Na^+] change, the two inverse linear correlations paralleled one other.

Table 4H.1: Demographics and performance characteristics of Ironman triathletes

	Mean ± Std Dev	Range	n
Age (years)	34.2 ± 8.2	18 – 66	181
Height (cm)	180.6 ± 7.0	161 –195	144
Weight (kg)	77.5 ± 9.4	54 –105	162
BMI (kg/m^2)	23.7 ± 2.2	18.8 – 31.7	144
Overall Race Time (min)	760 ± 95	535 – 969	179
Swim Time (min)	71 ± 11	50 – 104	157
Bike Time (min)	395 ± 41	313 – 508	138
Run Time (min)	290 ± 57	172 – 638	178

Table 4H. 2: Estimation of fuel substrate utilization during an Ironman triathlon

	Swim	**Bike**	**Run**	**Total**
Distance (km)	3.8	180	42.2	
Ave Time (min)[1]	71	395	290	
Ave Speed (km/h)	3.2	27.3	8.7	
Energy Requirements (kcal/kg/min)[2]	0.156	0.169	0.135	
Average Weight (kg)[1]	77.5	77.5	77.5	
Total Energy Requirements (kcal)	858	5174	3034	
Estimated RER	0.90	0.85	0.85	
Total Energy from carbohydrates (kcal)	600	3622	1517	
Total Energy from fats (kcal)	258	1552	1517	
Total carbohydrate utilised (g)	143	862	361	1366
Total fat utilised (g)	28	171	167	366
Endogenous carbohydrate utilised (g)[3]				728
Total Endogenous substrate utilised (g)				**1094**

[1]Values are from Table 1

[2]Nutrition, Weight Control and Exercise. F.I. Katch and W.D. McArdle. Lea and Febiger 1983 (Philadelphia) (Appendix B)

[3]Estimated carbohydrate ingested is approximately 800 ml/hr of a 7% carbohydrate drink during the cycle and the run legs of the race (685 min). Therefore 638 g of exogenous carbohydrate was subtracted from the total estimated carbohydrate utilised during the race.

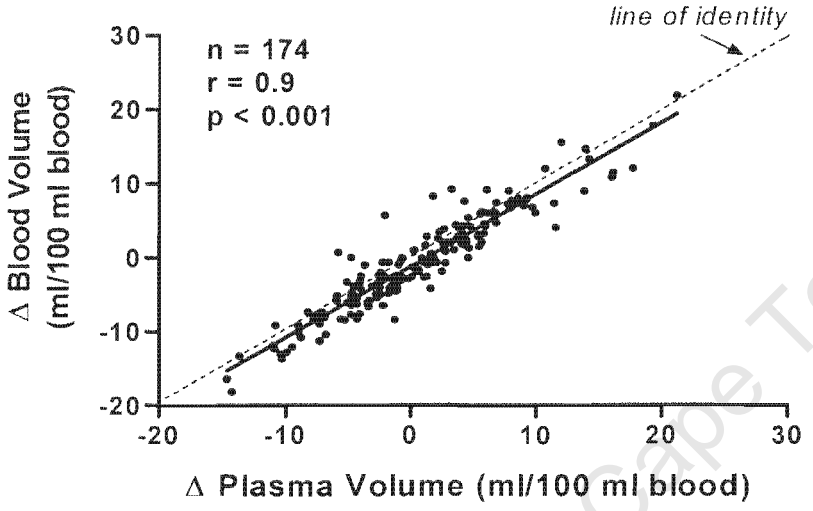
Table 4H.3: Changes in body fluid parameters

Variable	Pre-Race	Post-Race	Change (post - pre race)	%Change (post - pre race)	P-value
Blood Volume (ml)	100	99.4 ± 6.6 (181) (81.9 to 121.9)	-0.6 ± 6.6 (181) (-18.1 to 21.9)	-0.6 ± 6.6 (181) (-18.1 to 21.9)	Ø
Cell Volume (ml)	42.0 ± 2.8 (181) (32.7 to 48.3)	40.9 ± 2.8 (174) (33.4 to 47.3)	-1.2 ± 2.3 (174) (-7.5 to 7.8)	-2.6 ± 5.5 (174) (-17.8 to 20.5)	< 0.001
Plasma volume (ml)	58.0 ± 2.8 (181) (51.7 to 67.3)	58.4 ± 6.4 (174) (43.8 to 79.5)	0.5 ± 6.5 (174) (-14.6 to 21.2)	***1.0 ± 11.2 (174)*** (-24.4 to 37.2)	0.4
Serum [Na^+] (mmol/L)	141.2 ± 1.5 (181) (136.7 to 146.6)	142.1 ± 3.0 (177) (134.7 to 152.0)	0.8 ± 3.4 (177) (-9.2 to 10.2)	***0.6 ± 2.4 (177)*** (0.5 to -6.8)	< 0.001
Bodyweight Registration (kg)	77.6 ± 9.4 (179) (52.5 to 103.2)	74.6 ± 9.0 (181) (53.1 to 101.0)	-3.0 ± 1.6 (179) (-7.8 to -0.6)	***-3.8 ± 1.9 (179)*** (-8.2 to -1.1)	< 0.01
Bodyweight Start (kg)	78.5 ± 9.4 (177) (54.6 to 104.5)	74.6 ± 9.0 (181) (53.1 to 101.0)	-3.9 ± 1.4 (177) (-8.6 to -0.9)	-4.9 ± 1.7 (177) (-9.3 to -1.2)	< 0.0001

*All values reported as mean ± SD (n) followed by the range.

Figure 4H.1: Relationship between blood and plasma volume change from pre to post race

A



B

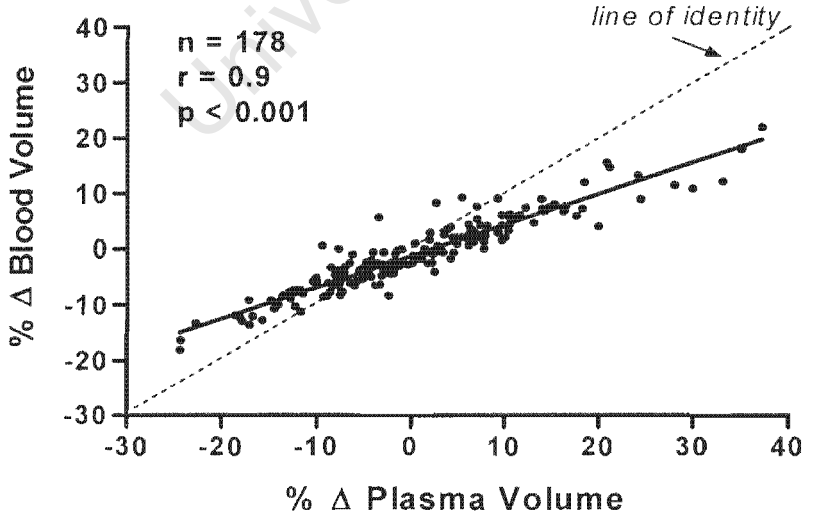


Figure 4H.2: Bodyweight and plasma volume changes at race registration, race start and registration corrected for fuel utilization.

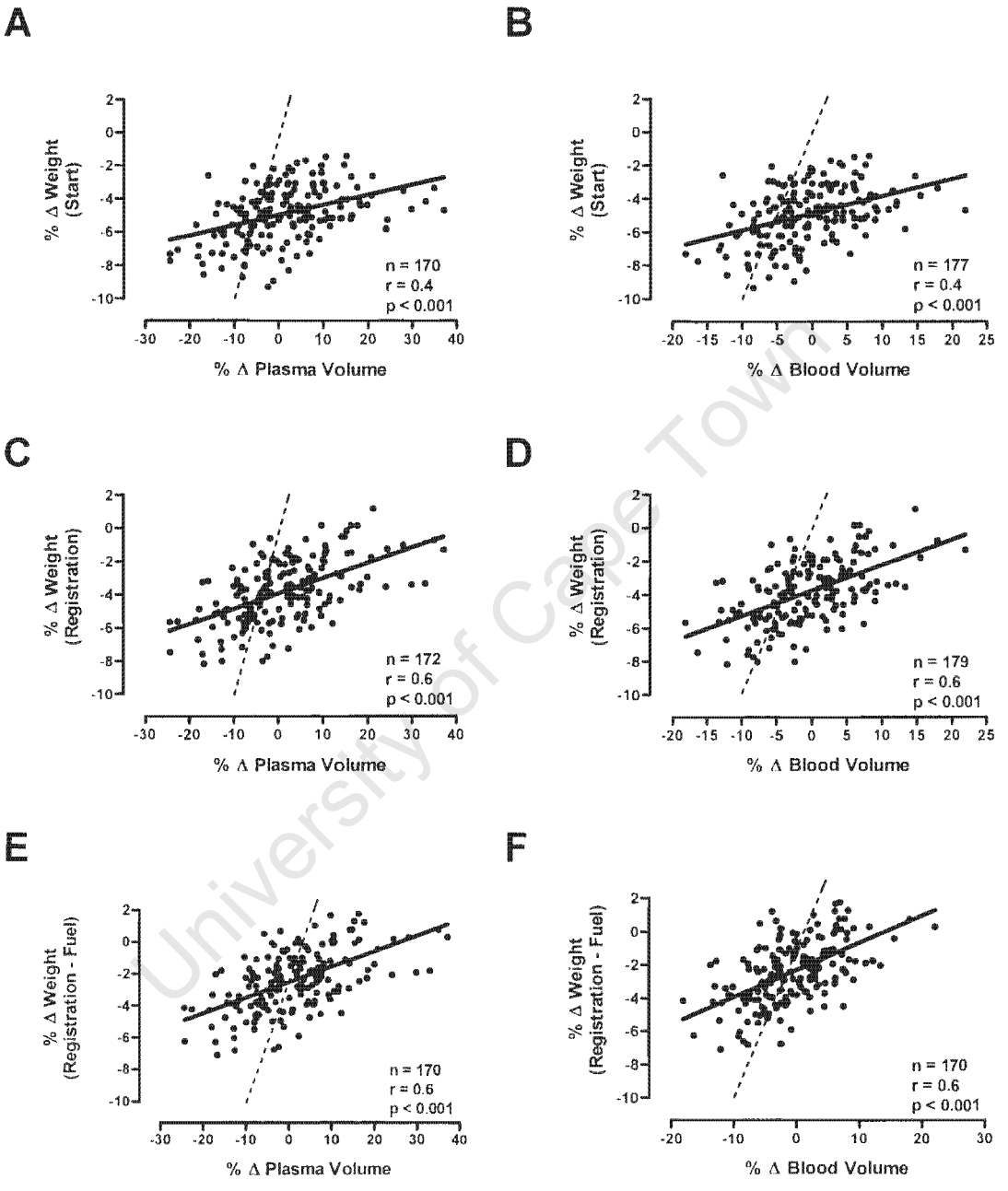


Figure 4H.3: Changes in bodyweight and serum [Na^+] with plasma volume change from pre to post race.

