# THE EFFECT OF CREATINE AND GLYCEROL INDUCED HYPERHYDRATION ON RUNNING ECONOMY IN MODERATE AND HOT ENVIRONMENTAL CONDITIONS

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# Table of Contents

# I. Abstract

The primary objective of this study was to investigate the effects of a hyperhydration strategy on running economy in cool and hot conditions. The hyperhydration regimen consisted of a combination of Creatine and glycerol which previously has been shown to each have a hyperhydrating effect and if applied together this effect is additive. Seven well-trained athletes were invited to participate in this study which included a pre- and post-supplementation experimental trial consisting of running at an intensity of 60% $VO_2$max for 30 minutes in moderate ($T_a$ = 10.5 ± 0.2 ℃; RH = 72.0 ± 1.1%) and another 30 minutes in hot ($T_a$ = 34.72 ± 0.19 ℃ and RH = 71.5 ± 0.9%) conditions with a 30 min break in between during which subjects were given water to replace any fluid lost in the first part of the trial. Between the last two experiments subjects followed a seven-day supplementation regime, i.e. consuming a daily dose of 11.4 g of $Cr{\cdot}H_2O$ (equivalent to 10g of Creatine) and one dose of glycerol ($1g{\cdot}kg^{-1}$ BM) on the day of the final experiment. The measurements of total body water (TBW) indicated that not all subjects responded to the hyperhydration regimen. Hence, only the subjects considered responders were taken into analysis (n=7). In this group of subjects we found an increase of 0.8 ± .0.3 kg in BM, 0.64 ± 0.18 L in TBW of which 0.20 ± 0.12 L could be assigned to an increase in extracellular water (ECW), and 0.44 ± 0.09 L were due to an increase in intracellular water (ICW). No significant changes could be observed in thermoregulatory (core body temperature), cardiovascular (heart rate) or pulmonary responses ($O_2$ consumption, $CO_2$ production, minute ventilation, respiratory exchange ratio), and rating of perceived exertion. Nevertheless, in the trial after the supplementation the subjective perception of heat, i.e. thermal comfort, was significantly reduced after 5 and 10 minutes in the bout at 35℃. As no change in $VO_2$ could be observed, we conclude that there is no negative impact of a slightly increased body weight on oxygen consumption. Compared to previous studies, this study could not achieve a hyperhydration sufficient enough to observe positive thermoregulatory and cardiovascular responses as reported by other studies investigating water pre-loading. These differences are possibly due to a change in the administration protocol of glycerol.

## II.a List of Abbreviations

| | |
|---|---|
| ACSM | American College of Sports Medicine |
| ADH | anti-diuretic hormone |
| ANOVA | analysis of variance |
| BM | body mass |
| $CO_2$ | carbon dioxide |
| Cr | Creatine |
| $Cr \cdot H_2O$ | Creatine monohydrate |
| ECW | extracellular water |
| [Glu] | Glucose concentration |
| Gly | Glycerol |
| Hb | hour |
| HR | heart rate |
| Hb | Haemoglobin |
| ICW | intracellular water |
| kg | kilogram |
| L | litre |
| m | metre |
| min | minute |
| NATA | National Athletics Trainers' Association |
| $O_2$ | oxygen |
| $VCO_2$ | carbon dioxide production |
| VE | minute ventilation |
| $VO_2$ | oxygen uptake |
| $VO_2max$ | maximal oxygen uptake |
| RE | running economy |
| RER | respiratory exchange ratio |
| RH | relative humidity |
| RPE | ratings of preceived exertion |
| s | second |
| s.d. | standard deviation |
| SV | Stroke volume |
| $T_a$ | ambient temperature |
| TBW | total body water |
| $T_c$ | core body temperature |
| ThCom | thermal comfort |

## II.b List of tables

## II.c List of figures

# 1. Introduction

## 1.1 Running Economy

In modern athletics, long distance runners constantly seek to improve their performance in order to reach their maximal performance level. Hence, training and race preparation should focus on the improvement of physiological factors that in the past have been shown to positively affect endurance running performance. It is influenced by several factors which among others are: maximal aerobic power ($VO_2$max), muscle fibre type and distribution, blood lactate accumulation threshold (Coyle et al., 1991), relative exercise intensity, i.e. the fraction of $VO_2$max used while running (% $VO_2$max) (Kozlowski et al., 1985, Weston et al., 2000), and running economy (RE) (Bassett and Howley, 2000, Saunders et al., 2004a). All these factors have been discussed in the scientific literature. In this case the focus was put on running economy as this is mostly influenced by the intervention undertaken in this study.

