The influence of glycerol hyperhydration on run performance within an Olympic distance triathlon

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Abstract

This study was designed to determine the impact of glycerol hyperhydration, compared with a placebo hyperhydration, on the run performance during an Olympic distance triathlon. Ten competitive triathletes (mean peak oxygen consumption, VO_2 peak = 65.5 ± 5.5 ml.kg.$^{-1}$min^{-1}) undertook two simulated Olympic Distance Triathlons in 31° C and 61% relative humidity. The trials were split into two work phases: a fixed workload phase comprising a 18-20 min swim and a 60 min cycle and, a self regulated time trial run over 10 kilometres conducted on a treadmill. One hundred and fifty min prior each trial, either a glycerol solution (1 g.kg.$^{-1}$ body mass (BM) in a 4% carbohydrate – electrolyte drink) or a placebo of equal volume of the 4% carbohydrate-electrolyte solution was ingested over one hour. The total fluid intake in each trial was 23 ml.kg.$^{-1}$ BM. A randomised, double blind, cross over design was used.

Due to either 1) the arduous nature of the trials 2) the side effects associated with the ingestion of glycerol 3) or the combination of the two aforementioned reasons, only five of the 10 subjects completed the final 10 km self regulated time trial for both treatments. Only the data obtained from these five subjects were reported in this study.

Glycerol ingestion expanded body water over the placebo by 154 ml (26%). At 60 and 90 min after the start of drinking, urine output was significantly higher with glycerol than placebo treatment (216.4, 366.4 ml vs 81.0, 242.0 ml, respectively) but significantly higher at 120 min in the placebo (421.6 ml vs 131.2 ml).

There were no significant differences in heart rate and rectal temperature during the swim and cycle phases. However, there were significant increases in heart rate (at 5, 10, 15, 25 and 30 min) and rectal temperature (at 5, 20 and 30 min) during the 10 km run in the glycerol trial.

The mean 10 km run time for the placebo trial was 40 min 21 sec (± 2.9 min) while the glycerol trial was 39 min 22 sec (± 2.0 min). The mean difference of 2.1% in finishing time between trials was not significant. Three of the five subjects in the glycerol trial improved their 10 km time by 7.0, 2.4 and 2.7%, respectively. The finishing time for one subject did not change for both trials while another subject had deteriorated by 2.3% in the glycerol trial. In the glycerol treatment, five subjects complained of bloating and nausea while only one subject complained of feeling unwell in the placebo treatment.

Data from this study have shown that glycerol hyperhydration did not significantly improve performance while plasma volume expansion and subsequent lower rectal temperature and lower heart rates were not evident. The exact mechanisms of how glycerol hyperhydration can improve performance warrant further investigation.

STATEMENT OF AUTHORSHIP

Except where explicit reference is made in the text of the thesis, this thesis contains no material published elsewhere or extracted in whole or in part from a thesis by which I have qualified for or been awarded another degree or diploma. No other person's work has been relied upon or used without due acknowledgement in the main text and bibliography of this thesis.

Signed ……..

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CHAPTER 1: INTRODUCTION

In sports or activities lasting up to 10 minutes in duration, temperature regulation and body fluid changes are of little consequence to performance if athletes are well hydrated (Gisolfi, 1996). However, as exercise extends beyond 10 minutes, both body fluid changes and temperature regulation become important factors limiting efficient performance (Gisolfi, 1996). In cool conditions, the thermoregulatory system is quite efficient in dissipating excess body heat. This is possible because a temperature gradient exists between the ambient temperature and the skin temperature thereby allowing internal heat to be lost to the environment via evaporation, radiation, conduction and convection. However, hot and humid conditions combined restrict the body's capacity to dissipate heat via evaporation.

Several studies have shown significant impairment in aerobic endurance performance of 3-5%, even at low levels of hypohydration ranging from 1-5% reduction in body mass (BM) (Sawka et al., 1985; Armstrong et al., 1985; Walsh et al., 1994; Moquin and Mazzeo 2000). This drop in performance can increase to 20 - 30% if losses of 4 - 5% in BM are experienced (Saltin and Costill, 1988; Wilmore and Costill, 1997). As a consequence, there are serious limitations on one's ability to perform in the heat if hypohydration is severe. Dehydration can have a significant deleterious effect on several physiological measures including a decrease in plasma volume, an increase in exercise heart rate (cardiac drift), and reduced stroke volume (Armstrong 2000). As a result cardiac output is compromised, core temperature increases during exercise and maximal exercise capacity is reduced (Nadel et al., 1980; Armstrong et al., 1985; Candas et al., 1988; Sawka and Pandolf 1990; Walsh et al., 1994; Murray 1996; Sawka and Coyle 1999). Coyle and Montain (1992) reported that a net one litre loss of body water due to sweat loss corresponded to an increase in exercise heart rate by eight beats, a reduction in cardiac output by one litre per minute and an increase in core temperature by 0.3° C.

As athletes often train and compete in extreme environmental conditions such as high heat and humidity and may lose substantial fluid, a number of pre- and intra-event hydration strategies and methods have been proposed. The aim of these strategies is to minimise fluid loss and potentially improve performance capacity by lessening the possible cardiovascular and thermal strain.

Numerous studies have investigated the effects of pre-event hyperhydration on thermoregulation and exercise performance. These studies are outlined in a review by Latzka and Sawka (2000). The majority of these studies have shown no significant change in core and skin temperature (Latzka et al., 1998; Hitchins et al., 1999) while only a few studies have reported increases in sweat rates (Nielsen 1974; Lyons et al., 1990) as a result of pre-event hyperhydration.

Hyperhydrating is a strategy whereby a large volume of fluid (400-600 ml) is consumed prior (1-2 hr) to an exercise bout. Hyperhydrating pre-exercise is used to either delay the onset of hypohydration (Latzka et al., 1997) or bring an athlete to a fluid balance if the hydration status is unknown. Hyperhydrating by means of doubling fluid intake for the week prior an event may also see some extra fluid gain (Burke 2000). Pre-exercise hyperhydration has also been suggested as a possible mechanism to expand plasma volume (PV) and maintain or enhance performance (Fellmann 1992). The transient increase in PV has been shown to suppress heart rate drift while promoting better heat transfer in a warm environment (Nose et al., 1990). It may be hypothesised that plasma expansion, possibly by hyperhydrating, could improve performance via the stabilising of heart rates and maintenance of core temperature. Although pre-exercise hyperhydration has been shown to increase total body water, this does not always correspond to an increase in PV (Latzka and Sawka, 2000).

Increases in PV have been induced by methods other than hyperhydration. Coyle et al. (1990) expanded PV via venous injection of a 6% dextrin solution in saline. This method increased SV and as a result, VO_2 max was increased slightly (4%) and performance improved in untrained men. Other studies involving the maintenance of PV through the infusion of isotonic saline or albumin have found lower heart rates and lower core temperature while exercising (Deschamps et al., 1989). Fellmann (1992) also suggested that an increased PV can lead to enhanced performance by inducing better muscle blood flow perfusion and by increasing SV and maximal cardiac output.

One hyperhydrating strategy that has recently received attention as a possible means of reducing hypohydration is the use of glycerol. However, the results of studies on the effects of glycerol on increasing PV and retaining fluid are variable. Fluid retention using glycerol prior to exercise can vary from 400 to 700 ml (Riedesel et al., 1987; Wendtland et al., 1987; Lyons et al., 1990; Seifert et al., 1995; Hitchins et al., 1999; Anderson et al., 2001) and recently to 1000 ml (Coutts et al., 2002) over other hyperhydration solutions.

While there is limited evidence to support glycerol as a possible plasma volume expander (Hitchins et al., 1999; Coutts et al., 2002), the influence of glycerol ingestion, and the accompanying retention of fluid, has been shown to improve exercise performance.

Recently Hitchins et al. (1999) used glycerol as a hyperhydrating strategy and reported an increase of approximately 600 ml in total body water over that achieved with a control solution and a 5% improvement of performance in a 30 min time trial by eight competitive cyclists. Montner et al. (1992) also noted a reduced heart rate and a 21% improvement in performance in cycling time to exhaustion in competitive cyclists with the use of glycerol as a hyperhydrating agent. To date, there is only one study that has investigated the potential performance effect of glycerol on triathlete performance (Coutts et al., 2001). Coutts et al. (2001) demonstrated a reduced decrement in performance time following glycerol hyperhydration compared to ingestion of a placebo fluid when exercising on a hot day in comparison to a warm day. The mean Olympic distance triathlon (swim 1.8 km, cycle 40 km, run 10 km) (ODT) times were 5.7% (8 min) faster during the hot day and 1.5% slower on the warm day following the glycerol treatment. The authors concluded that glycerol hyperhydration offers a physiological defence against the detrimental effects of exercise-induced dehydration in stressful hot environmental conditions. No physiological data were collected during the triathlon to explain the exact mechanisms through which glycerol hyperhydration enhanced performance; although it should be noted that glycerol ingestion resulted in a non-significant increase in PV of 21.2% over that observed for the placebo condition on the warm day.

Coutts et al. (2002) also reported that the majority of the ODT performance improvements occurred during the 10 km run leg phase on the hot testing day. This supports the recent change of the triathlon format allowing drafting on the cycle phase while a greater emphasis has been placed on the run phase. The unique format of this study will attempt to control the work output (and thus physiological responses) in the swim and cycle phases with both treatments in both trials however, allow a maximal self-regulated effort in the final 10 km run. Therefore, the athlete capable of enduring the first two phases (swim and cycle), with minimal thermal strain and limited dehydration as a consequence of the glycerol hyperhydration treatment, is more likely to have a distinct physiological and performance advantage during the run compared with the placebo treatment.

Purpose

The purpose of this study was to evaluate the effectiveness of glycerol as a pre-race hyperhydration strategy and its influence on the run performance during an Olympic distance triathlon.

Hypotheses

The following hypotheses were tested:

1. Glycerol-induced hyperhydration will result in significant plasma volume expansion compared with the placebo treatment.
2. The ingestion of glycerol will reduce thermal strain during the swim, cycle and run phases.
3. The ingestion of glycerol will reduce cardiovascular strain during the swim, cycle and run phases.
4. Glycerol ingestion will improve the time trial run performance compared with that of the placebo treatment.

The desired outcomes of this study were to:

1. provide evidence to determine the effect of glycerol hyperhydration on the performance of this target population;
2. test the effects and potential side effects of glycerol hyperhydration on thermoregulation and hypohydration during endurance exercise;

Delimitations

Subject population:

Ten highly trained triathletes were selected to complete two simulated triathlons in warm, humid conditions. Each triathlete was selected on the basis of their best time to complete an Olympic Distance Triathlon (ODT). To satisfy this selection criteria, subjects must have completed an ODT under 2 hrs: 20 min (females) or under 2 hrs: 5 min (males).

Limitations

Control of baseline training and nutritional status:

An educational approach was used to control the training and nutrition status of the subjects prior to the trials.

Respiratory/metabolic fluid losses/gain and absorption rates

Respiratory/metabolic fluid losses/gain and absorption rates were assumed to be similar between subjects: It was also assumed that fluid gain and losses were similar for all subjects between trials.

Sixty minute time trial in the cycle phase

The 60 min cycle time trial did not mimic actual race demands during an ODT.

Pacing strategy run trial

During a 10 km running race, most experienced athletes would calculate the pace at each kilometre to gauge their effort. Clearly this strategy would enable the athlete to pace the run in a controlled manner rather than stopping due to excessive fatigue towards the end of the race. Given that time was displayed on the monitor, it is possible that many of the subjects were able to ascertain their pace and run within their limits rather than running faster if they felt less cardiovascular and thermoregulatory strain than the previous trial.

Inaccuracy of fluid intake during run

Cool water was ingested during the run on an *ad-libitum* basis in accordance with general instructions.

Heat acclimatisation:

Acclimatisation was not controlled prior to participation in the study. All subjects were assumed to have a certain degree of acclimatisation during the summer months of competition leading into the study.

Motivation

Motivation for some subjects to complete two ODT in hot, humid conditions approximately a week apart for a small financial reward may not have been sufficient incentive to perform maximally in the 10 km run.

Self-awareness

The subjects may have been unable to understand the differences in subjective ratings and to link these to physiological responses between trials. This may have affected the finishing time for the 10 km run.

CHAPTER 2: LITERATURE REVIEW

Physiological responses to triathlon competition

Introduction

The triathlon is a multi-disciplined sport requiring an athlete to sequentially compete in open water swimming, road cycling and road running disciplines. Triathlons are divided into several distinct categories based on the distances covered in each discipline. A sprint distance triathlon consists of a 750 m swim, a 20 km cycle and a 5km run. At the extreme end of the triathlon continuum is the Ironman triathlon, which consists of a 3.8 km swim, a 180 km cycle followed by a 42 km run. An Olympic Distance Triathlon (ODT) requires the athlete to swim 1.5 km, cycle 40 km and run 10 km. Times to complete an ODT can range from 1 hr 50 min to 2 hrs 20 min depending on the level of development of the athlete and accuracy of the measured distance.

To cover an ODT in the shortest possible time, a triathlete must possess a highly developed oxygen transport system capable of producing a high-energy output for prolonged periods. However, a triathlete is also required to perform each discipline at an optimal pace without causing undue fatigue that will influence the next discipline. Therefore, the potentially deleterious effects of swimming on cycling and cycling on running must be considered.

Aerobic capacity and performance

Numerous studies have documented the aerobic capacity (VO_2 max) of triathletes for each triathlon discipline measured during single and simulated testing protocols. In general, the highest VO_2 max values have been elicited by triathletes during treadmill running while cycle ergometry can be expected to be between 3 to 6% less (O'Toole and Douglas, 1995). Mean VO_2 max values for tethered swimming are 13-18% less than those measured in treadmill running by trained triathletes (Roalstad 1989; Schneider et al., 1990; O'Toole and Douglas, 1995). However, Pannier et al. (1980) noted that VO_2 max during cycling was 8-11% less than treadmill running in elite cyclists.

Training responses

Several authors have surmised that the amount of muscle mass involved in the task, combined with the economy and efficiency of movement, will invariably affect the maximal oxygen consumption value elicited (Roalstad, 1989; Schneider et al., 1990; O'Toole and Douglas, 1995). Therefore, the exercise mode and volume of training will have a substantial impact on the oxygen consumption values obtained in these tests. It appears that the majority of triathletes come from an endurance running background and therefore tend to elicit higher VO_2 max values running on the treadmill than for the other disciplines (O'Toole et al., 1989a). In support of this, Bunc et al. (1996) showed that young elite Czech triathletes had physiological characteristics similar to middle distance runners. However, a triathlete who was previously an elite cyclist may achieve higher VO_2 max values on a cycle ergometer than treadmill running (O'Toole et al., 1989). A similar pattern has emerged for elite swimmers. Schneider et al. (1990) noted that the highest swimming VO_2 max relative to running (99.3%) was displayed by a subject whose competitive experience was in swimming. This relationship was also evident for an experienced cyclist who obtained a VO_2 max relative to running of 99.7%. These mode-specific differences in VO_2 max values between triathletes and elite single sport athletes may be explained by the greater training volume in a particular mode which appeared to be related to the triathlete's previous athletic background (Millard-Stafford et al., 1991). In general, elite single sport athletes will achieve higher VO_2 max values on the modality that is specific to their training history.

The variability in VO_2 max elicited by triathletes can be large (Schneider et al., 1990), and low values have been reported in triathletes whose performance was exceptional. For example, O'Toole et al. (1987) reported a man with a VO_2 max of 52.2 $ml.kg^{-1}.min^{-1}$ finished the Hawaii Ironman in less than 12 hr and a woman with a VO_2 max 56.1 $ml.kg^{-1}.min^{-1}$ finished third. These data suggest that other factors besides a high VO_2 max is required to perform successfully in an ultra-endurance triathlon.

It has been suggested that VO_2 max values are also poorly related to actual triathlon performance times. In an effort to assess the relationship of the laboratory test variables and the cycle race times, O'Toole et al. (1989b) concluded that the mean intra-event cycle VO_2 value of 57.4 $ml.kg.^{-1}min^{-1}$ was inversely related to bike finish. Only intra-event peak VO_2 and cycling economy were related to bike finish. This result confirms that maximum cycling VO_2 values obtained in laboratory testing are not predictive of

performance in actual competition. In support of the data reported by O'Toole et al. (1989b), Dengel et al. (1989) reported that swimming, cycling and running times during a half Ironman distance triathlon (1.9km swim, 90km cycle, 21km run) were not significantly related to the individual event specific VO_2 max values. Environmental factors may however have contributed to these results, as dry bulb temperature was 21.1ºC at the beginning of the swim and rose to be 32.2 ºC at the end of the run. The elevated environmental conditions would have forced some of the subjects to take a more conservative approach to the race due to the increased thermal load.

Research has also shown that the time dedicated to training any one of the three triathlon disciplines has a direct relationship on the specific training responses and adaptations. Therefore, discipline specificity has a direct influence on VO_2 max values. Kohrt et al., (1987) examined the longitudinal responses of training in swimming, cycling and running, and reported that the magnitude of improvements in VO_2 max in triathletes depends on the training volume in each discipline. They concluded that their results supported the theory of specificity of training in triathletes.

It is important to note that a triathlete will spend less time training in each discipline than a single sport athlete. However, the reported discipline specific VO_2 max values are only marginally lower than those of single sport athletes (O'Toole et al., 1989; Bunc et al., 1996). This may suggest a general cardiovascular cross training effect from one sport to another. The extent to which the very specific peripheral muscular adaptations from the swim, cycle and run in a triathlete contribute to discipline specific VO_2 max remains unclear. It has been proposed that peripheral adaptations to three different disciplines can lead to greater storage and utilisation of glycogen stores while discipline specific adaptations may also have an important role in the utilisation and clearance rates of muscle and blood lactate (Noakes, 1991). Mean VO_2 max values obtained from discipline specific testing are importantly dependent on the specific training and genetic potential of the triathlete (O'Toole and Douglas, 1995).

The intra-event fractional utilisation of maximal aerobic capacity (%VO_2 max) is related to both the VO_2 max and economy of motion (Dengel et al., 1989). In an effort to determine the relationships between selected metabolic measurements and triathlon performance, Dengel et al. (1989) observed that the best indicator of success in each of the three disciplines in the triathlon was the ability to use a lower fraction of VO_2 max at a sub-maximal workload during swimming, cycling and running.

The data reported by Dengel et al. (1989) are supported by those of Sjodin and Svendenhag (1985) and Malhotra et al. (1984) who also found significant relationships between economy and performance in marathons and long distance time trialling, respectively. The results in these studies suggest that other physiological variables such as the ability to use a smaller fraction of the athlete's VO_2 max at the same workload (economy) might also be important factors in determining an athlete's success in a triathlon. These studies support the premise that the proportion of the event-specific percentage VO_2 max that can be sustained for a prolonged period is closely related to success in triathlon races of long distances.

Although the measurement of VO_2 max will often provide an insight to the endurance capacity, the metabolic measurements at sub-maximal exercise intensities have been shown to be better predictors of overall endurance performance (Sjodin and Svendenhag, 1985). The anaerobic threshold (AT) concept reflects the highest percentage of VO_2 max the athlete can sustain without metabolic acidosis or accumulation of lactic acid occurring (Wilmore and Costill, 1994). The anaerobic threshold is also often arbitrarily recognised in the research on triathlons as equivalent to either 2 or 4 millimole lactate per litre of oxygen consumed. AT represents the point at which blood lactate accumulation begins and rises exponentially. However, the detection of ventilatory threshold (VT) is the point where there is a systematic increase in VE/VO2 without a concomitant increase in VE/VCO2 (Wilmore and Costill, 1994). It is important to note that much of the literature addressing the anaerobic threshold concept use similar terminology interchangeably, although there are physiological differences between the measures.

In triathlon racing, the sustainable energy output and the lactate or ventilatory thresholds expressed as a percentage of VO_2 max can vary between the three disciplines (O'Toole and Douglas, 1995). Lactate thresholds (4 $Mmol.L^{-1}$) for triathletes have been reported to be between 72 and 88% for cycling and 80 to 85%VO_2 max for treadmill running (O'Toole et al. 1989; Khort et al. 1989) while Bunc et al. (1996) reported running VT at 82% VO_2 max for young male and female triathletes. Recently, the Maximal Lactate Steady State concept (MLSS) has been considered more important to success than VT and LT, as VT and LT represents an intensity lower than what is maintained during competition (Jones and Carter 2000). The MLSS is the highest attainable intensity where blood lactate concentration does not accumulate and rise exponentially over time and importantly, without an appreciable decrement in performance. Carter and Jones (2000)

consider this the 'gold standard' measure of endurance exercise capacity. However, to date there have been no studies focused on the measurement and determination of MLSS during simulated laboratory conditions, or in the field, for triathletes.

A triathlete's ability to compete at a high percentage of their VO_2 max is a major determinant of successful endurance performance (De Vito et al., 1995; Zhou et al., 1997). The velocity, or pace, at this percentage is also a crucial determinant of successful endurance performance. However, the energy demands between two athletes, with the same VO_2 max value while competing at the same cycle velocity, or running pace, can be quite different. At any given sub-maximal speed, whether it is swimming, cycling or running, the athlete with greater economy of movement consumes less oxygen to perform the task (McArdle, Katch and Katch, 1991). Better economy can be considered to be an advantage to endurance performance (Jones and Carter, 2000).

Not only is it important to be skillful in each of the disciplines, during the triathlon both cycling and running will be affected by the accumulation of residual neural fatigue and thermal and cardiovascular strain from the previous discipline. Fatigue and the resultant reduction in coordination (force/power) and muscle strength will increase energy demands and thus have an impact on economy. Indeed, Dengel et al. (1989) concluded that the important determinants of success during a triathlon were good economy during swimming and running.

Most studies have focused on the physiological characteristics of successful triathlon performance measured in the laboratory while a few have assessed the relationship between a triathlete's race performance and any single or a combination of physiological variables. Schabort et al. (2000) set out to predict race time from laboratory testing in national French triathletes four days after they had competed in an ODT. All triathletes underwent a maximal swimming test, an incremental cycling test until exhaustion and a maximal running test. Data pertaining to submaximal steady state measures such as oxygen uptake, blood lactate and heart rate were also measured during the cycling and running tests. Schabort et al. (2000) concluded that the five most significant ($P<0.01$) predictors of triathlon performance were blood lactate measured at steady state cycling at 4 $W.kg^{-1}$ BM ($r = 0.92$), blood lactate while running at 15 $km.h.^{-1}$ ($r = 0.89$), peak power output while cycling ($r = 0.86$), peak treadmill velocity ($r = 0.85$) and VO_{2peak} during cycling ($r = 0.85$).

In summary, most studies conducted on triathletes are directed towards testing the capacity of the oxygen transport system as a single measure of aerobic performance. These single test studies have shown that triathletes have VO_2 max values comparable with single sport endurance athletes. It has been reported that the VO_2 max values from a heterogenous group of recreational to moderately trained triathletes correlates highly with success and overall finish times for short distance triathlons including ODT. However, when studying a homogeneous group of elite athletes other variables appear to influence performance than VO_2 max (Schabort et al., 2000). Importantly, a high oxygen uptake does not ensure triathlon success. It has been shown that other factors associated with performance should be considered. They include: 1) anaerobic threshold 2) fractional utilisation of VO_2 max and 3) economy of effort. This suggests that physiological factors other than maximal oxygen uptake may play a greater role in racing success. Although it is important to highlight the aerobic parameters that play a part in endurance performance, the ability of the athlete to be able to generate ATP anaerobically for sprint finishes and surges (a pace faster than an athletes' average velocity for the distance) during the race are also crucial factors to success.

Factors limiting triathlon performance

The decrease in economy of motion in the sequential triathlon disciplines has been the subject of many studies. Most of the research has been directed at the influence of prior swimming and cycling on the biomechanical and cardiovascular responses that occur during running.

Kreider et al. (1988b) concluded that successful triathlon performance seems to be affected by the extent to which a high percentage of VO_2 max could be sustained in the face of the decreased performance economy. It appears that regardless of the physiological variables attained in the single test of each discipline, the introduction of sequential disciplines increases the physiological cost and reduces the performance efficiency of the subsequent discipline. Therefore, the triathlete will invariably start the cycling and running discipline event under some physiological stress. These stresses may change depending on the fitness level of the athlete and specific training in event-to-event transition (Kreider et al., 1988b).

There have been several studies that have examined the effects of sequential disciplines on athlete function during a triathlon. Marino and Goegan (1993) recorded a 54%

increase in the mechanical work (624.4 J vs 395.1 J) after running 9 km following a 40 km bike race compared to a run only condition. They also noted that stride length was the differentiating factor in the lower running velocities (-38%) after the 40 km bike race. This also led to greater internal mechanical work (reduction in efficiency) following a 40 km bike race than in the run only condition. Millet and Vleck (2000) reported that a more forward leaning trunk influenced by sensorimotor perturbations could affect running economy and thus energy cost, post-cycling. Schneider and Pollack (1991) also reported similar residual effects of cycling on running when comparing the control group with the sequential group.

However, Hue et al. (1997) observed no differences in biomechanical variables such as stride length and frequency during running following cycling. In keeping with the previously mentioned studies, Hue et al. (1997) also noted that the oxygen cost was greater in the run with the cycle/run treatment compared with the run alone. The increase in oxygen consumption, or reduction in economy induced by a preceding period of exercise, has been supported by other studies in the literature (Kreider et al., 1988; Guezennec et al., 1995). The increase in VO_2 cost following cycling could be associated with changes in thermoregulation, increased dehydration and the corresponding reduction in plasma volume. As a result of these factors, heart rate will increase and an upward drift in ventilation and oxygen consumption will result (Guezennec et al., 1995).

In an effort to minimise the effects of cycling on running, Kreider et al. (1988a) directed the triathletes to begin the run at a lower intensity during the first ten minutes (55 to 65 to 75 % VO_2 max) and then progressively increase running speed to 85% of VO_2 max. This strategy appeared to minimise the residual effect of prior cycling. In support of the previous study by Kreider (1998a), Danner and Plowman (1995) concluded that the 45 min of cycling at 70% VO_2 max significantly decreased running efficiency as indicated by a higher VO_2 cost. This is also supported by Hue et al. (1997), who suggested that the VO_2 increase could be of an “organic system” origin due to dehydration and hyperthermia (cardiac drift).