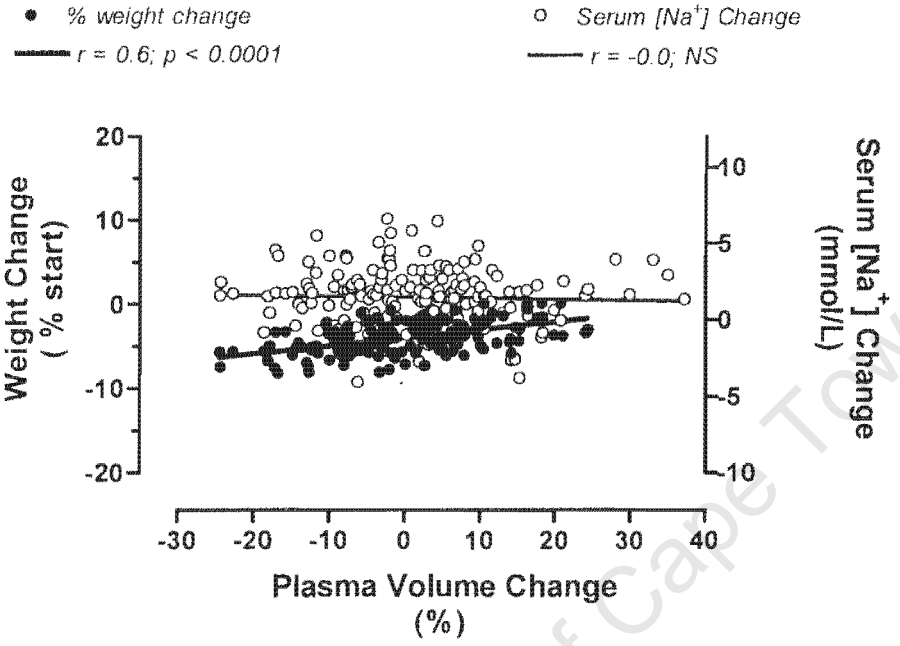
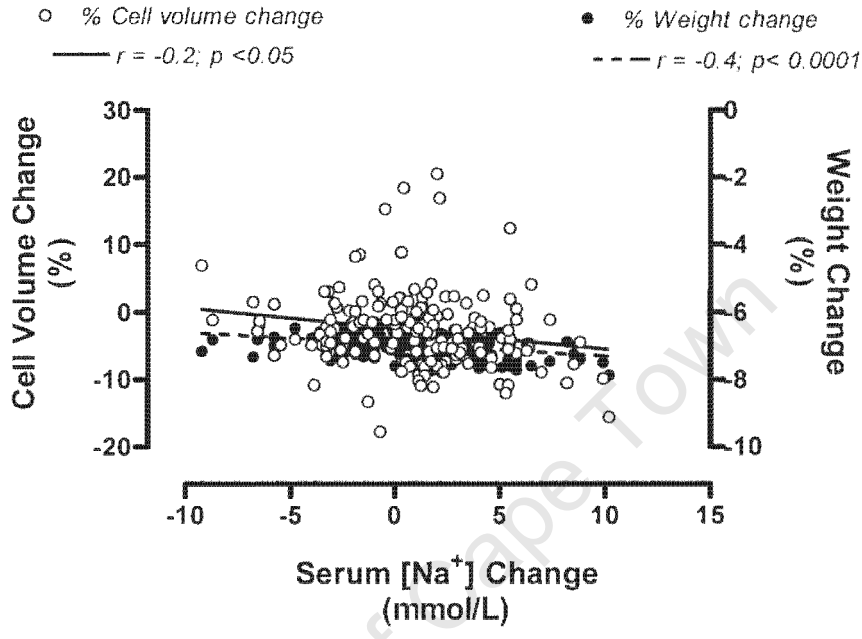


Figure 4H.4: Changes in cell volume and bodyweight with serum [Na^+] change from pre to post race.



DISCUSSION

The first important finding of this study is that bodyweight loss does not approximate the loss of circulating plasma volume in male athletes completing an Ironman triathlon. Although regression correlations confirm a significant linear relationship between these two variables, the 1% average increase in plasma volume associated with a 4% average decrease in bodyweight suggests that bodyweight and plasma volume are neither interchangeable nor regulated on the same absolute scale.

We designate the bodyweight measurement obtained at race registration as most representative of these athletes' "normal" weight. The registration bodyweight also corresponds to the time in which blood sampling for serum [Na^+] and plasma volume determinations were obtained. We hypothesise that the 0.9 kg weight gain from registration to race start represents carbohydrate and fluid loading, combined with reduced training immediately pre-race. This hypothesis is supported by earlier studies which report that runners and triathletes who consume a high carbohydrate diet 2-3 days before the race increase their total body water content to weigh 0.1 -2.4 kg greater on race day [23 24]. Furthermore, during exercise when bodyweight loss is corrected for metabolic substrate utilisation during exercise, the regression line between bodyweight and plasma volume angles closer towards the hypothesised line of identity. This suggests that the endogenous water production of carbohydrate and water provides an internal water source which alters the slope of the relationship between bodyweight and plasma volume [3;25-27].

Athletes who lose ~4.1% bodyweight during two hours of thermal exposure lose 1.4 litres of total body water while a weight loss of the same magnitude produced by two hours of vigorous exercise reduces total body water by only 0.2 litre [28]. These findings provide further evidence to support the conclusion that substrate utilisation may liberate an internal water supply that preserves body water content despite significant loss of bodyweight. Thus, body mass can decrease without a change in total body water [8] and total body water can increase without any measurable change in body mass [29].

Body fluids will shift into and out of the vascular space in response to hydrostatic, oncotic and osmotic gradients during exercise [8;30;31]. Hydrostatic forces at the onset of exercise produce a significant decrease in plasma volume [8]. This initial decrease in blood and plasma volume is greater in the heat then in the cold [8] and more pronounced in women than in men [32]. In male marathon runners, a 6.5% decrease in plasma volume occurs during the first 6 km of the race but remains unchanged thereafter: this suggests that the body reaches a "new equilibrium" during continuous endurance exercise [32]. In events of longer duration, however, fluid that is hydrostatically relocated into inactive muscles [33] may be redistributed back into the vascular space by changing oncotic and osmotic pressures.

The expansion of plasma volume, despite bodyweight loss from zero to 5.5%, is documented in athletes competing in prolonged endurance events beyond the standard marathon distance (>42.2 km) [14-19]. At or below the standard marathon distance, however, plasma volume generally declines in greater proportion to bodyweight loss [32;34-39]. These disparate findings may be explained by a difference in exercise intensity. While elite athletes' can generally complete a standard marathon running at their lactate threshold [39], the majority of athletes who complete marathons and Ironman triathlons compete at exercise intensities below the threshold for lactate accumulation [40]. At exercise intensities at or below 60% of VO_2 maximum, plasma volume contraction is less than 5% [41] suggesting that osmotic and/or oncotic pressures may be able to overcome the hydrostatic forces associated with low to moderate exercise intensities to maintain or even increase plasma volume during exercise. At exercise intensities at or above the threshold for lactate accumulation, however, plasma volume contraction from hydrostatic forces generally exceeds 10% [41] Thus, these results suggest that plasma volume can be maintained despite significant bodyweight loss during prolonged endurance activity if exercise intensities are kept well below the onset of lactate accumulation.

Twenty-four hours after completion of single bout of exercise or heat exposure can induce an isotonic expansion of both plasma [42-44] and interstitial fluid volume [29]. This combined extracellular fluid compartment expansion could serve as a "plasma volume reserve" [45] to maintain circulating blood volume and offset water lost through sweat. Ladell (1955) made early reference to this "free circulating water" and stated that until this ~two litre fluid "reserve" were used up bodily function would be unimpaired [46].

This hypothesised "fluid reserve" is likely contained within the interstitial fluid of the extracellular fluid compartment [8;47]. Water lost through sweat is drawn from the interstitial fluid surrounding the sweat glands and replenished by the filtration of fluid from local capillaries [48]. Thus, we assume that plasma volume is maintained in this cohort of Ironman triathletes from fluid shifts which occur dynamically during exercise. Metabolic water production, substrate utilisation and depletion of this "plasma volume reserve" may account for the disparate relationship between plasma volume and bodyweight loss in this study group. Only after the endogenous water supply is exhausted might bodyweight change better reflect absolute changes in plasma volume at exercise intensities below the threshold for lactate accumulation.

The second important finding of this study is that serum [Na^+] is only slightly elevated despite 3.8% bodyweight loss. This finding suggests that the body regulates serum sodium concentration and plasma osmolality over bodyweight during prolonged endurance activity. Field studies confirm that a bodyweight loss of between 2-4% results in the maintenance of serum sodium concentration within the normal range and that bodyweight loss outside of this range generally results in dysnatremia [9 11]. Although the 0.8 ± 3.4 mmol/L increase in serum [Na^+] from pre and post race is statistically significant, this 0.6% elevation in serum [Na^+] is not of physiological or clinical significance.

The body defends cell volume during exercise through the maintenance of tonicity. Although the intracellular compartment comprises 60-66% of total body water [8;10], fluid shifts protect intracellular fluid volume until fluid loss exceeds the body's ability to cope

with rising tonicity levels [31]. Since sweat is hypotonic compared with plasma, the maintenance of tonicity during exercise requires fluid loss to exceed hypotonic fluid intake. This results in a net bodyweight loss in order to maintain plasma osmolality within the normal physiological range. Since serum [Na^+] is not correlated with % plasma volume change, it appears that tonicity and plasma volume are independently regulated.

The third important finding of this study is that cell volume is inversely correlated with serum [Na^+] change. The progressive loss of red blood cell volume with a linear increase in serum [Na^+] change has been reported previously [31;49;50]; thereby prompting scientists to refer to the erythrocyte as an "osmometer" [49]. In general, an osmolality increase of ~7.5 mOsmol/kg H_2O can elicit a measurable decrease in red blood cell volume [49]. Furthermore, a bodyweight loss greater than 5% has been shown to produce a 3% decrease in cell volume [13]. These data concur with our present findings, where a 4% mean bodyweight loss corresponds to a mean 3% decline in cell volume in this cohort of Ironman triathletes. This ~3% loss of red cell volume does not seem physiologically relevant as an absolute volume, but considering that the intracellular compartment contains double the volume contained within the extracellular compartment, this "shifting" of water can contribute a significant water source into the extracellular space.

There are limitations when comparing plasma volume measurements both between and within study trials. Dill and Costill's Hct/Hb method is used in this analysis to measure changes in plasma volume [21]; a method which has been shown to be valid when plasma osmolality changes are within -1 and +13 mOsmol/kg/H_2O [51]. This "indirect "method of plasma volume is also in good agreement with "direct" plasma volume measurements using the Evans blue dye technique [52]. The Evans blue dye technique may not be suitable for use during exercise, however, because of the potential for dye leakage out of the vascular space [53].

Postural changes may impart significant variation in plasma volume measurement [42]. Because pre-race measurements were determined in either a sitting or supine position and post-race measurements were obtained mainly supine, a maximum error of 1.5% in blood and plasma volume measurement may exist [54]. If the maximum allowable error in body posture is conceded in this analysis, the variation in plasma volume would range between +2.5% to -0.5%. This small change will not appreciably alter the results or conclusions of this paper although, ideally, all blood sampling should have occurred 20 minutes after quiet rest in the supine position.

CONCLUSION

Plasma volume and serum sodium concentration are maintained in Ironman triathletes, despite significant (3.8%) bodyweight loss during the course of the race. This suggests that the body protects osmolality and circulating blood volume during prolonged endurance exercise; with bodyweight serving as an indirect marker of homeostatic alterations in fluid balance. Therefore, bodyweight is *not* an accurate surrogate of fluid balance homeostasis and should be viewed with caution if used as a regulatory tool for monitoring water and sodium balance during an Ironman triathlon.

Reference List

1. Coyle EF. Fluid and fuel intake during exercise. *J.Sports Sci.* 2004;**22**:39-55.

2. Convertino VA, Armstrong LE, Coyle EF, Mack GW, Sawka MN, Senay LC, Jr. *et al.* American College of Sports Medicine position stand. Exercise and fluid replacement. *Med Sci.Sports Exerc* 1996;**28**:i-vii.

3. Cheuvront SN, Carter R, III, Sawka MN. Fluid balance and endurance exercise performance. *Curr.Sports Med.Rep.* 2003;**2**:202-8.

4. GSSI. Gatorade Sports Science Institute Fluid Calculator. http://www.gssiweb.com/tackleheat/fluidcalc/fluidcalc_calculator.htm . 2005.

5. PowerBar. PowerBar event nutrition calculator. http://www.powerbar.com/NutritionResource/ToolsArticles/ . 2005.

6. Casa, D. J. USATF Self-Testing Program for Optimal Hydration. http://www.org/groups/Coaches/library/hydration/USATFSelfTestingforOptimalHydration.pdf . 2003.

7. Dill DB, Bock AV, Edwards HT. Mechanisms for dissipating heat in man and dog. *Am.J Physiol* 1933;**104**:36-43.

8. Maw GJ, Mackenzie IL, Taylor NA. Human body-fluid distribution during exercise in hot, temperate and cool environments. *Acta Physiol Scand.* 1998;**163**:297-304.

9. Hew-Butler T, Verbalis JG, Noakes TD. Updated Fluid Recommendation: Position Statement from the International Marathon Medical Directors Association (IMMDA). *Clin.J.Sport Med* 2006;**16**.

10. Verbalis JG. Disorders of body water homeostasis. *Best.Pract.Res.Clin.Endocrinol.Metab* 2003;**17**:471-503.

11. Speedy DB, Noakes TD, Kimber NE, Rogers IR, Thompson JM, Boswell DR *et al.* Fluid balance during and after an ironman triathlon. *Clin.J.Sport Med.* 2001;**11**:44-50.

12. Robertson GL. Abnormalities of thirst regulation. *Kidney Int.* 1984;**25**:460-9.

13. Astrand PO,.Saltin B. Plasma and cell volume alterations after prolonged severe exercise. *J Appl.Physiol* 1964;**19**:829-32.

14. Milledge JS, Bryson EI, Catley DM, Hesp R, Luff N, Minty BD *et al.* Sodium balance, fluid homeostasis and the renin-aldosterone system during the prolonged exercise of hill walking. *Clin.Sci.(Lond)* 1982;**62**:595-604.