RE is generally defined as the relative oxygen cost ($mL \cdot kg^{-1} \cdot min^{-1}$) for a given velocity of submaximal running. In other words, RE is the amount of oxygen used per kilogram body mass per minute for a certain exercise work load, in this case running. It has comprehensively been discussed in the literature (for reviews, see (Saunders et al., 2004a, Berg, 2003)). The lower the RE in a runner for a given running pace, the more efficient the runner is. His (or her) energy demand is lower (as given through lower oxygen cost) than that of a runner with a higher RE at the same pace. This is obviously a desirable state as the more efficient runner is more likely to be able to keep up the pace for a longer time, hence enabling him to perform better than the runner with a higher RE. In the past, the role of RE in running performance has been investigated by many researching groups. A relation from RE has been drawn to the success of African runners as their RE rates are significantly lower than those of Caucasian runners (Weston et al., 2000) giving them the ability for a better performance and possibly explaining their dominance in long distance running. Other studies further demonstrated that an improvement in RE is associated with an

enhancement of running performance (Pollock, 1977, Di Prampero et al., 1993, Svedenhag and Sjodin, 1985). For example, Pollock found as early as 1977 that American elite distance runners use a lower percentage of $VO_2$max compared to their colleagues considered good runners (Pollock, 1977). They put together a cohort of subjects that according to their endurance running performance capability were assigned to groups (20 elite runners: $VO_2$max = 79 $mL \cdot kg^{-1} \cdot min^{-1}$ cf. 8 good runners: $VO_2$max = 69.2 $mL \cdot kg^{-1} \cdot min^{-1}$). After submaximal and maximal treadmill testing including cardiopulmonary and respiratory measurements, results showed superior characteristics for HR, % VO2max as well as submaximal and maximal working capacity in the elite runners group. In conclusion they found that these elite athletes are more efficient at submaximal velocities giving them a considerable advantage during endurance running competition by having a better running economy (Pollock, 1977). Besides training, RE is influenced by several other factors which are of biomechanical, physiological, environmental, and anthropometrical nature (Saunders et al., 2004a). As probably environmental (ambient temperature, altitude, etc.), biomechanical (flexibility, elastic stored energy, ground reaction forces, etc.) and physiological ($VO_2$max, metabolic factors, etc.) factors are the most important among these, these have been the focus of attention of how to improve the individual's RE through training and/or other interventions. Many different ways of improving RE have been described in the past. Several studies established that strength training has a positive effect on RE, and hence running performance. Among these are studies which found that untrained individuals could improve their RE significantly by heavy resistance training (McCarthy et al., 1995, Marcinik et al., 1991, Hickson et al., 1988). Simultaneous strength and endurance training have also been shown to improve RE and hence running performance (Paavolainen et al., 1999). Furthermore, many studies on the influence of altitude on RE have been undertaken. It was shown that altitude training led to a better running performance which in those studies were found to be reliant on improved RE (Katayama et al., 2003, Saunders et al., 2004b).

### 1.2 Running economy and Hydration Status

One of the physiological determinants of RE is hydration status. Therefore, a number of studies have investigated the effects of water load on RE and/or endurance performance (Easton et al., 2007, Lyons et al., 1987, Kern et al., 2001). As dehydration and hyperhydration have both been intensively studied they will be introduced separately.

### 1.2.1 Dehydration and Exercise Performance

During exercise the skeletal muscle produces a significant amount of heat causing the core body temperature ($T_c$) to rise. This heat can be lost via two main mechanisms. Firstly, through non-evaporative heat loss, i.e. radiation, convection and conduction, heat can be lost to the environment. In high ambient temperatures which often occur at major running events, such as the Olympic summer games in Atlanta 1996, Sydney 2000, Athens 2004 and Beijing 2008, this mechanism is not available to the body. Once the surrounding environment reaches a temperature higher than the skin temperature, the heat transfer is reversed and heat is taken up by the body. In this case and secondly, the main way of cooling is through evaporative heat loss. This means that through the excretion and evaporation of water by the sweat glands of the skin heat is lost, cooling the skin and the blood in the subcutaneous vessels down.

The ‘cardiovascular model’ of dehydration explains the performance impairment in prolonged exercise. Through these mechanisms heat is lost allowing the body to cool, but with the loss of water (dehydration) plasma volume is also reduced. This leads to a decrease in the venous return to the heart and hence a smaller stroke volume of the heart. In order to maintain cardiac output and thus ensure sufficient circulation of blood, the heart rate is increased (‘cardiac drift’) (Ekelund, 1967). Eventually, the limitation of maximum heart rate will not be able to compensate for the loss in stroke volume and result in a decrease of cardiac output and aerobic performance (Rowell, 1986). Several studies support this idea of impaired performance due to hypo- or dehydration (Gonzalez-Alonso et al., 1995, Montain et al., 1998, Cheuvront et al., 2005, Below et al., 1995). Nevertheless, there is a lively debate in the literature whether dehydration negatively affects performance or not (Sawka and Noakes, 2007).