The difference in the increase in energy cost of running with and without cycling beforehand can also be dependent on the standard of the triathlete. Millet et al. (2000) noted a significant difference between middle level (regional and national level) and elite triathletes (three in World top 12 while others in World top 50) in the cost of running after the fatiguing effects of cycling. The cost of running decreased by 3.7% for the elite

triathletes while the middle level triathletes had an increase in the energy cost of running of 2.3% following cycling. They concluded that energy cost differences may be due to the fatigue of inspiratory muscles and alterations in muscle tendon stiffness and may be related to training status (Millet and Vleck, 2000). Ultimately, run times alone, when compared to various combinations of swim and/or cycle beforehand, have been affected by biomechanical factors together with cardiovascular and thermoregulatory strain (Hausswirth et al., 1999).

Heat gain and/or thermoregulatory strain begins as early as the swim and consequently affects the cycle and run performances (Kreider et al., 1988b). Only a few studies have focused on minimizing the heat load prior to cycling. Chadwick et al. (1998), simulated an ODT in 32°C and 65% RH and found that even with the use of a wetsuit, swimming in relatively warm water did not influence the thermoregulatory responses of the triathlete on the subsequent cycling and running stages compared with a normal swimming suit. Although it was found that core temperature was not significantly different, mean body temperature and mean skin temperature were higher in the wet suit. Trappe et al. (1995) reported similar findings when swimming in a wet suit compared with conventional competitive swimming suit in water ranging from 20.1 to 25.6 °C.

Biomechanical differences between cycling and running coupled with changes in muscle activation due to fatigue should also be considered as limiting factors in performance during the run. Witt (1993) documented the change of electromyographic (EMG) activity from running to cycling and then to running again. Witt (1993) concluded that even though contraction type and muscle length were considerably different between the two disciplines, the muscle activation pattern during running after cycling was disturbed to the point that instabilities in running frequency were observed. Importantly, when compared to the first run, the EMG activation pattern from the running to the cycling did not return to normal values throughout the second run. Although there is some evidence that an overlap in musculature used for cycling and running exist, biomechanical and EMG analysis suggests that ranges of motion, lengths of muscles and type and speed of contraction are different between the two modes of exercise (O'Toole and Douglas, 1995). This could possibly account for some of the decrease in running efficiency observed previously.

Miura and Matoba (1998) observed an increase in oxygen uptake, heart rate and shorter stride length as well as biochemical disturbances with sequential disciplines. They

concluded these perturbations indicated decreased running economy when cycling was performed prior to the run.

Clearly much research suggests that specific transition training is a fundamental component of the triathlon. The ability to successfully combine the cycle and run section through a smooth transition from one exercise mode to another results in improved running economy and ultimately, less physiological and sensory fatigue.

Thermoregulatory response to a triathlon

Heat production from physical exertion in a hot, humid environment can exceed the body's capacity to dissipate heat. Ultimately, optimal performance is compromised unless strategies are undertaken to minimise the resultant effects of dehydration and thermal strain. It must be appreciated that the combination of three disciplines in series will have an accumulative effect on core temperature. Kreider et al. (1988a) while researching the cardiovascular and thermal responses of triathlon performance also concluded that triathlon performance elicited cardiovascular and thermal adjustments not experienced when performing the disciplines separately.

In another study, Kreider et al. (1988b) reported significant cardiovascular and thermoregulatory stress while monitoring the cardiovascular and thermoregulatory responses to control versus simulated triathlon performance. In the simulated triathlon the subjects performed a 0.8 km swim in a pool at competition speed, cycled for 75 min (70% of VO_2 max,, over approximately 40km at an average of 191 Watts) and then ran for 40 min (70% of VO_2 max at a mean triathlon 10km time). Pool temperature was approximately 23°C while the cycling and running were performed in a temperature controlled room at 29°C with fans to cool the athletes. These data were compared with control cycling and running at the same intensities as those previously cited. During the simulated triathlon, less work was performed in the cycling session (17% or 32 W reduction). Briefly, swimming beforehand precipitated a lower mean ventilation (5%), lower mean VO_2 (5.5%), lower mean %VO_2 max (4%), lower mean stroke volume and cardiac output, and a higher rectal temperature in the simulated cycling session when compared with the control session. Overall, as the simulated triathlon cycling session continued the subjects could not maintain the control cycling work output. Kreider et al. (1988b) concluded that the rise in core temperature elicited in the swim had apparently induced the thermoregulatory and cardiovascular adjustment to become evident earlier

during the triathlon cycling session. These adjustments precipitated less work output in order to minimise cardiovascular and thermal stress in the cycle and the ensuing run. In the later stages of the run several athletes experienced heat complications exhibited by complaints of abdominal and/or leg cramps.

Guezennec et al. (1995) reported a mean body mass loss due to fluid loss of two litres for the ODT (swimming = 800 g, cycle = 200 g, run = 1000g) while running alone only precipitated a BM loss of 600 g. There was also a significantly higher mean haematocrit value at the end of the ODT than the run alone. This suggests a progressive haemoconcentration throughout the triathlon and a resultant decrease in plasma volume. Plasma volume had decreased at the end of the triathlon by 14.43% while the control run only incurred a 6.75% decrease in plasma volume.

However, lower haematocrit may have also been associated with better finishing times with some triathletes. Nagao et al. (1992) suggested that expanded blood volumes in triathletes could augment the improved efficiency of the heart and in combination, improve movement economy in all modes during a race. This suggests that plasma expansion or hyperhydrating to transiently reduce the haematocrit could improve performance via the stabilising of heart rates and maintenance of core temperature.

Summary

In summary, success in a prolonged endurance event such as a triathlon requires an optimal combination of biomechanical and physiological variables. Nevertheless, triathletes undergo unique cardiovascular, neurological, thermoregulatory and haemodynamic changes not experienced by the single discipline athlete. These changes have the effect of reducing mechanical efficiency and increasing the physiological cost of the next discipline. Only the swimming discipline remains unaffected. It is also important to note that thermoregulatory and associated cardiovascular responses to a triathlon, in combination with environment conditions, play an ever-increasing role in determining performance outcome (Hausswirth et al., 1999). Indeed, research has established that exercise in the heat and the resultant physiological responses such as hypohydration and hyperthermia, are limiting factors to performance (MacDougall et al., 1974; Nadel et al., 1980; Armstrong et al., 1985; Candas et al., 1988; Sawka and Pandolf, 1990; Walsh et al., 1994; Murray, 1996; Hargreaves and Febbraio, 1998; Sawka and Coyle, 1999). Therefore, any strategies that minimise the affect of hypohydration and reduce the chance

of hyperthermia will have major benefits in the final stages of the ODT, namely the 10 km run.

Physiological responses to exercise in the heat

Heat production

Maintenance of body heat is essential for life. This rate of heat production is proportional to the metabolic rate and can be affected by sympathetic stimulation, hormones, body temperature and exercise (McArdle, Katch and Katch, 1991). Of all the mechanisms that increase the metabolic rate, and thus heat production, it is exercise than can have the most dramatic effect.

The metabolic rate can increase up to 5-20 times that of basal level during exercise (McArdle, Katch and Katch, 1991). In spite of this large increase in the metabolic rate, less than 25% of all energy produced by the contracting muscles is used to produce work with the remaining 75% appearing as heat in the muscles (Åstrand and Rodahl, 1986). The heat produced by the working muscles must be dissipated to restore body heat balance. If this heat transfer exceeds the body's capacity to remove heat, core temperature can rise to life threatening levels. Normal resting core temperature can range between 36.8 - 37.4 °C however, when exercising core temperature reaches 44 - 45 °C, death usually ensues (Hughson, 1992).

As a consequence of the generation of heat, exercise would last a short time if it were not for the well-developed ability of the body to dissipate this thermal load. Regulation of body temperature and fluids are processes critical not only to training and competition but also for survival. Many deaths have occurred in sports or activities where athletes have suffered hyperthermia and heat stroke (Sawka and Pandolf, 1990). Therefore, maintenance of body temperature and fluid balance is central for optimal cardiovascular and thermoregulatory function.

Regulation of temperature

Responses to exercise and heat

Body temperature is regulated by mechanisms that attempt to keep heat production and heat loss in balance. The area responsible for this control is the anterior portion of the hypothalamus called the pre-optic region. It is this area that controls the loss of heat and heat promotion (Tortora and Anagnostakos, 1987). Neurons in the pre-optic area fire either rapidly or slowly depending on the increase or decrease in blood temperature and information from peripheral thermal receptors in the skin. Impulses are sent to either the heat-losing or heat-promoting centre. The heat loss centre is mainly driven by parasympathetic nervous stimulation while the heat promoting centre is driven primarily by the sympathetic nervous system (Tortora and Anagnostakos, 1987). In the case of an increase in heat by either exercise and/or combined with environmental heat, rapid impulses from the pre-optic region stimulate the vasodilation of blood vessels and the activation of the sweat glands to produce more perspiration.

At the onset of exercise, heat is generated via an increase in metabolism and muscle activity. The rate of muscle heat production is also a function of exercise intensity (Astrand and Rodahl, 1986; Nadel, 1992), the environmental temperature and the relative contributions of evaporative and dry heat exchange (Sawka and Pandolf, 1990).

When heat produced by exercise exceeds the body's ability to remove heat, the body's core temperature will increase. There are four mechanisms by which heat loss is directed from the core to prevent over-heating and hyperthermia: evaporation, convection, conduction and radiation. The two most basic mechanisms by which excess heat is dissipated are: 1) dramatic increase in peripheral skin blood flow increasing heat transfer from the core to the shell and 2) the activation of sweat glands with the evaporative of sweat taking heat from the body and causing an evaporative heat loss (Åstrand and Rodahl, 1986).

Skin is the primary organ for heat exchange. During exercise as much as 75% of the heat loss is achieved by the evaporation of sweat on the skin. The redistribution of blood from central to peripheral circulation facilitates the removal of heat from the core. In extreme heat stress, 15-25% of the cardiac output passes through the skin (McArdle, Katch and Katch, 1991). The increased peripheral skin blood flow will consequently reduce central blood volume (BV) and thus stroke volume (Fortney and Vroman, 1985). This will have a

direct influence on the heart rate as it will drift upward in direct response to the continual drop in central BV (Sawka et al., 1985; Rowell, 1986). Sweating promotes fluid losses while reductions in BV cause hypovolemia. With the shift of blood flow to the skin away from the muscle to dissipate heat, the average arteriovenous oxygen difference as well as VO_2 max can be reduced (Caldwell et al., 1984). Muscle and blood lactic acid levels as well as accelerated liver and muscle glycogenolysis have also been documented to increase due to the redirection of blood flow to the skin surface to dissipate heat to the environment and the reduced blood flow to the liver (Febbrario et al., 1994; Hargraves et al., 1996; Moquin and Mazzeo, 2000).

However, when the ambient temperature is high and blood volume is decreased by sweat loss during prolonged exercise, the body has great difficulty meeting the demands for high blood flow to both muscle and skin. As a result, skin blood flow is likely to be compromised to allow central venous pressure and muscle blood flow to be maintained but as a consequence there is reduced heat loss which causes the core temperature to rise to life threatening levels. At this point, the body is programmed to protect central blood volume (CBV) and cardiovascular function at the expense of thermoregulation (Murray, 1992). Ultimately, cardiovascular demands will take precedence over thermoregulatory demands (Rowell, 1983).

The maintenance of blood pressure (by peripheral vasoconstriction), CBV and core temperature are crucial to the ability to perform for prolonged bouts of exercise in the heat. When the cardiovascular demands of exercise override the need for thermoregulation, heat dissipation can decline and subsequently core temperature may increase to a limit at which performance will begin to decline (Nadel, 1992). To add to the thermal strain, exercise in hot and humid environments hampers evaporative cooling due to the high vapour pressure of ambient air. As a consequence, hyperthermia appears to be the critical determinant of exercise performance in the heat when hypohydrated (MacDougall et al., 1974; Armstrong et al., 1985; Hargreaves and Febbraio, 1998). However, it is assumed that PV expansion induced in response to heat acclimatisation will partially compensate for the peripheral displacement of blood volume that accompanies exercise in the heat and this will subsequently allow greater maintenance of cardiac output (Nose et al., 1990).

An improved ability to dissipate heat, via an expansion of PV allowing greater heat loss through increased skin blood flow, will lead to reduced heat storage. This will result in

decreased rectal temperature and may lead to reductions in HR as HR and rectal temperature have been found to be closely linked (Pichen et al., 1988). Thus any strategies that minimise the rise in core temperature and maintain BV during exercise in the heat are likely to contribute to cardiovascular and thermoregulatory stability and enhanced performance (Hargreaves and Febbraio, 1998).

The endocrine system

The endocrine system plays a major role in balancing body fluids and monitoring changing fluid levels. Total Body Water (TBW) represents approximately 50 – 70% of body weight while 66% of body fluid is located in the intracellular fluid (ICF) and 33% is located in the extracellular fluid (ECF). The ECF can be further divided into interstitial and plasma spaces. Stability in the various fluid compartments provides the basis for homeostasis however, not all fluid volumes are static but are in constant flux from one compartment to another (Sawka and Coyle, 1999). Exercise, heat exposure or both, can modify the net volumes and water turnover rates between fluid compartments. Hormones that mediate total body plasma levels, electrolytes and blood pressure thus regulate body fluids and core temperature include anti-diuretic hormone (ADH) and aldosterone (ALD) (Guyton 1991).

ADH promotes water conservation by increasing the water permeability of the kidneys thereby reducing excretion of urine. During exercise in the heat, ADH is stimulated by the increase in blood plasma osmolality brought on by a decrease in TBW and the concentration of electrolytes. Both sodium and potassium, and their principle anion (chloride) are primarily responsible for the elevated plasma tonicity (hyperosmolality) during hypohydration (Sawka, 1992). Aldosterone, and the associated renin angiotension system, is an arteriolar vasoconstrictor that increases peripheral resistance and thus increases blood pressure plus sodium retention and water conservation (Tortora and Anagnostakos, 1987).

Atrial naturetic peptide (ANP) opposes the renin-angiotensin-aldosterone system. ANP is released in response to an increase in blood volume and pressure and as a result it inhibits the release of renin from the juxtaglomerular apparatus while also directly reducing the aldosterone release from the adrenal glands. The effect of these actions decreases sodium re-absorption and lowers blood pressure and volume. ADH, ANP, aldosterone and renin angiotesin regulate the kidney's ability to maintain homeostasis. The combined actions of

these hormones minimise the risk of hypohydration during heavy sweating and prolonged bouts of exercise. These hormone systems contribute to renal and sweat gland retention of fluid and electrolytes during exercise (Tortora and Anagnostakos, 1987). Increases in ADH and ALD to protect BV, BP and cardiac output (Q) have been shown to occur in hypohydrated subjects (Nadel et al., 1980) while fluid ingestion has attenuated ADH responses (Armstrong et al., 1994).

Physiological responses to heat

Factors that can effect performance

Acclimatisation

Acclimatisation refers to the process of change to which an individual adapts to a new environment and initiates mechanisms that enable them to cope effectively (Gunning, 1994). Repeated exposure to hot environments coupled with exercise results in reduced thermal and cardiovascular strain and improved capacity for exercise (Armstrong and Maresh, 1991). The improved tolerance to exercise in the heat is known as heat acclimatisation. The primary benefit of heat acclimatisation results from metabolic, biochemical, heamatologic and cardiovascular adaptations (Armstrong, 1998).

The majority of literature reporting the changes associated with heat acclimatisation after 14 days of strenuous exercise in hot conditions has observed: lower resting core temperature (Buono et al., 1998), lower core temperature threshold at the at onset of sweating, increased heat loss via radiation, increased PV, decreased heart rate, decreased core temperature during exercise (Sawka et al., 1983), decreased skin temperature, increased sweat rate (Nadel et al., 1974) altered metabolic fuel utilisation, increased maximal oxygen consumption, improved exercise economy and increased sympathetic nervous system overflow (Sawka and Pandolf, 1990; Armstrong and Maresh, 1991). Early adaptation (five days) includes improved cardiovascular function, plasma volume expansion, reduced heart rate, and increased peripheral/muscle blood flow (Armstrong and Maresh, 1991). Ratings of perceived exertion also decrease due to the proportional reduction in thermoregulartory and CV strain.

The rate of decay of these adaptations is affected by the number of exposures per week, the number and format of training sessions and the degree to which core body

temperature is elevated. Individuals with a high VO_2 max will lose heat acclimatisation adaptations slower than untrained individuals with a low VO_2 max (Armstrong, 1998).

Physical Training

Physical training has been found to offer a 'partial heat acclimation' (Armstrong and Maresh, 1991) therefore training induced adaptations are similar to those observed during heat acclimatisation (Terrados and Maughan, 1995). As a consequence, a trained individual will respond better than an untrained individual to heat stress.

A trained subject will have a higher initial PV than untrained (Sawka et al., 2000). This enables greater fluid losses before PV drops below pre-training levels (Fellman, 1992). This suggests that total body water (TBW) will take longer to drop in trained than untrained subjects before a level where cardiovascular and thermal regulation efficiency is compromised. Thus it would appear that trained individuals have a distinct advantage compared with untrained and the physiological responses to exercise in the heat.

Armstrong (1998) observed individuals with high VO_2 max (>60 $ml.kg^{-1}.min^{-1}$) exhibit superior heart rate and rectal temperature responses and reach a stable heat acclimation state faster than an individual with a low VO_2 max (40 $ml.kg^{-1}.min^{-1}$). Individual variability as well as the level of regular physical training (Pandolf et al., 1988) are other factors that must be considered when examining heat acclimatisation. Wells et al. (1987) noted that cardiovascular conditioning and acclimatisation results in lower core temperatures, elevated sweat rates, and increased heat tolerance in both trained men and women.

Age

The majority of studies reporting differences observed between young and the elderly in terms of heat regulation may only reflect the reduction in training volume, and lower maximal VO_2 max and not changes due to ageing (Armstrong, 1998; Kenney, 1993). Kenney (1993) concluded that the ability to exercise in hot climates is less a function of chronological age than functional capacity (VO_2 max) and physiological health status. However body size can also be a factor affecting water turnover as TBW is markedly affected by body composition (ie lean body mass vs fat mass) (Sawka and Coyle, 1999). Therefore, differences between men and women and adults and children and aged in reference to TBW and body composition should be acknowledged.

Gender

Men sweat at a greater rate than women with the same exercise type, intensity and environmental conditions (Rehrer and Burke, 1996) while possessing a higher surface area to BM ratio (Kenney, 1985). Females tend to produce less sweat than men for a comparable heat exercise load (efficient sweaters) and have more heat activated sweat glands per unit skin area than men. Although women start sweating at higher skin and core temperatures, heat tolerance is similar with men when compared with relative loads in a hot environment (Åstrand and Rodahl, 1986).

The menstrual cycle (MC) represents another factor that can influence temperature regulation independent of exercise for women. Generally, changes in basal body temperature usually confirm ovulation. This is generally a drop in core temperature by 1°C while rises of up to 0.5 °C in core temperature during the second half of the MC (luteal phase) from the time of ovulation to menstruation have been noted (Frye and Ely, 1996).

Other studies have shown varied thermoregulatory responses during different MC phases. Grucza et al. (1993) noted a small increase in rectal temperature at the follicular phase (FP) in rectal temperature and significantly greater values in mean skin, mean body temperature and heart rate than in the luteal phase (LP) but no difference in sweat rates. The oral contraception pill (OCP) has also been shown to induce an upward shift in the threshold for heat loss responses and increased the body core temperature at rest and during exercise (Rogers and Baker, 1997). Further research is needed to confirm changes in the thermoregulatory and cardiovascular responses during exercise in the heat during MC and OCP ingestion.

Dehydration/Hypohydration

Dehydration is defined as the process of dynamic body water loss or the transition from euhydration to hypohydration. Hypohydration, or a reduction in total body water (TBW), has been shown to increase thermal strain and heat storage during exercise (Sawka et al., 1985). The greater heat storage is compromised by the reduced sweat rate (thus less evaporative heat loss) and reduced skin blood flow for a given core temperature (Sawka, 1992). Studies investigating the effects of hypohydration have shown that hypohydration affects several physiological responses such as decreased plasma volume, increased

exercise heart rates (cardiac drift), reduced stroke volume, increased core temperature during exercise and a reduction in maximal exercise capacity (Nadel et al., 1980; Candas et al., 1988; Sawka and Pandolf, 1990; Walsh et al., 1994; Murray, 1996; Sawka and Coyle, 1999).

Nadel et al. (1980) observed that a reduced cardiac output (Q) at a given intensity of exercise while hypohydrated resulted in either greater oxygen extraction and/or greater anaerobiosis in the contracting muscles. Nadel et al. (1980) also noted that splanchnic and renal blood flow are also compromised proportional to the increase in exercise intensity, and this relationship was shifted to lower intensities during exercise in the heat.

It appears that much of the literature on hypohydration is focused on heat storage and dissipation and the relationship this has to core temperature, sweat rate and plasma volume. Small changes in the body's ability to deal with heat, and exercise, will ultimately affect performance.

Core temperature

When heat produced by exercise exceeds the body's ability to remove heat, the core temperature will increase. Sweating is critical to the maintenance of core temperature. However, during exercise induced thermal stress the decrease in TBW via the sweating mechanism can compromise the body's inability to dissipate heat. Core temperature is also highly dependent on the state of hydration (Sawka et al., 1985; Montain and Coyle, 1992). Both hypohydration and losses in TBW can result in hyperthermia (Montain and Coyle, 1992). Walsh et al. (1994), however, noted no changes in core temperature during exercise while hypohydrated. The lack of difference in core temperature between subjects in this study may also be explained by the low level of dehydration experienced by the subjects (1.8%). In contrast to these findings, Pichen et al. (1988) observed that the greater the dehydration the more elevated the oral (core) temperature. In general, a number of studies have noted that body mass decreases due to exercise induced dehydration of up to 5% are only associated with increases in rectal temperature of 1º C or less (Greenleaf and Castle, 1971; Hamilton et al., 1991; Montain and Coyle, 1992).

It is also important to recognise that reductions in rectal temperature induced by heat are considered a classic sign of heat acclimatisation. Resting and sub-maximal rectal temperature can be accompanied by a reduction in the absolute rise in rectal temperature

(Hahn et al., 1986). Therefore any reduction in the resting rectal temperature will provide a subsequent drop in the peak rectal temperature by the same margin. This suggests a physiological lowering of the "setpoint" of the body (Hahn et al., 1986) and greater heat storage capacity. Eventually, the rise in core temperature as a result of an increased intensity of exercise coupled with environmental conditions becomes a limiting factor in performance (MacDougall et al., 1974).

Sweat responses

Sweating is a vital thermoregulatory response that occurs at the expense of intra- and extra-cellular fluid (plasma). If fluid intake does not match sweat loss, sweating results in dehydration, which in turn will adversely affect CV and thermoregulatory responses (Murray, 1992). Individual sweat rates and fluid losses vary widely. Body size, gender, individual differences in sweat gland size and number, the rate of sweat production, the training and fitness level of the individual, exercise intensity, environmental conditions and individual metabolism all influence sweat rate (Rehrer and Burke, 1996). While some individuals exhibit sweat rates of up to 2-3.7 $L.hr^{-1}$ (Armstrong et al., 1986), sweat rates of 1 $L.hr^{-1}$ are more common (Sawka, 1992). Although sweat losses constitute the main response to an increase in metabolic heat and therefore reduction in TBW, insensible losses via respiration must also be considered where possible. Mitchell (1972) calculated that physically active individuals lose 2-5 $ml.min^{-1}$ from the respiratory tract during strenuous exercise.

Hypohydration has been shown to increase sweating threshold, core temperature and decreased sweating sensitivity in a graded manner with increased body water loss (Montain et al., 1995). Therefore the sweat rate is lower for a given core temperature, and the potential for heat dissipation via sweat evaporation is reduced (Sawka and Pandolf, 1990). This is mainly due to the proportional increase in core temperature relative to the body water loss experienced during exercise induced heat stress.

Studies have also shown that the threshold and sensitivity for both sweating and skin blood flow are altered by hypovolemia and hyperosmolality (Candas et al., 1988; Sawka and Pandolf, 1990). Both hypovolemia and hyperosmolality increase with hypohydration, while prolonged exercise in the heat without hydration has been shown to lead to hyperosmotic hypovolemia. Hypohydration has been shown to increase average sweating threshold by 0.06°C for each percentage of body water loss (Sawka and Pandolf, 1990).

Sweat losses will reduce plasma volume while the associated affect of hypohydration will reduce the ability for the body to sweat effectively to minimise heat gain.

Plasma volume

The reduction in plasma volume due to sweating negatively affects performance during exercise in a hot environment. Studies have shown that as much as 10 – 20 % or greater drop in blood plasma can occur during prolonged exercise (Robinson et al., 1995). Sweating increases the fluid being drawn from the interstitial space to maintain heat dissipation while the osmotic pressure within the muscle and the build up of accumulated metabolites such as lactate, continually attracts more fluid (Sawka et al., 2000). As a result, some fluid is also lost from the intracellular compartment to make up the fluid loss. Red cell shrinkage is consistent with hyperosmotic plasma changes during exercise (Sawka et al., 1984; Dengal et al., 1992) as intracellular water contributes to the interstitial component to maintain blood plasma. Sawka (1992) suggests that at low volumes of body water loss, the deficit primarily comes from the ECF but as these water losses increase, a proportionally greater percentage of the water deficit comes from the ICF compartment.

Mechanisms that minimise further loss of plasma include the increase of plasma protein osmotic pressure, differences in peripheral vasoconstriction in active and inactive tissues and elevated lymph flow (Convertino, 1987). ADH and ADL also contribute to the retention of fluid and maintenance of BV. Other factors that have been suggested to maintain PV during exercise include: metabolic water production (Pivarnick et al., 1984; Sawka, 1992), release of water complexed to glycogen (Sawka, 1992) and water redistribution from skeletal muscle (Sawka, 1992). The interaction of these factors provides adequate circulating BV to meet both the cardiovascular and thermoregulatory demands of exercise.