15. Williams ES, Ward MP, Milledge JS, Withey WR, Older MW, Forsling ML. Effect of the exercise of seven consecutive days hill-walking on fluid homeostasis. *Clin.Sci.(Lond)* 1979;**56**:305-16.

16. Gastmann U, Dimeo F, Huonker M, Bocker J, Steinacker JM, Petersen KG *et al.* Ultra-triathlon-related blood-chemical and endocrinological responses in nine athletes. *J.Sports Med.Phys.Fitness* 1998;**38**:18-23.

17. Stuempfle KJ, Lehmann DR, Case HS, Hughes SL, Evans D. Change in serum sodium concentration during a cold weather ultradistance race. *Clin.J.Sport Med.* 2003;**13**:171-5.

18. Glace BW, Murphy CA, McHugh MP. Food intake and electrolyte status of ultramarathoners competing in extreme heat. *J.Am.Coll.Nutr.* 2002;**21**:553-9.

19. Fallon KE, Broad E, Thompson MW, Reull PA. Nutritional and fluid intake in a 100-km ultramarathon. *Int.J Sport Nutr.* 1998;**8**:24-35.

20. Sharwood K, Collins M, Goedecke J, Wilson G, Noakes T. Weight changes, sodium levels, and performance in the South African Ironman Triathlon. *Clin.J.Sport Med.* 2002;**12**:391-9.

21. Dill DB,.Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl.Physiol* 1974;**37**:247-8.

22. Drabkin DL,.Austin JH. Spectrophometric studies II: Preparation from washed cells, nitric oxide, hemoglobin and sulfhemoglobin. *J Biol Chem* 1935;**112**:51.

23. Riley WJ, Pyke FS, Roberts AD, England JF. The effect of long-distance running on some biochemical variables. *Clin.Chim.Acta* 1975;**65**:83-9.

24. Speedy DB, Noakes TD, Rogers IR, Thompson JM, Campbell RG, Kuttner JA *et al.* Hyponatremia in ultradistance triathletes. *Med.Sci.Sports Exerc.* 1999;**31**:809-15.

25. Pastene J, Germain M, Allevard AM, Gharib C, Lacour JR. Water balance during and after marathon running. *Eur.J Appl.Physiol Occup.Physiol* 1996;**73**:49-55.

26. Rogers G, Goodman C, Rosen C. Water budget during ultra-endurance exercise. *Med Sci.Sports Exerc* 1997;**29**:1477-81.

27. Refsum HE, Tveit B, Meen HD, Stromme SB. Serum electrolyte, fluid and acid-base balance after prolonged heavy exercise at low environmental temperature. *Scand.J.Clin.Lab Invest* 1973;**32**:117-22.

28. Kozlowski S,.Saltin B. Effect of sweat loss on body fluids. *J Appl.Physiol* 1964;**19**:1119-24.

29. Patterson MJ, Stocks JM, Taylor NA. Sustained and generalized extracellular fluid expansion following heat acclimation. *J.Physiol* 2004;**559**:327-34.

30. Sanders B, Noakes TD, Dennis SC. Sodium replacement and fluid shifts during prolonged exercise in humans. *Eur.J.Appl.Physiol* 2001;**84**:419-25.

31. Nose H, Mack GW, Shi XR, Nadel ER. Shift in body fluid compartments after dehydration in humans. *J Appl.Physiol* 1988;**65**:318-24.

32. Myhre LG, Hartung GH, Nunneley SA, Tucker DM. Plasma volume changes in middle-aged male and female subjects during marathon running. *J.Appl.Physiol* 1985;**59**:559-63.

33. Nygren AT,.Kaijser L. Water exchange induced by unilateral exercise in active and inactive skeletal muscles. *J.Appl.Physiol* 2002;**93**:1716-22.

34. Costill DL, Cote R, Fink W. Muscle water and electrolytes following varied levels of dehydration in man. *J.Appl.Physiol* 1976;**40**:6-11.

35. Rocker L, Kirsch KA, Heyduck B, Altenkirch HU. Influence of prolonged physical exercise on plasma volume, plasma proteins, electrolytes, and fluid-regulating hormones. *Int.J.Sports Med.* 1989;**10**:270-4.

36. Maron MB, Horvath SM, Wilkerson JE. Acute blood biochemical alterations in response to marathon running. *Eur.J.Appl.Physiol Occup.Physiol* 1975;**34**:173-81.

37. Whiting PH, Maughan RJ, Miller JB. Dehydration and serum biochemical changes in marathon runners. *Eur.J Appl.Physiol* 1984;**52**:183-7.

38. Cohen I,.Zimmerman AL. Changes in serum electrolyte levels during marathon running. *S.Afr.Med J.* 1978;**53**:449-53.

39. Myhre LG, Hartung GH, Tucker DM. Plasma volume and blood metabolites in middle-aged runners during a warm-weather marathon. *Eur.J.Appl.Physiol Occup.Physiol* 1982;**48**:227-40.

40. Laursen PB,.Rhodes EC. Factors affecting performance in an ultraendurance triathlon. *Sports Med.* 2001;**31**:195-209.

41. Wilkerson JE, Gutin B, Horvath SM. Exercise-induced changes in blood, red cell, and plasma volumes in man. *Med.Sci.Sports* 1977;**9**:155-8.

42. Senay LC, Jr.,.Pivarnik JM. Fluid shifts during exercise. *Exerc.Sport Sci.Rev.* 1985;**13**:335-87.

43. Yang RC, Mack GW, Wolfe RR, Nadel ER. Albumin synthesis after intense intermittent exercise in human subjects. *J.Appl.Physiol* 1998;**84**:584-92.

44. Nagashima K, Mack GW, Haskell A, Nishiyasu T, Nadel ER. Mechanism for the posture-specific plasma volume increase after a single intense exercise protocol. *J.Appl.Physiol* 1999;**86**:867-73.

45. Pivarnik JM, Leeds EM, Wilkerson JE. Effects of endurance exercise on metabolic water production and plasma volume. *J.Appl.Physiol* 1984;**56**:613-8.

46. Ladell WBSS. The effects of water and salt intake upon the performance of men working in hot and humid environments. *J Physiol* 1955;**127**:11-46.

47. Costill DL, Cote R, Fink W. Muscle water and electrolytes following varied levels of dehydration in man. *J Appl.Physiol* 1976;**40**:6-11.

48. Fortney SM, Nadel ER, Wenger CB, Bove JR. Effect of blood volume on sweating rate and body fluids in exercising humans. *J.Appl.Physiol* 1981;**51**:1594-600.

49. Kolka MA, Stephenson LA, Wilkerson JE. Erythrocyte indices during a competitive marathon. *J.Appl.Physiol* 1982;**52**:168-72.

50. Costill DL, Branam L, Eddy D, Fink W. Alterations in red cell volume following exercise and dehydration. *J.Appl.Physiol* 1974;**37**:912-6.

51. Greenleaf JE, Convertino VA, Mangseth GR. Plasma volume during stress in man: osmolality and red cell volume. *J.Appl.Physiol* 1979;**47**:1031-8.

52. Jimenez C, Melin B, Koulmann N, Allevard AM, Launay JC, Savourey G. Plasma volume changes during and after acute variations of body hydration level in humans. *Eur.J.Appl.Physiol Occup.Physiol* 1999;**80**:1-8.

53. Feigenbaum MS, Welsch MA, Mitchell M, Vincent K, Braith RW, Pepine CJ. Contracted plasma and blood volume in chronic heart failure. *J.Am.Coll.Cardiol.* 2000;**35**:51-5.

54. Maw GJ, Mackenzie IL, Taylor NA. Redistribution of body fluids during postural manipulations. *Acta Physiol Scand.* 1995;**155**:157-63.

A summary of fluid balance physiology: proposed fluid recommendations for marathon runners

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INTRODUCTION

Controversy exists regarding optimal fluid guidelines for athletes engaging in different sports. Most published recommendations emphasize the detrimental consequences of dehydration [1-4] while more recent reports warn of the morbid consequences of hyperhydration [5-7]. Accordingly, individualized recommendations emphasizing a balance between the two extremes have evolved [8-10]. These revised guidelines, however, continue to promote static recommendations for what in reality are very dynamic athletic situations.

Marathon running epitomizes a dynamic situation that requires a constant adjustment to constantly changing homeostatic requirements. Real-time assessments of fluid and sodium homeostasis are physiologically represented by changes in plasma osmolality. Pituitary AVP secretion is stimulated when plasma osmolality increases by only 1-2%, representing the body's attempt to prevent dehydration by decreasing kidney water excretion. After maximal antidiuresis is achieved, thirst is then stimulated in order to replace water losses that are in excess of the ability of AVP-stimulated antidiuresis to conserve body water. In general, the osmotic threshold for thirst is 5-10 mOsm/g H_2O higher than that of AVP secretion. Thus, thirst is stimulated with decreases in body water of approximately 1.7-3.5%. [11-13]. Concomitant performance decrements and cardiovascular strain are also documented when baseline body fluid losses exceed approximately 2% [4].

The failure of athletes to replace 100% of bodyweight losses from ad libitum fluid intake has been well-described as "involuntary" [14;15] or "voluntary" dehydration [16-18]; this

phenomenon has resulted in the devaluation of thirst as a "poor" indicator of body fluid needs. The combination of laboratory with recent field data (see Table 1 and subsequent discussion), however, suggests that the body primarily defends plasma osmolality, and NOT blood or extracellular fluid volume during prolonged endurance exercise. Since it is well established that thirst is stimulated in response to changes in tonicity in most land mammals [11;13;19-22], it seems reasonable to conclude that thirst would be the predominant physiological and dynamic regulator governing fluid balance during exercise.

This article will focus on the physiology of normal fluid balance as the ultimate guide towards to evolution of optimal fluid recommendations. Six physiological considerations of fluid and sodium balance will be detailed followed by six practical recommendations. The neuroendocrine regulation of homeostasis will be emphasized in defense of the behavioral drives for thirst and sodium palatability during dynamic activities such as prolonged endurance exercise. The behavioral drives to maintain fluid and sodium balance are evolutionary stable, essential for the safety and survival of the species, and deeply rooted within the human genetic makeup [23].

PHYSIOLOGICAL CONSIDERATIONS OF FLUID AND SODIUM BALANCE:

1. Bodyweight is not an accurate surrogate for body fluid volume during prolonged exercise.

The field of exercise science promotes fluid replacement at 100% of bodyweight losses under the premise that sweat loss is an accurate surrogate for changes in body fluid volume [1;2;4;8;24-28]. This recommendation - to externally defend bodyweight during exercise - remains entrenched in the literature despite initial observations in 1932 stating that voluntary water consumption replaced only 56% of sweat lost during exercise [29]. Research on ad libitum fluid intakes over the last 74 years have repeatedly verified that humans, when given free access to fluids, replace no more than 75% of net water losses during physical activity [15-17;30-32]. Abdominal distress, nausea and vomiting

have been reported in highly trained distance runners who have tried to match fluid intakes to sweat rates [17;33]. The ongoing discrepancy between thirst, satiation and the inability to inherently maintain bodyweight during prolonged endurance activity suggests that ***bodyweight is only a marker of body fluid homeostasis and is not regulated in itself***. The remarkable capacity of the body to maintain circulating plasma volume despite large deficits in bodyweight due to "sweat losses" during prolonged endurance exercise exemplifies the complex regulation of body fluid volume and distribution [34] that are unrelated to the maintenance of bodyweight.

2. Blanket fluid guidelines with fixed ranges will *not* protect the diverse population currently participating in athletic events.

The American College of Sports Medicine's (ACSM) current guidelines promote a fluid intake of 600-1,200 ml/h; a range largely based on laboratory studies performed on elite male athletes [1]. The popularity of marathon running increased markedly at the time these guidelines were released, with charity running groups enticing more recreational runners into the sport. Slower, more inexperienced, female athletes heeded the upper limit of the ACSM guidelines and developed exercise-associated hyponatremia (EAH) by ingesting more fluid than they could excrete over the course of a marathon run [35;36]. In 2001, the International Marathon Medical Directors Association (IMMDA) approved guidelines lowering the "acceptable" range to 400-800 ml/h to adjust the upper limit and protect smaller athletes (mainly female) from overdrinking [7].