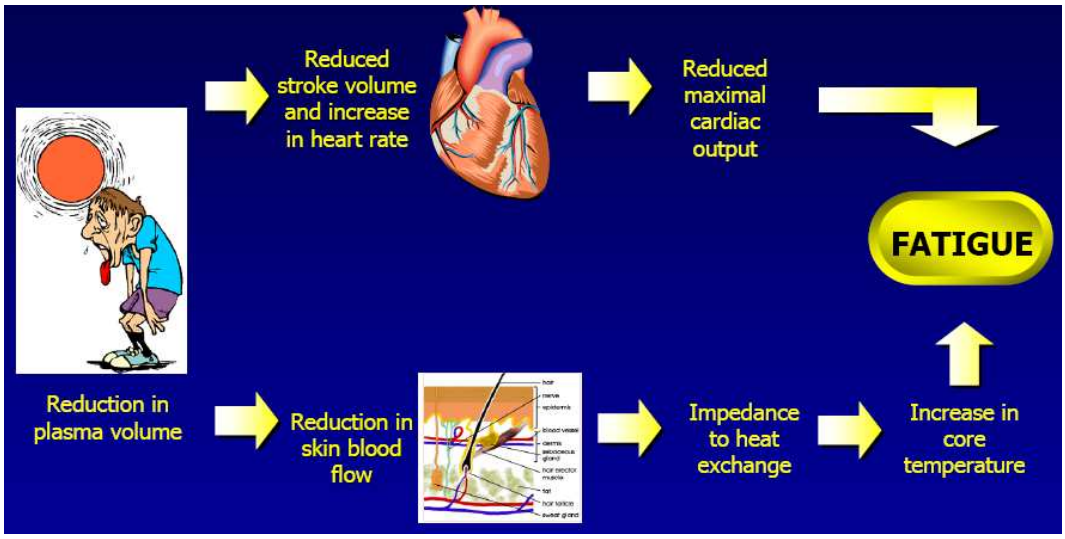


Figure 1.1 Cardiovascular model of dehydration

And even if dehydration does that the question remains what factor or factors limit endurance exercise. Many ideas have been published that could possibly explain the phenomenon of fatigue. And there are also numerous studies contradicting the hypotheses. First of all, one should define what is meant by the term fatigue. This has been done by Hultman who states that fatigue is 'the failure to maintain an expected power output' (Hultman et al., 1986). The negative effect of high ambient temperatures on exercise performance is well known. But there is a reasonable debate about the underlying mechanisms which limit performance and no final conclusion has been drawn as yet (Noakes and Gibson, 2004). Most of the research done in this area focused on the periphery as the cause of fatigue (Noakes and Gibson, 2004). Hence, many studies described below investigated possible mechanisms that would explain the inability to keep up physical activity at a certain level in the heat over time. Some studies therefore looked at the availability of energy substrates within the muscles. These have demonstrated that under different conditions of exercise (heat, cold) the ATP concentration never decreases to less than 50% of the resting level, and muscle glycogen as well as PCr were not related to the onset of fatigue in working muscle (Gonzalez-Alonso and Calbet, 2003). Pitsiladis & Maughan were led to the same conclusion by their results, finding that fatigue occured before carbohydrate stores were depleted (Pitsiladis and Maughan, 1999). These results were supported by another study which reported that after exercise to exhaustion the muscle glycogen content was highest in an ambient temperature of 40℃, compared to 3 and 20℃ (Parkin et al., 1999). Hence, it is

difficult to say the peripheral factors that means mechanisms that are beyond the neuromuscular junction, are the only cause of fatigue underlying heat stress. Paying tribute to this, there is more and more evidence suggesting that the origin of fatigue is at a higher level than the skeletal muscle itself. It was first suggested by Newsholme that an increase in tryptophan blood levels increases the concentration of the neurotransmitter 5-hydorxytryptamine in some neurons that regulate motor activity in the brain and could therefore lead to central fatigue (Newsholme et al., 1987, Newsholme and Blomstrand, 2006). Furthermore, from their study Nielsen et al. concluded that the physical endurance for exercise in hot, dry environments appears to be limited by the attainment of a critical core body temperature (Nielsen et al., 1993). As cardiac output and leg blood flow were not reduced and energy substrate utility or availability was not changed, the authors concluded that the rise in $T_c$ per se, and not circulatory failure, were the determining factor for exhaustion under heat stress (Nielsen et al., 1993). Moreover, Nielsen & Nybo found that during prolonged maximum voluntary muscle contraction the ability to generate muscle force is decreased with an increase in temperature. Hence, an increased $T_c$ could be impairing the capacity of the brain to recruit muscle fibres rather than an impairment of skeletal muscle function (Nielsen and Nybo, 2003). This knowledge has led to further investigation and eventually to the development of the central governor theory which states that during prolonged exercise in hot ambient conditions the brain reduces muscle fibre recruitment in order to maintain the integrity of the organism (Noakes, 2001).