Hypovolemia

Exercise induced increases in core temperature and the decreases in the interstitial fluid associated with sweat losses, induce plasma hypovolemia. Hypovolemia reduces TBW and thus compromises cardiovascular function (reduction in SV, frequency and Q) and thermoregulation (reduced sweat rates and reduction in threshold) during exercise (Convertino, 1987; Sawka, 1992). This has an effect of increasing plasma osmotic

pressure (hyperosmolality) in proportion to the decrease in TBW while resting (Sawka et al., 2000). Hypovolemia coupled with the shift of blood flow to the skin effectively reduces the central venous pressure and cardiac output. This has a major impact on the ability to maintain and support metabolism and thermoregulation during exercise heat stress (Sawka, 1992).

Studies have shown that as much as 7 – 12 % or greater reduction in blood plasma can occur in prolonged moderate exercise over several days (Convertino et al., 1980). It has also been shown that PV is restored to baseline levels after 10-20 min of cessation of exercise (Senay, 1979).

Exercise has a transient effect on plasma volume and restoration of PV is highly dependent on the length or duration of the exercise bout, coupled with fluid intake.

Hypohydration and performance

Exercise and the likely onset of dehydration affect cardiovascular and thermal function. The majority of studies have shown impairment in exercise performance even at low to moderate levels of dehydration ranging from 1-5% reduction in BM (Sawka et al., 1985; Armstrong et al., 1985; Walsh et al., 1994; Moquin and Mazzeo, 2000). It has been observed for every 1 litre reduction of body water due to sweat there is an corresponding increase in exercise heart rate by eight beats, a reduction in cardiac output by 1 L per minute and an increase in core temperature by 0.3° C (Coyle and Montain, 1992). Hyperthermia appears to be the critical determinant of exercise performance in the heat when hypohydrated (MacDougall et al., 1974; Armstrong et al., 1985; Hargreaves and Febbraio, 1998) while cognitive performance has been shown to be adversely influenced by body water deficits (Gopinathan et al., 1988).

Montain and Coyle (1992) investigated the influence of graded dehydration on hyperthermia and cardiovascular drift during exercise with endurance cyclists exercising at 62-67% VO_2 max for two hours in 33°C, 50% RH. The four treatments during exercise were, no fluid (NF), small, moderate and large amounts of fluid, which equated to 20, 48 and 81% of the fluid lost in sweat while exercising, respectively. There was a decline in BM of 4.2, 3.4, 2.3 and 1.1%, respectively, due to the treatment at the completion of the exercise. Montain and Coyle (1992) concluded that the magnitude of increase in core temperature and HR and the decline in SV are graded in proportion to the amount of

dehydration accrued during exercise. Increasing fluid intake during exercise attenuates the elevations in osmolality and sodium concentration rather than expanding PV. Fluid ingestion attenuates hyperthermia by promoting greater skin blood flow with the greatest blood flow to the skin occurring with the larger volumes of fluid ingested.

Oxygen transport and uptake have been observed to be contributing factors to the decrement of performance in a hypohydrated state during exercise for some studies but not for others (Armstrong et al., 1985; Dengal et al., 1992; Walsh et al., 1994; Hargreaves et al., 1996; Moquin and Mazzeo, 2000). In general, it appears that a fluid deficit equivalent to 3% BM is necessary to induce a decrement on VO_2 max (Sawka and Pandolf, 1990). Caldwell et al. (1984) also suggests that increases in blood viscosity due to a fluid deficit of 3% BM may alter the pulmonary ventilation perfusion balance and thus, limit maximal oxygen uptake.

Moquin and Mazzeo (2000) examined the effects of dehydration on the lactate threshold and performance time to exhaustion in moderately trained women. They observed that the lactate threshold (LT) occurred at a significantly lower relative percentage of VO_2 max when dehydrated even though the difference in blood lactate concentration at LT between trials was not significant. The lower exercising percentage VO_2 max also decreased the time to exhaustion at a low level of dehydration (1.5%) compared with euhydrated controls. Moquin and Mazzeo (2000) also noted lactate concentrations correlated highly with norepinephrine and epinephrine concentration with both hydration trials and these changes may have contributed to the shift in the lactate threshold. In agreement, Hargreaves et al. (1996) found that dehydration resulted in a greater rate of muscle glycogen utilisation compared with rehydration however, the increase in circulating epinephrine levels did result in greater lactate production during dehydration. Febbrario (2001) in reviewing the literature in the alterations in energy metabolism during exercise and heat stress, found that the majority of studies have shown an increase in muscle glycogen utilization and blood lactate production while exercising aerobically and anaerobically during heat stress.

In contrast, Dengal et al. (1992) found no alteration in cardiorespiratory or blood lactate responses with hypohydration of up to 5.6% caused by exercise and diet manipulation. Small but statistically nonsignificant differences were noted in time to exhaustion in favor of euhydrated over hypohydrated levels of 3 and 6% of BM. The short duration test protocol used in a thermoneutral environment could have attributed to these blunted

responses. It is important to note that plasma glucose levels were significantly elevated at a hypohydration level of 5.6%. Dengal et al. (1992) suggest that this may be due to an increase in circulating catecholamine concentration and the effects of stimulating glucose production in the liver by increasing glycogenolysis. However, blood lactate levels were no different regardless of hydration status suggesting glucose utilisation may have decreased due to the inhibition of insulin mediated glucose uptake by muscles and adipose tissue.

In a benchmark study correlating decrements in performance with dehydration, Armstrong et al., (1985) investigated performance in 1,500, 5,000 and 10,000 m with hypohydration levels of 1.6, 1.9 and 2.1 % of BM. The levels of dehydration resulted in a corresponding reduction in plasma volume of 9.9, 12.3 and 9.9%, respectively. Running performance times decreased by 5% for 5,000 and 10,000 m and 3% for 1,500 m. Armstrong et al. (1985) concluded that even small amounts of dehydration (exercise induced) would augment hyperthermia in warm environments. This conclusion is consistent with a study by Walsh et al. (1994) in which a low (1.8%) level of dehydration resulted in a 30% decrement in time to exhaustion during high intensity cycling at 90% VO_2 max after cycling for 60 min at 70% VO_2 max. Walsh et al. (1994) also observed a significant increase in RPE and ADH associated with the dehydration in the no fluid trial. The provision of fluid prior to and during the trial in which performance was enhanced was due to the decreased RPE and not from reductions in any of the physiological parameters reported. These findings suggest that fluid ingestion, compared to no fluid, may positively alter cognitive and sensory aspects more so than physiological responses when exercising to exhaustion. Decreases in maximal physical work capacity due to a 3-5% BM loss induced by exercise, diuretic, or sauna bathing, was also observed by Caldwell et al. (1984).

Methods used to establish the level of dehydration might in fact determine the physiological responses in some studies. Caldwell et al. (1984) observed that the method used to induce dehydration may influence the changes in PV and plasma osmolality. Caldwell et al. (1984) concluded that dehydration through exercise alone resulted in a reduction in weight without a decrease in PV while dehydration by sauna resulted in a reduction in BM as well as PV. While providing advice for fluid replacement during marathon running, Noakes and Martin (2002) noted that that the level of dehydration cannot be simply determined (calculated) by the difference in weight from pre- and post-

race. It has been suggested that the weight loss incurred during exercise includes up to 1 kg of metabolic fuel that is irreversibly oxidised during exercise and metabolic water dissociated from liver and muscle glycogen oxidation. The physiological vagaries in establishing the true level of dehydration can place serious limitations on the interpretations made by many studies.

The maintenance of cardiovascular and thermoregulatory function during exercise in the heat is exacerbated by dehydration. The immediate response to the increase in core temperature involves an increase in skin blood flow and sweat rate thereby allowing the transfer of heat from the muscles to the skin to be dissipated to the environment. Dehydration causes a reduction in skin blood flow and sweat rate as the body tries to conserve BV. This leads to an inevitable rise in core temperature and impairs physiological responses while the capacity for exercise in a hot environment is reduced unless fluid is administered. Ultimately, decreases in anaerobic capacity, muscular endurance, maximal aerobic power and physical work capacity are observed (Sawka and Pandolf, 1990).

Hypervolemia

Plasma volume (PV) is known to be enhanced and sustained by exercise training and heat acclimatisation (Latzka et al., 1997). Plasma volume expansion (PVE), or hypervolemia, is considered one of the major adaptations of heat acclimation. The maintenance of PV by training and acclimatisaton has been shown to maintain BV, thus SV and Q is maintained while increasing skin blood flow (Fortney et al., 1981; Coyle et al., 1986). However, PV increases are not permanent. PVE has been shown to reach a peak and then decline during 8-14 days of heat acclimatisation (Armstrong and Maresh, 1991). However, this mechanism can be blunted with extended training and PV may decline as training continues (Armstrong and Dziados, 1986).

Studies involving the maintenance of PV through the infusion of isotonic saline or albumin during exercise have been found to significantly lower HR and improve thermoregulatory function (Fortney et al., 1981; Deschamps et al., 1989; Hamilton et al., 1991) although some studies have found no effect of this strategy on exercise heart rates (Nose et al., 1990; Watt et al., 2000). However, the artificial expansion of PV does not necessarily enhance performance (Sawka et al., 1983; Deschamps et al., 1989) or lower the perception of effort (Deschamps et al., 1989). Watt et al. (2000) artificially induced a

PVE of 13% and did not find improved exercise performance in the heat. Watt et al. (2000) concluded that the observed PVE might not be critical in determining the changes in thermoregulation and exercise performance in the heat.

In contrast to the findings outlined above, the notion that an artificially increased PV can enhance performance in a temperate environment is supported by Coyle et al. (1990). Coyle et al. (1990) observed that acute PVE with dextran solution following detraining restored approximately 50% of their trained state VO_2 max. Therefore, blood volume contributes directly to the increases seen in VO_2 max as a response to training. Coyle et al. (1990) also noted that excessive haemodilution due to PVE decreased maximum oxygen uptake and concluded that a PVE of 200-300ml appears to be a limit to induce VO_2 max improvement in untrained men. In contrast to untrained subjects, Warburton et al. (1999) investigated whether PVE (6% dextran) with elite endurance trained cyclists (VO2 max: 68.9 ml.kg. $^{-1}min^{-1}$) could lead to further enhancement of VO_2 max and endurance performance. The PVE of 547 ml contributed to haemodilution to an extent that oxygen transport, VO_2 max while performances were unchanged. Warburton et al. (1999) concluded that further expansion of BV provided no advantage in elite cyclists who already possess high BV and a high VO_2 max compared with moderately trained men (Watt et al., 2000). However it has been shown that PV expansion to levels of 500-600 ml above normal results in an excessive haemodilution and a subsequential decline in VO_2 max (Coyle et al., 1990) and arterial oxygen content (Helyar et al., 1996).

Changes in PV and BV by exercise, via heat production and/or environmental conditions, support the idea of expanding plasma volume to enhance thermoregulatory and CV function, and thus performance. Although most studies have shown significant decrements in performance due to the reduction in PV by hypohydrating, many fail to find appreciable performance improvement by artificially inducing PVE. There have also been numerous studies investigating the effects of pre-event hyperhydration on PVE, thermoregulation and exercise performance (Latzka and Sawka, 2000). The majority of these studies in this review have shown no significant change in PV, core and skin temperature and performance. On the other hand, in studies investigating the detrimental effects of hypohydration on performance, it appears that hyperhydrating to minimise PV decreases is a method that could attenuate the thermal and cardiovascular strain and possibly maintain performance in the heat (Lyons et al., 1990; Freund et al., 1995; Rio-

Sanz et al., 1996). However, for these hyperhydrating methods to be successful they are highly dependent on the stomach and gut to absorb the fluid.

Gut transport and absorption

Factors affecting fluid absorption

Gastric emptying is stimulated by two principal factors: nerve impulses in response to stomach distention, and stomach gastrin released in responses to certain types of foods. In the presence of gastrin, the lower esophageal sphincter contracts, the motility of the stomach increases, and the pyloric sphincter relaxes. The resultant effect of these actions is stomach emptying (Tortora and Anagnostakos, 1987). Over a period of two to six hours the stomach empties all its contents. Although the stomach does absorb some water, electrolytes, certain drugs (aspirin) and alcohol, the main site of hydrolysis of carbohydrates, proteins and fats occurs in the small intestine (Leiper, 2001). The gastric emptying of liquids is quicker than achieved following ingestion of solids. Solutions with low energy content empty more quickly than do those of that are more energy dense (Leiper, 2001). Water uptake from the intestinal lumen is caused by the absorption of solutes, creating an osmotic gradient suitable for net water absorption. A hypo-osmotic solution (relative to plasma) may slightly improve net intestinal absorption compared to an isotonic solution (Rehrer, 1996).

Osmolality of fluids similar to that of blood (280 – 300 mosmol.kg $^{-1}$) are termed 'isotonic' and are preferred as this osmolality assists in the transport of nutrients and fluid between the gut lumen and the blood. Beverages with a greater carbohydrate content and osmolality are referred to as 'hypertonic' and slow the rate of gastric emptying (Rehrer et al., 1992; Pearce, 1996; Maughan, 2000). A net secretion of water into the intestine will result from the ingestion of a hypertonic solution and this could possibly exacerbate dehydration (Maughan, 2000). The effect of the consumption of food and its content (fat, protein and CHO) with fluid will cause a hyperosmolar solution which can also delay gastric emptying leaving athletes feeling full (Pearce, 1996).

Although water is the best choice when replacing fluid lost via sweat during exercise, carbohydrate-electrolyte drinks address the issues of fluid retention and preservation of BV, replacement of muscle glycogen, maintenance of blood glucose levels and delays the onset of fatigue (Pearce, 1996). Plain water has been shown to remove the osmotic drive

to drink (Nose et al., 1988) while cool drinks (15-20 ºC) are not only preferable as they are refreshing and promote the ingestion of large volumes but also provide a heat sink that slightly attenuates core temperature, reduces sweat rates and increases heat storage compared with warm and no fluid (Gregory et al., 1997).

The carbohydrate content of most sport drinks is formulated to produce a solution that is isotonic with human serum, while ensuring that suitable osmotic gradients cause net water absorption in the small intestine (Leiper, 2001). Highly concentrated CHO solutions with a high osmolality decrease the net rate of intestinal absorption of water from a beverage containing CHO (Rehrer, 1996). The ingestion of beverages containing CHO has been shown to increase performance to the greatest extent compared to water while a carbohydrate intake of 30-60 g per hr has been shown to provide additional fuel when muscle stores become depleted (Coyle and Montain, 1992).

Drinks containing 4-6% carbohydrate can deliver fluid and CHO at rates which are likely to benefit performance (Pearce, 1996). The addition of sodium to CHO beverages enhances the palatability of the drink, boosts the osmotic drive to drink, replaces sweat losses and promotes retention and absorption of the fluid consumed (Pearce, 1996; Hargreaves, 1996; Maughan, 2000). The major electrolytes that are added to sports drinks are potassium, chloride, phosphate and magnesium. Their main function is to provide the body with essential minerals for muscle contraction, nerve conductivity, control the osmosis of water between body compartments, and help maintain acid-base balance for normal cellular activities (Totora and Anagnostakos, 1988).

The upper limits for fluid replacement during exercise heat stress are set by the maximal gastric emptying rates. Studies have shown that fluid ingestion rates of 900 – 1000ml per hr are well tolerated and readily emptied from the stomach during cycling and running (Rehrer et al., 1990) while the upper limits for fluid replacement during exercise heat stress at maximal gastric emptying rates are between 1 and 1.5 $L.hr^{-1}$ (Noakes et al., 1991). However, the emptying rate may also be highly variable among individuals (Noakes et al., 1991; Mitchell et al., 1991). It has also been demonstrated that stomach distention promotes greater gastric emptying while ‘priming’ the stomach for optimum delivery of fluid during exercise. However, subsequent fluid intake may be crucial when replacing sweat losses and maximising fluid replacement during exercise (Burke, 1996).

Despite the importance of gastric emptying rates, performance has been shown to decrease while trying to match fluid ingestion rates with sweat losses. Robinson et al. (1995) concluded that if sweat rates are to be matched precisely, performance might be impaired if the ingestion of too much fluid is consumed. Feeling bloated due to the ingestion of the large volume of fluid intake may have been responsible for performance decrement while cycling at 85% VO_2 max for 1 hr in 20 ºC (Robinson et al., 1995).

The gastric emptying rates are reported to decrease during high intensity (>75% VO_2 max) exercise (Rehrer, 1996), hypohydration and heat strain. Leiper et al. (2001) showed that even intermittent exercise in moderate conditions would slow down gastric emptying. This is further exacerbated by hypohydration and heat strain (Sawka, 1992). It has been shown that dehydration at levels greater than 3% decrease in body mass can cause gastrointestinal distress and decreased gastric emptying (Neufer et al., 1989; Rehrer et al., 1990). While disturbances in gastrointestinal function during exercise may be the result of redistribution of blood from the splanchnic to muscle and skin for oxygen and substrate transport and thermoregulation, respectively (Fallon, 2000).

It appears that the rate of gastric emptying is highly dependent upon the carbohydrate content, volume ingested, fluid temperature (Murray, 1996), the intensity of exercise and the state of hydration (Sawka et al., 1985; Montain and Coyle, 1992). Gastric emptying is also influenced by a number of other factors including the initial volume of fluid in the stomach, the density of the fluid (osmolality), and the emotional status of the individual (Pearce, 1996). Fluid replacement is crucial to the maintenance of plasma volume and thus thermal balance and cardiovascular stability.

Hydration strategies to minimise cardiovascular and thermal strain

Exercise performance can only be optimised when dehydration is minimised (Murray, 1996). It has been well established that performance is impaired even at low levels of dehydration. Therefore, it is crucial that scientifically substantiated, practical guidelines are instigated before and during exercise to reduce the likely resultant cardiovascular and thermal strain and thus maintain exercise capacity.

The main benefits of sufficient fluid replacement during exercise is to maintain cardiac output and allow adequate skin blood flow to increase in order to promote heat

dissipation thus preventing hyperthermia (Montain and Coyle, 1992). Fully replacing sweat losses during exercise reduces the risk of hyperthermia and improves exercise performance by maintaining CV function (Murray, 1996).

Clearly, during physical exercise in the heat it would be beneficial to match fluid intake with sweat losses. Most athletes will commonly dehydrate by 2-8% of their BM during exercise related heat stress (Sawka, 1992) while it has been shown that despite the intervention of pre-, intra- and post- event drinking strategies, most team sport athletes are only able to replace 47-75% of BM decreases due to sweat losses (Burke et al., 1997). The value of maintaining euhydrated and minimising dehydration is well illustrated by the studies of Montain and Coyle (1992), Armstrong et al. (1985), Sawka et al. (1985) and Walsh et al. (1994). These studies recognised cardiovascular, thermoregulatory and exercise performances were optimised by replacing at least 80% of sweat losses during exercise. It is also crucial to note that with the ingestion of fluid, subjective ratings can reduce the perception of exertion at a given workload (Montain and Coyle, 1992). This has important implications to the motivation required for all out efforts at the end of several studies to measure the performance improvements.

To maximise fluid balance and reduce cardiovascular and thermal strain, fluids should be consumed before, during and post activity. Recently, Sports Medicine Australia (SMA, 2002) have provided guidelines and strategies to reduce the incidence of heat related illnesses. SMA recommend ingestion of at least 7-8 ml of cool fluid per kg of BM (~ 400-500 ml) no more than 2 hrs before exercising to promote adequate hydration and allow time for excess fluid to be excreted. It has also been suggested that 400-600 ml of cool fluid can be consumed 10-20 min before exercising in the heat (McArdle, Katch and Katch, 1991). This can prime the stomach and promote faster empty rate (distention). Both hyperhydrating methods can delay the onset of dehydration, however, due to differences in individual tolerance and in gastric emptying it is recommended that these strategies are practiced at training and not at competition.

Sport Medicine Australia (SMA) (2002) also recommends that during exercise athletes should ingest at least 3 ml of cool fluid per kg of BM (150 – 200 ml) every 15-20 min. Maintenance of euhydration by fluid replacement throughout exercise has been shown to attenuate hyperthermia and prevent the decline in SV and Q (Hamilton et al., 1991). Importantly, rehydrating during exercise is more efficient in maintaining plasma volume and osmolality and in reducing the hormonal response (Brandenburger et al., 1989).

Hyperhydration and performance

Hyperhydrating is a strategy in which a large volume of fluid (400-600 ml – or a volume that will be tolerated by the individual) is consumed prior (1-2hrs) to an exercise bout. Hyperhydration strategies have been used to delay the onset of water body deficit when sweat losses are not fully replaced (Latzka et al., 1997) during prolonged exercise or exposure to environmental extremes (Freund et al., 1995). Hyperhydration strategies may be useful for those individuals who poorly match rates of fluid losses (high sweat rates) to fluid intake and in situations where the opportunity for fluid intake is limited (Burke, 1996). Hyperhydrating may also bring the athlete to a fluid balance (euhydrated) if the hydration status is unknown.

A limited number of studies have shown that hyperhydration can delay the onset of hypohydration by increasing the body's storage of water (Freund et al., 1995), increase the sweating rate (Lyons et al., 1990), reduce the sweating threshold while also providing a smaller rise in core temperature (Lyons et al., 1990; Rio-Sanz et al., 1996). Ingestion of fluid one hour before exercise lowered core temperature and heart rate during exercise compared when no fluid was consumed (Greenleaf and Castle, 1971; Montain and Coyle, 1992). However, there have been many studies that either verify or refute the reported benefits of hyperhydration over euhydration in minimising thermoregulation and exercise performance in the heat (Latzka and Sawka, 2000; Sawka et al., 2001).

In particular, some hyperhydration infusion studies have noted lower core temperatures but not decreased heart rates during exercise. Sawka et al. (1983) found lower heart rates but no difference in skin or rectal temperature during treadmill exercise after albumin infusion. Likewise Fortney et al. (1981) found an increased SV and lowered HR but no change in core temperature compared to a euhydrated state with blood infusion. Also Nadel et al. (1980) reported that hyperhydration did lower heart rates during exercise but did not affect esophageal temperature.

Unfortunately, any advantages gained due to the transitory effects of hyperhydration tend to be quickly lost due to the efficiency of the kidneys in eliminating excess fluid (Freund et al., 1995). Performance can also be affected due to the gastric discomfort of the excessive fluid intake while some athletes may experience frequent voiding of urine before the race and in some cases, during competition. Recent studies have suggested that adding glycerol to the ingested fluid may delay the excretion of the excess fluid and

thereby prolong the hyperhydrated state (Riedesel et al., 1987; Lyons et al., 1990; Konisberg et al., 1995).

Hyperhydration with glycerol has been shown to induce significant hypervolemia (Jimenez et al. 1999) while others have found increases in PV (Freund et al., 1995; Gleesen et al., 1996; Hitchins et al., 1999; Coutts et al., 2002). However, many other studies using glycerol-induced hyperhydration have reported no change in PV (Riedesel et al., 1987; Murray et al., 1991; Freund et al., 1995; Montner et al., 1996; Latzka et al., 1997; Grice et al., 1997; Latzka et al., 1998; Lamb et al., 1999) over carbohydrate, orange juice and water hyperhydrating treatments. This is mainly due to lack of change in hemoglobin and haematocrit values which subsequently suggest no change in PV (Dill and Costill, 1974). Most of the research investigating the hyperhydrating effects of glycerol has shown that the increased fluid tends to be distributed in all body compartments (Lyons et al., 1990; Lyons and Riedesel, 1993). On the basis of these data, expansion or maintenance of PV resulting from the ingestion of glycerol could minimise the affects of exercise induced cardiovascular and thermal strain and subsequently, enhance exercise performance. The following section will explore the physiological responses associated with the ingestion of glycerol while also highlighting the contradictory nature of the research methodology pertaining to performance improvements.

Glycerol

Mechanisms of action of glycerol

Glycerol is an uncharged, endogenous metabolite that freely permeates most body spaces when given orally with water (Lin, 1977). Initially, glycerol was used clinically as a potent osmotic dehydrating agent for the treatment of elevated intracranial pressure while also being effective in lowering intraocular pressure in glaucoma and shrinking the brain during neurosurgical procedures (Frank et al., 1981). Glycerol infusion and ingestion has been given close examination in both research and clinically (osmotherapeutic intervention) for over 60 years (Frank et al., 1981).

Glycerol is a naturally occurring trivalent alcohol that constitutes the structural core of the triglycerol molecule in man. Glycerol ingestion can cause blood serum glycerol to rise from 0.05 to 15-20 $mmol \cdot L^{-1}$. This rise is associated with increased liver gluconeogenesis, increased blood osmolality and increased urinary glycerol excretion (Robergs and Griffin, 1998).

Glycerol is involved in intermediate metabolism via two major pathways. After phosphorylation by glycerol kinase, approximately 70-90% of glycerol is oxidised by glycerol-3-phosphate dehydrogenase to dihydroxyacetone phosphate. This then enters the Embeden-Meyerhof pathway at the level of glyeraldehyde-3-phosphate. At this point glycerol's fate may be involved in gluconeogenesis or glycolysis (via pyruvate). The remaining 10-30% of glycerol combines with free fatty acids to form triglycerides (Robergs and Griffin, 1998). Glycerol supplies 4.32 $calories.g^{-1}$ when oxidized to carbon dioxide and water (Frank et al., 1981). Glycerol is also cleared in the kidneys by a combination of metabolic conversion, passive diffusion from the filtrate into the cells and interstitium of the tubule cells (Frank et al., 1981; Robergs and Griffin, 1998).

About 80% of glycerol metabolism occurs in the liver while 10-20% occurs in the kidney. This distribution corresponds to the primary locations of glycerol kinase. A small amount of glycerol kinase has been found in intestinal mucosal and brown adipose tissue and has also been identified in skeletal muscle. Glycerol phosphate dehydrogenase is predominantly found in skeletal muscle, the liver and adipose tissue. Free glycerol occurs by the de-esterification of triacylglycerols (Robergs and Griffin, 1998).

In the process of gluconeogenesis, moderate quantities of glucose can be formed from amino acids and the glycerol portion of fat molecules. Glycerol may be converted into glyceraldehyde-3-phosphate, which may also be resynthesised into glucose or form pyruvic acid. In this way glycerol can enter the glycolytic pathway as an energy source. The fate of pyruvic acid and the energy pathway it takes depends on the availability of oxygen. Gluconeogenesis is stimulated by cortisol and thyroxine but can also be stimulated by epinepherine, glucagon and growth hormone (Guyton, 1996).