Recently, mathematical models have proposed that these ranges may not be ideal to cover the current population of runners participating in marathon events [37]. Close inspection of a marathon field today illustrates the variety of shapes, sizes and speeds that must be taken into consideration when formulating fluid intake guidelines. For example, the average weight of 7,299 runners registering for the 2005 Comrades Marathon was 73 kg. The lightest competitor weighed 43 kg while the heaviest runner weighed 119 kg. The fastest runner averaged 16.4 km/h (winning time 5:27) while the slowest runners ran at a 7.4 km/h pace (the official 12 hour cut-off pace). The 11,728

runners who completed the 89.2 km Comrades Marathon ran, on average, 49 minutes slower during the second half of the race than they ran the first half of the race with a minimum and maximum range of -11 (negative split) and 180 minutes (positive split) respectively. The wide variation between split times exemplifies the late-race functional deterioration that has been described elsewhere [38] and highlights the fact that modern marathon running is a dynamic situation, including participants with widely diverse physical attributes and fitness levels. An even wider spread of running speeds and bodyweights would be expected in a standard 42.2 km marathon, as the Comrades Marathon is more than double the distance, requires a qualifying time for entry, has a defined cut-off and is held over a grueling terrain; all of which precludes the participation of unfit and novice runners. Conversely, relatively unfit and untrained runners can often complete a standard 42 km marathon, amplifying the wide range of participant profiles.

The three main factors governing fluid loss during exercise are mass (bodyweight), running speed (metabolic rate) and ambient temperature [26;27]. Race day temperatures in the New York City Marathon have varied between 1°C to 29°C and have fluctuated as much as 17°C from start to finish [39]. Therefore, it would seem very unlikely that ANY single "range" could successfully encompass this wide spectrum of weather conditions, bodyweights and running speeds that characterize modern day marathon running.

3. Drinking to thirst will preserve plasma osmolality within the normal range and maintain intracellular volume and homeostasis.

Thirst is a subjective sensation characterized by a deep-seated desire for water [11;20;23] and is generally associated with oral sensations such as dryness, irritation and an "unpleasant taste" in the mouth [21;22;40]. Thirst has been quantified using geometric and visual analogue scales [11;21;40;41] and documented to increase when plasma osmolality is 5-10 mOsm/kg above the threshold for AVP secretion; **well within the normal physiological range of ~280-295 mOsm/kg H_2O** [11;22;42].

Plasma osmolality (P_{Osm}) is maintained within this narrow range to protect intracellular volume. Serum sodium concentrations [Na^+] mirror plasma osmolality [43] because sodium is the principle solute of extracellular fluid; thus [Na^+] and plasma osmolality are viewed as interchangeable in this discussion, although the appropriate calculation is: $P_{Osm} = 2 \times [Na^+] + [BUN] + [glucose]$ (all in mmol/L) [13].

The maintenance of intracellular volume is of vital importance for cell function and survival. Hypertonicity causes intracellular dehydration; a bodyweight loss of 5.5% during prolonged severe exercise elicits a reduction in red cell volume by 3.2% [44]. A reduced intracellular volume can reduce the rates of glycogen and protein synthesis [45] and hypertonicity beyond serum sodium concentrations of 160 mmol/L can cause encephalopathy and death [46]. Conversely, hypotonicity causes intracellular expansion. Although high cell volume can stimulate glycogen and protein synthesis, excessive and acute cell swelling from serum sodium levels below 125 mmol/L can lead to hyponatremic encephalopathy, noncardiogenic pulmonary edema and death [47].

Thirst and arginine vasopressin (AVP) secretion are intimately related: synergistically lowering elevations in plasma osmolality by stimulating fluid intake and promoting antidiuresis. The threshold for AVP release (~280-285 mOsm/kg H_2O) is normally set 5-10 mOsm/kg H_2O below that for thirst (~290-295 mOsm/kg H_2O), despite significant variation between individual set points [11]. This evolutionary design liberates individuals from constantly seeking water, as thirst is only stimulated when antidiuresis is maximal and hypertonicity exceeds the capacity of the kidneys to cope with rising tonicity. The strength of an intact thirst mechanism is exemplified in patients with the disorder of diabetes insipidus, either due to inadequate AVP secretion or an impaired kidney response to AVP, where a normal plasma osmolality is maintained by stimulated thirst despite the excretion of up to 20 liters of fluid per day [11]. Oropharyngeal metering [48-50]and stomach fullness [21;22;33] provide inhibitory feedback to terminate drinking more rapidly than the return of P_{Osm} to normal levels, perhaps as a safety measure to prevent overdrinking.

The strength and precision of the thirst mechanism has been demonstrated both at rest and during exercise in young adults. In one study involving seven men an increase in P_{Osm}, in response to hypertonic saline infusion, promoted a dipsogenesis proportional to the increase in P_{Osm}; with 82% of the total volume of water consumed within the first 5 minutes of rehydration with the immediate cessation of intake due to "stomach fullness"[22]. In a separate fluid deprivation study, 65% of total water intake was consumed during the first 2.5 minutes of rehydration in five males [21]. Subjective ratings of dry mouth and plasma protein concentrations returned to baseline levels in 2.5 minutes, plasma volume returned to baseline levels within 5 minutes and serum sodium concentrations begin to decrease within 5 minutes; returning to baseline values within 12.5 minutes. This initial drinking bout was also interrupted by sensations of "stomach fullness". During prolonged endurance exercise, eight female and ten male athletes responded to graded levels of hypohydration with ad libitum fluid intakes sufficient enough to restore plasma osmolality, replace sweat losses and attenuate thermal and circulatory strain [17;30;31].

Thus, humans respond to increases in serum $[Na^+]$ with AVP secretion, thirst and water intake that occur when P_{Osm} is still well within normal ranges. The precision and magnitude of the thirst drive is related to ancestral areas of the brain that are associated with vegetative functions such as the hunger for air and food, micturition and pain [51]. To assume that the thirst drive would be an "inaccurate index" of fluid balance during exercise (the dynamic homeostatic imbalance alternatively described as the "flight or fight" response) would seem contradictory to the evolution of our species.

Although there is concern that thirst due to intracellular dehydration or extracellular volume depletion may be confused with the sensations of a dry mouth (xerostomia) during exercise, thirst due to xerostomia has ***not*** been shown to lead to severe polydipsia [41]. The sensation of a dry mouth can promote more frequent drinking episodes, but total fluid intake is similar, or counter regulated by increased urinary output, in tested clinical and experimental settings [52]. Recent studies on xerostomia, thirst, and interdialytic weight gain have not factored in osmolality and therefore do not

provide convincing evidence that a dry mouth without changes in P_{Osm} can contribute to excessive fluid intakes or to hyponatremia from fluid overload without inappropriate AVP secretion [53-56].

4. The body defends plasma osmolality and volume over bodyweight. "Involuntary dehydration" is a homeostatic mechanism designed to protect plasma osmolality and cellular volume in response to hypotonic sweat losses.

The reluctance of man to voluntarily replace fluids at amounts equivalent to bodyweight losses during exercise was recognized in 1933, when Dill hypothesized that humans only drunk as much fluid to maintain constant osmolarity of the extracellular fluid [32]. This "failure" of man to replace 100% of body fluid losses, generally using bodyweight as a surrogate marker of body fluid volume, has been subsequently recognized as "voluntary" or "involuntary" dehydration; this has had the effect of minimizing the importance of thirst as an adequate indicator of body fluid needs [15;16 14].

Actual field study data form multiple endurance studies indicates that the maintenance of bodyweight will actually lead to reductions in serum sodium concentrations from pre- to post-race (Table 1). Most athletes who lose less than 2% of bodyweight experience a decrease in serum $[Na^+]$ [57;58]. Athletes who gain weight progress from normonatremia to hyponatremia [59]. In contrast, plasma osmolality is maintained within normal ranges (±3 mmol/L) when percent bodyweight losses are between 2-4% [60-67]. Whereas most athletes who lose more than 4% of bodyweight demonstrate increases in serum $[Na^+]$ above normal ranges [44;68-71]. Thus, the cumulative data in Table 1 suggests that the body defends plasma osmolality over body fluid volume, as reflected by changes in bodyweight, during prolonged endurance activity lasting between 2.7 to 38.2 hours, and can maintain plasma osmolality and therefore intracellular volume, best between 2-4% cumulative bodyweight losses. Acute bodyweight changes below 2% or above 4% cannot be compensated for internally, and may lead to dysregulation of serum $[Na^+]$ with resultant clinical symptomatology.

Concordant with these field data, three laboratory studies replacing fluids to 100% of bodyweight losses confirm a reduction in serum sodium concentrations during prolonged endurance exercise lasting between 2 and 6 hours [72-74]. Although these decreases in serum sodium concentrations were generally within normal physiological ranges, fluid replacement at or above 100% does not appear to offer any performance benefits [73;75].

Advocates of replacing 100% of bodyweight losses argue that cardiovascular drift – the downward drift in central venous pressure and stroke volume with concomitant rise in heart rate – commences with a 1% decrease in bodyweight losses [76]. Subsequent studies normalizing blood volume while inducing hypohydration have confirmed, however, that cardiac drift during exercise can and often does result from factors other than hypovolemia [76;77]. The corresponding increase in heart rate with progressive dehydration is accompanied by a proportional decrease in stroke volume which serves to maintain cardiac output [77]. These reductions in stroke volume are more related to elevations in core temperature, circulating catecholamine concentrations and heart rate than due to the redistribution of blood flow or circulating blood volume [78]. Only at degrees of dehydration beyond ~ 3% is cardiac output significantly diminished as a result of a reduction in circulating blood volume [78;79]. This bodyweight loss exceeds the threshold level at which AVP secretion and thirst are generally stimulated.

Equations predicting ad libitum fluid intake from multiple regression analyses identify plasma osmolality as the primary factor driving voluntary fluid intake during exercise or under stressful environmental conditions; with plasma volume and subjective symptoms of secondary importance [15;40]. Thus, under acute situations involving sweat sodium losses, the body will defend osmolality and intracellular volume through fluid shifts from the extracellular (primarily interstitial) fluid compartments [80-82] until ~4% bodyweight losses through sweating accrue; after which intracellular fluid volume is compromised and an external fluid supply is necessary.

Athletes rehydrating with either water or an electrolyte-containing beverage restore 68% and 82% of fluid losses, respectively, after an exercise-induced dehydration of 2.3% [14]. Changes in free water clearance followed changes in plasma osmolality and rehydration with only water restored plasma osmolality to control (isotonic) levels. Conversely, extracellular fluid space was fully restored only through consumption of the electrolyte-containing beverage. Ad libitum fluid intake did not match 100% bodyweight losses during 3 hours of rehydration with either beverage (hence the "involuntary" dehydration), illustrating the immediate defense of the body to preserve plasma osmolality first and only secondarily intravascular volume as reflected by bodyweight changes.

Metabolic water formation, from the combustion of fat and carbohydrate combined with the liberation of glycogen bound water, may contribute an internal water source to offset sweat losses and sustain internal fluid balance during prolonged or intense physical activity [83-85]. The activation or inactivation of internal sodium stores has also been hypothesized as a mechanism aiding in the defense of P_{Osm} during prolonged endurance exercise [86;87]. Data from 2,135 weighed competitive athletic performances demonstrate that athletes who overhydrate, underhydrate or euhydrate during prolonged endurance races generally maintain serum sodium concentrations within normal ranges (Figure 1 reprinted with permission) [86]. Despite this wide variation in bodyweight changes, 80% of the athletes in this large and diverse pool maintained P_{Osm} and intracellular volume during prolonged continuous athletic activity.

Thus, the combination of laboratory work with field data requires a re-examination of the phrase "voluntary dehydration" to more accurately reflect the preservation of plasma osmolality (and therefore intracellular volume) over extracellular and plasma volume, secondary to sweat NaCl losses during endurance exercise. The body does **not** defend ECF and plasma volume over plasma osmolality during an acute exercise bout, and the immediate replacement of body fluids at 100% of bodyweight losses is not beneficial to performance. AVP secretion and thirst are stimulated when P_{Osm} increases 1-4%; well within the physiological ranges of homeostatic compensation. Performance and cardiovascular decrements are clearly documented when bodyweight losses exceeds

4%, however this range is well beyond the protective capacity of the synergistic AVP and thirst mechanisms, whose primary physiological interaction is to preserve osmolality within relatively narrow physiological ranges. Only after plasma osmolality is stabilized will the body then activate mechanisms to *gradually* restore plasma volume by increasing sodium consumption and fluid intake over the next 24 hours [20;88].

5. Plasma volume contraction, resulting from the preservation of plasma osmolality during exercise, will be restored over a period of 24 hours by hormonal mechanisms which will increase the palatability for sodium containing foods and beverages. When sodium is ingested, plasma volume will gradually return to baseline levels.

The primary rationale for the inclusion of sodium into rehydration beverages is to aid in the restoration of plasma volume [45] rather than prevent the development of exercise-associated hyponatremia [5]. The presence of a sodium appetite is equivocal in humans [89-91], but studies on sodium palatability and preference have revealed significant associations with sodium loss and deficiency [88;90;92-97]. A peculiar theme echoing throughout these studies is the delayed expression of the attractiveness of sodium-containing items after cumulative sodium losses, particularly in studies involving sweat sodium losses after exercise [88;95].