Obviously from these conclusions, in order to maintain the power output while exercising for a prolonged period of time in the heat it is very desirable to delay and attenuate the rise in $T_c$ for as long as possible. Therefore, one has to come up with strategies that promote and enhance the mechanisms of heat loss described earlier. According to the cardiovascular model of dehydration sweating causes a decrease in plasma volume and consecutively to a reduction in skin blood flow. This negatively affects the convective heat loss mechanism as not enough blood is exposed to the external environment to effectively attenuate the rise in $T_c$. According to the hypothesis explained above, this will eventually lead to fatigue. A reduction in plasma volume is also accompanied

with a decrease in venous return to the heart which results in a reduced cardiac output and a reduction of the evaporative cooling mechanism and thus performance is further impaired. In order to maintain plasma volume at an adequate level and prevent the reduction in skin blood flow and the associated rise in $T_c$ the rationale for fluid ingestion during exercise is widely accepted. While exercising in the heat this would maintain plasma volume and reduce the cardiovascular strain asserted on the heart. There would also be sufficient body water available to sustain adequate sweat production and accordingly, sufficient evaporative cooling. Furthermore, the uphold of skin blood flow would enhance convective heat loss and additionally improve thermoregulatory function. Hence, it is proposed that by fluid ingestion one can improve athletic performance (Convertino et al., 1996). There has been a tremendous debate about the most effective fluid uptake strategy (Convertino et al., 1996, Noakes, 2001). The American College of Sports Medicine (ASCM) created guidelines, suggesting specific fluid intake (ACSM, 1975). These guidelines are regularly revised and adjusted accordingly. The most recent guideline advises to prevent a weight loss of more than 2% BM due to dehydration, giving specific amounts of water to drink according to body mass and running speed (Sawka et al., 2007). Another organisation, the National Athletic Trainers' Association (NATA) suggests a fluid replacement of 200 to 300 mL every 10 to 20 minutes while exercising, also advising that drinking should be amended to the individual (Casa et al., 2000). These guidelines are not always practical as there are some limitations preventing the athlete from consuming the advised amounts of fluids. These could be race strategy, unwell-being of the athlete while running and drinking, impracticality of supplying sufficient drinks, etc. Moreover, over drinking might result in a reduction in blood sodium concentration below 130 $mmol \cdot L^{-1}$ and ultimately lead to hyponatraemic encephalopathy, in the worst case even to death (Noakes, 2005). Although this is the case for very slow runners who consume vast amounts of fluids (Almond et al., 2005).

### 1.2.2 Hyperhydration

Considering the detrimental effects of dehydration for running performance mentioned above, it is obvious to conclude that a hyperhydrated state is

desirable when exercising in the heat, providing sufficient water during exercise to keep $T_c$ down, prolonging fatigue. One approach described in the literature was by expansion of acute plasma volume. An attenuation of the rise $T_c$ during exercise in the heat has been described by Forney et al. (1988) which they concluded came from the maintenance of central blood volume, leading to an increase in skin blood flow and an associated increase in convective heat loss. Using the same method, giving saline intravenous solution led to an attenuation of the rise $T_c$ and increase in heart rate (Deschamps et al., 1989). Furthermore, it was demonstrated that a plasma volume expansion using dextran solution (which is a banned substance by the World Anti Doping Agency WADA, see prohibited list 2009) can also lead to an increase in cycling performance by more than 10% (Luetkemeier and Thomas, 1994). Nevertheless, the medical expertise, equipment and time required for an intravenous infusion, make this method very inconvenient for use in the field. Even more, it has been put on the World Anti Doping Agency (WADA) prohibited list and hence its usage is not allowed. Additionally, other studies do not agree with these findings (Grant et al., 1997, Watt et al., 2000) putting even more doubt on the application of this method. Achieving hyperhydration by ingesting large amounts of water has been shown ineffective in the past as most excess water is excreted via the kidneys (Freund et al., 1995). Hence, hyperhydrating agents have to be used to successfully ‘pre-load’ the body with water.

One of these agents is Creatine (α-methylguanidine acetic acid), a compound naturally synthesized in the body from the amino acids arginine, glycine and methionine. Most of the stored Cr originates from food, mainly meat and is stored in the muscle tissue. In the form of Phosphocreatine (PCr) it is a major source of muscle energy during short term (2-30s), high intensity muscle contraction. Therefore, it has also been used as an ergogenic aid, taken up in the form of Creatine Monohydrate ($Cr \cdot H_2O$), especially in short duration, high intensity exercise (Demant and Rhodes, 1999). Creatine has been known to increase the specific heat capacity of the body by retaining intracellular water (ICW) (Kern et al., 2001, Kay and Marino, 2000). By which mechanism this effect is mediated remains to be clarified. Haussinger and colleagues (1993) suggested the retention of water being due to osmotic effects, cell swelling and