Initial research into the mechanisms of glycerol action were not only focussed on man but also on cats (Larsen, 1963), dogs (Winkler et al., 1969), rabbits (Wade, 1981) and recently horses (Schott et al., 2001). Given the powerful osmotic properties of glycerol and its documented ability to retain fluid within the body compartments, it has been suggested to be a possible hyperhydrating agent and plasma volume expander (Lyons and Riedesel, 1993). This suggestion not only has positive implications for the retention of fluid but could theoretically minimise thermoregulatory and cardiovascular strain during exercise in hot environments.

Effect of glycerol on fluid retention

Many studies investigating hyperhydration strategies attempt to increase total body water (TBW) by over drinking water or carbohydrate-electrolyte solutions (Latzka and Sawka, 2000). The cumulative amount of fluid retained is calculated by subtracting the total amount of fluid ingested from the accumulated urine volume. It is assumed that the amount not excreted is retained in the body in different fluid compartments.

However, problems exist when hyperhydrating with common beverages such as water and water-electrolyte solutions. Most of these attempts have only produced a transient expansion of TBW as the kidneys rapidly clear the majority of the fluid consumed (Freund et al., 1995). Many athletes may also be discouraged to ingest a large amount of fluid before exercise due to the fear of gastro-intestinal discomfort and inconvenience of frequent urination, not only prior to the event, but also during the event.

Early studies with rats using a 5% glycerol solution have reported a 50% increase in fluid retention over that achieved from ingesting water (Lyons and Riedesel, 1993). Although the actual fluid compartment volume data presented in this study were limited due to possible experimental error and the use of such a small animal, the relative change in

volume was important. It was concluded that the fluid significantly expanded the intra-cellular fluid (ICF) while values for extra-cellular fluid (ECF), plasma volume (PV) and interstitial fluid (ISF) were unchanged for both glycerol and water.

Over the last 10 years human research has clearly demonstrated that fluid retention can be achieved by drinking a dilute liquid solution containing glycerol. Recent studies have shown increases in fluid retention using glycerol prior to exercise can vary from 400 to 700 ml (Riedesel et al., 1987; Wendtland et al., 1987; Lyons et al., 1990; Seifert et al., 1995; Hitchins et al., 1999; Anderson et al., 2001) and recently 1000 ml (Coutts et al., 2002) over other hydration treatments. Contradictory data have been presented by Murray et al., (1991) who found no indication of a hyperhydrating effect with a 4% and 10% glycerol solution over that observed with a water and carbohydrate-electrolyte treatment. This result may have been due to the beverages being consumed during the exercise and not before exercise along with the ingestion of a small dose rather than a large dose of glycerol as used by most other related studies (Riedesel et al. 1987 Arnall and Goforth, 1993; Freund et al. 1995; Montner et al., 1996; Latzka et al., 1997; Grice et al. 1997; Latzka et al., 1998).

Glycerol has also been shown to enhance fluid retention long after its ingestion. Koenigsberg et al. (1995) found retention of 746 ml 49 hrs post-ingestion using small multiple doses of glycerol (and a large volume of water and orange juice) over that achieved following ingestion of water and orange juice alone. Glycerol doses ranged from 0.3 $g.kg^{-1}$ to 1 $g.kg^{-1}$ while the total fluid intake of 51 $ml.kg^{-1}$ was spread over an ingestion period of 13 hr. This strategy may have positive implications for the timing of glycerol ingestion prior to and during exercise bouts where fluid balance is crucial to performance.

Interestingly, there is also evidence that fluid retention and increases in TBW occur following ingestion of a control/placebo solution comprised of carbohydrate electrolyte sports drinks (Coutts et al., 2002) orange juice and water (Wendtland et al., 1997) and water (Latzka et al., 1997). However, the hyperhydration was notably smaller than that achieved following ingestion of glycerol. A possible limitation of these studies may be lack of tight control of the baseline pre-hydration status of the subjects. These data also represent supportive evidence that hyperhydration, and thus fluid retention, can occur with solutions other than glycerol. Some studies have reported the sodium content of ingested fluids (Riedesel et al., 1987; Freund et al., 1995; Hitchins et al., 1999; Anderson

et al., 2001; Coutts et al., 2002) thereby acknowledging that sodium will also play a role in fluid retention and can assist in the hyperhydration process. This has implications when differentiating subtle variations in physiological responses and performance improvements (Lamb et al., 1999) from the glycerol treatment with the control.

The majority of published research has also shown that hyperhydration with glycerol increases plasma osmolality (Gleeson et al., 1986; Riedesel et al., 1987; Murray et al., 1991; Freund et al., 1995; Seifert et al., 1995; Inder et al., 1998; Hitchins et al., 1999; Lamb et al., 1999; Anderson et al., 2001) which, attenuates any reduction in plasma antiduretic hormone (ADH) concentration induced by ingesting large volumes of fluid thereby enabling greater fluid retention. As a consequence, there is lower free water clearance and greater fluid retention for glycerol hyperhydration over that observed following ingestion of other solutions. Gleeson et al. (1986) noted an increase in plasma osmolality after glycerol ingestion exceeded 18 $mOsmol.kg^{-1}$ and remained elevated through the exercise period over that achieved after ingestion of a diluted flavoured drink. It should be noted, however, that plasma osmolality for both the glucose and placebo treatments also increased 5-8 $mOsmol.kg^{-1}$ during the exercise phase. Montner et al. (1996) reported a decrease of 5 $mOsmol.kg^{-1}$ initially and then an increase during exercise of 14 $mOsmol.kg^{-1}$ with water placebo hydration. The glycerol solution increased plasma osmolality by 7 $mOsmol.kg^{-1}$ after hydration and a further increase of 3 $mOsmol.kg^{-1}$ during exercise. Lyons et al. (1990) proposed that the smaller increase in plasma osmolality during exercise, as a result of glycerol induced hyperhydration, may be due to the maintenance of PV by other fluid compartments (ICF, ISF, ECF). Exercise diluted the normal rise in plasma osmolality associated under resting conditions. In contrast Murray et al. (1991) reported an increase in osmolality for glycerol during exercise. To add to the dilemma in the published data, it has also been suggested that as glycerol is being reabsorbed, there is an increase in the concentration gradient in the medullary collecting duct of the kidney causing more water to be reabsorbed (Freund et al., 1995; Robergs et al., 1998).

While most studies suggest that plasma osmolality and the associated hormonal responses have a direct influence on urine output and thus fluid retention, others imply a direct effect on reabsorption of glycerol by the renal tubules, independent of ADH concentration and the associated responses. Although small differences in ADH and ALD concentrations have been reported by some research, their relevance is disputed due to the

limitations in measurement sensitivity and individual variability to different treatments (Murray et al., 1991; Freund et al., 1995). Despite the lack of evidence to support its significance relative to the retention of fluid, Murray et al. (1991) reported a slight increase in ADH values during exercise with the highest occurring with glycerol treatment.

Although ADH has been implicated as a possible mechanism for the resultant fluid retention, atrial nutrietic peptide (ANP) may also be a considered as a potential factor contributing to glycerol induced fluid retention. ANP opposes the renin-angiotensin-aldosterone system and may play a role in inhibiting the retention of fluid (including ADH and ALD) and decreasing blood volume and pressure. However, increases in ANP concentration have only been observed with control treatment and not with glycerol treatment (Anderson et al. 2001) or remained unchanged (Freund et al., 1995).

Plasma arginine vasopressin (AVP) increases have also been reported (Freund et al., 1995; Anderson et al., 2001) after the ingestion of glycerol and during exercise. These data suggest transient changes occur in plasma osmolality due to the presence of hypertonic fluid in the gut and the changes in plasma osmolality during exercise. Anderson et al. (2001) proposed that the ingestion of a hypertonic solution such as glycerol would in fact cause net fluid movement into the gut lumen rather than movement into other fluid compartments. As a consequence, PV would initially fall which may increase plasma osmolality and vasopression concentration. Glycerol ingestion may reduce urine output as the fluid remains in the gut and is absorbed more slowly than the absorption and subsequent excretion of the control solution. However, Inder et al. (1998) found that the use of either the AVP analog desmopressin (DDAVP) or glycerol before training or competition did not provide any benefit over conventional fluid replacement in highly trained triathletes exercising for 1 hr at ~70% $V0_2$ max. It was noted that although there was a trend for a reduction in urine volume using DDAVP, this did not reach statistical significance.

Clearly the majority of the literature is supportive of glycerol's ability to retain fluid while the exact mechanisms responsible for fluid retention are not completely understood. Freund et al. (1995) proposed that fluid retention may be due to an increase in the medullary concentration gradient of the kidney, thereby forcing enhanced reabsorption of

water in the distal tubules and collecting ducts of the kidney. This is quite possible given the inconclusive hormonal data presented in many studies.

Urine output

Glycerol hyperhydration has been shown to decrease urine output when compared with the ingestion of water, a carbohydrate solution or orange juice (Riedesel et al., 1987; Lyons et al., 1990; Murray et al., 1991; Hitchins et al., 1999; Anderson et al., 2001; Scheett et al., 2001; Coutts et al., 2002). However, some studies (Latzka et al., 1998; Lamb et al., 1999) have noted no difference in urine output between carbohydrate or water and glycerol hyperhydration after the drinking phase (Miller et al., 1983; Murray et al., 1991; Arnall and Goforth, 1993; Latzka et al., 1997) or during exercise (Montner et al., 1996). This apparent conflict may have been due to the short duration between the ingestion of fluid before exercise. Exercising so soon after the ingestion of fluid may diminish any notable fluid retention if adequate time was not given time for the fluid to be absorbed in the gut.

Some studies report higher urine outputs soon (peak=60 min) after ingesting a glycerol solution (Freund et al., 1995; Hitchins et al., 1999; Scheett et al., 2001) while others several hours later (Reidesel et al., 1987). Two hours after fluid ingestion Koenigsberg et al. (1995) noted a large increase in urine output with placebo (73.3% larger volume) compared with glycerol and the significant differences in urine volume persisted throughout the 49 hr post-ingestion period.

The majority of research has shown that glycerol-induced hyperhydration reduces urine output. Urine output reflects the increased amount of fluid retained. However, the clearance of urine is directly related to the tonicity, timing and amount of fluid ingested and the pre-hydration status of the subjects (Rehrer, 1996). To reduce the biological changes in TBW between trials, Freund et al. (1995) placed behavioural restrictions on subjects before experimental trials and baseline blood volume and TBW measurements. Therefore, true experimental effects were reported rather than differences in baseline hormonal concentrations, renal function and hydration status. This method reduced any "baseline" variation within subjects and between trial fluctuations and ensures data and research credibility.

In an effort to standardise hydration status of all subjects prior to testing, Anderson et al. (2001) had subjects ingest five $ml.kg^{-1}$ of water upon waking and consume a standardised diet (14 MJ; 70% CHO) 24 hr prior to the test. Similar to Anderson et al. (2001), Scheett et al. (2001) instructed subjects to ingest between 200 and 500 ml of fluid 24 and four hours prior to the test session, respectively. The subjects also ate a plain bagel (31 g carbohydrate, 163 Kcals) upon arrival to the laboratory. Murray et al. (1991) only encouraged subjects to drink before reporting to the laboratory test while Hitchins et al. (1999) standardised both diet and training 24 hr leading into the two performance trials. Training, diet and fluid intake was confirmed with recording sheets. Other studies had subjects acting as their own control (Lyons et al., 1990; Montner et al., 1996) and/or advised to abstain from strenuous physical activity and standardise their diets 48 hr prior to testing (Miller et al., 1983; Gleeson et al., 1986; Lamb et al., 1999; Scheett et al., 2001; Coutts et al., 2002).

Many studies focusing on the changes in TBW and fluid retention via glycerol induced hyperhydration report a notable decrease in urine output compared with other ingested fluids. Timing and amount of fluid ingested and food consumed varied considerably between studies and thus compromised the nutritional and hydration status of the participants. Ultimately, limitations will always exist where there is inadequate control of hydration status of subjects. Hydration status varies considerably from day to day. While data may suggest uniform hydration status between trials, individual hydration status should be established over several days of monitoring urine osmolality (Parisotto, 2002). The lack of uniform nutritional status will distort whether real change has occurred due to the treatment.

Timing of glycerol ingestion and other fluids

The timing of glycerol ingestion is crucial to the detection of changes in plasma osmolality and thus fluid retention before and during exercise. The hormones ADH and ALD directly respond to any subtle changes in plasma osmolality and body fluid. The primary role of ADH is osmoregulation or regulation of the body fluid by osmotic pressure. ADH begins to take effect 20 to 60 min after fluid is ingested with noticeable fluid output. ALD is a slower acting hormone than ADH, taking several hours before the effects of its action are visible (Guyton, 1991). Changes in plasma osmolality will reflect both the resultant absorption rates and the tonicity of the fluid.

Timing of the hydration phase when comparing glycerol over other treatments is important because the hydration status at the start of exercise will vary with different hyperhydrating periods (Latzka and Sawka, 2000). Latzka and Sawka (2000) noted if exercise starts 1 h post drink, then the hydration status at the start of exercise will be similar between glycerol and water hyperhydration. However, if the exercise time is at 2-3 hrs post drink the glycerol hyperhydration trial will begin with 400 – 700 ml greater TBW.

Timing of the fluid ingested before exercise often ranges between 30 min (Miller et al., 1983) and four hours (Inder et al., 1998). The majority of studies however, have timed fluid ingestion to be between 1-3 hrs prior to exercise (Reidesel et al., 1987; Lyons et al., 1990; Seifert et al., 1995; Montner et al., 1995; Grice et al., 1997; Hitchins et al., 1999; Scheett et al., 2001; Anderson et al., 2001; Coutts et al., 2002). Inder et al. (1998) concluded that the four hours leading into the exercise period may negate any possible effect of glycerol on hydration status (hyperhydration) and therefore result in no physiological or thermal advantage over water on exercise performance. Clearly the longer the time before exercise begins after fluid ingestion the greater fluid clearance. This will occur whether the ingested fluid is accompanied by glycerol or not.

The time allocated to ingest the total volume of glycerol and control solutions in the available literature ranged from 15 min (Anderson et al., 2001) to 30 min (Hitchins et al., 1999) to 1 hr (Coutts et al., 2002) and paced over 30 min (Freund et al., 1995). One hour prior to exercise Montner et al. (1996) had set doses every 15 min for 90 min while Murray et al. (1991) had subjects ingest glycerol solution every 15 min during the exercise. In an effort to minimise the effects of progressive dehydration some subjects were given either water (Gleeson et al., 1986 100ml every 15 min; Miller et al., 1983 300ml at 30 min intervals) or a carbohydrate solution (Montner et al., 1986; Anderson et al., 2001) during exercise. Montner et al. (1995) made $3ml.kg^{-1}$ BM of a dextrose solution (5%) available every 20 min while Anderson et al. (2001) supplied 3.5 $ml.kg^{-1}$ BM of CHO-electrolyte (6%) every 15 min. Latzka et al. (1997) encouraged the subjects to choose either cool water or a commercial electrolyte beverage during rest and exercise but it should be noted that quantities drunk were not reported in this study.

Most of the literature reviewed involving glycerol ingestion does not standardise the timing and ingestion rates prior to the exercise bout and exercise. It is also quite possible that the intra-exercise drinking strategies utilised in the literature may have negated the

possible physiological effects of glycerol solution compared with other treatments. Maughan et al. (1996) reported that fluid ingested during prolonged low intensity exercise will minimise the effects of dehydration and maintain cardiovascular and thermoregulatory function better than no water. Further, the ingestion of CHO-electrolyte drink has clearly additional benefits over water due to the availability of exogenous glucose for the muscle thereby limiting the deterioration of performance.

Dosage (concentration) and type

The majority of studies evaluating glycerol effectiveness have used approximately 0.8 to 1.5 g of glycerol .kg^{-1} BW (Riedesel et al., 1987; Lyons et al., 1990; Murray et al., 1991; Freund et al., 1995; Montner et al., 1995; Seifert et al., 1995; Wendtland et al., 1997; Latzka et al., 1997; Inder et al., 1998; Latzka et al., 1998; Hitchins et al., 1999; Anderson et al., 2001; Scheett et al., 2001; Coutts et al., 2002). Riedesel et al. (1987) examined the effects of three different dosages of glycerol (0.5, 1 and 1.5 $g.kg^{-1}$ BM) on the retention of a dilute saline solution (1%) in orange juice 21.4 $ml.kg^{-1}$ BM (concentration unknown) ingested over 40 min. All three dosages resulted in reduced urine output compared with the control (mean accumulated urine volume for control was 94% vs 73% with glycerol) with the 1 and 1.5 $g.kg^{-1}$ BM resulting in a significantly greater amount of fluid retention then the control treatment. All dosages of glycerol increased water retention after the second hour while only the lower dose was not statistically significant. Reidesel et al. (1987) could not explain, however, why a 50% increase in glycerol solution (1 vs 1.5 $g.kg^{-1}$ BM) did not result in 50% greater water retention.

Glycerol is usually consumed with a large volume of fluid (20-30 $ml.kg^{-1}$ BM) either consisting of water (Riedesel et al., 1987; Arnall and Goforth, 1993; Freund et al., 1995; Montner et al., 1996; Latzka et al., 1997; Grice et al., 1997; Latzka et al., 1998), carbohydrate solution of varying concentration (Murray et al., 1991; Meyer et al., 1995; Grice et al., 1997; Hitchins et al., 1999; Anderson et al., 2001; Coutts et al., 2002) or orange juice (Lyons et al., 1990; Koenigsberg et al., 1995; Wendtland et al., 1997). Methods for determining total fluid intake volume accompanying glycerol have been related to various indices including subject's body weight (Gleeson et al., 1986; Lyons et al., 1990; Grice et al., 1997; Wendtland et al., 1997; Hitchins et al., 1999; Anderson et al., 2001; Coutts et al., 2002), fat-free mass (Arnall and Goforth, 1993; Latzka et al., 1997; Latzka et al., 1998) and TBW volume (Freund et al., 1995). In an effort to establish glycerol hyperhydration as an effective rehydrating agent over water, Scheett et al. (2001)

used a fluid intake volume equal to the total fluid loss in a dehydration session prior to the test. Some solutions had sweeteners such as saccharine (Miller et al., 1983; Coutts et al., 2002), aspartame (Murray et al., 1991; Freund et al. 1995; Koenigsberg et al., 1995; Montner et al., 1996; Latzka et al., 1998; Scheett et al., 2001) or both (Gleeson et al., 1986) added to mask the flavour, colour and sweetness of the drink.

However, not all studies ingested large volumes of fluid with the glycerol. Gleeson et al. (1986) used 1 $g.kg^{-1}$ BM with only 400 ml water while Miller et al. (1983) mixed 1 $g.kg^{-1}$ BM with 300ml of water. Anderson et al. (2001) made corrections for glycerol density when duplicating the volume for the other fluid treatment. Even though Inder et al. (1998) had subjects ingest 1 $g.kg^{-1}$ BM of glycerol with only 500 ml of water during breakfast, an extra 300 ml skim milk, 250 ml of orange juice and a further 200 ml of water was also consumed along with 60 g corn flakes, two slices of wheat grain toast, 1 medium banana, 10 g margarine and 14 g of jam. The total amount of fluid ingested (1250 ml) was considered to be inadequate to produce a state of hyperhydration and could possibly explain the lack of physiological and performance benefits of glycerol observed compared with water. Only Murray et al. (1991) reported the subjects having a "light lunch" 3-4 hr before the exercise session.

Absorption in the gut is regulated by the osmotic gradients of the solutions. The percentage of glycerol in solution will increase the fluid's tonicity (osmolality) and thus affect the absorption rates in the gut. Several studies had fluid concentrations of 4-5% glycerol (Hitchins et al., 1999; Anderson et al., 2001; Coutts et al., 2002) while some had concentrations up to 17.5% (Gleeson et al., 1986; Koenigsberg et al., 1995). Inder et al. (1998) had a combination of several fluids including water and glycerol, orange juice and milk as a hyperhydration solution. Anderson et al. (2001) concluded that difference in concentration, and thus osmolality, might affect absorption rates. Anderson et al. (2001) observed that the fluid ingestion by the control group was isotonic (114 mosmol) which allowed absorption to be rapid. On the other hand, the glycerol based fluid (611 mosmol) was hypertonic and this may have delayed the rate of absorption. The osmotic load in the gut and the incomplete absorption of the glycerol was confirmed by the osmotic-induced diarrhea 24 hrs later. Hence, urine output was greater with the control and to a lesser extent with glycerol. Freund et al. (1995) also reported the osmolar load of the glycerol solution (777 mosmol) but failed to associate the relevance of the solutions osmolality and the rate of absorption in the gut.

While only a few studies acknowledged the osmolar load of the ingested solutions, food intake and its effect on the rate of gastric emptying must also be considered. Vist and Maughan (1994) demonstrated that the energy density of both food and fluid combined is of far greater significance in determining the rate of gastric emptying than fluid alone. Therefore, the combination of the type of fluid and food will increase the osmolality and energy density. This has the potential to vastly affect the rate of absorption and thus directly contribute to the increases in plasma osmolality and reduction in urine volume.

The majority of the literature conforms to the standard glycerol dosage and accompanying volume of fluid. While the overall effectiveness will vary widely among people (Wagner, 1999), glycerol is well tolerated and safe at an oral dosage of 1 $g.kg^{-1}$ BM every six hours (Lin et al., 1977). Koenigsberg et al. (1995) also concluded that the volume, timing and concentration of glycerol, including other fluid intake, requires additional exploration and modification to meet the specific needs of the situation.

Glycerol and its effect on body fluid compartments

Plasma volume

The ingestion of glycerol solution as a hyperhydration strategy has been purported to act as a possible plasma volume expander which has the potential to attenuate thermal and cardiovascular strain while exercising in the heat (Riedesel et al., 1987; Lyons et al., 1990; Freund et al., 1995; Jimenez et al., 1999).

Most studies have found similar differences and changes in hemoglobin and haematocrit concentration and have assumed no change in PV following glycerol ingestion when compared to carbohydrate, orange juice and water hyperhydrating treatments (Riedesel et al., 1987; Murray et al., 1991; Freund et al., 1995; Montner et al., 1996; Latzka et al., 1997; Grice et al., 1997; Latzka et al., 1998; Lamb et al., 1999). Gleeson et al. (1986), however, have shown that glycerol did increase PV to a greater extent (10%) in the pre-exercise period compared with glucose or placebo treatment. Gleeson et al. (1986) also noted that PV dropped considerably with all treatments despite 100 ml of water being ingested every 15 min during exercise. However, PV changes with glycerol and glucose ingestion were less over a longer duration than placebo during the trial. This suggests greater fluid maintenance. Coutts et al. (2002) also reported a 21.2% increase in PV with

glycerol ingestion compared with placebo solution. This change was significant and it was surmised that the plasma volume change might have been due to the change in ambient temperature or a combination of high ambient temperature and glycerol loading. Similar to the findings of Coutts et al. (2002), Hitchins et al. (1999) also reported a mean 2.8% increase in PV with glycerol ingestion compared with placebo. However, the small change in PV was not significant between trials during the hydration phase and exercise phase. Clearly, the majority of research has shown that increases in PV due to glycerol ingestion have been equivocal.

While there is a measurable and transient increase in TBW with the ingestion of glycerol, there is little research into the exact body fluid compartment in which the fluid is retained. Lyons et al. (1990) suggests that all compartments (interstitial, extracellular and intracellar) were involved with glycerol and carbohydrate-electrolyte hyperhydration. The increases were primarily in the ICF and ISF compartments with little effect on PV. Lyons et al. (1990) concluded that excess fluid in these compartments after both hyperhydration strategies maintained sweating and minimised the elevation of core temperature during exercise and thermal stress during exercise in hot, humid conditions. The increase in ISF and ICF by glycerol ingestion over water is also supported by Seifert et al. (1995). Seifert et al. (1995) also hypothesised that these increases may have been responsible for the lower core temperature during exercise.

Latzka and Sawka (2000) suggests that if plasma expansion is one result of hyperhydration then any small increase in PV should be proportionate to the change in TBW. Latzka and Sawka (2000) commented that if plasma constitutes 7.5% of TBW, then a TBW increase of 600ml (equally distributed between ICF and ECF) observed by some studies would increase plasma volume by 45ml. Such a small increase in plasma volume could exceed the measurement's resolution. Although Coutts et al. (2002) observed a 175 ml increase in PV with glycerol ingestion compared with placebo, they concluded that the expansion of this order is unlikely to account for the protection against the effects of exercising in the heat.

It has been suggested that the water retained by the glycerol would be available for the maintenance of PV as water is lost via sweating. The extent in which performance is .improved by glycerol hyperhydration remains to be substantiated (Riedesel et al., 1987). Even with the injection of ADH, along with the ingestion of 2000 ml of water that

resulted in fluid retention and hyperhydration, PV only had a minor increase (Nadel et al., 1980).

For the body to maintain isotonicity within the fluid compartments, fluid shifts would accompany the movement of glycerol into the fluid spaces. Lyons and Riedesel (1993) suggest that fluid being distributed intracellulary with glycerol would minimise the stimulation of volume and osmotic receptors and therefore it would seem that ICF has a greater potential to act as a fluid reservoir rather than plasma volume expansion. This suggests intra-cellular fluid distribution (Nadel et al., 1980).

Summary of glycerol effects on blood measures

In conclusion, the majority of studies show no significant changes or differences in the hemoglobin and haematocrit concentration and therefore no increases in PV with glycerol ingestion compared with other hyperhydrating solutions. The extent to which PV, ISF and ICF are modified by changes in TBW has yet to be established.