A study by Takamata et al [88] confirms that the body protects osmolality over extracellular volume following seven hours of intermittent exercise at 35°C. Elevated serum sodium concentrations following exercise increased the palatability for water and decreased the pleasantness of concentrated sodium beverages during the first hour of rehydration. Plasma osmolality returned to baseline levels three hours after rehydration had begun, at which time the palatability rating for sodium containing beverages significantly increased over baseline levels. The palatability of highly concentrated sodium beverages continued to increase at 6, 17 and 23 hours following the cessation of exercise which correlated with significant increases in aldosterone secretion, but not AVP or P_{Osm}. The authors concluded that "increased H_2O palatability is only associated

with osmotically induced thirst and thus contributes to body fluid osmoregulation. In contrast, extracellular (ECF) thirst accompanied by an increased Na^+ preference appears after a delay of many hours after osmoregulatory responses occur and may contribute to ECF volume regulation".

Thus, the palatability for sodium is delayed following exercise until plasma osmolality is restored to normal levels from the ingestion of, and preference for, plain water. Only after plasma osmolality returns to baseline levels - through the ingestion of plain water to dilute elevated serum sodium concentrations - will the body seek sodium (by a palatability increase) to return plasma volume to baseline levels in the succeeding hours following the exercise bout. This is presumably facilitated by the natriorexogenic and dipsogenic actions of aldosterone and angiotensin II, which are known to stimulate these actions [23]. This phenomenon has also been supported by studies of exercising students, with low sweat rates, who expressed a decreased avidity for sodium when given high doses of salt before exercise [94], and has also been clearly demonstrated in rats made hypovolemic by polyethylene glycol injection [20]. Figure 2 illustrates that hypovolemic rats, on a normal sodium diet, will respond to isotonic hypovolemia by first ingesting only water until the decline in plasma osmolality inhibits water intake. Thereafter, the rats will drink saline until plasma osmolality increases, which will again stimulate water intake. The rat will then alternate between saline and water consumption until plasma volume is returned to baseline levels; maintaining a stable plasma osmolality throughout the gradual return to euvolemia. Thus, it has been shown in both animals and humans that sodium ingestion is necessary to restore plasma volume once osmolality has been stabilized. This restoration normally progresses over the course of 24 hours after the hypovolemic insult.

Since sodium is clearly necessary to restore and maintain plasma volume after sweat sodium losses from exercise, the beneficial effect of sodium ingestion during exercise is a topic of substantial debate. Studies have shown that consumption of hypotonic sodium containing beverages do not prevent the development of hyponatremia in athletes replacing 100% fluid loses or less [72;74;98] or in polydipsic psychiatric patients

[99;100], because most of the sodium ingested is rapidly lost through the urine [74;101]. In conditions of fluid overload, however, a mild blunting of serum sodium decline occurs with consumption of sodium containing beverages. This blunting does not eliminate the development of hyponatremia in athletes who continue to overdrink, however [59].

There are no studies documenting that sodium ingestion during exercise provides a clinical, physiological or performance benefit [60;102]. On the contrary, there are several studies linking sodium ingestion with *negative* physiological and performance effects [103-107]. Konikoff et al. supplemented normal dietary intakes with 10.2 g (173 mmol) of sodium three days prior to a 2 hour exercise bout (60% VO_2Max on a cycle ergometer) with fluid intake matching sweat rate. The authors concluded that the "salt loading" did not have a beneficial effect on temperature and fluid balance and in fact, elicited undesirable effects including significant increases in bodyweight, heart rate and rectal temperature [105]. Furthermore, the extra sodium was rapidly excreted in the urine, suggesting that the body had no need for the extra NaCl. Similarly, Pitts and Consolazio administered 9 g of NaCl to 11 subjects just prior to a ten-mile march. Water was replaced at equal volumes to sweat loss every 2 miles. The supplemental sodium ingestion also led to elevated heart rates and rectal temperatures, along with complaints of "gastrointestinal uneasiness" [103]. Performance in a VO_2Max test was impaired after rapid infusion of 30ml/kg of isotonic saline 30 minutes prior to the exercise bout [107]. There was a consistent increase in ventilation after every exercise work level following rapid saline infusion, as well as significant reductions in forced vital capacity and forced expiratory volume. All of these subjects felt more fatigued at maximal effort and reported sensations of "increased leg tightness" These authors hypothesized that the symptoms were due to increased intramuscular tissue pressure and resultant edema from the saline infusion.

Thus, sodium supplementation has no documented advantages when consumed during exercise, with over-replacement exhibiting detrimental cardiorespiratory and thermoregulatory effects during exercise. Since sodium is necessary for plasma volume restoration in the 24 hours following exercise, once plasma osmolality has been

normalized, electrolyte-containing food and beverages should be freely available after exercise and ingested according to palatability and tolerability.

6. Conditions where changes in plasma volume alter the set-point between thirst and tonicity: advanced age, the syndrome of inappropriate anti-diuretic hormone secretion (SIADH) and exercise in extreme heat or cold.

It is well documented that individuals over the age of 65 years demonstrate a reduced thirst drive - with corresponding decreases in fluid intake - in response to rising tonicity, when compared to younger adults [108-112]. Evidence suggests that this "reduced thirst drive" alternatively represents an upward shift in the operating set point rather than an age-related defect in osmoregulation [109;113]. Studies confirm that healthy older men demonstrate appropriate, although delayed, reflex adjustments serving to maintain osmotic homeostasis during conditions of hypertonic hypovolemia [109] and hypervolemia [114], when the rehydration period is *sufficiently long* (3 hours). Osmotic stimulation of AVP has been shown to elicit a three-fold greater increase per unit rise in plasma osmolality in older men [112] but follows the same pattern as that of younger men when exposed to similar osmotic stimuli [111;112;114]. Thus, evidence suggests that it is not a reduction in the osmotic drive that is primarily responsible for the attenuation of thirst during aging, but rather a reduction in the sensitivity of cardiopulmonary baroreceptors to plasma volume expansion that may contribute to an upward shift in the operating set point [108;110]. The diminished inhibitory effect of central volume expansion on thirst may result from an age-related impairment of plasma volume maintenance and expansion; perhaps from the inability to mobilize or maintain proteins in the vascular space [113;115]. This impairment in older males to expand or maintain plasma volume has been demonstrated in situations of hypertonic saline infusion [111;114], thermal dehydration [113] and during repeated exercise/heat stress [108;115]. The inability of most elderly people to expand plasma volume may necessitate elevation of the osmotic thirst threshold because age-related declines in renal function could concomitantly facilitate the development of fluid overload hyponatremia if lower, more "physiological", osmotic thresholds continued to exist.

Exercise in cold conditions may also elevate the osmotic threshold for which thirst and AVP secretion are stimulated. Subjects hypohydrated by ~3-4% experience a decline in both AVP secretion and perceived thirst sensations when entering a cold (4°C) chamber, despite resting plasma osmolalities above 295 mosmol/kgH_2O [116]. These subjects relate significantly lower thirst ratings at 40 and 60 minutes of treadmill exercise - when euhydrated and hypohydrated - in cold compared to temperate environments (27°C), despite similar plasma osmolalities. Thus, the authors of this study hypothesize that thirst and AVP secretion are "attenuated" from central volume expansion, secondary to peripheral vasoconstriction in the cold. These findings suggest that the osmotic operating set point in cold conditions could be elevated due to increased central blood volume.

Conversely, non-osmotic pituitary AVP secretion, leading to dilutional hyponatremia and SIADH, may cause a downward resetting of the osmotic threshold for thirst [117]. The presence of thirst at low osmolalities has been hypothesized to pathologically contribute to the development of hyponatremia [118], as patients with chronic SIADH relate the sensation of thirst and report daily fluid intakes similar to healthy individuals. The study by Smith et al. 2004 demonstrates, however, that SIADH patients with chronic dilutional hyponatremia (average serum sodium concentration: 125.8 ± 2.7 mmol/L) respond appropriately to osmotic challenge by hypertonic saline infusion. Baseline plasma osmolalities and the osmotic thresholds for both thirst and AVP release were 20 mosmol/kgH_2O lower in the SIADH group when compared to a group of healthy matched controls. Thirst ratings, elevations in AVP, urine osmolalities and fluid intakes were similar between the two groups with parallel elevations in plasma osmolality. Perceived ratings of thirst returned to baseline levels in both groups after 30 minutes of drinking and fluid consumption led to an immediate fall in thirst ratings within five minutes in both cohorts. Thus, the suppression of thirst in the SIADH group was similar to that of the control group, suggesting that the neural inhibition of thirst and drinking is preserved in patients with SIADH. These laboratory-based conclusions have been verified in the field setting, where marathon runners diagnosed with exercise-associated

hyponatremia reportedly consumed fluids in excess of excretion rates not because they were thirsty, but because they were fearful of becoming dehydrated [119]. The sensation of thirst should not be confused with the act of drinking. The act of drinking occurs from regulated (drinking to physiological thirst) as well as unregulated (social, habitual, palatability) fluid intake which may be governed by factors (marketing, commercialism) unrelated to the osmotic set point[13].

Work performance at the initial stages of acclimatization during exercise in extreme heat may improve with "forced" fluid intakes - beyond thirst - at volumes equivalent to sweat loss [103;120;121]. The environmental temperatures in these trials were extreme, ranging from 38ºC [103] to 46ºC [120;121] and involved a limited number of subjects. Subjects in the Bean and Eichna trial [121] ingested water equivalent to weight lost (1200 ml) and were able to complete five work cycles "without great difficulty" on the first day of exercise compared to subjects receiving only enough fluid to quench thirst (600 ml). On the fourth day of exercise, however, the degree of acclimatization - as measured by heart rate, rectal temperature and blood pressure - was similar between the two groups. The one subject in the Pitts et al. [103] trial also reported less fatigue and had lower rectal temperatures when "forced" to drink water equal to sweat losses. The fact that the rectal temperatures between ad libitum fluid replacement and "full" replacement varied greatly between trials (~0.3º to 1ºC) suggests that initial acclimatization requires fluid intakes beyond thirst to accommodate plasma volume expansion. After plasma volume is appropriately expanded, the need to ingest fluid beyond ad libitum intakes is not necessary to maintain similar rectal temperatures, heart rates and blood pressures. Alternatively, Moroff and Bass 1965 [120] administered a water pre-load of 2000 ml to non-acclimatized subjects and reported that when these subjects were water loaded, they had lower rectal temperatures and pulse rates compared to when not pre-loaded. Acclimatized subjects given the same pre-load, however, displayed lower heart rates with similar rectal temperatures for reasons that are unclear. Thus, initial exercise in extreme heat may require **transient** fluid intakes beyond thirst to facilitate the expansion of plasma volume - and heat acclimatization - irrespective of the current operating osmotic set point.

PRACTICAL RECOMMENDATIONS:

1. Drinking to thirst is the body's *dynamic* physiological fluid calculator and in most cases will protect athletes from the hazards of both over and underdrinking by providing real-time feedback on tonicity.

2. A *static* fluid calculator can provide an estimate of body fluid losses thereby providing a numeric range as a *generalized* strategy for fluid replacement during racing and training.

3. Athletes are advised to understand their individualized fluid needs through use of a static fluid calculator but ALWAYS defer to physiologic cues to increase fluid intake (thirst) or decrease fluid consumption (increased urination, bloating, weight gain) while running.

4. Water, sodium and glucose (in foods and or beverages) should be freely available at fluid replacement stations, spaced 1.6 km (minimum) to 5 km (maximum) apart. The quantity and amount of food and fluid consumed should be guided by individual palatability and tolerance for such items.

5. Calibrated scales along a marathon course should be at the discretion of the medical team; a weight loss of >4% or any weight gain constitutes justification for medical consultation.

6. Exercise in extreme heat (> 38°C) may require hydration beyond thirst during the initial days of heat acclimatization while advanced age (> 65 years) and cooler environmental temperatures (< 5°C) may elevate the operating set point for the stimulation of thirst.