a consequent increase in protein synthesis. In contrast to this, it has also been suggested that the ICW increase is mediated by an up regulation of protein synthesis followed by an increase in cell water content (Kreider et al., 1998). The beneficial effects, i.e. reducing thermoregulatory and cardiovascular responses (e.g. heart rate, rectal temperature, sweat rate) of this water retention during prolonged exercise in the heat were demonstrated by Kilduff et al. (Kilduff et al., 2004). Research has been undertaken to elucidate the mechanisms underlying Cr uptake into skeletal muscle. It is now well established that a Cr transporter exists which can be found in many tissues and is regulated by many different mechanisms (Snow and Murphy, 2001). A study has shown that Cr uptake into the skeletal muscle during supplementation can be increased by 60% if about 360 g of carbohydrates are consumed shortly after the Cr supplement (Green et al., 1996). Another study has demonstrated that Cr uptake during supplementation can be increased by submaximal exercise (Robinson et al., 1999). This affect can be significantly increased by adding 93 g of simple sugars to the supplement (Robinson et al., 1999). Although exact mechanisms remain unclear, the results from above mentioned studies and other experiments (Haugland and Chang, 1975, Odoom et al., 1996) suggested that insulin plays a significant role in the Cr uptake by a stimulation of the Cr transporter mediated via the sodium-potassium pump acitivity (Steenge et al., 2000). Steenge et al. (Steenge et al., 2000) also investigated the effects of the Cr/glucose supplementation (90 g carbohydrates) compared to the addition of protein to the supplement and therefore decreasing glucose content to about 50 g. This alteration resulted in a non-significantly different uptake of Cr into the muscle although insulin response is slightly decreased (Steenge et al., 2000).

Another well described compound applied to increase total body water is glycerol (1,2,3-propanetriol). It is also naturally synthesised in the body and distributed within and between all cells of the body, except from the cerebral spinal fluid and aqueous humor (Tourtellotte et al., 1972, Lin, 1977). Its hyperhydrating ability has also been described (Riedesel et al., 1987, Magal et al., 2003), but in contrast to Creatine, glycerol is thought to expand extracellular water (ECW), and effectively minimising the exercise induced reduction in

plasma volume (Murray et al., 1991). The question by which mechanisms this occurs has not been answered yet. Several researchers proposed the idea that the retention is due to higher levels of ADH concentrations associated with glycerol ingestion (Freund et al., 1995). Previous reports have only shown small differences that only approach statistical significance in ADH concentration levels and glycerol and water inventions (Freund et al., 1995). However, another mechanism suggested which would explain the water retention is the action of glycerol on the kidneys. Sommer et al. (1993) reported that glycerol when at normal blood levels is filtered but then almost completely passively reabsorbed in the proximal and distal renal tubules of the kidney. During exogenous glycerol ingestion the glycerol blood concentration increases. More glycerol is reabsorbed in the tubules and hence, through an osmotic gradient, more water is reabsorbed leading to a higher plasma volume (Kruhoffer and Nissen, 1963). Taking these results into account, a few studies concluded that glycerol reduces thermal and cardiovascular strain during exercise in the heat if it is administered 2-3 hours prior to exercise (Anderson et al., 2001, Lyons et al., 1990, Montner et al., 1996). Lyons et al. (1990) went further to conclude that these beneficial effects are due to the maintenance of blood volume and cutaneous blood flow. Montner and colleagues (1996) reported furthermore, that a 23% increase in time to exhaustion was observed after the ingestion of glycerol. Nevertheless, not all studies come to the same conclusion. For example, Murray et al. (1991) and Latzka et al. (1998) were unable to show beneficial effects of glycerol ingestion during exercise in hot conditions. One fact that has to be considered when looking at the efficacy of glycerol is the way it is taken up into the body. To date, very little is known about exact mechanisms, but the finding that in rats the efficiency of a glycerol drink can be enhanced by adding glucose to the drink is very noteworthy (Wapnir et al., 1996). Furthermore, it is now widely accepted that the hyperhydrating effect of Gly can be observed as soon as 60 to 90 min after consumption (Goulet, 2009). However, this recent review on the topic of glycerol absorption clearly states that there is a lack of knowledge in this area and more research, especially on humans, has to be undertaken (Goulet, 2009).

Concluding, it can be said that Creatine or glycerol can be described as a hyperhydrating agent leading to a water retention and hence, an increase in total body water of about 400-800 mL (Kilduff et al., 2004, Montner et al., 1996). Easton et al. went one step further and were the first to apply Gly additionally to Cr and could recently demonstrate in their study that a combined application of the two hyperhydrating agents has an additive effect. This results in a higher total body water with an increase in ICW and ECW compared to each agent applied alone (Easton et al., 2007). Easton et al showed further that a combined Creatine and glycerol supplementation leads to a hyperhydrated state reducing cardiovascular and thermal strain during prolonged exercise in the heat while not impacting on exercise performance (Easton et al., 2007).

### 1.2.3 Effects of hydration status on Running Economy

Obviously, the hydration status is closely related to body mass. The more water in the body, the higher the body mass. The body mass has an impact on performance, as the higher the body mass is the higher is the oxygen demand of the skeletal muscle as more weight has to be moved by them. In contrast to this, one study has demonstrated that a dehydration of 5% body mass has no effect on running economy whatsoever (Armstrong et al., 2006). So far, no study has been looking into the effects of hyperhydration on running economy. It is necessary to investigate whether this effect of weight gain can be overcome by prolonging the ability to perform at a lower $VO_2$ level induced by the effects of hyperhydration, i.e. by attenuating heart rate, maintaining cardiac output, and a reduction in the rise of core body temperature.