Physiological measures in response to glycerol hyperhydration

Most research has identified several key physiological variables that are compromised by dehydration. They include: the reduction of plasma volume and sweat rates and the increase in heart rate and core temperature (Sawka and Pandolf, 1990). Studies measuring the effect of hyperhydration prior to exercise on performance have shown a decrease, delay and even attenuation of the detrimental effects of dehydration (Greenleaf and Castle, 1971; Montain and Coyle, 1992; Freund et al., 1995; Jimenez et al., 1999). In most studies glycerol induced hyperhydration has been shown to increase fluid retention (Riedesel et al., 1987; Wendtland et al., 1987; Lyons et al., 1990; Seifert et al., 1995; Hitchins et al., 1999; Anderson et al., 2001) TBW and in some cases increase PV, over other hyperhydrating solutions (Coutts et al., 2002). The increase in TBW and PV is tenuously assumed to decrease the thermoregulatory and cardiovascular strain experienced during exercise in hot and humid conditions.

Only three studies (Lyons et al., 1990; Seifert et al., 1995; Anderson et al., 2001) have actually reported a decrease in core/rectal temperature during exercise as a result of glycerol hyperhydration while many have not (Gleeson et al., 1986; Murray et al., 1991;

Montner et al., 1996; Wendtland et al., 1997; Latzka et al., 1997; Latzka et al., 1998; Lamb et al., 1999; Hitchins et al., 1999; Scheett et al., 2001).

Lyons et al. (1990) recorded a significant decrease ($p<0.01$) in rectal temperature following 30 min of moderate (60% $V0_{2max}$) exercise succeeding glycerol ingestion over other two hydration regimens. Lyons also reported a significant difference between glycerol and the other two regimens at 30-60 min ($P<0.05$) and 60-90 min ($P<0.01$). Seifert et al. (1995) reported a 0.3ºC decrease while Anderson et al. (2001) noted a mean 0.4ºC decrease ($p<0.05$) in rectal temperature towards the end of 90 min steady state cycling at 98% of lactate threshold in dry heat. In an effort to explain the decrease Lyons et al. (1990) suggested that glycerol might have a physiological effect on the temperature regulation centre and/or sweating mechanism. It is important to note that any small change in rectal/core temperature may be an independent and critical factor limiting exercise performance in the heat.

If increases in core/rectal temperature are to be observed during exercise in the heat, skin temperature may rise or fall depending on the priority of blood flow. Blood flow will be either directed to the surface to dissipate heat from the muscle and core, via sweat losses, or used to preserve adequate CBV for maintenance of SV, HR and cardiac output during prolonged exercise.

The potential for increasing sweat rate and sweat rate threshold have been previously reported as a result of fluid hyperhydration. Studies in which hyperhydration strategies have been employed have shown sweat rates to increase, decrease or remain unchanged. Lyons et al. (1990) observed a higher sweating rate during exercise (33%) by untrained non-heat acclimatised subjects following the ingestion of glycerol compared to that observed following the ingestion of water. Anderson et al. (2001) recorded higher forearm skin blood flow (FBF) with no differences in skin and muscle temperature, catecholamines, muscle glycogenolysis, lactate accumulation, adenine nucleotide and PCr degradation. Anderson et al. (2001) also noted a late decrease in forearm blood flow (FBF) in the control group compared with the glycerol treatment. Anderson et al. (2001) concluded that blood flow may have been redirected to maintain mean arterial BP at the expenses of skin blood flow and that the reduced FBF may attenuate SV and thus increase HR.

Most studies found no thermoregulatory benefit (Murray et al., 1991; Latzka et al., 1997; Wendtland et al., 1997; Latzka et al., 1998; Hitchins et al., 1999; Scheett et al., 2001) and no sweat loss differences (Murray et al., 1991; Montner et al., 1996; Hitchins et al., 1999; Scheett et al., 2001; Coutts et al., 2002) with glycerol hyperhydration over other hydration treatments. Lamb et al. (1999) confirmed that prehydration with glycerol provided no physiological (HR, rectal temperatures, sweat rates and RPE) or performance advantage over pre-hydration with 6% CHO. These data are supported by Montner et al. (1996) who also concluded no thermoregulatory advantage (including sweat rates) with glycerol hyperhydration over aspartame-flavoured water. Montner et al. (1996) acknowledged however, that glycerol could extend endurance in temperate conditions (23.5 –24.5^{o}C and 25-27%RH). Interestingly, Inder et al. (1998) did not record any thermoregulation or cardiovascular responses, only the consequences of glycerol ingestion and the potential effect of glycerol hyperhydration on performance. Latzka et al. (1998) concluded that glycerol provides maintenance of euhydration state and may delay the affects of hypohydration.

Exercise in the heat has been shown to alter oxygen consumption, RER, heart rates, epinepherine, blood lactate and glucose concentrations. Only two studies have noted marginal decreases in heart rates (3-5 b.min^{-1}) during exercise (Montner et al., 1995; Anderson et al., 2001) preceded by glycerol hydration while the majority have not reported any effect (Riedesel et al., 1987; Murray et al., 1991; Seifert et al., 1995; Latzka et al., 1997; Wendtland et al., 1997; Latzka et al., 1998; Hitchins et al., 1999; Lamb et al., 1999; Scheett et al., 2001). Lyons et al. (1990) concluded there were no significant differences in heart rates for all drinking regimens due to great variance in the fitness level of the subjects.

Gleeson et al. (1986) observed similar RER values during exercise following both glycerol and control hyperhydration thereby suggesting carbohydrate and fat oxidation was similar. However, RER values did increase over time for both glycerol and control fluid treatments. The increase in RER noted by Gleeson over the time of the performance measure is supported by many others (Miller et al., 1983; Montner et al., 1986; Murray et al., 1991; Hitchins et al., 1999).

It has been suggested that glycerol ingestion may have a direct influence on substrate utilisation during exercise and provide a glucogenesis effect via the liver (Lin, 1977) or act to a limited extent as an oxidative substrate for working muscles. In an effort to

explain further the potential for glycerol to alter substrate metabolism, Gleeson et al. (1986) found no improvement in performance with glycerol ingestion prior to exercising to exhaustion at 73% VO_2 max compared to that observed following glucose ingestion and concluded that exogenous glycerol provided negligible contribution to exercise energy production. This finding is consistent with that of Miller et al. (1983) while Anderson et al. (2001) found no differences in muscle glycogen utilization, circulating epinephrine and lactate production between glycerol and dilute cordial ingestion. Glycogen usage was the same during exercise following glycerol feeding compared with water ingestion. Both treatments also failed to alter (increase/change) serum glucose, FFA, lactate and RER. Blood lactate and glucose concentration during exercise have also been shown to be similar between glycerol hyperhydration and other hyperhydration solutions (Montner et al., 1986; Murray et al., 1991; Hitchins et al., 1999; Anderson et al., 2001).

Much of the literature has shown that man cannot utilise glycerol as a gluconeogenesis substrate rapidly enough, over endogenous glucose, to serve as a fuel source during strenuous exercise. Therefore, glycerol ingestion pre-exercise does not appear to significantly spare muscle glycogen and improve work capacity in man. Importantly, the majority of studies have shown that glycerol-induced hyperhydration has no physiological benefit over other hyperhydrating solutions that could explain the mechanisms that enhance performance.

Thirst/Thermal ratings and RPE

Given that most of the objective physiological and biochemical measures have been unable to confirm conclusively a thermoregulatory and cardiovascular advantage of glycerol compared with other hyperhydrating treatments, it is possible that a subjective rating may establish positive support for glycerol hyperhydration over other hydrating regimens. It is possible that glycerol hyperhydration might have the potential to reduce the perceptions of thermal load and thirst while reducing the perceived effort during the exercise period.

Unfortunately, only a few studies have reported differences in RPE, thirst and thermal rating with glycerol hyperhydration over other hydrating regimens (Murray et al., 1991; Montner et al., 1995; Hitchins et al., 1999; Lamb et al., 1999). Murray et al. (1991)

suggested that a reduced rating for thirst might have negative implications for fluid consumption for those exercising in the heat and thus exacerbate dehydration.

Performance effects

Glycerol induced hyperhydration has been clearly shown to effectively retain more fluid than that achieved by ingesting other hyperhydrating solutions (Riedesel et al., 1987; Wendtland et al., 1987; Lyons et al., 1990; Seifert et al., 1995; Hitchins et al., 1999; Anderson et al., 2001; Coutts et al., 2002) while there is limited evidence to support glycerol as a possible plasma volume expander. However, the direct influence of glycerol ingestion on exercise performance is not completely understood. Any physiological benefits, thermal or cardiovascular, following glycerol-induced hyperhydration must be supported by a performance improvement before the regimen can be recommended to athletes.

Some studies did not report performance measures (Riedesel et al., 1987; Freund et al., 1995; Koenigsberg et al., 1995) and only measured the physiological potential and mechanisms of glycerol-induced hyperhydration over other hyperhydrating solutions. Several other studies had fixed exercise durations without a maximal performance phase (Miller et al., 1983; Lyons et al., 1990; Murray et al., 1991; Latzka et al., 1997) or exercised to volitional exhaustion (Montner et al., 1995; Latzka et al., 1998). The majority of the fixed exercise time durations ranged between 60 min to 150 min.

Recent studies have used a split protocol to determine any possible benefits glycerol hyperhydration has over other hyperhydrating treatments. The split protocol combines a measurable fixed work output and duration with a self-regulated maximal performance test. Two studies have employed a time trial (Hitchins et al., 1999; Anderson et al., 2001), and one an incremental test governed by a set exercise protocol (Inder et al., 1998). In an effort to mimic maximal performance one study interestingly combined a 3x30 min cycling regimen at 73% VO_2 max and a 600 revolution performance test (Seifert et al., 1995). In support of a self regulated performance test, Jeukendrup et al. (1996) reported that protocols for measuring endurance performance at a fixed workload to exhaustion were not reliable and that a time trial is a more reliable and consistent performance test. Foster et al. (1993) also suggested that athletes who are free to regulate their muscular power output achieved greater physiological responses than the work pattern dictated by a set protocol. Even with a set protocol as performance test, Inder et al. (1998) found no

differences in the time to exhaustion following glycerol ingestion. Inder et al. (1998) concluded that while the performance test was too short, the four hour delay in commencing the exercise after glycerol ingest may have negated any potential beneficial effect of the glycerol hyperhydration regimen.

Some of the studies reviewed only provided moderately hot (Montner et al., 1995) to thermoneutral (Inder et al., 1998) environments to evaluate the CV and thermoregulatory responses to glycerol hyperhydration. These conditions may have created insufficient heat stress to induce significant levels of dehydration and thermoregulatory and CV stress. Lack of measurement sensitivity may also have accounted for the inability to detect subtle differences in responses to the treatments. A small number of studies have also made an effort to mimic specific competition and/or training environments to confirm glycerol's advantage in the field rather than in the laboratory. Coutts et al. (2002) had subjects complete two ODT's (swim, cycle and run) in the field on a warm (WBGT 25.4ºC) and hot day (WBGT 30.5ºC) while Grice et al. (1997) had open water swimmers perform a high intensity endurance session in a 25°C 50 m pool for two hours.

An early study by Montner et al. (1995) showed a 21 - 24% improvement in endurance time when exercise was performed at a workload equivalent 60% max power output following pre-exercise glycerol hyperhydration. Montner et al. (1995) concluded that there was substantial variability in the performance improvements observed between individuals and the poor selection of workload may have affected the results. Although there were differences in the fixed work period (30 min vs 90 min), intensity (90%LT vs 73% VO_2 max) and a slight difference in environmental conditions (32 ºC/60%RH vs 35ºC/30%RH), Hitchins et al. (1999) and Anderson et al. (2001) both reported a 5% improvement in a 30 min and 15 min cycling time trial, respectively, following glycerol hyperhydration. Hitchins et al. (1999) in an effort to explain the performance improvements proposed that reductions in serum blood osmolality or electrolyte and its effect on ionic gradients across cell induced by glycerol hyperhydration may have delayed the onset of either muscle or central fatigue.

Anderson et al. (2001) concluded that the increase in performance was mediated by the attenuation in thermoregulatory and cardiovascular strain, and not by changes in muscle metabolism. Coutts et al. (2002) demonstrated a reduced decrement in performance time following glycerol hyperhydration compared to ingestion of a placebo fluid when exercising on a hot day in comparison to the warm day. The ODT mean times were 5.7%

(8 min) faster following the glycerol treatments during the hot day and 1.5% slower on the warm day. They concluded that glycerol hyperhydration offers a physiological defence against the detrimental effects of exercise-induced dehydration in heat stressful environmental conditions. However, no physiological data were collected during the triathlon that could explain the exact mechanisms of glycerol hyperhydration on performance. Scheett et al. (2001) reported a significantly increased time to exhaustion (12.6%) with glycerol over water solution following a rehydration strategy while Seifert et al. (1995) recorded a 8% improvement in time to complete 600 revolution time trial (355 vs 385 sec).

One study showed a greater performance improvement with other hyperhydration treatments than with glycerol. Lamb et al. (1999) reported a non-significant 5.3% improvement with 6% CHO over glycerol hyperhydration in endurance time. Lamb et al. (1999) concluded that glycerol prehydration offered no apparent physiological or performance advantage over prehydration with 6% CHO solution.

There is a considerable amount of variability in the performance test, designated exercise intensity, mode of exercise, physical and trained status of subjects and environmental conditions employed in the various studies that have investigated the effects of glycerol hyperhydration on exercises performance. These variables may partly account for the inconsistencies in the results of the studies. Wagner (1999) and Freund et al. (1995) suggested glycerol hyperhydration might be most effective for those competing in ultra distance sports in hot, humid conditions and should be the focus of future studies.

Motivation to perform also becomes a critical factor that should not be overlooked in comparing the performance improvements in the self regulated performance test while in the laboratory or the field.

Side effects of glycerol

The side effects of glycerol ingestion such as increased irritability of muscles, relaxation of the gall bladder sphincter and increased force and amplitude of intestinal contraction have been well documented (Frank et al., 1981; Robergs and Griffin, 1998). The majority of published research has indicated the presence of some side effects including gastro-intestinal upset (eg bloated and nausea) (Coutts et al., 2002), vomiting (Murray et al., 1991; Latzka et al., 1997; Inder et al., 1998), headaches (Gleeson et al., 1986) and

lightheadedness (Murray et al., 1991) after the ingestion of glycerol. Gleeson et al. (1986) suggested that the side effects may have been due to high dosage (17.5% glycerol) and noted that there was great individuality and variability in response to glycerol induced hyperhydration. No studies reported side effects with the control solution.

Latzka et al. (1997) reported on one occasion two of the eight subjects became nauseated (and vomited) after drinking a glycerol solution. This response required the trial to be aborted and repeated on another day with one of nine removed from study due to an inability to ingest glycerol. Latzka et al. (1997) and Murray et al. (1991) fed subjects glycerol during exercise, which may have delayed gastric emptying and caused subjects to complain of gastrointestinal symptoms but with no headaches (dilute solution). Only one study (Anderson et al., 2001) noted several subjects reporting diarrhea 24 hrs post trial as a result of glycerol-induced hyperhydration. This scenario may have been due to the high osmolality (hypertonic) of the glycerol solution (> 600 mosmol/L).

In support of the ingestion of the glycerol solution, two studies reported that participants did not experience any side effects (Montner et al., 1996; Hitchins et al., 1999) or failed to report possible side effects (Freund et al. 1995; Seifert et al. 1995). In one study one subject complained with the ingestion of both glycerol and water solution (Koenigsberg et al., 1995). It is quite possible that the glycerol and the accompanying volume of fluid may make some participants feel bloated and sick. Individual tolerance to glycerol while exercising may also contribute to the side effects. Clearly any reported side effects could negate any possible performance benefits to glycerol hyperhydration whether they be thermal or cardiovascularly mediated.

Conclusions

The numerous studies investigating the physiological and performance impact of glycerol-induced hyperhydration have reported mixed conclusions. Many of the responses are highly dependent on a multitude of interacting factors. They include: pre-test hydration status, the acclimation and training status of the participants, the performance environment and exercise intensity coupled with performance time. The timing of the exercise performance after the hydration phase also appears to be a major factor that must be considered in light of the research conclusions.

Often the differences in methodologies employed in studies do not mimic actual training or competition conditions. It is in these conditions that an athlete would employ glycerol-induced hyperhydration as a strategy to reduce the thermoregulatory and cardiovascular strain. It would be these conditions that should expose any performance improvements over other hyperhydration solutions.

The overall findings into the effectiveness of glycerol hyperhydration over other hyperhydrating treatments have been equivocal. Research both supports and refutes the effectiveness of glycerol as a hyperhydrating agent and as a performance enhancer. The majority of the literature has shown glycerol ingestion has no physiological/metabolic advantage compared with other ingested solutions that could explain the subtle improvements in performance in a few studies.

Wagner (1999) in his review, noted many methodological differences might be responsible for the disparity between glycerol hyperhydration research. This includes the subjects starting the exercise too soon after glycerol ingestion, not exercising long enough, low exercise intensity, performance versus exhaustion tests and their reliability and the environmental conditions inadequate to stress the cardiovascular and thermoregulatory systems. The reported inconsistencies in study design are supported by Burke and Deakin (2000), Williams (1998) and Latzka and Sawka (2000). The questionable pre-test hydration status of subjects before the trials and the level of dehydration during exercise may also confound the results (Latzka et al., 1997) while further research into the extra water weight and its effect on running economy (Williams, 1998) and cycling uphill (Burke and Deakin, 2000) may also be needed.

Currently, the theoretical advantages of increased sweat rate and a greater capacity for heat dissipation, and the attenuation of cardiovascular and thermoregulatory strain are not consistently observed with a hyperhydrating strategy. Glycerol hyperhydration has been purported as a possible PV expander although there are only a few studies in the literature to support this. Clearly, with only a few studies observing performance benefits the use of glycerol as a hyperhydration strategy warrants further investigation.

CHAPTER 3: METHODS AND PROCEDURES

Subjects

Ten competitive triathletes (7 male, 3 female) were recruited for this study. Recruitment was based on personal best (PB) performances over the Olympic distance triathlon (ODT). The selection criteria required males and females to have a PB of less than 2 hr 5 min and 2 hr 20 min, respectively. Subjects were highly trained with a mean maximum oxygen uptake of 65.5 ± 6.6 $ml.kg^{-1}. min^{-1}$ for males and 61.4 ± 4.2 $ml.kg^{-1}. min^{-1}$ for females. All had extensive training backgrounds and were training regularly while competing at the national level.

Each subject signed an informed consent form (Appendix A) in accordance with the University of Canberra Committee for Ethics in Human Research and the Australian Sports Commission Human Ethics Committee and were given a detailed explanation of the procedures. All subjects were financially compensated for participating in this study.

Methods

Experimental Design

The study used a double blind, randomised and crossover design to determine the effect of glycerol ingestion on a simulated triathlon that mimicked an ODT performance. The independent variables were hyperhydration type (glycerol, placebo) and time. Each ODT trial was preceded by either oral ingestion of 1g. kg^{-1} BM glycerol dissolved in 22g. kg^{-1} BM (4% CHO) sports drink (ISOSPORT™) solution or, 23g. kg^{-1} BM sports drink (ISOSPORT ™) mixed in cool water (~15º C). The glycerol hyperhydration procedure was similar to those employed in other studies (Miller et al., 1983; Lyons et al., 1990; Montner et al., 1996; Latzka et al., 1997; Latzka et al., 1998; Hitchins et al., 1999; Anderson et al., 2000; Coutts et al., 2002).

One to two weeks leading up the trial, each athlete underwent two familiarisation sessions. These sessions were used to: 1) determine the cycling intensity to be used during the trials and to individually adjust the bicycle in preparation for the trials; and 2) familiarise the athlete with the treadmill and the operation of the touch screen to self

regulate the running speed during the 10 km time trial. Both familiarisation sessions were conducted in controlled laboratory conditions (30º C/60%RH).

Diet and Training

The subjects were asked to complete a training log and dietary food intake record for three days leading up to each of the trials. All subjects were instructed by a nutritionist on how to estimate portion size and measure fluid intake. The log provided a comprehensive profile of the training (frequency, duration, intensity and type) and dietary intake of each subject (macronutrients). On the basis of this profile, each subject was his or her own control for the first trial and was instructed to match their food intake and training program leading into the second trial. Given this directive, both diet and training were similar for each subject for three days prior to the two ODT performance trials in order to ensure similar muscle and liver glycogen storage levels (Coyle et al., 1986) and levels of fatigue. Recording sheets were also supplied (and completed) which allowed crosschecking of diet, training and fluid intake prior to both trials. Each subject was also given an extensive dietary analysis and counselling session by a qualified nutritionist after both trials as a part of their compensation for participating in this study. Table 1 represents the mean daily nutrient and energy intake of macronutrients ($g.day^{-1}$) over the 3 days prior to the ODT.

Table 1. Mean daily macronutrient and energy intake of macronutrients ($g.day^{-1}$) over the 3 days prior to the ODT

	Kilojoule (kJ)	CHO	Fat	Protein
Glycerol	16141.2***	548.2	126.0	156.4***
	(5041.4)	(128.5)	(80.2)	(32.0)
Placebo	15472.2	526.2	111.5	141.2
	(6694.8)	(221.2)	(86.9)	(34.6)

Values are means and (SD).
**** denotes a significant difference between trials: $p < 0.001$.*

Performance Trials

Each subject arrived at the laboratory at the same time of the day for both trials, three hours prior to the actual triathlon simulation. This time period was used to reduce any short-term disruptions that may have impacted upon the physiological and performance responses elicited in the trials. All subjects were instructed to standardise their hydration

and training status and diet during the three days prior both performance trials. Subjects were tested either at 7 am or 10 am and were requested not to consume any food, drink tea or coffee on the morning of the trials (other than what they consumed in the previous trial).

Athletes were tested during March and April (beginning of autumn) where the average ambient temperature was 15 – 23 ° C. It was assumed that all subjects came into the trial heat acclimatised as a result of competition undertaken over the preceding summer months. The study was conducted in Canberra at an altitude of approximately 601 m above sea level.

Subjects undertook two simulated ODTs with two different hydration treatments (ie glycerol vs placebo). The cycle and run phases were conducted in a laboratory at approximately 31° C (± 0.9%) and 61% (± 4.0%) relative humidity (RH). The swim phase was undertaken in either a 25 or 50 m pool (27.2 – 28.2° C). The length of the pool was constant for all subjects in both trials. No two trials were more than 10 days apart and a minimum of 7 days apart.

The swim phase was completed at a controlled velocity while the cycle phase was undertaken at a fixed power output. The 10 km run was completed at a self-regulated work rate (time trial). By controlling both the swim and cycle effort it was possible to assume a similar physiological cost/effort for both trials preceding the run portion of the ODT. This was a crucial control to accurately measure changes in the true performance test (run time trial) and account for the influence of the effects of energy demands, thermoregulatory and cardiovascular responses from the preceding activities.

It is important to note that during the first trial, the swim phase was a self-regulated performance time trial and the cycling phase was a pre-determined, but adjustable, power output. These standardised work outputs were established in the first trial and were replicated as precisely as possible for the second trial.

The time trial run was used to evaluate the potential performance enhancement induced by glycerol compared to the placebo. The fixed work output in the swim and cycle phases allowed the measurement of physiological variables at a comparable known output between trials. The format of the study was chosen as it combined the responses to both a measured, fixed work output and a maximal performance response.

Procedures

Body mass

Subjects were weighed on calibrated digital scales accurate to 20 g (Model DS-410, Teraoka Seiko Co., Ltd, Japan) dressed in only underwear. The subjects were instructed to stand still and look straight ahead (Norton and Olds, 1996).

Height

Stretched height was measured using a stadiometer (Holtain Ltd, Crymych, Dyfed, England) to a resolution of ± 0.1 cm. The heel, buttocks and upper part of the back of the subject were positioned against the stadiometer and their feet remained on the ground during the measurement. The stadiometer was lowered down onto each subject's head in the Frankfort plane. The subjects were instructed to remain still, look straight ahead and hold a deep breath during the measurement (Norton and Olds, 1996).

Sum of skinfolds

Each subject underwent anthropometric measures to calculate the sum of seven skinfolds (biceps, triceps, subscapular, abdominal, supraspinale, mid thigh and medial gastrocnemius) to be calculated. Skinfolds were measured using the method endorsed by the International Society for the Advancement of Kinanthropometry (ISAK) by a Level 2 ISAK accredited anthropometrist. Harpenden calipers were used to measure skinfolds (Model: HSK-BI, British Indicators, West Sussex, UK).

Measurements during the trials

Oxygen uptake (VO_2) was measured between 5-10, 25-30, 50-55 min during the cycling phase and between 5-10, 15-20, 25-30 min during the run phase of each trial. The subjects breathed through a suspended rubber mouthpiece and a two-way Hans Rudolph valve with a nose clip positioned to prevent the intake of air through the nose. Oxygen consumption was calculated at 30 s intervals from expired air collected in a custom built automated Douglas bag gas analysis system (Australian Institute of Sport, ACT, Australia). Inspired air was drawn directly from the room while expired air passed through 50 mm diameter low resistance respiratory tubing into Douglas bags of 100 L capacity.

The Douglas bags were automatically rotated at 30 s intervals. After each 30 s, an expired gas sample was obtained, oxygen and carbon dioxide concentrations were measured using an Ametek N-22M electrochemical oxygen sensor (Model S3A) and a Ametek P-61b infrared carbon dioxide sensor (Applied Electrochemistry, Ametek Instruments, Pittsburgh, PA), respectively. Both sensors were calibrated prior to, and during the trials, with known high, middle and low calibration gases (O_2:18-15%;CO_2:3-5%) of alpha grade. The sample was then pumped into a previously calibrated 600 L Collins chain-compensated gasometer (Warren E. Collins Inc. Braintree, MA) ('Tissot' tank) for direct measurement of ventilatory volume (V_E). The ventilatory volume was corrected to standard temperature, pressure, dry (STPD) conditions and oxygen and carbon dioxide concentrations were then used to calculate oxygen uptake and carbon dioxide production (McArdle, Katch and Katch, 1991). Typical error of measurement (TEM) for maximal oxygen consumption was 1.25%.

The gas analysis system was interfaced to an IBM compatible computer by Optical Rotary Encoders (RS 341-597, Berne, Switzerland) that calculated the rate of oxygen consumption, carbon dioxide production, minute ventilation and the respiratory exchange ratio every 30 s from conventional equations (Withers et al., 2000) which were expressed as per min values.