FINAL WORD:

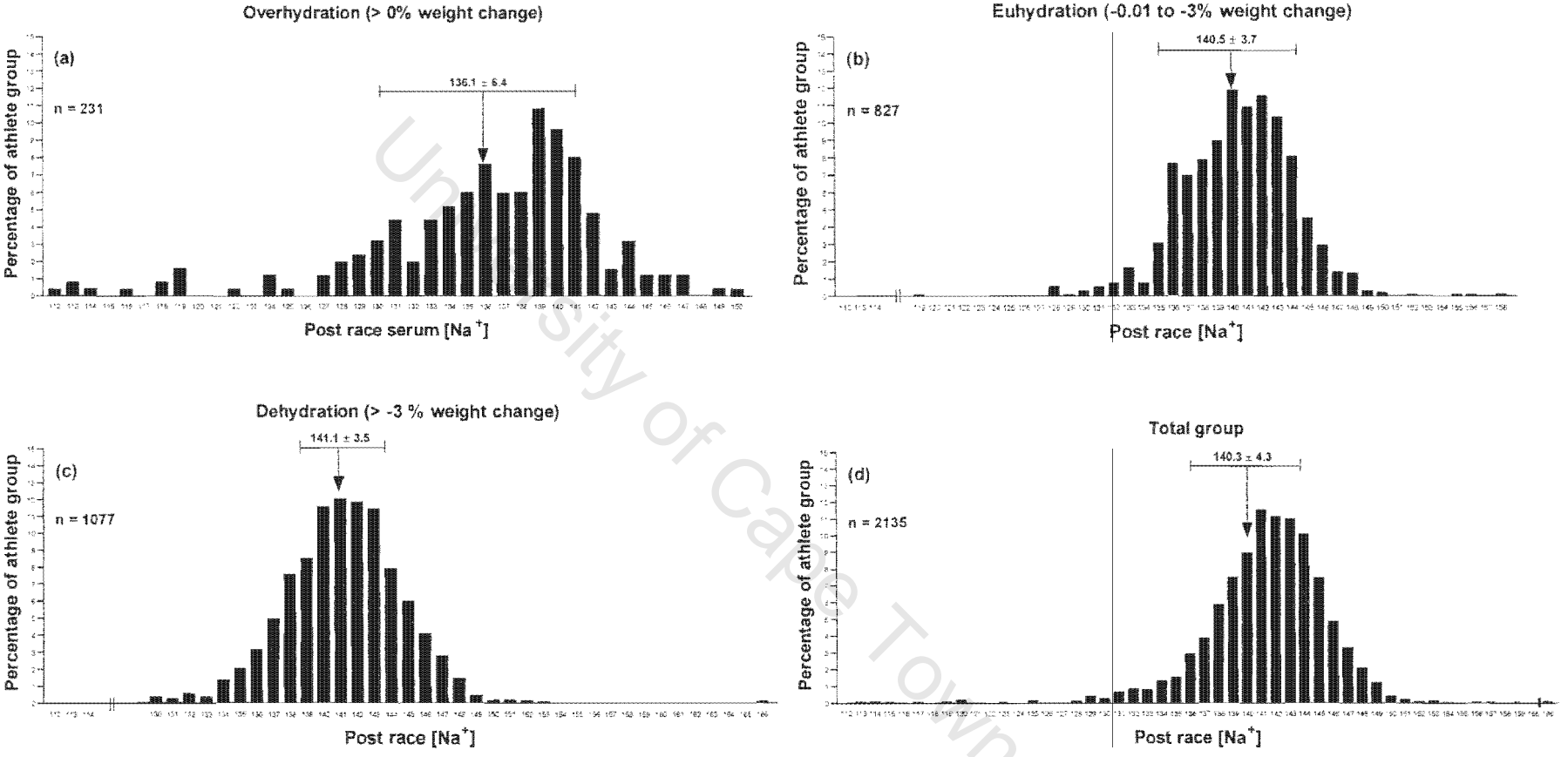
Marathon running is a serious endeavor that requires a personal commitment to sustained physical training. The hazards of marathon running have been highlighted by the recent deaths of four novice female runners from exercise-associated hyponatremia. Understanding individual fluid requirements **before** tackling a 42.2 km race is pivotal toward ensuring the successful and safe completion of such an event.

Although our society revolves around rules and algorithms to guide us through different situations, individuals should not be confined to these rules in a dynamic setting. There are no shortcuts toward great achievement, and marathon running is no exception. Clinicians and scientists must resist handing out unrealistic "blanket advice" to individuals seeking simple answers, but rather should encourage athletes to explore, understand and be flexible toward their own needs. By providing guidelines and advice on how to appropriately understand individual fluid replacement needs, we can eliminate future fluid balance problems by avoiding the temptation to generalize one rule for every situation and every athlete.

Table 1. Field Study Data: Serum Sodium and Bodyweight Change

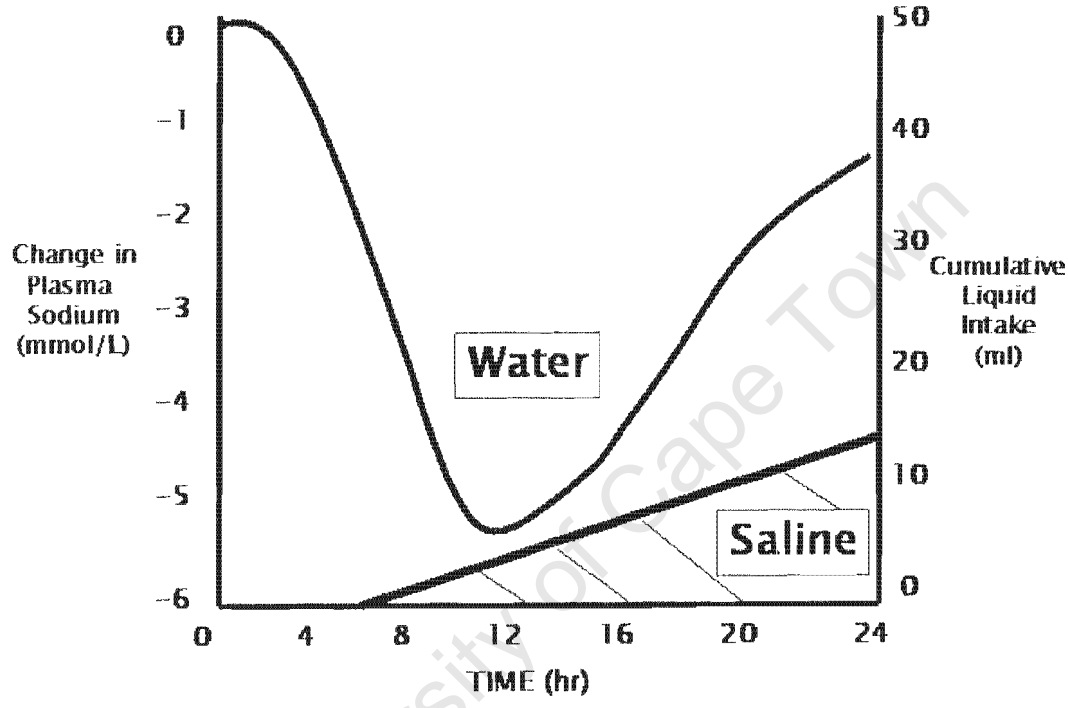
Study	Race distance (km)	[Na+] pre-race (mmol/L)	[Na+] post-race (mmol/L)	[Na+] change (pre-post race)	Bodyweight loss (%)	Finish time (hour)
Twerenbold 2003 (N=13)	*~41 run*	*137 ± 1*	*133 ± 2*	*-4*	*2 ± 1 weight gain*	*4*
Glace 2002 (N=13)	*160 run*	*144*	*140*	*-4*	*1*	*26*
Gerth 2002 (N=51)	*100 run*	*137 ± 5*	*131 ± 2*	*-6*	*1*	*14*
Hew-Butler (unpublished) (N=33)	109 cycle	139 ± 3	138 ± 3	-1	2 ± 1	5 ± 1
Stuempfle 2003 (N=20)	161 snow race	141 ± 1	138 ± 2	-3	2	38 ± 7
Nelson 1989 (N=45)	42 run	139 ± 0	142 ± 0	3	3	4
Refsum 1973 (N=41)	90 ski	142	141	-1	3	8
Cohen 1978	42 run	139 ± 2	142 ± 2	3	3	3
Noakes 1976 (N=13)	160 run	144 ± 1	140 ± 3	-4	3	16 ± 6
Kavanagh 1977 (N=9)	42 run	146 ± 2	148 ± 2	2	3	4 ± 1
Hew-Butler (unpublished) (N=82)	56 run	139 ± 3	138 ± 3	-1	4 ± 1	6 ± 1
Hew-Butler 2006 (N=413)	226 triathlon	141 ± 2	141 ± 3	0	4 ± 2	12 ± 2
Maron 1975 (N=6)	42 run	141 ± 1	141 ± 1	0	4 ± 1	3 ± 0
Gastmann 1998 (N=9)	*453 triathlon*	*138 ± 4*	*133 ± 4*	*-5*	*5*	*26*
Rocker 1989	*42 run*	*144*	*149*	*5*	*5*	*3*
Beckner 1954	*42 run*	*141*	*156*	*7*	*5*	*No time*
Riley 1975	*32 - 42 run*	*143 ± 1*	*148 ± 1*	*5*	*5*	*4*
Astrand 1964 (N=6)	*85 ski*	*144*	*152*	*8*	*6*	*9*

Figure 1. Distributions of post race [Na^+] in 2135 overhydrated, euhydrated and dehydrated athletes (reprinted with permission from Noakes TD).



(a) vs (b) $P < 0.001$
(a) vs (c) $P < 0.001$
(b) vs (c) $P < 0.001$

Figure 2. Changes in [Na^+] and cumulative fluid intake in hypovolemic rats (after subcutaneous injection of 30% polyethylene glycol solution) with free access to water and concentrated saline solutions over a 24 hour period (redrawn from Stricker EM and Verbalis JG 1988 with permission from Verbalis JG).



Reference List

1. Convertino VA, Armstrong LE, Coyle EF, Mack GW, Sawka MN, Senay LC, Jr. *et al.* American College of Sports Medicine position stand. Exercise and fluid replacement. *Med Sci.Sports Exerc* 1996;28:i-vii.

2. Coris EE, Ramirez AM, Van Durme DJ. Heat illness in athletes: the dangerous combination of heat, humidity and exercise. *Sports Med.* 2004;34:9-16.

3. Cheuvront SN, Carter R, III, Sawka MN. Fluid balance and endurance exercise performance. *Curr.Sports Med.Rep.* 2003;2:202-8.

4. Coyle EF. Fluid and fuel intake during exercise. *J.Sports Sci.* 2004;22:39-55.

5. Hew-Butler TD, Almond CS, Ayus JC, Dugas JP, Meeuwisse WH, Noakes TD *et al.* Consensus Statement of the 1st International Exercise-Associated Hyponatremia Consensus Development Conference, Cape Town, South Africa 2005. *Clin.J.Sport Med* 2005;15:208-13.

6. Noakes TD. Overconsumption of fluids by athletes. *BMJ* 2003;327:113-4.

7. Noakes TD. Fluid Replacement during Marathon Running. *Clin.J.Sport Med* 2003;13:309-18.

8. Casa, D. J. USATF Self-Testing Program for Optimal Hydration. http://www.org/groups/Coaches/library/hydration/USATFSelfTestingforOptimalHydration.pdf . 2003.

9. GSSI. Gatorade Sports Science Institute Fluid Calculator. http://www.gssiweb.com/tackleheat/fluidcalc/fluidcalc_calculator.htm . 2005.

10. PowerBar. PowerBar event nutrition calculator. http://www.powerbar.com/NutritionResource/ToolsArticles/ . 2005.

11. Robertson GL. Abnormalities of thirst regulation. *Kidney Int.* 1984;25:460-9.

12. McKinley MJ,.Johnson AK. The physiological regulation of thirst and fluid intake. *News Physiol Sci.* 2004;19:1-6.

13. Verbalis JG. Disorders of body water homeostasis. *Best.Pract.Res.Clin.Endocrinol.Metab* 2003;17:471-503.

14. Nose H, Mack GW, Shi XR, Nadel ER. Role of osmolality and plasma volume during rehydration in humans. *J.Appl.Physiol* 1988;65:325-31.

15. Greenleaf JE. Problem: thirst, drinking behavior, and involuntary dehydration. *Med.Sci.Sports Exerc.* 1992;24:645-56.

16. Greenleaf JE,.Sargent F. Voluntary dehydration in man. *J.Appl.Physiol* 1965;20:719-24.

17. Cheuvront SN,.Haymes EM. Ad libitum fluid intakes and thermoregulatory responses of female distance runners in three environments. *J.Sports Sci.* 2001;19:845-54.

18. Hubbard RW, Sandick BL, Matthew WT, Francesconi RP, Sampson JB, Durkot MJ *et al.* Voluntary dehydration and alliesthesia for water. *J.Appl.Physiol* 1984;57:868-73.

19. McKinley MJ, Cairns MJ, Denton DA, Egan G, Mathai ML, Uschakov A *et al.* Physiological and pathophysiological influences on thirst. *Physiol Behav.* 2004;81:795-803.

20. Stricker EM,.Verbalis JG. Hormones and Behavior. *American Scientist* 1988;76:261-7.

21. Rolls BJ, Wood RJ, Rolls ET, Lind H, Lind W, Ledingham JG. Thirst following water deprivation in humans. *Am.J.Physiol* 1980;239:R476-R482.