## 1.3 Aim of the study

The hypothesis of the study is that the higher water load in athletes outweighs the disadvantages of the associated higher body mass as fatigue due to dehydration is prolonged, hence resulting in a better running performance by improving running economy, i.e. reducing oxygen uptake. Therefore, the present study was designed to investigate the cardiopulmonary and

thermoregulatory effects of hyperhydration induced by a novel Creatine and glycerol supplementation in well trained male athletes in cool (10℃, RH = 70%) and hot conditions (35℃, RH = 70%) usually encountered in major sporting events (e.g. Olympic Summer Games, World Championships, etc.).

The Creatine/Glycerol loading protocol has been altered compared to Easton et al. (2007). It remains to be seen whether the new protocol leads to the same increase in total body water, intracellular body water and extracellular water and hence the associated beneficial thermoregulatory and cardiovascular effects.

# 2. Methods

## 2.1 Subjects

Initially, twelve subjects participated who were healthy endurance trained male athletes. After careful consideration of individual circumstances 3 subjects were excluded from analysis (for more details see results section page 24). The other nine subjects were divided in a responders (n = 7) and non-responders (n = 2) group according to current literature (Kilduff et al., 2003) The average age of the responder-group subjects (n = 7, unless otherwise stated) was 22 ± 3 years, height was 1.77 ± 0.06 m with an average weight of 72.0 ± 8.9 kg. The subjects had an average maximum oxygen uptake ($VO_2max$) of 58.04 ± 6.44 $mL \cdot kg^{-1} \cdot min^{-1}$. An interview prior to this study confirmed that the subjects were fully informed of any risks and discomforts associated with the experiments and informed that they could withdraw at any point without any explanation before giving their written confirmed consent to participate. The interview also ensured that all subjects did not take any creatine in the 8 weeks prior to the start of the study. All experiments were approved by the University of Glasgow Ethics Committee.

## 2.2 Experimental Design

The $VO_2max$ was determined for all subjects essentially as described by Fudge et al. (2007) during an initial discontinuous incremental test to volitional exhaustion in normal laboratory conditions (room temperature RT = 20-21℃, relative humidity 30-40%) on a motorized treadmill (PPS Med, Woodway, Germany) at 1% grade. The subjects started running at 8 $km \cdot h^{-1}$ for three minutes in order to reach a steady state. After this first bout treadmill speed was reduced to 4 $km \cdot h^{-1}$ for four minutes as a recovery period. In the next exercise bout the treadmill speed was set to 10 $km \cdot h^{-1}$ for three minutes. After another recovery period of 4 minutes, the speed was increased to 12 $km \cdot h^{-1}$. This procedure was repeated with 2 $km \cdot h^{-1}$ increments in running speed until volitional exhaustion of the subject. During the test heart rate was recorded at

the end of each bout using heart rate monitors (Polar Sports Tester, Polar, Finland) and expired gas samples (30 s collection time at the end of each bout) were taken using Douglas bag collection technique and analyzed for % $O_2$ and % $CO_2$ (Servopro 4100 Gas Purity Analyzer, Servomex, UK) as well as analyzed for volume and temperature of expired gases. Barometric pressure was measured using a standard mercury barometer. Respiratory values were calculated using the collected data. Running speed at 60% of $VO_2$max (exercise intensity) was calculated using the linear relation between treadmill speed and $VO_2$. This relatively low intensity was chosen in order to ensure that all subjects could finish the experiment in the heat whereas it is high enough to observe possible adaptations in cardiopulmonary or thermoregulatory parameters to the supplementation. As shown in figure 2.1, before the actual experimental trials described below the subjects completed familiarization trials following the experimental protocol until the variability of $VO_2$ of two consecutive tests was less than 5%. According to Becque et al. (1993) normal variability of $VO_2$ at submaximal $VO_2$-intensities is 4.3%. Therefore, a variability of more than 5% implies that standardisation has not been achieved and hence, to ensure that measurements can be considered reliable subjects should be re-tested until one can exclude a familiarization effect or other physiological reasons affecting measurements other than the intervention itself. At least three days after the last familiarization visit subjects reported back to the laboratory for the first experimental trial, i.e. a pre-supplementation trial. After this baseline test, all subjects commenced the hyperhydration treatment comprising creatine and glycerol. For this, subjects consumed twice daily (afternoon and evening) a solution of 11.4 g of $Cr{\cdot}H_2O$ (Creatine 6000-ES, Iovate Health Sciences Research Inc., Canada), mixed in 150 mL sugar-free juice concentrate 850 mL of warm water (about 50℃) and allowed to cool to r oom temperature before consumption. This supplementation regimen was followed for 7 days. On the day of the post-supplementation test 1 g per kg body mass glycerol (Aldrich Chemical, Milwaukee, WY, USA) was added to the mixture and consumed 5 hours prior to the test. In order to prevent degradation of Cr, each supplement was prepared fresh each time before consumption. The subject was given a temperature pill (HQInc., USA*)* about 8-12 h prior to each of test allowing core body temperature ($T_c$) to be measured, also see (Easton et al., 2007).