Familiarisation and preliminary testing session protocols

To determine the optimal cycling workload for the performance trial it was necessary for all the subjects to take part in a pre-trial Graded Cycle Test (GCT). The results of the GCT were used to determine the work rate on the cycle ergometer that corresponded to a blood lactate concentration of approximately 4 mmol.L^{-1} during the first trial.

After an initial individual warm-up, the subject began the GCT by cycling for 5 min at an intensity equivalent to 3 W.kg $^{-1}$ BM. This was increased by 1 W.kg $^{-1}$ BM every five min until a blood lactate concentration of 4 mmol.L $^{-1}$ was reached. The power output at the measured blood lactate of 4 mmol.L $^{-1}$ was used as a guide to the determination of each subject's cycling intensity in the first trial (Heck et al., 1985). In the first trial the power output was matched as close as possible to the 4 mmol.L $^{-1}$ obtained in the GCT. However, to compensate for the fatigue effects of the swim prior to the run, the load could be adjusted on the basis of the subject's own perception of the time trial effort for

the full 60 min during the ODT. Therefore, the wattage could be either increased or decreased depending on the subject's fatigue prior to starting the time trial.

Cycling was performed on a LODE Excalibur electromagnetically braked ergometer (Lode, Groningen, The Netherlands) during the GCT and both performance trials. The bicycle was equipped with a racing seat, low profile handlebars and pedals for clipless shoes. The power outputs could be manually adjusted during cycling independent of changes in pedalling frequency in both trials. Subjects wore their own shoes during the trials. Before the GCT and both performance trials the cycle ergometer was adjusted according to the subject's bicycle dimensions and preferred position.

Running was conducted on a previously calibrated treadmill (HYENA, Australian Institute of Sport, Australia) set at a 1% grade (to simulate wind resistance). A familiarisation session was conducted to instruct each athlete on the use of the touch screen monitor, and to reduce anxiety and possible learning effect encountered with each performance test. The touch screen allowed the subject to manually control the treadmill speed during the run portion of the simulated triathlon.

To further reduce anxiety-mediated physiological responses, each subject inserted the mouthpiece used for the collection of expired gas and walked through the sequence of the performance phases. The familiarisation session also simulated the procedures for weighing in, blood withdrawal and measurement of rectal temperature.

Each familiarisation session was conducted in a climate chamber that was set at 30°C and 60% relative humidity with a fan providing air speed at approximately 5 $m.sec^{-1}$.

The performance trials

Before each performance trial all subjects consumed the same breakfast, which consisted of muffins and bananas, while no fluid was consumed. The quantity of food was consistent for both trials. Subjects began hyperhydrating at around 30 min (-150 min) after breakfast in which they were asked to void their bladder and provided a urine sample (mid-stream) to determine pre-test hydration status. Subjects were then weighed on calibrated digital scales (Model DS-410, Teraoka Seiko Co., Ltd, Japan).

Hyperhydration phase

After breakfast, and 150 min before the performance trial commenced, all subjects had 60 min (between -150 and -90 min) to drink a measured amount of fluid equating to 23g. kg^{-1} of BM.

The fluid ingested for the treatment trial consisted of 1g (glycerol). kg^{-1} BM dissolved in 22g.kg^{-1} BM (4% CHO) sports drink (ISOSPORT™) solution. For the placebo trial 23g.kg^{-1} BM of sports drink (ISOSPORT ™) mixed in cool water was consumed. The solutions were chosen on the basis of pilot work by Hitchins et al. (1999) who found that taste of the glycerol and placebo drinks, namely ISOSPORT™, was indistinguishable. To maximise hyperhydration and minimise any side effects, the timing of the ingestion of fluid matched those used by Hitchins et al. (1999). Subjects sat in a thermally neutral environment (approximately 21°C/50%RH) during breakfast, the hydration phase and the period leading up to the performance trial (180 min).

Urine samples were collected every 30 min during the pre-performance hyperhydrating period (-180, -150, -120, -90, -60, -30 and 0 min) and post-run period to determine urine osmolality and output volume. Urine osmolality was determined using a Micro Osmometer (Model 3MO Advanced instruments Inc, Norwood, USA.). Changes in total body water were calculated by subtracting accumulated urine volume at each point from total, initial fluid intake. Insensible water loss, metabolic water and sweat losses were assumed to be similar between performance tests (Sawka, 1992).

Blood collection and analysis

Venous blood samples (12 mL) were collected at -150 min (baseline), prior to the swim (0 min) and post-run in both trials. All venous blood samples were withdrawn from a superficial forearm vein with stasis (tourniquet) by a trained nurse using a Terumo Surflo 21 G winged infusin set (Terumo-Elkton MD) via a luer adaptor (Griener Labortechnik, Frickenhausen, Germany). Subjects stood for at least 20 min prior to all venous blood withdrawals. This controlled for influence of posture on plasma volume (Hagan et al., 1978). Plasma osmolality was determined using a Micro Osmometer (Model 3MO Advanced instruments Inc, Norwood, USA.). Percentage changes in blood volume and plasma volume were calculated from hemoglobin and haematocrit values according to the formula of Dill and Costill (1974).

Venous blood samples were drawn into a 4 mL and an 8 mL K3 EDTA Vacuette (Griener Labortechnik) and stored for approximately 60 min prior to analysis. The 8 mL sample was allowed to clot (in a tube containing SST Gel and clot activator), spun at 4000 rev.min^{-1} for 5 min and then analysed on a Hitachi 717 automatic analyzer (Boehringer-Mannheim, Japan). The 4 ml sample was directly analysed on a Bayer H*3 Haematology analyser (Bayer Diagnostics, Tarrytown NY, USA). Blood samples were analysed for sodium, potassium, chloride and hemoglobin concentration, and haematocrit, in the biochemistry laboratory at the Australian Institute of Sport.

Arterialized finger tip blood samples were taken manually using an autolet fitted with a platform and lancet. Blood samples were withdrawn pre- and post-swim and every 10 min thereafter during the cycle and run phases. Twenty five microlitres (μL) of whole blood was pipetted into 50 μL of lysing agent making two parts lysing agent to one part blood (total 75 μL). The blood was then analysed on a calibrated YSI 2300 Stat-Plus glucose and lactate analyser (YSI – Yellow Springs Instruments, OH) for blood lactate and glucose levels. The remaining blood was analysed for pH and bicarbonate concentrations in a Ciba-Corning 278 blood gas system (Ciba Corning, Boston, USA).

Temperature measurement

A rectal probe (Model 401, Yellow Springs Instruments, Yellow springs, OH) was inserted 10 cm beyond the external sphincter for measurement of rectal temperature. The subjects inserted the rectal probe with the aid of a water-soluble lubricant (KY Lubricating Jelly, USA). The rectal probe was inserted before the swim and remained in place during the cycle and run phase. The rectal probe was connected to an eight-channel switch box (Zentemp 5000, Digital Thermometer, Australia). Rectal temperature was measured pre- and post-swim phase and recorded every 5 min during the cycle and the run phase. The trial was terminated if the participant's rectal temperature exceeded 39.5°C while displaying signs of heat intolerance, or when the athlete chose to discontinue the test.

Fluid loss changes

Fluid losses were determined from measured changes in body mass from measurements taken at pre-test, pre-swim, post-swim, post-bicycle ride and post-run on calibrated digital scales (Model DS-410, Teraoka Seiko Co., Ltd, Japan) and was corrected for fluid intake

and urine output volume. In the cycle phase, participants were weighed after urine output while during the run phase they were weighed before the urine output. Therefore, BM changes were corrected after the final weigh in. No correction was made for respiratory water loss or metabolic fluid changes (assumed negligible/equal in both trials). Other sources of error in the calculation of true sweat losses for swimmers include water absorbed through the skin, accidental ingestion and unreported urine losses due to the diuretic stimulation of water immersion (Cox et al., 2002).

Heart rate

Heart rate was measured immediately pre- and post-swim and every 5 min during the cycle and run phases using a Polar Heart Rate monitor (Polar Vantage, Finland). The heart monitor was firmly strapped to the chest (below the pectorals) of each subject after the swim phase and remained attached until completion of the run phase.

Subjective ratings

Subjects were asked to indicate their Rating of Perceived Exertion (RPE) on a 6-20 scale (6 = no effort, 20 = maximal exertion; Borg, 1982), thermal sensations on a 0-8 scale (0 = unbearably cold, 8 = unbearably hot; Young et al., 1987) and thirst, 0-10 (0 = not thirsty-10 very thirsty; Murray et al., 1991). All subjective measures were verbally acknowledged following the swim phase and at 10 min intervals during the cycle and run phase. Participant's comments (e.g., bloated feeling, nausea) were also recorded.

Laboratory environment

The temperature and humidity of the chamber was recorded every 5 min and subtle fluctuations were noted and corrected.

The Swim Phase

The swimming time trial was conducted in an indoor 25 or 50 m chlorinated pool (water temperature ranged from 27.2 – 28.2°C) during March/April. All subjects swam in their swimming costume for both trials.

In the first trial the subjects were instructed to swim for as far as possible for 20 min (performance race pace). The subjects were then stopped at the nearest 100 m after completing approximately 18 min of swimming (given that each 100 m took 1:10 to 1:40

min to complete). During trial two, the participants were informed of their swim pace by visual signals and adjusted their pace accordingly to match that achieved in trial one. This ensured that the distance and velocity remained constant for both trials. A standard warm-up was employed in which time and distance were also recorded in trial one and repeated in trial two. Heart rate, RPE, thermal and thirst rating pre- and post-swims were also recorded in both trials. Upon completion of the swim the subjects jogged 80 m (approximately 20 s) to the climate chamber.

The 60 minute Cycle Phase

Upon arrival in the heat chamber the subjects towelled down, weighed and then rectal temperature was recorded. The subject's own shoes were attached to the bicycle with the helmet placed on the handlebars prior to the athlete entering the chamber. Heart rate, cadence, power output (W), T_{rec} and chamber temperatures were recorded at 5 min intervals. RPE, blood lactate, pH, Glucose, HCO_3 (bicarbonate), and thermal and thirst ratings, were recorded every 10 min. During the bike ride cool water was supplied (10 $mL.kg^{-1}$ BM) and consumed *ad libitum*. This was thought to mimic an athlete's normal fluid consumption during a race. The total fluid consumed during the bike ride was noted. Oxygen consumption (VO_2) was measured between 5-10, 25-30, 50-55 min during the cycling phase. Oxygen uptake values were averaged for each five-minute period and were expressed per min for each sample.

Before and during trial two, each participant was briefed on the amount of work required in the swim and cycle phase. This allowed the participants to be familiar with the effort from the previous trial, and provide uniformity in total work (and thus physiological demands) between the trials.

The 10 kilometre time trial run

After the cycle phase, the subjects towelled down and were then weighed. Subjects then put on their running shoes and walked two metres to the treadmill (approximately 5-10 s). They then proceeded to increase the treadmill speed until their individual 10 km race pace was reached.

The final assessment of performance required the participants to run as intensely as possible for the 10 km. This protocol allowed the subjects to mimic actual competition

conditions and thereby provide a more valid reflection of performance. Each subject was verbally motivated to elicit the best possible performance on the run. This remained constant for each subject.

The subjects were given visual feedback on the distance covered and the time taken on the touch screen. Expired air samples were collected between 5-10, 15-20, 25-30 min during the run session. Oxygen consumption values were averaged for each five-minute period and were expressed per min for each sample.

During the run, 150 mL of cool water was given at 2.5 km, 5 km and 7.5 km to simulate drink stations. After the run, the participants were encouraged to empty their bladder (if possible) to measure the remaining volume of urine. Data were collected up to and including the slowest finishing time of the 10 km run. Statistical analyses on individual differences within trials were taken at 30-35 min depending on the fastest finishing time and variable.

Oxygen Uptake

Post-test

Two weeks after the final performance trial, the subjects completed a discontinuous maximal treadmill test. The maximal treadmill test protocol was comprised of three minute stages with a one minute recovery between stages to allow for blood collection. Treadmill speed began at 10 $km.hr^{-1}$ and was increased 2 $km.hr^{-1}$ every stage while a 1% grade remained constant. The test was terminated when the subject reached volitional exhaustion. The peak rate of oxygen uptake (VO_2 peak) was defined as the highest oxygen uptake a subject attained during two consecutive 30 s sampling periods. Data from this test were used to ascertain the consistency of effort in the cycle phase and to determine changes in run time trial as a percentage of VO_2 peak for both trials.

Data analysis

Statistical analysis

Descriptive statistics [mean, ± standard deviation (SD)] were determined for all variables. Due to only five of the ten subjects actually finishing the 10 km performance run, a within-subjects repeated measure *t*-test (two-tailed) was used to analyse the data (Hopkins – personal communication, 2002). The t-test determined whether differences in the means between treatments were statistically significant. The alpha level of significance for all tests was $P < 0.05$. ($t=2.776$, $p<0.05$, $t= 4.604$, $p<0.01$).

CHAPTER 4: RESULTS

Final number of participants

Only five of the 10 subjects completed the final 10 km self regulated time trial on both test occasions. Reasons for non-completion were either due to 1) the arduous nature of the trials 2) the side effects associated with the ingestion of glycerol or 3) the combination of the two afore mentioned reasons. Only the data obtained from the five subjects who completed both the run time trials have been reported in this study.

Summary of performance data

Swim phase

There were no differences in the mean distance (1260 ± 54.8 m) covered during the swim between treatments. These data reflect uniformity in physiological effort in the swim phase between trials. These data reflect consistency in physiological effort in the swim between trials.

Cycle phase

The mean cycling output remained constant with no significant differences in the mean fixed workload (206 ± 28.2 W; range 158 – 232 watts) and cadence (placebo 94.4 ± 16.2 – glycerol 95 ± 16.5) between treatments. These data reflect consistency in total work in the cycle phase for both trials

Run phase

The mean 10 km run time for the placebo trial was 40 min 21 sec (± 2.9) while the glycerol trial was 39 min 22 sec (± 2.0). This represents a mean difference of 2.1% in finishing time between treatments. Three of the five subjects in the glycerol trial improved their 10 km time by 7.0, 2.4 and 2.7%, respectively. The finishing time for one subject did not change for both trials while another subject had deteriorated by 2.3% in the glycerol trial. However, the mean differences in the 10 km performance time were not significant between treatments.

The average run pace was 1.5% faster in the glycerol trial (3.96 min. km.$^{-1}$) compared with the placebo trial (4.04 min. km. $^{-1}$).

Reported side effects

Five out of ten subjects in the glycerol trial complained of feeling either bloated or nauseous during the run. Four of the five who did complain in the glycerol trial did not complete the 10 km run. However, only one subject felt unwell during both trials.

Physiological and anthropometric characteristics of subjects

The mean age, anthropometric characteristics and maximal oxygen uptake values of the five subjects are reported in Table 2.

Table 2. Physical characteristics of subjects (n=5)

	Age (years)	Weight (kg)	Height (cm)	Sum of 7 skinfolds (mm)	VO_2 max ($ml.kg^{-1}.min^{-1}$)	VO_2 max ($L.min^{-1}$)
Subject 1	22.6	77.6	189.2	43.9	69.6	5.40
Subject 2	29.2	50.5	163.7	65.2	61.2	3.09
Subject 3	22.5	68.0	179.1	52.4	69.9	4.75
Subject 4	22.4	76.2	187.3	48.2	68.7	5.23
Subject 5	28.8	68.6	172.1	53.4	57.9	3.97
Mean	**25.1**	**68.2**	**178.3**	**52.6**	**65.5**	**4.49**
SD	**3.6**	**10.8**	**10.6**	**8.0**	**5.5**	**1.0**

The mean value for body mass (BM) pre-trial for the glycerol trial was 68.2 ± 10.8 kg while BM prior to the placebo trial was 68.3 ± 10.8 kg. There were no significant differences between means in baseline body mass between trials ($p > 0.05$).

Diet

The mean daily kilojoule (kJ) intake three days prior to the glycerol trial was 16141 ± 5041 kJ while daily placebo trial intake for the same period was 15472 ± 6876 kJ. Mean daily protein intake three days prior to the trials for glycerol and placebo was 156.4 g ±

32.0 and 141.2 ± 34.6 g, respectively. Mean daily carbohydrate intake for glycerol and placebo trials was 548.2 ± 128.5 and 526.2 ± 221.2 g, respectively. Mean daily fat intakes for glycerol and placebo trial were 126. ± 80.2 and 111.5 ± 86.9 g, respectively. The contribution of energy from macronutrients to total energy consumed for the glycerol trial was approximately 17% protein, 27% fat and 56% carbohydrate. Corresponding figures for the placebo trial were 17% protein, 26% fat and 57% carbohydrate.

There were significant differences between trials for mean energy intake and the mean protein intake during the three days prior to the ODT ($p < 0.01$). There were no significant differences in the mean carbohydrate and fat intake between trials, collected three days prior to the ODT.

Urine and blood parameters

Table 3. Mean values for selected blood and urine parameters for the pre- and post-hydration phase and at the end of the run for the glycerol and placebo group (n=5).

	Pre-hydration		Post-hydration		Post-run	
	Glycerol	**Placebo**	**Glycerol**	**Placebo**	**Glycerol**	**Placebo**
Plasma osmolality ($mOsmol.L^{-1}$)	292.0 (7.4)	290.2 (4.2)	294.8 (4.8)*	281.8 (7.7)*	300.0 (7.3)*	293.2 (6.3)*
Urine osmolality ($mOsmol.L^{-1}$)	562.8 (271.8)	634.4 (335.3)	601.0 (170.2)*	156.0 (121.6)*	607.0 (133.0)	510.4 (85.1)
Haemoglobin ($g.dL^{-1}$)	14.8 (1.0)	15.2 (0.7)	14.7 (1.0)	14.6 (0.5)	15.1 (0.8)	15.2 (0.6)
Haematocrit (ratio)	0.45 (0.02)	0.47 (0.03)	0.45 (0.03)	0.45 (0.03)	0.45 (0.00)	0.46 (0.00)
Sodium ($mmol.L^{-1}$)	140 (0)	137.4 (2.5)	137.8 (0.8)	135.6 (4.1)	140.6 (1.7)	141.4 (1.1)
Potassium ($mmol.L^{-1}$)	4.4 (0.3)	4.2 (0.2)	4.1 (0.3)	4.1 (0.2)	4.1 (0.1)	4.1 (0.3)

*Values are means, (SD), * Significant difference between treatments.*

The data presented in Table 3 will be commented upon in two ways. Firstly, descriptive comments will be made on the mean differences between treatments for the measures taken at each individual collection point (pre-hydration, post-hydration, post-run) and the changes over time. Secondly, comments will only be made where there is a statistically significant effect of the treatment upon selected variables.

Pre-hydration

There was a 17% (71.6 $mOsmol.L^{-1}$) difference in the mean pre-hydration urine osmolality between trials. There were, however, no significant differences in the treatment means for plasma and urine osmolality, haematocrit, hemoglobin, sodium and potassium prior to the hydration phase (Table 3).

Post hydration

Plasma osmolality

At the end of the hydration phase, the mean plasma osmolality for the glycerol trial was 294.8 ± 4.8 $mOsmol.L^{-1}$ while for the placebo trial it was 281.8 ± 7.7 $mOsmol.L^{-1}$ (Table 3).This change represents a 1% (2.8 $mOsmol.L^{-1}$) increase in plasma osmolality at the completion of the pre-hydration phase compared with post-hydration phase for the

glycerol trial. The placebo trial had a 2.9% (8.4 mOsmol.L^{-1}) decrease in plasma osmolality at post-hydration compared with pre-hydration. At the end of the hydration phase there were significant differences between the treatment means in plasma osmolality ($p < 0.01$) (Table 3).

Urine osmolality

Mean urine osmolality increased by 6.4% (38.2 mOsmol.L^{-1}) for the glycerol trial while the placebo trial had a 75.5% (478.4 mOsmol.L^{-1}) reduction at post-hydration compared with pre-hydration. There was a significant difference between the treatment means at post-hydration compared with pre-hydration phase ($p<0.05$).

Haematocrit

The mean haematocrit concentration in the glycerol trial remained unchanged while the mean haematocrit in the placebo trial increased by 4.3% at post-hydration phase compared with pre-hydration phase.

Plasma volume

Mean plasma volume increased by 3.4% in the placebo trial while the mean plasma volume in the glycerol trial increased by 0.6% at post-hydration compared with the pre-hydration phase. There were no significant differences between the treatment means for plasma volume post-hydration.

Potassium

The mean potassium concentration for the glycerol and placebo trials decreased at the end of the post-hydration phase by 6.8% and 2.4% compared with pre-hydration phase, respectively.

Post run

Plasma osmolality

At the end of the run phase plasma osmolality for glycerol was 300.0 ± 7.3 mOsmol.L^{-1} while placebo was 293.2 ± 6.3 mOsmol.L^{-1}. This represents a 2% (6 mOsmol.L^{-1})

increase from the pre-swim (post-hydration) osmolality for the glycerol trial and an increase of 3.9% (11 mOsmol.L^{-1}) for the placebo trial.

At the end of the run phase there were significant differences between treatment means in plasma osmolality ($p < 0.01$) (Table 3).

Urine osmolality

The mean urine osmolality post run for the glycerol trial was 607 ± 133.2 mOsmol.L^{-1} while placebo was 510 ± 85.1 mOsmol.L^{-1}. The difference represents a 326% increase in mean urine osmolality in the placebo trial at the end of the run compared with the post-hydration phase. There was a 9.9% increase in mean urine osmolality in the glycerol trial at the end of the run compared with the post-hydration phase.

No significant differences between treatment means were observed.

Potassium

The mean potassium concentration for the glycerol trial and placebo trial remained unchanged at the end of the run phase compared with the post-hydration phase.

Urine and plasma changes during the hydration phase and the run

Urine output/volume

Urine output peaked at -60 min (366.4 ± 180.3 ml) and decreased to 96.8 ± 30.2 ml prior to the swim phase in the glycerol trial (Figure 1). In the placebo trial the mean urine output peaked at -30 min (421.6 ± 161.5 ml) and decreased to 248.4 ± 176.8 ml prior to the swim phase. There were significant differences in the mean urine output at -90 min ($p < 0.05$), -60 min ($p < 0.05$) and -30 min ($p < 0.05$) between treatments (Figure 1).

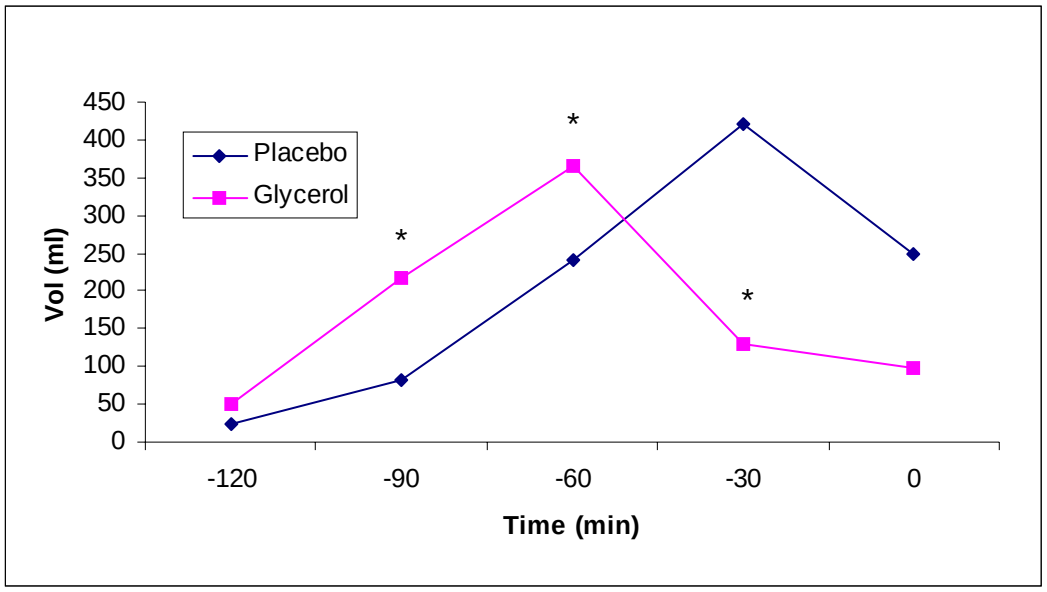


Figure 1: Mean urine volume during hydration phase

** = Significant difference between treatments (p >0.05)*

The hydration phase in the glycerol trial resulted in the mean total body water (TBW) increasing by 701.8 ml ± 353.1 ml. The placebo trial resulted in a mean TBW increase of 547.6 ml ± 353.4 ml (Figure 2). The mean difference of 154.2 ml (26%) suggests greater retention of fluid by the ingestion of glycerol compared with the placebo trial. The subjects retained an average of 45.0 % of total fluid intake in the glycerol trial compared to 35.3 % in the placebo trial post-hydration.

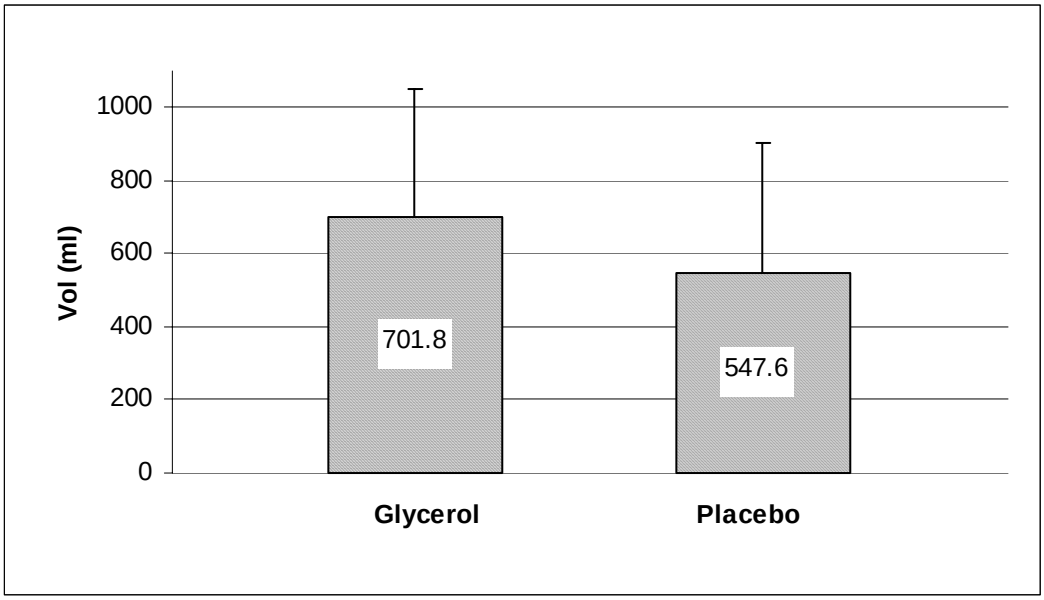


Figure 2: Mean fluid retention post-hydration phase

There were no significant differences in the mean fluid retention between treatments ($p > 0.05$). Mean percentage of accumulated urine volume outputs at -120, -90, -60, -30 and 0 min pre-exercise for the glycerol trial was 3.2, 17.0, 40.4, 48.8 and 55 respectively. The mean percentage of accumulated urine volume outputs in the placebo trial was 1.4, 6.5, 21.9, 48.9 and 64.7, respectively.