22. Phillips PA, Rolls BJ, Ledingham JG, Forsling ML, Morton JJ. Osmotic thirst and vasopressin release in humans: a double-blind crossover study. *Am.J.Physiol* 1985;248:R645-R650.

23. Fitzsimons JT. Angiotensin, thirst, and sodium appetite. *Physiol Rev.* 1998;78:583-686.

24. Barr SI. Effects of dehydration on exercise performance. *Can.J.Appl.Physiol* 1999;24:164-72.

25. Broad EM, Burke LM, Cox GR, Heeley P, Riley M. Body weight changes and voluntary fluid intakes during training and competition sessions in team sports. *Int.J.Sport Nutr.* 1996;6:307-20.

26. Cheuvront SN, Haymes EM, Sawka MN. Comparison of sweat loss estimates for women during prolonged high-intensity running. *Med.Sci.Sports Exerc.* 2002;34:1344-50.

27. Barr SI,.Costill DL. Water: can the endurance athlete get too much of a good thing? *J.Am.Diet.Assoc.* 1989;89:1629-32, 1635.

28. Galloway SD. Dehydration, rehydration, and exercise in the heat: rehydration strategies for athletic competition. *Can.J.Appl.Physiol* 1999;24:188-200.

29. Vernon HM,.Warner CG. The influence of the humidity of the air on capacity for work at high temperatures. *J.Hyg.* 1932;32:431-63.

30. Maresh CM, Gabaree-Boulant CL, Armstrong LE, Judelson DA, Hoffman JR, Castellani JW *et al.* Effect of hydration status on thirst, drinking, and related hormonal responses during low-intensity exercise in the heat. *J.Appl.Physiol* 2004;97:39-44.

31. Armstrong LE, Maresh CM, Gabaree CV, Hoffman JR, Kavouras SA, Kenefick RW *et al.* Thermal and circulatory responses during exercise: effects of hypohydration, dehydration, and water intake. *J.Appl.Physiol* 1997;82:2028-35.

32. Dill DB, Bock AV, Edwards HT. Mechanisms for dissipating heat in man and dog. *Am.J Physiol* 1933;104:36-43.

33. Costill DL, Kammer WF, Fisher A. Fluid ingestion during distance running. *Arch.Environ.Health* 1970;21:520-5.

34. Senay LC, Jr.,.Pivarnik JM. Fluid shifts during exercise. *Exerc.Sport Sci.Rev.* 1985;13:335-87.

35. Hew TD, Chorley JN, Cianca JC, Divine JG. The incidence, risk factors, and clinical manifestations of hyponatremia in marathon runners. *Clin.J.Sport Med.* 2003;13:41-7.

36. Almond CS, Shin AY, Fortescue EB, Mannix R, Wypij D. Hyponatremia among Runners in the Boston Marathon. *N.Engl.J Med* 2005;352:1550-6.

37. Montain SJ, Cheuvront SN, Sawka MN. Exercise associated hyponatraemia: quantitative analysis to understand the aetiology. *Br.J.Sports Med.* 2006;40:98-105.

38. Cade R, Packer D, Zauner C, Kaufmann D, Peterson J, Mars D *et al.* Marathon running: physiological and chemical changes accompanying late-race functional deterioration. *Eur.J.Appl.Physiol Occup.Physiol* 1992;65:485-91.

39. Cheuvront SN,.Haymes EM. Thermoregulation and marathon running: biological and environmental influences. *Sports Med.* 2001;31:743-62.

40. Engell DB, Maller O, Sawka MN, Francesconi RN, Drolet L, Young AJ. Thirst and fluid intake following graded hypohydration levels in humans. *Physiol Behav.* 1987;40:229-36.

41. Wright M, Woodrow G, O'Brien S, King N, Dye L, Blundell J *et al.* Polydipsia: a feature of peritoneal dialysis. *Nephrol.Dial.Transplant.* 2004;19:1581-6.

42. Valtin H. "Drink at least eight glasses of water a day." Really? Is there scientific evidence for "8 x 8"? *Am.J.Physiol Regul.Integr.Comp Physiol* 2002;283:R993-1004.

43. Kratz A, Siegel AJ, Verbalis JG, Adner MM, Shirey T, Lee-Lewandrowski E *et al.* Sodium status of collapsed marathon runners. *Arch.Pathol.Lab Med.* 2005;129:227-30.

44. Astrand PO,.Saltin B. Plasma and cell volume alterations after prolonged severe exercise. *J Appl.Physiol* 1964;19:829-32.

45. Shirreffs SM,.Maughan RJ. Rehydration and recovery of fluid balance after exercise. *Exerc.Sport Sci.Rev.* 2000;28:27-32.

46. Riggs JE. Neurologic manifestations of electrolyte disturbances. *Neurol.Clin.* 2002;20:227-39, vii.

47. Ayus JC, Varon J, Arieff AI. Hyponatremia, cerebral edema, and noncardiogenic pulmonary edema in marathon runners. *Ann Intern.Med* 2000;132:711-4.

48. Figaro MK,.Mack GW. Regulation of fluid intake in dehydrated humans: role of oropharyngeal stimulation. *Am.J.Physiol* 1997;272:R1740-R1746.

49. Salata RA, Verbalis JG, Robinson AG. Cold water stimulation of oropharyngeal receptors in man inhibits release of vasopressin. *J.Clin.Endocrinol.Metab* 1987;65:561-7.

50. Seckl JR, Williams TD, Lightman SL. Oral hypertonic saline causes transient fall of vasopressin in humans. *Am.J.Physiol* 1986;251:R214-R217.

51. Egan G, Silk T, Zamarripa F, Williams J, Federico P, Cunnington R *et al.* Neural correlates of the emergence of consciousness of thirst. *Proc.Natl.Acad.Sci.U.S.A* 2003;100:15241-6.

52. Steggerda FR. Observations on the water intake in an adult man with dysfunctioning salivary glands. *Am.J Physiol* 1941;132:517-21.

53. Brunstrom JM. Effects of mouth dryness on drinking behavior and beverage acceptability. *Physiol Behav.* 2002;76:423-9.

54. Brunstrom JM, Tribbeck PM, MacRae AW. The role of mouth state in the termination of drinking behavior in humans. *Physiol Behav.* 2000;68:579-83.

55. Bots CP, Brand HS, Veerman EC, Korevaar JC, Valentijn-Benz M, Bezemer PD *et al.* Chewing gum and a saliva substitute alleviate thirst and xerostomia in patients on haemodialysis. *Nephrol.Dial.Transplant.* 2005;20:578-84.

56. Bots CP, Brand HS, Veerman EC, Valentijn-Benz M, Van Amerongen BM, Valentijn RM *et al.* Interdialytic weight gain in patients on hemodialysis is associated with dry mouth and thirst. *Kidney Int.* 2004;66:1662-8.

57. Glace BW, Murphy CA, McHugh MP. Food intake and electrolyte status of ultramarathoners competing in extreme heat. *J.Am.Coll.Nutr.* 2002;21:553-9.

58. Gerth J, Ott U, Funfstuck R, Bartsch R, Keil E, Schubert K *et al.* The effects of prolonged physical exercise on renal function, electrolyte balance and muscle cell breakdown. *Clin.Nephrol.* 2002;57:425-31.

59. Twerenbold R, Knechtle B, Kakebeeke TH, Eser P, Muller G, von Arx P *et al.* Effects of different sodium concentrations in replacement fluids during prolonged exercise in women. *Br.J.Sports Med.* 2003;37:300-3.

60. Hew-Butler TD, Sharwood K, Collins M, Speedy D, Noakes T. Sodium supplementation is not required to maintain serum sodium concentrations during an Ironman triathlon. *Br.J.Sports Med.* 2006;40:255-9.

61. Stuempfle KJ, Lehmann DR, Case HS, Hughes SL, Evans D. Change in serum sodium concentration during a cold weather ultradistance race. *Clin.J.Sport Med.* 2003;13:171-5.

62. Nelson PB, Ellis D, Fu F, Bloom MD, O'Malley J. Fluid and electrolyte balance during a cool weather marathon. *Am.J.Sports Med.* 1989;17:770-2.

63. Refsum HE, Tveit B, Meen HD, Stromme SB. Serum electrolyte, fluid and acid-base balance after prolonged heavy exercise at low environmental temperature. *Scand.J.Clin.Lab Invest* 1973;32:117-22.

64. Cohen I,.Zimmerman AL. Changes in serum electrolyte levels during marathon running. *S.Afr.Med J.* 1978;53:449-53.

65. Noakes TD,.Carter JW. Biochemical parameters in athletes before and after having run 160 kilometres. *S.Afr.Med.J.* 1976;50:1562-6.

66. Kavanagh T,.Shephard RJ. On the choice of fluid for the hydration of middle-aged marathon runners. *Br.J.Sports Med.* 1977;11:26-35.

67. Maron MB, Horvath SM, Wilkerson JE. Acute blood biochemical alterations in response to marathon running. *Eur.J.Appl.Physiol Occup.Physiol* 1975;34:173-81.

68. Gastmann U, Dimeo F, Huonker M, Bocker J, Steinacker JM, Petersen KG *et al.* Ultra-triathlon-related blood-chemical and endocrinological responses in nine athletes. *J.Sports Med.Phys.Fitness* 1998;38:18-23.

69. Rocker L, Kirsch KA, Heyduck B, Altenkirch HU. Influence of prolonged physical exercise on plasma volume, plasma proteins, electrolytes, and fluid-regulating hormones. *Int.J.Sports Med.* 1989;10:270-4.

70. Beckner GL,.Winsor T. Cardiovascular adaptations to prolonged physical effort. *Circulation* 1954;9:835-46.

71. Riley WJ, Pyke FS, Roberts AD, England JF. The effect of long-distance running on some biochemical variables. *Clin.Chim.Acta* 1975;65:83-9.

72. Barr SI, Costill DL, Fink WJ. Fluid replacement during prolonged exercise: effects of water, saline, or no fluid. *Med Sci.Sports Exerc* 1991;23:811-7.

73. McConell GK, Burge CM, Skinner SL, Hargreaves M. Influence of ingested fluid volume on physiological responses during prolonged exercise. *Acta Physiol Scand.* 1997;160:149-56.

74. Vrijens DM,.Rehrer NJ. Sodium-free fluid ingestion decreases plasma sodium during exercise in the heat. *J Appl.Physiol* 1999;86:1847-51.

75. Maresh CM, Bergeron MF, Kenefick RW, Castellani JW, Hoffman JR, Armstrong LE. Effect of overhydration on time-trial swim performance. *J.Strength.Cond.Res.* 2001;15:514-8.

76. Montain SJ,.Coyle EF. Fluid ingestion during exercise increases skin blood flow independent of increases in blood volume. *J.Appl.Physiol* 1992;73:903-10.

77. Heaps CL, Gonzalez-Alonso J, Coyle EF. Hypohydration causes cardiovascular drift without reducing blood volume. *Int.J.Sports Med.* 1994;15:74-9.

78. Gonzalez-Alonso J, Mora-Rodriguez R, Coyle EF. Stroke volume during exercise: interaction of environment and hydration. *Am.J.Physiol Heart Circ.Physiol* 2000;278:H321-H330.

79. Gonzalez-Alonso J, Calbet JA, Nielsen B. Muscle blood flow is reduced with dehydration during prolonged exercise in humans. *J.Physiol* 1998;513 (Pt 3):895-905.

80. Maw GJ, Mackenzie IL, Taylor NA. Human body-fluid distribution during exercise in hot, temperate and cool environments. *Acta Physiol Scand.* 1998;163:297-304.

81. Costill DL, Cote R, Fink W. Muscle water and electrolytes following varied levels of dehydration in man. *J.Appl.Physiol* 1976;40:6-11.

82. Sanders B, Noakes TD, Dennis SC. Sodium replacement and fluid shifts during prolonged exercise in humans. *Eur.J.Appl.Physiol* 2001;84:419-25.

83. Kozlowski S,.Saltin B. Effect of sweat loss on body fluids. *J Appl.Physiol* 1965;19:1119-24.

84. Pastene J, Germain M, Allevard AM, Gharib C, Lacour JR. Water balance during and after marathon running. *Eur.J Appl.Physiol Occup.Physiol* 1996;73:49-55.

85. Rogers G, Goodman C, Rosen C. Water budget during ultra-endurance exercise. *Med Sci.Sports Exerc* 1997;29:1477-81.

86. Noakes TD, Sharwood K, Speedy D, Hew T, Reid S, Dugas J *et al.* Three independent biological mechanisms cause exercise-associated hyponatremia: evidence from 2,135 weighed competitive athletic performances. *Proc.Natl.Acad.Sci.U.S.A* 2005;102:18550-5.