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Week 1 | Day 1<br>Familiarization test | Day 2 | Day 3 | Day 4 | Day5 | Day 6 | Day 7 |
| Week 2 | Day 8<br>pre-supplementation | Day 9 | Day 10 | Day 11 | Day 12 | Day 13 | Day 14 |
| | | supplememtation regimen | | | | | |
| Week 3 | Day 15<br>post-supplementation | | | | | | |

Figure 2.1 Schematic experimental design

## 2.3 Experimental Procedures

The subject reported to the lab after a 3 hour fast, having consumed 500 mL of liquid one hour before and having refrained from alcohol, caffeine, and strenuous exercise in the 24 hours before. The whole procedure is shown in figure 2.2. Firstly, a urine sample was collected from the subject prior to taking the pre-test nude body mass. Body water compartments were measured using a Bodystat Multiscan 5000 bioimpedance analyzer (Bodystat Ltd., Isle of Man, UK) as previously prescribed by Easton et al. (Easton et al., 2007). This method allows total body water (TBW) and extracellular water (ECW) to be estimated, and hence intracellular water (ICW) can be deduced from those values. After the measurement the subject sat up for 10 and a 21G cannula was inserted into a superficial vein of the anti-cubital fossa of the right arm. A 10 mL blood sample was taken. The venous cannula was kept patent by flushing it with 10 mL of isotonic saline solution between samples. Prior to entering the environmental chamber a heart rate monitor (Polar Sports Tester, Polar, Finland) was attached to the subject. The subject was further equipped with an accelerometer (3dNX, Biotel Ltd. , Bristol, UK) as described previously by Fudge and colleagues (Fudge et al., 2007). Then the subject was moved to the climate chamber (ambient temperature of $T_a$ = 10.5 ± 0.2 ℃ and a relative humidity of RH = 72.0 ± 1.1 %). Firstly, the subject was asked to stand on the treadmill for a 2-minute rest period during which a Douglas bag collection of the whole duration was taken as well as a record of heart rate (HR) and $T_c$ at the end of this period. The subject exercised at an intensity of 60% $VO_2$max for 30 minutes at 1% treadmill grade. Every five minutes HR, ratings of perceived

exertion (RPE), thermal comfort and $T_c$, were recorded. RPE was recorded using the Borg category scale (Borg, 1982). Thermal Comfort was taken using the scale as shown in the appendix page 70. Furthermore, at the end of each 5-minute interval a 1-minute collection of expired gases using Douglas bags was taken and analyzed for oxygen and carbon dioxide content, as well as temperature and volume of expired gases. After finishing the first exercise bout the subject was removed from the chamber and another blood sample was retrieved from the subject after a 10-minute sitting down period. The subjects nude body mass was taken and during the last 20 minutes of the 30-minute resting period at normal room temperature, the fluid loss was replaced by giving the subject the equivalent amount of water. Then another bout followed the first one at the same intensity (60% $VO_2max$), but this time at $T_a$ = 34.72 ± 0.19 ℃ and RH = 71.5 ± 0.9 %. Data collection was taken in the same manner as in the first bout of exercise, but without the rest period at the beginning. After this 30 minute bout, the subject was removed from the chamber and nude body weight was taken once again. Moreover, another blood sample was obtained as described above.

### 2.4 Blood analysis

Blood obtained from the subject was divided into two parts. One part of the blood was pipetted into $K_3$EDTA coated tubes (duplicates). From these tubes 400 µL were taken out and mixed into 800 mL of ice-cold perchloric acid (0.3 $mol \cdot L^{-1}$) for rapid deproteinization, centrifuged (10 min, 13000 rpm, HettichMicrocentrifuge, Germany) and analyzed for glucose using standard enzymatic spectrometrical methods (Mira Plus, ABX Diagnostics, Montpellier France) as described by Maughan (1982). The blood from the $K_3$EDTA tube was analyzed for haemoglobin (cyanmethemoglobin method, Sigma, Chemical Company Ltd., Dorset, UK) and packed cell volume (conventional microhaematocrit method).