Urine osmolality

Table 4. Mean urine osmolality (mOsmol.L^{-1}) before and during the hydration phase

Measure	Time					
	Pre	**-120 min**	**-90 min**	**-60 min**	**-30 min**	**0 min**
Glycerol	562.8	597.0	360.4	365.6	487.4*	601.0*
	(271.8)	(316.6)	(303.1)	(267.0)	(167.1)	(170.2)
Placebo	634.4	861.8	734.0	247.4	88.0*	156.0*
	(335.3)	(242.7)	(390.1)	(252.9)	(32.0)	(121.6)

*All values are means, SD = ( ). * Significant difference between treatments, $p<0.05$.*

There were significant differences in the mean urine osmolality between treatments at -30 min ($p < 0.05$) and 0 min ($p < 0.01$).

Post cycle

Urine output

Cycle phase

Only one subject was able to produce a urine sample post cycle for the placebo trial (268 mL) while subjects in the glycerol trial were unable to produce a urine sample.

Run phase

Mean post-run urine output was significantly (24%) greater in the glycerol trial than the placebo trial (82 ± 0.1 ml vs 62 ± 0.1 ml) ($p < 0.01$).

Physiological responses and performance data

Table 5. Mean values (± SD) for physiological measures obtained during the simulated ODT (n=5).

		Pre-swim		Post-swim		Post-cycle		Post-run	
	Units	**Glycerol**	**Placebo**	**Glycerol**	**Placebo**	**Glycerol**	**Placebo**	**Glycerol**	**Placebo**
Rectal temp	(ºC)	37.0 (0.1)	37.2 (0.3)	37.7 (0.5)	37.8 (0.2)*	38.5 (0.6)#	38.4 (0.3)*#	39.7 (0.5)#	39.6 (0.4)#
Heart rate	($b.min^{-1}$)	77 (18)	78 (22)	144 (21)	145 (16)	149 (15)*	149 (17)*	186 (6)*	183 (10)*
Blood lactate	($mmol.L^{-1}$)	1.5 (0.4)	1.6 (0.4)	3.4 (1.1)*	3.1 (0.9)*	3.3 (1.4)*#	3.0 (0.6)*#	5.7 (1.4)#	5.7 (1.7)#
pH		7.401 (0.020)	7.396 (0.017)	7.357 (0.032)	7.365 (0.024)	7.408 (0.019)	7.390 (0.031)	7.423 (0.021)	7.425 (0.042)
Blood glucose	($mmol.L^{-1}$)	4.6 (0.7)	4.8 (0.6)	4.5 (0.9)	4.2 (0.7)	4.7 (1.4)*	3.8 (1.2)*	6.3 (0.7)*	5.6 (0.8)*
Bicarbonate	($mmol.L^{-1}$)	26.6 (2.9)	25.9 (1.4)	23.0 (4.4)	20.0 (4.5)	24.2 (2.2)#	21.5 (2.0)#	20.6 (2.5)#	19.9 (5.4)#
Body mass	(kg)	68.6 (10.8)	68.5 (10.9)	68.5 (10.8)*	68.4 (10.9)*	67.8* (10.7)	67.6* (10.8)	66.8* (10.5)	66.7* (10.6)
Sweat losses	(mL)			70 (94)	62 (86)	1299 (215)	1405 (174)	1462 (296)	1348 (162)
Distance	(m)			1260 (54.8)	1260 (54.8)				
Power output	(W)					206 (28.2)	206 (28.2)		
Run pace	($min.km^{-1}$)							3.96 (0.2)	4.04 (0.3)

** =Significant difference within treatments, p<0.05.*

 # =Significant difference within treatments, p<0.01.

The data presented in Table 5 will be commented upon in two ways. Firstly, comments will be made on the comparison of the mean differences within treatments. Secondly, comments will only be made on the statistically significant effects of the treatments for selected variables between treatments. The final data collection point used for the statistical analysis for rectal temperature, heart rate, RPE and thermal and thirst rating (Table 6) during the run was at 35 min. While the final collection point used for the statistical analysis for blood lactate, blood glucose and bicarbonate concentration and pH during the run was at 30 min.

Rectal temperature

Swim phase

Differences in the mean rectal temperature in the pre- and post-swim phase were non significant within treatments.

Cycle phase

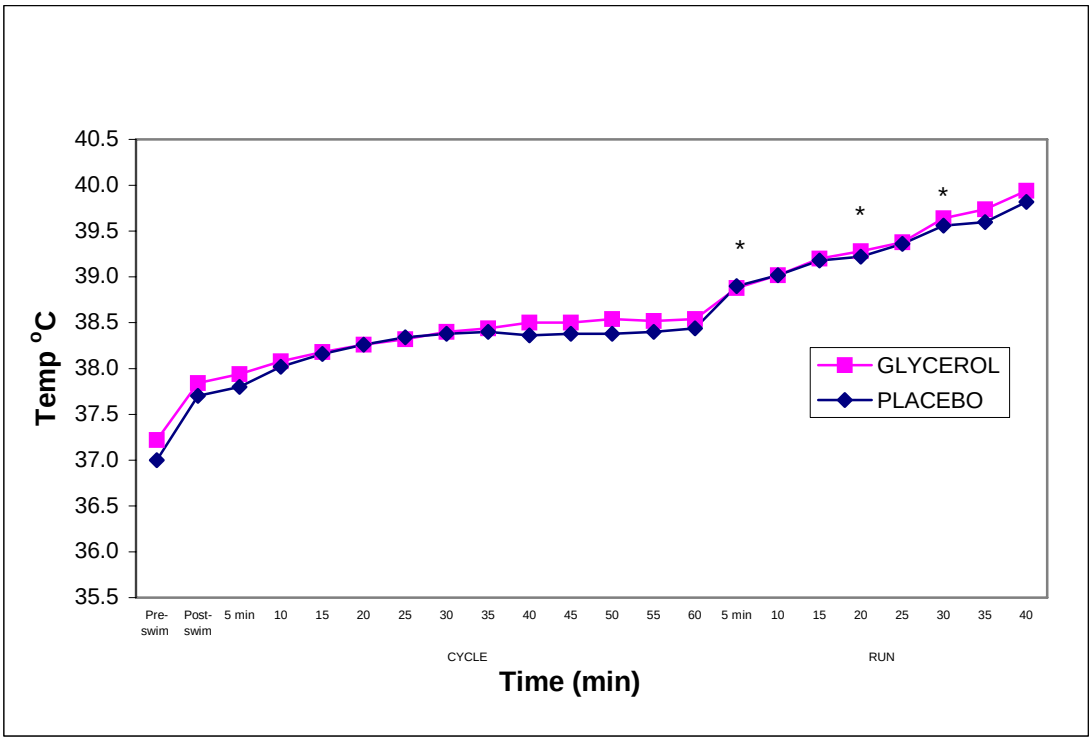
The mean rectal temperatures measured before the cycling phase had risen slightly (glycerol 38.4 ± 0.4 °C – placebo 38.5 ± 0.6°C) since post-swim measurement. Rectal temperature also rose by 1.2% (0.7°C) for both the glycerol and placebo trial from the swim phase to the end of the cycle phase.

There were no significant differences in the mean rectal temperature throughout the cycle phase within treatments. However, there was a significant difference at the change over time in rectal temperature from post-swim to the end of the cycle phase in the placebo trial ($p < 0.05$) but this change was not evident in the glycerol trial.

Run phase

Mean rectal temperature rose 3.1% (38.4 ± 0.3 °C to 39.6 ± 0.4 °C) from the end of the cycle phase to the end of the run phase for the placebo trial (Figure 3). Mean rectal temperature also rose 3.1% (38.5 ± 0.6 °C to 39.7 ± 0.5 °C) from the end of the cycle phase to the end of the run phase for the glycerol trial. The mean rectal temperature at 5, 20 and 30 min in the run phase was significantly higher in the glycerol trial than the placebo ($p < 0.05$).

The change in the mean rectal temperature from the end of the cycle phase to the end of the 10 km run was significantly different ($p < 0.01$) for both the glycerol and placebo trials.



*=*Significant differences in the means between treatments. Values are means.*

Figure 3: Mean rectal temperature during the ODT

Heart rate

Swim phase

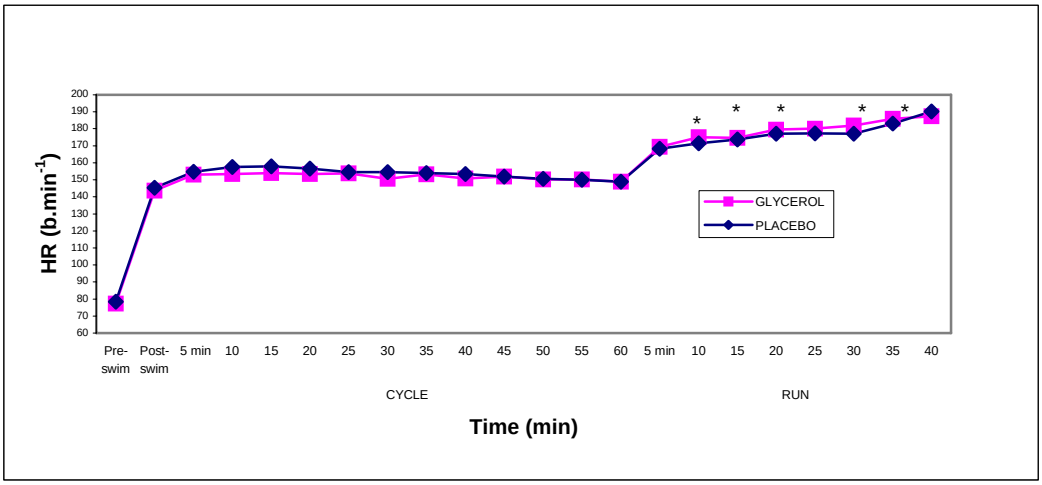
The differences in the mean heart rate between treatments in the pre- and post-swim phase were non significant.

Cycle phase

Mean heart rates were similar for both trials throughout the cycle phase (Figure 4). There were no significant differences in the mean heart rate during and at the end of the cycling phase between treatments ($p > 0.05$).

Run phase

Mean heart rates rose by 24.8% for glycerol (149 – 186 b.min^{-1}) while placebo rose by 22.8% (149 – 183 b.min^{-1}) from the end of the cycle phase to the end of the run phase. There was a significant increase in the mean heart rate during the run at 5, 10, 15, 25 and 30 min in the glycerol trial ($p < 0.05$). The change in heart rates from the end of the cycle phase to the end of the 10 km run was significantly different ($p < 0.05$) for both the glycerol and placebo trials.



**= Significant difference in means between treatments ($p < 0.05$). Values are means.*

Figure 4: Mean heart rates during the ODT

Blood parameters

Pre-swim

There was a nonsignificant difference of 6.2% (0.1 mmol.L^{-1}) between the pre-swim mean blood lactate concentration in the glycerol trial compared to the placebo trial. The difference in mean blood pH for both trials was also nonsignificant. The blood glucose concentration was 4.3% (0.2 mmol.L^{-1}) lower in the glycerol trial compared to the placebo trial and there was a 3.7% (0.7 mmol.L^{-1}) difference in mean bicarbonate concentration.

Post-swim

At the end of the swim phase there was a significant difference of 8.9% (0.3 mmol.L^{-1}) in the post-swim mean blood lactate concentration in the glycerol trial compared with the placebo trial. Significant differences were also evident in the mean blood lactate concentration post-swim within treatments ($p < 0.05$).

There was a nonsignificant 6.7% (0.3 mmol.L^{-1}) difference in post-swim mean blood glucose concentration following the glycerol trial compared with the placebo trial.

The mean bicarbonate concentration was 13.1% (3.0 mmol.L^{-1}) higher post-swim in the glycerol trial compared with the placebo trial. This difference was, however, not significantly different.

Cycle Phase

At the end of the cycling phase there was an 8.6% (0.3 mmol.L^{-1}) increase in mean blood lactate concentration in the glycerol trial compared with the placebo trial. This difference was statistically significant. The difference in mean blood pH during the cycling phase was non significant within treatments.

The mean blood glucose concentration in the placebo trial dropped by 9.6% (0.4 mmol.L^{-1}) while the mean blood glucose concentration rose by 4.3% (0.2 mmol.L^{-1}). These differences were not statistically significant. The mean bicarbonate concentration in the placebo trial rose by 7% (1.5 mmol.L^{-1}) while the mean bicarbonate concentration in the glycerol trial rose by 5% (1.2 mmol.L^{-1}) at the end of the cycling phase compared with the start of the cycling phase. These differences were not statistically significant.

Run phase

The mean blood lactate concentration at the end of the run phase was similar in both trials. The blood lactate concentration measured at the end of the run phase was significantly greater than that obtained at the end of the cycle phase for both the glycerol and placebo trials ($p < 0.01$).

The mean blood glucose concentration was 12.5 % higher (0.7 $mmol.L^{-1}$) in the glycerol trial compared with the placebo trial at the end of the run phase. This difference was not statistically significant. Significant differences were also noted between the mean blood glucose concentration at the end of the cycle phase and the end of the 10 km run for both the glycerol and placebo trials ($p < 0.05$).

At the end of the run phase there was a nonsignificant difference within the mean bicarbonate concentrations for the two treatment conditions. However, there were significant differences in the mean bicarbonate concentration from the end of the cycle phase to the end of the 10 km run for the glycerol trial ($p < 0.01$).

Plasma osmolality

Plasma osmolality for the glycerol trial increased by 2 % (6.0 $mOsmol.L^{-1}$) from pre-swim compared to post-run while the plasma osmolality for the placebo trial increased by 3.8% (11.0 $mOsmol.L^{-1}$) (Table 3). These differences were not statistically significant. There were significant differences between the mean plasma osmolality from the end of the cycle phase to the end of the 10 km run for the glycerol trial ($p <0.05$) and for the placebo trial ($p<0.001$) (Table 3).

Body mass

Swim phase

The mean BM decrease for the placebo trial was 62.0 ± 0.1 g (range: +80 to –140 g) while the glycerol trial resulted a mean BM decrease of 70.0 ± 0.1 g (range: +60 to –200 g). This represents a 12.9% difference in mean values between trials. Mean percentage decrease in total fluid loss was 2.2% for the glycerol trial and 2.0% for the placebo trial. None of these differences were statistically significant.

Cycle Phase

The mean decrease in BM from the beginning to the finish of the cycle phase was 1.1% for both the glycerol and placebo trials (Table 5). The differences within treatments were statistically significant ($p < 0.05$).

Run phase

At the end of the run phase BM decreased significantly for both treatments compared to the post-cycle ($p < 0.05$). The decrease in BM was 1.16% for the glycerol trial and 1.15% for placebo trial. The calculation in BM changes includes sweat losses, urine losses and fluid intake during the cycle and run phase.

Fluid intake and sweat losses

Cycle phase

The mean fluid intake during the cycle phase was 632.8 ± 106 ml for the glycerol trial and 633.2 ± 109 ml for the placebo trial. There was a 106 ml lower sweat loss in the glycerol trial compared with the placebo trial (NS).

Run phase

The mean fluid intake during the run phase for both the glycerol and placebo trial was exactly 390.0 mL. The glycerol trial had a 7.8% (114 ml) greater mean sweat loss compared with placebo trial (Table 5) (NS).

Mean estimated total sweat loss over the entire simulated triathlon for glycerol and placebo trials was 2.831 ± 0.394 kg (range = 3.194 to 2.278 kg) and 2.815 ± 0.321 kg (range = 3.092 to 2.3 kg), respectively. The mean total BM decrease represents a mean fluid deficit (dehydration) of 2.6 ± 0.2% for placebo trial and a 2.6 ± 0.3% for the glycerol trial. Mean fluid deficit was corrected for fluid intake and urine output. These data reflect consistency in fluid intake in the cycle and run phase between trials.

Subjective ratings

Table 6. Mean subjective data during the simulated ODT (n=5).

	Pre-swim		Post-swim		Cycle phase		Run phase	
	Glycerol	**Placebo**	**Glycerol**	**Placebo**	**Glycerol**	**Placebo**	**Glycerol**	**Placebo**
RPE	8.8 (2.3)# *	8.2 (1.3)# *	13.0 (1.0)*	14.4 (0.9)*	13.7 (2.1)*	14.5 (0.9)*	17.4 (1.1)*	17.7 (1.6)*
Thermal rating	3.1 (0.5)	2.3 (1.4)	4.2 (0.3)	4.9 (0.7)	4.9 (0.7)*	5.0 (0.8)*	6.4 (0.7)*	6.7 (1.0)*
Thirst rating	0.7 (1.1)	0.5 (1.1)	1.6 (1.5)	1.6 (2.1)	4.3 (1.9)*	5.2 (2.0)*	6.4 (1.7)*	6.2 (2.1)*

*# = post warmup, * Significant difference within treatments, p<0.05.*

Swim phase

There were significant differences in the mean RPE at pre- and post-swim within treatments ($p < 0.05$). However, there were no significant differences in the mean thermal and thirst ratings within treatments.

Cycle phase

There were no significant differences in the mean RPE, mean thirst and thermal ratings at the post-swim phase to the end of the cycling phase.

Run phase

There were significant differences in the mean RPE, thermal and thirst rating at the end of the cycle phase to the end of the run phase within treatments ($p < 0.05$).

Oxygen consumption

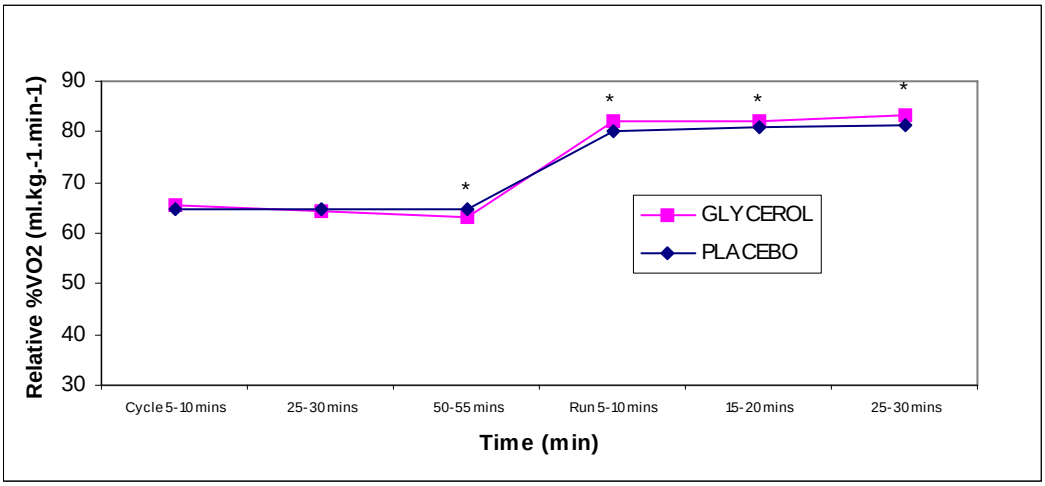
Cycle phase

The average amount of oxygen consumed (expressed as a percentage of VO_2 relative to body weight) throughout the cycle phase remained unchanged for the glycerol trial while it decreased by 3.2% (2.5 ml.kg. $^{-1}$. min $^{-1}$) at the end of placebo trial. There were significant differences in the mean percentage of relative oxygen consumption at 50-55

min in the cycling phase between treatments ($p < 0.05$). These data were expressed relative to body weight measured after the swim phase.

Run phase

From the start of the cycling phase to the start of the run phase there was a 19.5% (15.6 ml.kg.$^{-1}$ min^{-1}) increase in relative oxygen consumption in the placebo trial and a 23.2% (19.0 ml.kg.$^{-1}$ min^{-1}) increase in the glycerol trial. At the end of the run phase the mean percentage of relative maximal oxygen consumption increased by 1.8% (1.5 ml.kg.$^{-1}$ min^{-1}) for the glycerol trial while the mean percentage of relative maximal oxygen consumption increased by 1.3% (1.0 ml.kg.$^{-1}$ min^{-1}) for the placebo trial. There was a significant difference between treatments for the mean relative oxygen consumption at 5-10, 15-20, 25-30 min during the run phase ($p < 0.05$). Mean relative VO_2 max scores were corrected for body weight measured after the cycling phase.



*= *significant differences in the means within treatments ($p < 0.05$). Values are means.*

Figure 5: Mean relative oxygen consumption during the cycle and run phase

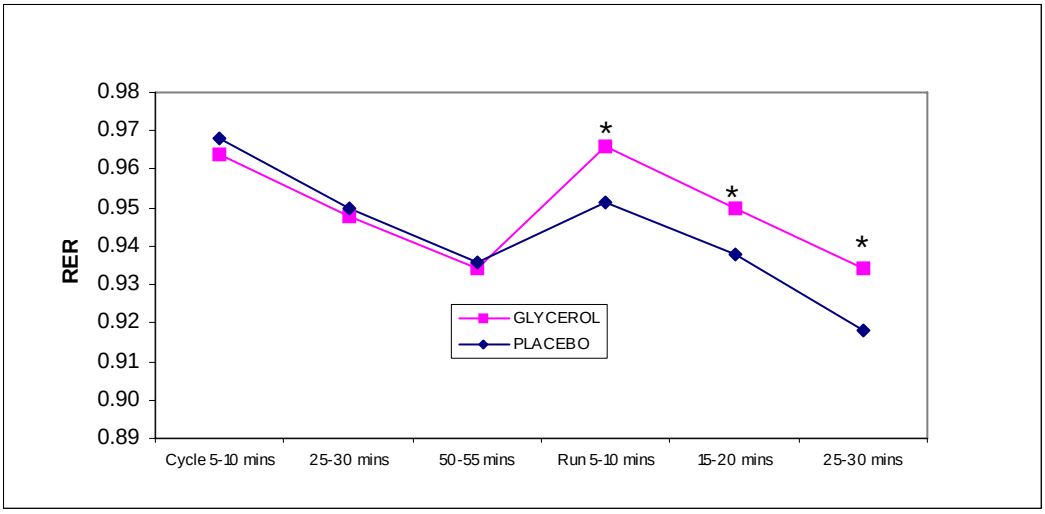
Respiratory Exchange Ratio (RER)

Cycle phase

The mean RER during the cycle phase for glycerol and placebo trials was 0.95 (Figure 6). The mean RER also dropped slightly with time over the cycle period. There were no significant differences in the mean RER during the cycling phase within treatments ($p > 0.05$).

Run phase

The mean RER changed significantly during the glycerol trial ($p < 0.05$) but not in the placebo trial (Figure 6).



**= Significant differences in the means within treatments ($p < 0.05$). Values are means.*

Figure 6: Mean RER during the cycle and run phase.

Laboratory conditions

Cycle phase

The mean temperature and relative humidity in the heat chamber in the cycle phase was similar in the glycerol (mean = 31.2 °C ± 0.2) and placebo trial (mean = 31.3°C ± 0.3). Mean relative humidity for the glycerol trial was 57.9 ± 1.6 % while placebo trial was 57.8 ± 1.0 %.

Run phase

There were no significant differences in mean temperature and relative humidity in the heat chamber between the two trials ($p > 0.05$). Mean temperatures during the cycle phase for glycerol and placebo were 31.1 ± 0.6 and 31.1 ± 0.4 °C, while the mean relative humidity was 58.6 ± 2.6 and 59.5 ± 2.1, respectively.

These data reflect consistency in environmental conditions in the cycle and run phase between trials.

CHAPTER 5: DISCUSSION

Introduction

The physiological rationale for this study was to increase TBW via the ingestion of glycerol and reduce the likely impact of exercise-induced dehydration on thermal and CV function thereby improving the run performance in the ODT. Despite the significant increase in fluid retention during the swim and cycle phases, the mean time to complete the 10 km self-regulated with glycerol ingestion was similar to that achieved in the placebo trial. Despite this lack of significance, 3 out of 5 subjects had improved times in the run using glycerol. The three subjects whose times improved were associated with increased average cardiac frequency, relative oxygen consumption, rectal temperatures, pH, blood lactate concentration and blood glucose concentration.

This discussion will focus on determining the extent to which the performance outcome observed in the 10 km run leg of the ODT was affected by the changes in fluid balance. Once the fluid balance status of the subjects has been explored, the discussion will examine the relationship between the observed fluid balance and the resultant metabolic and thermoregulatory responses that were measured during the 10 km run leg of the ODT.

During each trial, both the swim and cycle phases were rigidly controlled to elicit the same physiological effort and strain prior to the run. It was anticipated that the subjects would be able to complete the swim and cycle phases at close to race pace in the moderately hot conditions. In contrast, the final leg of the ODT, being the 10 km run phase, was to be completed in the shortest possible time and required near maximal effort. The 10 km run was the final assessment of performance that would determine the effectiveness of glycerol as an ergogenic aid compared with the placebo hyperhydration strategy. However, due to the combination of the arduous nature of the trials and the side effects associated with the ingestion of glycerol, only five of the 10 subjects completed the final 10 km self regulated time trial. It is acknowledged that the subjects who were unable to complete the 10 km run may have had an impact on the results if included and were therefore excluded from the analysis.