87. Milledge JS, Bryson EI, Catley DM, Hesp R, Luff N, Minty BD *et al.* Sodium balance, fluid homeostasis and the renin-aldosterone system during the prolonged exercise of hill walking. *Clin.Sci.(Lond)* 1982;62:595-604.

88. Takamata A, Mack GW, Gillen CM, Nadel ER. Sodium appetite, thirst, and body fluid regulation in humans during rehydration without sodium replacement. *Am.J.Physiol* 1994;266:R1493-R1502.

89. Verbalis JG. Clinical aspects of body fluid homeostasis in humans. In Stricker EM, ed. *Neurobiology of Food and Fluid Intake. Handbook of Behavioral Neurobiology*, pp 421-62. New York: Plenum, 1990.

90. Beauchamp GK, Bertino M, Burke D, Engelman K. Experimental sodium depletion and salt taste in normal human volunteers. *Am.J.Clin.Nutr.* 1990;51:881-9.

91. McCance RA. Experimental sodium deficiency in man. *Proc Royal Soc Bri* 1936;119:245-68.

92. Kochli A, Tenenbaum-Rakover Y, Leshem M. Increased salt appetite in patients with congenital adrenal hyperplasia 21-hydroxylase deficiency. *Am.J.Physiol Regul.Integr.Comp Physiol* 2005;288:R1673-R1681.

93. Yeomans MR, Blundell JE, Leshem M. Palatability: response to nutritional need or need-free stimulation of appetite? *Br.J.Nutr.* 2004;92 Suppl 1:S3-14.

94. Wald N,.Leshem M. Salt conditions a flavor preference or aversion after exercise depending on NaCl dose and sweat loss. *Appetite* 2003;40:277-84.

95. Leshem M, Abutbul A, Eilon R. Exercise increases the preference for salt in humans. *Appetite* 1999;32:251-60.

96. Leshem M. Salt preference in adolescence is predicted by common prenatal and infantile mineralofluid loss. *Physiol Behav.* 1998;63:699-704.

97. Leshem M,.Rudoy J. Hemodialysis increases the preference for salt in soup. *Physiol Behav.* 1997;61:65-9.

98. Meyer LG, Horrigan DJ, Jr., Lotz WG. Effects of three hydration beverages on exercise performance during 60 hours of heat exposure. *Aviat.Space Environ.Med.* 1995;66:1052-7.

99. Goldman MB, Nash M, Blake L, Petkovic MS. Do electrolyte-containing beverages improve water imbalance in hyponatremic schizophrenics? *J.Clin.Psychiatry* 1994;55:151-3.

100. Worsening of hyponatremia with electrolyte-containing beverage. *Am J Psychiatry* 2004;161:374-5.

101. Vieweg WV, Rowe WT, David JJ, Spradlin WW. Oral sodium chloride in the management of schizophrenic patients with self-induced water intoxication. *J.Clin.Psychiatry* 1985;46:16-9.

102. Speedy DB, Thompson JM, Rodgers I, Collins M, Sharwood K, Noakes TD. Oral salt supplementation during ultradistance exercise. *Clin.J.Sport Med.* 2002;12:279-84.

103. Pitts GC, Johnson RE, Consolazio FC. Work in the heat as affected by intake of water, salt and glucose. *Am.J Physiol* 1944;142:253-9.

104. Ladell WBSS. The effects of water and salt intake upon the performance of men working in hot and humid environments. *J Physiol* 1955;127:11-46.

105. Konikoff F, Shoenfeld Y, Magazanik A, Epstein J, Shapira Y. Effects of salt loading during exercise in a hot dry climate. *Biomed.Pharmacother.* 1986;40:296-300.

106. Hargreaves M, Morgan TO, Snow R, Guerin M. Exercise tolerance in the heat on low and normal salt intakes. *Clin.Sci.(Lond)* 1989;76:553-7.

107. Robertson HT, Pellegrino R, Pini D, Oreglia J, DeVita S, Brusasco V *et al.* Exercise response after rapid intravenous infusion of saline in healthy humans. *J.Appl.Physiol* 2004;97:697-703.

108. Takamata A, Ito T, Yaegashi K, Takamiya H, Maegawa Y, Itoh T *et al.* Effect of an exercise-heat acclimation program on body fluid regulatory responses to dehydration in older men. *Am.J.Physiol* 1999;277:R1041-R1050.

109. Mack GW, Weseman CA, Langhans GW, Scherzer H, Gillen CM, Nadel ER. Body fluid balance in dehydrated healthy older men: thirst and renal osmoregulation. *J.Appl.Physiol* 1994;76:1615-23.

110. Stachenfeld NS, DiPietro L, Nadel ER, Mack GW. Mechanism of attenuated thirst in aging: role of central volume receptors. *Am.J.Physiol* 1997;272:R148-R157.

111. Phillips PA, Bretherton M, Johnston CI, Gray L. Reduced osmotic thirst in healthy elderly men. *Am.J.Physiol* 1991;261:R166-R171.

112. Phillips PA, Rolls BJ, Ledingham JG, Forsling ML, Morton JJ, Crowe MJ *et al.* Reduced thirst after water deprivation in healthy elderly men. *N.Engl.J.Med.* 1984;311:753-9.

113. Miescher E,.Fortney SM. Responses to dehydration and rehydration during heat exposure in young and older men. *Am.J.Physiol* 1989;257:R1050-R1056.

114. Stachenfeld NS, Mack GW, Takamata A, DiPietro L, Nadel ER. Thirst and fluid regulatory responses to hypertonicity in older adults. *Am.J.Physiol* 1996;271:R757-R765.

115. Zappe DH, Bell GW, Swartzentruber H, Wideman RF, Kenney WL. Age and regulation of fluid and electrolyte balance during repeated exercise sessions. *Am.J.Physiol* 1996;270:R71-R79.

116. Kenefick RW, Hazzard MP, Mahood NV, Castellani JW. Thirst sensations and AVP responses at rest and during exercise-cold exposure. *Med.Sci.Sports Exerc.* 2004;36:1528-34.

117. Smith D, Moore K, Tormey W, Baylis PH, Thompson CJ. Downward resetting of the osmotic threshold for thirst in patients with SIADH. *Am.J.Physiol Endocrinol.Metab* 2004;287:E1019-E1023.

118. Robertson GL. Syndrome of inappropriate antidiuresis. *N.Engl.J.Med.* 1989;321:538-9.

119. Hew T. Response to the Letter to the Editor by Roy J. Shephard. *Clin.J Sport Med* 2003;13:192-3.

120. Moroff SV,.Bass DE. Effects of overhydration on man's physiological responses to work in the heat. *J Appl.Physiol* 1965;20:267-70.

121. Bean WB,.Eichna LW. Performance in relation to environmental temperature. *Fed.Proc* 1943;2:144-58.

SUMMARY AND CONCLUSIONS

Although this thesis largely reflects my own work and the ideas of my supervisors, these small advancements in knowledge more accurately represent a global effort. The most valuable outcome from the 1st International Exercise-Associated Hyponatremia (EAH) Consensus Development Conference was the realization that people working together towards a common goal were far more productive than people working apart or antagonistically.

When this scientific expedition began, the etiology of EAH was still unresolved and widely debated. The consensus amongst a diverse group of researchers - encompassing nephrology, neuroendocrinology, internal medicine, cardiology, epidemiology, family practice and exercise physiology - that the etiology of EAH was primarily *dilutional* was the first major contribution derived from this thesis work. The orchestration of this highly interactive and collaborative evidenced-based review of the literature elevated the more "traditional" literature review to new heights.

The scientific contributions highlighted throughout the rest of these investigations broadly spanned the pathogenesis, treatment and prevention of both dysnatremias (hyponatremia and hypernatremia) associated with exercise. The endocrine regulation of fluid homeostasis during high intensity, steady-state and prolonged distance running were also evaluated to compliment our understanding of fluid dysregulation. Thus, although it was concluded that exercise-associated hyponatremia most likely resulted from a combination of sustained fluid intake which exceeded output, exercise associated hypernatremia seemed to result from an inability to tolerate oral fluid ingestion during prolonged endurance exercise.

Investigations on collapsed athletes revealed that a small bolus of hypertonic saline could rapidly reverse altered mental status changes in a triathlete with symptomatic EAH. Conversely, the administration of hypotonic fluids to collapsed hypernatremic ultramarathon runners - who were intolerant to oral fluid ingestion – seemed to be the

most appropriate treatment option for the "opposite" dysnatremia of exercise. In all other cases of symptomatic normonatremic or hypernatremic athletes, the efficacy of oral versus intravenous fluid administration was equivalent when the time to discharge was the main outcome variable. The presence of dysnatremia upon admission to the medical tent, however, predicted a delayed recovery in collapsed ultramarathon runners. This novel finding underscored the detrimental physiological consequences associated with serum sodium imbalance and fluid dysregulation.

The prevention of dysnatremia during prolonged endurance activity was addressed in both a prospective and retrospective study. Sodium supplementation or a placebo was administered to triathletes before a 226 km Ironman Triathlon. The ingestion of the supplemental sodium tablets did not alter serum sodium concentrations in athletes who lost weight during 12 hours of exercise, compared with athletes who consumed the placebo tablets. Therefore, sodium supplementation did not prevent hyponatrernia (nor promote hypernatremia) during prolonged endurance exercise and appeared unnecessary as a recommendation to "maintain" of serum sodium concentrations during an Ironman triathlon.

Similarly, in a retrospective study on Ironman triathletes, a 4% body weight loss did not adversely affect health following completion of this event. Because bodyweight is currently recommended as a surrogate measure of body fluid homeostasis, we wished to evaluate if changes in bodyweight accurately represented changes in both serum sodium concentration and plasma volume loss (the "true" physiological markers of fluid homeostasis) during prolonged endurance exercise. The 4% bodyweight loss, documented in these triathletes from pre- to post-race, corresponded to a 1% increase in both serum sodium concentration and plasma volume. Therefore, bodyweight loss was not an accurate measure of fluid homeostasis during prolonged endurance exercise and recommendations to fully replace bodyweight loss may cause - rather than prevent – the dysnatremias associated with exercise.

The preservation of serum sodium concentration, and not body mass, during exercise was further validated by our investigations of the endocrine regulation of fluid balance during high intensity, steady-state and prolonged endurance running. The most remarkable finding from these neuroendocrine studies was the documented linear relationships between post-run sweat and urine sodium concentrations versus post-run serum sodium concentrations following high intensity and steady-state running. The changes in sweat and urine sodium concentration appeared to be homeostatically mediated by changes in plasma arginine vasopressin (AVP) concentrations; thereby suggesting a regulatory link between osmoregulation and thermoregulation during exercise in staunch defense of fluid conservation.

Prolonged endurance running appeared to enhance non-osmotic or potentiate osmotic AVP secretion. This noteworthy finding would confirm the plausible role that AVP would play in the pathogenesis of EAH, if hypoosmolality were to develop during exercise from sustained fluid intake exceeding output. The positive correlation between AVP with the "other" neurohypophyseal hormone, oxytocin, was also a curious and unexpected phenomenon following prolonged endurance exercise. Since oxytocin has confirmed antidiuretic properties in humans, the possible pathological role of this nonapeptide in the genesis of EAH would likely require further investigation.

Another unique discovery from these endocrine investigations was the large increase in plasma brain natriuretic peptide (BNP) concentrations following prolonged endurance running, despite the presence of concomitant hypovolemia. When data from seven subjects participating in high intensity, steady-state and prolonged endurance running were combined, a positive linear relationship between BNP and urine osmolality Δ and a negative linear relationship between BNP and serum sodium concentration Δ support the potential contribution BNP may play in the regulation of serum sodium concentration during exercise.

This thesis then closed as it opened: with an interactive evidenced-based review that was subsequently published as the Updated Position Statement for Fluid Replacement

by the International Marathon Medical Directors Association. This Statement reflected the data presented in this thesis and proposed that fluid replacement guidelines be based upon the physiological maintenance of serum sodium concentration rather than on the non-physiological maintenance of bodyweight during prolonged endurance exercise.

Hence, the circle of knowledge presented in this thesis hopefully created a new framework for the continued investigation of fluid balance physiology and pathophysiology during exercise. When this dissertation on EAH began, "blanket range" fluid guidelines along with recommendations to drink "as much as possible" during exercise were advised. Over this four year period of study, however, individualized fluid guidelines and recommendations to ***not*** replace fluids in excess of bodyweight loss have replaced this erroneous advice. And although the scientific method does not directly embrace courage, passion, flexibility, opportunity or creativity in the human quest for knowledge; perhaps these attributes are what ultimately drive academic progress, novel ideas and a contagious delight in scientific discovery.