The effect of glycerol ingestion on fluid retention and plasma volume

The study was modeled on the previously demonstrated capacity of glycerol to increase total body water (Hitchins et al., 1999) and plasma volume (Riedesel et al., 1987; Lyons et al., 1990; Freund et al., 1995; Jimenez et al., 1999). The ingestion of glycerol in this study during a controlled pre-hydration period resulted in a significant increase in TBW, which is consistent with other studies (Riedesel et al., 1987; Wendtland et al., 1987; Lyons et al., 1990; Seifert et al., 1995; Anderson et al., 2001; Coutts et al., 2002). Importantly, however, ingestion of the placebo drink was also shown to result in a substantial increase in TBW but not as large an increase. This evidence of hyperhydration and the resultant increase in TBW by other ingested fluids is supported by some studies (Wendtland et al., 1997; Latzka et al., 1997; Coutts et al., 2002). However, hyperhydration in these studies was notably smaller than that achieved following ingestion of glycerol. A possible limitation in these studies and the current study might be lack of tight control of the baseline pre-hydration status of the subjects.

In the current study there were also similar differences and changes in haemoglobin and haemaotcrit concentration post-hydration in both treatments. In this study, there was no significant percentage change in PV, which is consistent with other studies (Riedesel et al., 1987; Murray et al., 1991; Freund et al., 1995; Montner et al., 1996; Latzka et al., 1997; Grice et al., 1997; Latzka et al., 1998; Lamb et al., 1999).

The reasons for the lack of significant response in PV in the five subjects in this study is likely to be due to many factors including small sample size, research design and methodology and pre-hydration status of subjects. It is also important to note that the influence of glycerol-induced retention of fluid on other compartments (ICF and interstitial) was not measured. Therefore, the possibility that glycerol did increase the ICF and interstitial compartments and not PV should not be ignored. The analysis of urine and venous blood suggests that subjects were slightly hypohydrated at the beginning of the performance testing despite efforts to maintain adequate hydration status. These data indicate that the average urine osmolarity obtained from the subjects under the placebo condition was 634 ± 335 mOsmol.l^{-1}. This value was higher than that considered to be indicative of a euhydrated population (Armstrong et al., 1994). Also the average urine osmolarity in the pre-hydration state for the glycerol trial was 563 ± 272 mOsmol.l^{-1} and this was higher than the accepted euhydrated value of less than 442 mOsmol kg^{-1}

(Armstrong et al., 1994). These data, therefore, suggest that the subjects may have presented to the placebo and glycerol trials in a slightly hypohydrated condition. The explanation of the apparent conflict in relative impact of the placebo and glycerol treatments observed in the current study in comparison to those reported in the literature needs to be explored further.

Changes in plasma osmolality and the associated hormonal responses primarily determines fluid retention and thus, urine output. The majority of published research, including the current study, have shown that hyperhydration with glycerol increases plasma osmolality (Gleeson et al., 1986; Riedesel et al., 1987; Murray et al., 1991; Freund et al., 1995; Seifert et al., 1995; Inder et al. 1998; Lamb et al., 1999; Hitchins et al., 1999; Anderson et al., 2001) which, attenuates the reduction in plasma antiduretic hormone (ADH) concentrations thereby enabling greater fluid retention. As a consequence, there is lower free water clearance and greater fluid retention for glycerol hyperhydration over that observed following ingestion of other solutions. The clearance of urine is also directly related to the tonicity, timing and amount of fluid ingested and the pre-hydration status of the subjects (Rehrer, 1996). In the current study, fluid retention and urine output was firstly attributed to the pre-trial hydration status (as determined by the initial urine and the plasma osmolality) and then by the changes in plasma osmolality.

It is apparent, therefore, that despite the extensive efforts to exert control over the field conditions under which the study was conducted, the slight hypohydration measured in the subjects during the glycerol and placebo trial may have masked the anticipated treatment effect of pre-hydrating with the glycerol solution.

Effects of glycerol on metabolic and thermal parameters

Although the differences in fluid balance between the placebo and glycerol trials were small, the relative effects of glycerol on metabolic and thermal parameters were obvious and require further discussion. It was hypothesized that the ingestion of glycerol would increase the reserve of fluid available to offset the impact of the dehydration that often results from participation in ODTs. The data obtained in the current study demonstrated that during the glycerol trial, the ODT resulted in a 2.6 ± 0.3% decrease in total body mass and a 1.8% decrease in calculated plasma volume. The level of hypohydration observed during the ODT was similar in the glycerol and the placebo trials. The failure to

observe a significant difference in the level of dehydration observed in the two trials is not unexpected. What is important is whether the remaining body fluid volume was sufficiently large in the glycerol trial to establish a protective effect upon thermal balance, metabolic function and ODT run leg performance.

Although data in the current study support the increase in TBW in the glycerol and placebo trials, the mean 154 ml larger increase in fluid retained by glycerol compared to the placebo did not provide sufficient evidence for changes in thermal, cardiovascular and biochemical parameters during the ODT. The implications of these data suggest that increases in TBW above baseline measures are not necessarily reflected in alterations in normal responses to exercise in the heat and improvements in performance. The idea that hyperhydration with glycerol produces a "reservoir" of extra fluid which can be called upon to offset losses in TBW and minimize thermal and cardiovascular strain, has yet to be proved.

The non-significant increase in cardiac frequency during the trials is consistent with data provided by Hitchins et al. (1999) but is in contrast to those of Montner et al. (1995). The difference between the data in the current study and that of Montner et al. (1995) might be due to the moderate testing conditions used in their investigation. The differences in cardiac frequency in the current study compared to others may also be a consequence of the testing methodology. The self-regulated performance protocol used in the current study, and by Hitchins et al. (1999), were adopted to mimic actual performance. This protocol is different to those studies that used fixed exercise durations without a maximal performance phase (Miller et al., 1983; Lyons et al., 1990; Murray et al., 1991; Latzka et al., 1997) or exercise to volitional exhaustion (Montner et al., 1995; Latzka et al., 1998) used by other studies. The testing methodology used in the current study may explain some of the differences in the cardiovascular and thermoregulatory variables compared with other studies recorded in the self-regulated (variable) performance phase of the current protocol.

There were no significant differences (reductions) in the mean heart rate and rectal temperature in the fixed workload phases of the ODT that might suggest attenuation in cardiovascular and thermoregulatory strain prior to the run between treatments. These findings are in direct contrast to those of Montner et al. (1995) and Anderson et al. (2001) in which marginal decreases in heart rate (3-5 $b.min^{-1}$) during exercise was observed. However, the current study did show significant differences in mean heart rate, rectal

temperature, relative oxygen consumption values, blood lactate concentration, serum glucose concentration and pH during the 10 km run between treatments. The differences in these parameters may reflect the higher intensity and effort achieved by the subjects in the 10 km performance run with glycerol ingestion compared to the placebo trial. The velocity in the final run was reflected by faster 1 km times (1.5%) and average 10 km completion times (2.1%). Although the effort had increased in the run phase in the glycerol trial, data prior to the run did not show significant reductions in thermal and cardiovascular strain that may have precipitated performance improvements.

Effects of glycerol on plasma volume

The ingestion of glycerol solution as a hyperhydration strategy has been purported to act as a possible plasma volume expander which has the potential to attenuate thermal and cardiovascular strain while exercising in the heat (Riedesel et al., 1987; Lyons et al., 1990; Freund et al., 1995; Jimenez et al., 1999). Plasma volume expansion can be observed with the changes to the percentage of red blood cell or haematocrit. If the change in pre-hydration haematocrit values compared with post-hydration were significant between the treatments, this may suggest some plasma volume expansion due to the hyperhydration strategy. However, the calculated change in plasma volume did not indicate expansion in plasma volume in the glycerol trial compared with the placebo. This result is in contrast to other studies claiming plasma volume expansion as a result of glycerol hyperhydration (Freund et al., 1995; Gleesen et al., 1996; Hitchins et al., 1999; Coutts et al., 2002) but provides additional support for many others who didn't (Riedesel et al., 1987; Murray et al., 1991; Freund et al., 1995; Montner et al., 1996; Latzka et al., 1997; Grice et al., 1997; Latzka et al., 1998; Lamb et al., 1999). The hypohydrated status of the subjects prior to the trials may explain the non-significant expansion of PV in the current study.

Effects of glycerol on rectal temperature

In the present study, a 0.6-0.7ºC rise in rectal temperature was observed post-swim. Several studies have concluded that the rise in core/rectal temperature is dependent on the level of induced dehydration (Sawka et al., 1985; Montain and Coyle, 1992). The rise in rectal temperature elicited in the swim was likely to increase thermoregulatory and cardiovascular strain into the cycling phase. These changes in temperature may have been

linked to the reduced work output in the cycling phase observed in the first trial compared with work output determined in the GCT test. The rise in rectal temperature during the cycle phase in the warm and humid conditions was not attenuated by the glycerol-induced hyperhydration. Despite the increase in the mean rectal temperature during the run, mean sweat rates were not significantly different between treatments.

Effects of glycerol on body mass

At the end of the performance run in both trials, there was a 2.6% decrease in body mass. While sweat losses were not significantly different at the end of the swim, cycle and run phase between treatments, the overall total sweat losses for the subjects were significantly lower in the glycerol trial. Coutts et al., (2002) also found no significant differences in sweat rates between treatments.

Effects of glycerol on fluid retention

The outcomes from this study have clearly demonstrated that glycerol-induced hyperhydration did result in significant differences in urine output at -90, -60 and -30 min prior to the ODT compared with a placebo solution. The treatment with glycerol also resulted in the retention of an average 702 mL while the placebo trial retained 548 mL. This amounts to a 26% difference in the retention of fluid between treatments. The gross 702 mL increase in fluid retention in the glycerol trial above baseline TBW is in agreement with other published literature. These studies have shown fluid retention using glycerol prior to exercise can increase baseline TBW from 400 to 700 mL (Riedesel et al., 1987; Wendtland et al., 1987; Lyons et al., 1990; Seifert et al., 1995; Hitchins et al., 1999; Anderson et al., 2001) and recently up to 1000 ml (Coutts et al., 2002) compared with other hyperhydration solutions. The current study also observed an increase in the average retention of fluid in the placebo trial of 543 mL and this is in agreement with the findings of Coutts et al. (2002).

In the post-hydration phase, urinary output volume slowly increased for both trials. In the glycerol trial mean urine output peaked early at -60 min (12.2 $mL.min^{-1}$) and steadily declined to 3.2 $mL.min^{-1}$ just prior to the test. The mean urinary output for the placebo trial peaked later at -30 min (14.1 $mL.min^{-1}$) with a small decline at 0 min (8.3 $mL.min^{-1}$). This trend in urine output is similar to that reported by Hitchins et al., (1999).

Koenigsberg et al. (1995) also noted large differences in urine output two hours following glycerol ingestion (73.3%) compared to placebo ingestion.

Effects of glycerol on plasma and urine osmolality

There were no significant differences in mean baseline plasma osmolality, haemoglobin and haemotacrit concentration between treatments, suggesting a uniform level of hydration (hypohydration) before both trials. In fully hydrated people, plasma osmolality can increase from 283 mOsmol.l $^{-1}$ to values exceeding 300 mOsmol.l $^{-1}$ in hypohydrated people (Sawka 1992). The average increase in plasma osmolality of 2.8 mOsmol.l $^{-1}$ post-hydration in the current study is in agreement with the majority of the literature reporting increases in plasma osmolality due to glycerol-induced hyperhydration (Gleeson et al., 1986; Riedesel et al., 1987; Murray et al., 1991; Freund et al., 1995; Seifert et al., 1995; Inder et al., 1998; Lamb et al., 1999; Hitchins et al., 1999; Anderson et al., 2001). In contrast, plasma osmolality in the placebo trial had reduced by an average 8 mOsmol.l $^{-1}$ by the end of the hydration phase. This response may suggest some plasma dilution as a direct response to the large volume of fluid ingested.

Urine osmolality can also reflect hydration status. Hydration status in the current study was largely determined using urine osmolality data. A value of less than 442 mOsmol.kg $^{-1}$ was considered to indicate a "well hydrated" status (Armstrong et al., 1994). The data collected indicates that the majority of the subjects were slightly dehydrated prior to both trials (glycerol: 562.8 ± 271.8 mOsmol kg. $^{-1}$ and placebo: 634.4 ± 335.3 mOsmol kg. $^{-1}$). This could explain the large retention of fluid for both glycerol and placebo trials. It was also observed that two out of the five subjects in the study retained more fluid in the placebo trial than the glycerol trial. Both of these subjects were in a slightly hypohydrated state according to their urine osmolality status prior to the placebo trial compared with the glycerol trial. The hydration status of the subjects may help explain the level of fluid retention rather than the suggestion that fluid retention was primarily caused by glycerol-induced hyperhydration. Therefore, fluid retention is highly dependent on the hydration status prior the trials. The lack of uniform pre-test hydration status is a major limitation in light of the interpretation of fluid retention and corresponding hyperhydration effect of the glycerol solution.

The disparity in hydration status was noted in the substantial differences in the mean urine osmolality and haematocrit between treatments for two subjects. Both subjects were completing their second trial in the study and these data suggest that the subjects could have altered their pre-trial preparation in anticipation of the physiological/emotional stress of the ODT. The change in hydration status may be in either a direct response to the physiological/thermoregulatory strain experienced in the first trial or a misunderstanding of the directions given by the researchers concerning the importance of matching pre-nutritional and hydration status for both trials.

Fluid absorption

Vist and Maughan (1995) have shown that the energy density, due to the combination of both food and fluid, largely determines the rate of gastric emptying. This in turn will increase the osmolality and energy density. This has the potential to significantly affect the rate of absorption and thus directly contribute to the increases in plasma osmolality and reduction in urine volume. The tonicity and energy density of the glycerol and placebo solutions were not measured in the current study however, differences in tonicity and energy density of ingested fluids were noted in other studies. Anderson et al. (2001) reported the difference in solution osmolality while Freund et al. (1995) only acknowledged the glycerol density.

The combined ingestion of food and a hypertonic solution has been shown to delay gastric emptying and resulted in a net flux of fluid into the small intestine rather that absorption (Rehrer et al., 1992; Pearce, 1996; Maughan, 2000). The differences in energy density and the hyperosmolar food in the glycerol trial may have impacted on the absorption of fluid in the gut and thus may have retarded the uptake of fluid. The possibility that some of the fluid remained in the gut rather than increasing TBW is also supported by Anderson et al. (2001). In contrast, however, Anderson et al. (2001) suggested that the combined effect of a hypertonic solution ingested with food and the associated increase in plasma osmolality, attenuates the reduction in plasma antiduretic hormone (ADH) concentrations thus, enabling greater fluid retention. Anderson et al. (2001) also observed osmotic induced diarrhea 24 hr post-test as a result of the influx of fluid into the gut. This has provided further evidence to support alterations in the absorption of fluid in the gut. It is important to note that the alteration in gut absorption could also have occurred in the placebo trial. In the current study food was consumed

prior to the fluid ingestion and this could quite possibly have delayed gastric emptying. In other studies gastric emptying may have also been delayed when food was consumed 3-4 hr prior to the exercise test (Murray et al., 1991) and while food was consumed with various types of fluid (Inder et al., 1998).

It is important to note that hypertonic fluids and large volumes of fluid can also hamper gastric emptying (Rehrer et al., 1992; Robinson et al., 1995; Pearce, 1996; Maughan, 2000). Clearly the large volumes of fluid ingested with the glycerol may not mimic actual competition or training hydration routines. It has been noted by some researchers that individuals could train their stomach (and gut) to cope with these extreme hyperhydrating volumes and frequency during training and before competition (Murray, 1996). Without directing specific drinking guidelines on how much and when each individual should comfortably ingest the fluid, one can only speculate on how quickly the fluid was absorbed and retained in the current study. Subjects may also have had no experience at hyperhydration methods and therefore had limited training of the gut to tolerate large volumes of fluid.

The ingestion of fluid, compared to abstaining from fluid ingestion, has been to shown to attenuate cardiovascular and thermoregulatory strain (Greenleaf and Castle, 1971; Montain and Coyle, 1992) while a deficit of approximately 2-3% in body mass due to sweat losses will result in a reduction in gastric emptying (Neufer et al., 1989; Rehrer et al., 1990). The subjects in both trials experienced a mean 1-1.1 % reduction in body mass due to sweating while cycling at 63-65% VO_2 max for one hour prior to the performance run. In the current study, subjects were provided with 10 $ml.kg^{-1}$ BW of fluid during the cycle phase and a total of 450 ml of fluid in the run phase. Gastric emptying would not have been assumed to have been decreased in the cycling phase as the intensity was below the threshold of 75% VO_2 max that has previously been shown to affect gastric emptying (Rehrer 1996). Also, fluid intake during the cycle phase could have minimised the drop in blood volume and therefore blunted any exercise induced cardiovascular and thermoregulatory alterations. This suggests little to minimal effect of dehydration on cardiovascular and thermoregulatory function occurred during the cycling phase. However, the greater effort elicited in the run (>80% VO2 max) at a higher level of dehydration (2.6%) may have prompted the reduction in gastric emptying and gastric distress experienced by most of the subjects. The reduction in gastric emptying may have been further exaggerated by the diminished splanchnic blood flow (Fallon, 2001).

Clearly, the combination of accumulated heat gain, exercise mode, intensity and duration coupled with the level of dehydration experience by the subjects suggests that gastrointestinal distress would be a possibility in the current study.

Side effects

Although most studies have reported no signs of discomfort or gastrointestinal distress as a result of the ingestion of a glycerol hyperhydration solution (Riedesel et al., 1987; Lyons et al., 1990; Feund et al., 1995; Montner et al., 1996; Hitchins et al., 1999; Anderson et al., 2001) some studies did report such gastro-intestinal distress (Gleeson et al., 1986; Murray et al., 1991; Latzka et al., 1997; Inder et al., 1998; Coutts et al., 2002). Hitchins et al. (1999) concluded that the ingestion of the dilute glycerol solution (5%) prior to exercise might have minimised such side effects. This is in complete contradiction to the current study in which the same dosage was employed as Hitchins et al. (1999). In the current study five out of ten subjects in the glycerol trial complained of feeling either bloated or nauseous during the 10 km performance run. Four of the five who did complain did not complete the 10 km. Only one subject reported feeling unwell in both the placebo and glycerol trial. It is crucial to note that five out of the original ten subjects recruited for the study failed to complete the 10 km performance run. Of those five who failed to complete the 10 km performance run, four withdrew from in the glycerol trial while only one withdrew from the placebo trial. The side effects and the resultant impact on the ability of the subject to complete the ODT have major implications in respect to whether one should advocate hyperhydrating with glycerol.

Conclusion

The purpose of this study was to determine the impact of glycerol hyperhydration over a placebo hyperhydration as a pre-race strategy and its influence on the run performance during an Olympic distance triathlon. The study supports the majority of the literature in which glycerol increases plasma osmolality and reduces urine output but provides little evidence to confirm glycerol as a potential plasma volume expander. Results have shown that there were no significant differences in cardiovascular and thermoregulatory variables prior to the 10 km run in both trials and there was also no significant difference in the 10 km run performance. Although this study did not further add to the specific understanding of the mechanisms of glycerol, the overall result and identified side effects

place real doubts on the practical use of glycerol as a hyperhydrating agent. The density and unusually large volume of fluid ingested in the glycerol trial seemed to hamper the ability to absorb much of the fluid and thereby affect the level of plasma volume, ICF, ISF and ECF expansion.

In conclusion this research did (a) not suggest performance improvements in Olympic distance triathlons and establish glycerol hyperhydration as an ergogenic aid; (b) provide further information on the effects of glycerol hyperhydration on thermoregulation and dehydration during endurance exercise which could help other sports where there is a high risk of dehydration (and thermal stress); (c) confirm present glycerol dosage guidelines and document any side-effects that may be present (d) ultimately provide information which will best prepare athletes for endurance competition by ensuring they are optimally hydrated and the effects of thermal stress is minimised. However, methodological differences in the study design, the timing of solution ingestion, hydration status of subjects and the environmental and physiological stress in many of the studies, including the current study, appear to play a major role in establishing glycerol as a potential hyperhydration agent and performance enhancer.

CHAPTER 6: RECOMMENDATIONS FOR FUTURE STUDIES

Hydration/nutritional and training status pre-trial

Subjects acting as their own control is fraught with many inconsistencies as highlighted by the reported significant differences in mean energy intake, protein intake and pre-trial hydration status between trials. It can be argued that the capacity to control the pre-test status for all the subjects for both trials is extremely difficult for any research project. Not only must the subjects try to mimic the exact nutritional intake, including hydration level, but also the training status three days prior to the trials. This must also take into consideration the individual degree of acclimatisation over the preceding weeks leading into the trials. The current study hypothesis is heavily reliant on the subjects being in the same hydration status for the efficacy of glycerol to be realised. Importantly, the hydration status prior to the trial will also play a major role in attenuating thermoregulatory stress and cardiovascular strain.

In an effort to minimise these limitations it is recommended in future studies that all subjects are given food and fluid to consume three days prior to the trial. Fluid balance should be verified by either plasma osmolality or urine specific gravity in the three days leading into, and on the trial day. On the day of the trial euhydration status must be confirmed and actioned upon before starting the trial. Only by taking on these measures will the issue of uniform training and nutritional status of subjects between trials be addressed.

Due to the ability of the placebo, namely sports drink, to provide a hyperhydrating/retention effect similar to that of the treatment, it has been suggested that another treatment be used as a control. The control group would be using water as a fluid to induce a hyperhydrating effect. This would clearly differentiate the treatments and their ability to minimise the effects of thermoregulatory stress and cardiovascular strain during an ODT.

Any subtle physiological difference that might have existed between treatments was not realised due to the similar volumes consumed during the cycle and run phase of the ODT.

The volume of water may have also minimised the dehydration level and thus, thermoregulatory/cardiovascular effect for both treatments.

Careful consideration must be given to the ability for the gut to absorb the volume of fluid. Therefore, it is important to control ingestion rates during the hydration phase. A formal schedule of when and how much fluid should be ingested would minimise the different absorption rates between trials.

Performance Test

A 10 km time trial in the field rather than a self-regulated laboratory time trial would address the issue of pacing in both trials.

The format of the cycle phase during an ODT has changed from a steady state effort (time trial) to a draft legal ride of intermittent intensity (stochastic). The unpredictable nature of the cycle phase can now have a dramatic effect on the performance demands prior to the run. A variable power output test that mimics the effects of drafting, surges and attacks similar to that of a cycling criterium would best suit the change in the cycling format during a ODT.

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APPENDIX A

CONSENT FORM

INFLUENCE OF GLYCEROL HYPERHYDRATION ON RUN PERFORMANCE DURING AN OLYMPIC DISTANCE TRIATHLON

I, give my consent to participate in the project referred to above on the following basis:

1. I understand the purpose of the project is to:

Determine the benefits of glycerol hyperhydration over a control hyperhydration and its influence on the run performance during an Olympic distance triathlon. The results of this research should (a) show performance improvements in olympic distance triathlons and establish glycerol hyperhydration as an ergogenic aid; (b) provide further information on the effects of glycerol hyperhydration on thermoregulation and dehydration during endurance exercise which could help other sports where there is a high risk of dehydration (and thermal stress); (c) confirm present guidelines to the usage of glycerol (d) and ultimately provide information which will best prepare athletes for endurance competition by ensuring they are optimally hydrated and the effects of thermal strain is minimised.

If the pre-race glycerol hyperhydration strategy is shown to improve the run performance over the control, then this would mean that the glycerol pre-race hyperhydration strategy has allowed the athlete to perform the run with reduced thermal strain and minimised the dehydrated state. This could have a distinct advantage to the final outcome of a race.

2. I understand that this is an experimental project, which will involve some form of exercise-induced physical stress and intrusion and may have minor/inconsequential effects upon me. I will be required to perform two simulated Olympic distance triathlons (male and female: 20 min swim, 60 min cycle, 10 km time trial run) one week apart. Only the swim and cycle legs will be at a specified intensity (near race pace) while the run will be performed at maximal effort. I have been advised that the cycle and run session will be performed in a heat chamber in hot and humid conditions (30°C, 60% relative humidity).

Preceding each simulated triathlon I will be either undergoing a control or a glycerol hyperhydration strategy. I understand that blood and urine samples will be taken at times during the study and a rectal probe will be inserted prior the swim leg to monitor body core temperature during the ODT.

I am aware that the level of discomfort and risk will be no greater than felt during a competitive Olympic distance triathlon. The level of discomfort is self-reported and monitored by myself. I have been told I will be asked on numerous occasions how I am feeling at each leg/session during each simulated triathlon. I fully understand the thirst and thermal rating and RPE. I am aware there is a slight risk of gastro-intestinal discomfort and infection however, I understand that all precautions will be made concerning reducing the possibility of it occurring. I am aware that I have the option to withdraw from any leg/session should I wish to at any time during their administration.

I have been informed that blood and urine will be taken from me by qualified personnel using fully sterilised equipment. The rectal probe will also be sterilised and inserted by myself.

3. I understand that my involvement in this study will be necessary on four separate occasions. I have been informed that all the testing could be completed in approximately 14-21 days. I have been advised that the study will be conducted during late March/early April whist I am in competitive phase of training.

4. I have been told that my interests will be protected in the following way:

All equipment used to puncture the skin or inserted into the body will be fully sterilised and all medical precautions will undertaken with the presence of a doctor on standby.

5. I know that if I wish to find out more about the study I can contact Gerald Van Ewyk at work on 256 3111.

6. I know if I wish to complain about the way I am being treated I can discuss the matter with Dr Alan Roberts (University of Canberra, Faculty of Applied Science) on 201 2931 or Dr Darren Smith (Physiology Department, AIS) on 252 1791.

7. I understand that I am participating in this study in a voluntary capacity and can withdraw at anytime without penalty.

8. I understand that I will be paid for my part in this study at standard rates.

9. I have no objection working with Gerald Van Ewyk

10. The aims of this research project have been thoroughly explained to me, and I have been told how it will be conducted and my role in it. I am willing to participate.

11. I understand that I do not have to answer all questions if I choose not to.

12. I am co-operating in this research project on condition that;

13. I have been given a copy of this form signed by me and the principle researcher, Gerald Van Ewyk and,

Any information I provide will be kept confidential; the results of the study will be used only for this research project; and the research will be available to me at my request.

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(subject)	(Principle researcher)	(witnessed by)