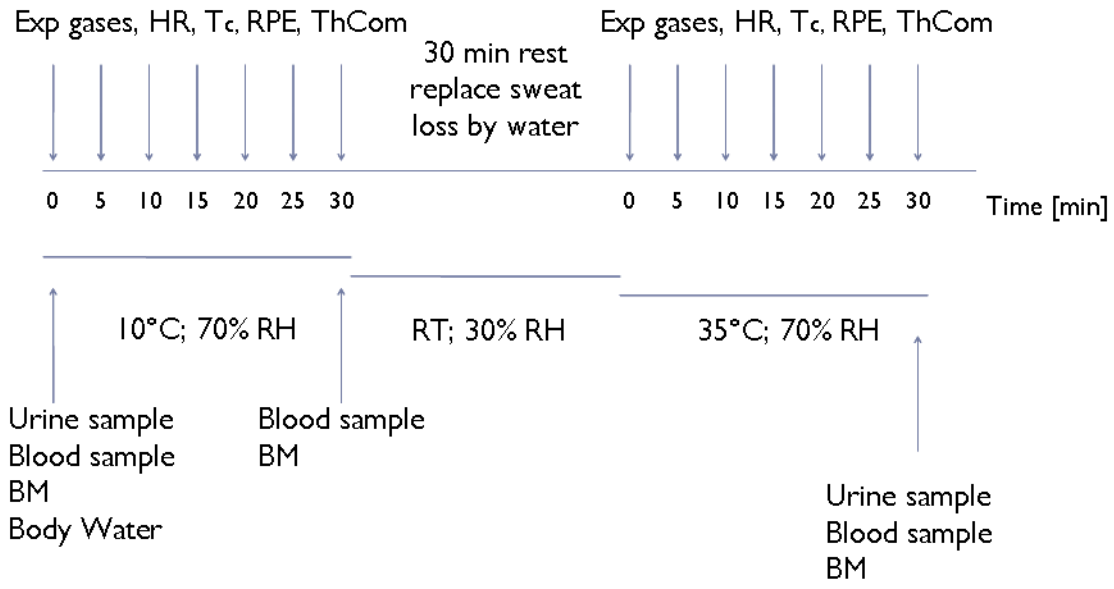


Figure 2.2 Schematic experimental procedure (Exp gases – expired gases; HR – heart rate; $T_c$ – core body temperature, RPE – Ratings of perceived exertion; ThCom – thermal comfort; RH – relative humidity; BM – body mass)

The other part (about 3 mL) of the blood was allowed to coagulate, centrifuged (25 min, 4000 rpm, Thermo IEC, Needham Heights, USA) and analyzed for plasma osmolality (Micro-Osmometer 3300, Vitech Scientific, West Sussex, UK). Calculations for plasma volume changes were done using the formula published previously (Dill and Costill, 1974). All measurements were taken in duplicates except the analysis for packed cell volume which was done in triplicates.

### 2.5 Data analysis

Data is expressed as the mean ± standard deviation (s.d.). Before further statistical analysis, the data was analyzed for the normality of distribution. Statistical significance was set at $p < 0.05$. Two-way analysis of variance (ANOVA) tests of repeated measures were used for the comparison of $VO_2$, $VCO_2$, $V_E$, RER, HR, $T_{core}$, RPE, Thermal Comfort and accelerometry data pre- and post-supplementation as well as looking for an effect within one trial over time before and after the supplementational period. In cases where significance was identified, the difference for the factor was further determined for each time

point using paired-sample t-tests. Changes in BM, TBW, ECW, ICW, urine osmolality, sweat rate and total sweat loss were tested for significance using the paired-sample t-test. Statistical analysis was carried out using SPSS 15.0 for Windows.

Power analysis for the calculation the minimum sample size, i.e. subject number, was done using the following formula:

$$n \approx (z_{1-\alpha}+_{z1-\beta})^2 \cdot 2 \cdot (s/d)^2$$

where $\alpha$ was set to 5% (significance level according to $p = 0.05$) and the Type II error level $\beta$ was set at 20% as the power of the test was set to 0.80 as a standard for adequacy.

| | Age [years] | Height [m] | Weight [kg] | BMI [kg·m$^2$] | $VO_2$max [ml·min$^{-1}$·kg$^{-1}$] |
|---|---|---|---|---|---|
| **Subjects** | 22 ± 3 | 1.77 ± 0.06 | 72.0 ± 8.9 | 22.9 ± 1.7 | 58.0 ± 6.4 |

Table 3.1 The physical characteristics and maximal oxygen uptake of the subjects considered responders. Data presented as mean ± s.d.

# 3. Results

## 3.1 Body Mass and Water Compartments

Twelve subjects completed the study according to the protocol described in the methods section. After careful consideration of all the factors involved, three subjects were excluded from the study as the individual circumstances did not guarantee standardization and hence reliability of the respective results. One subject competed in a marathon between the familiarization trials and the actual experimental trials. Another subject admitted to changing his diet, i.e. eating a lot more while being abroad during the creatine/glycerol supplementation, hence it cannot be guaranteed that the increase in weight is exclusively due to the supplementation protocol. The third subject experienced a severe cold and other health related issues and his results were hence discarded. The remaining nine subjects were furthermore divided into responders and non-responders to the supplementation. Since no urine was collected during loading regimen, Cr uptake could not be determined for each subject. Hence in this study, the criterion used to distinguish between responders and non-responders was based on Kilduff et al. where subjects with a body mass increase of less than 0.2 kg were considered non-responders (Kilduff et al., 2003).

The average age of the subjects (n=7, unless otherwise stated) was 22 ± 3 years, height was 1.77 ± 0.06 m with an average weight of 72.0 ± 8.9 kg. The subjects had an average $VO_2$max of 58.04 ± 6.44 mL·kg$^{-1}$·min$^{-1}$ (also see table 3.1). As shown in Figure 3.1, total Body Mass (BM) increased by 0.8 ± 0.3 kg on average after the one week supplementation protocol described in the

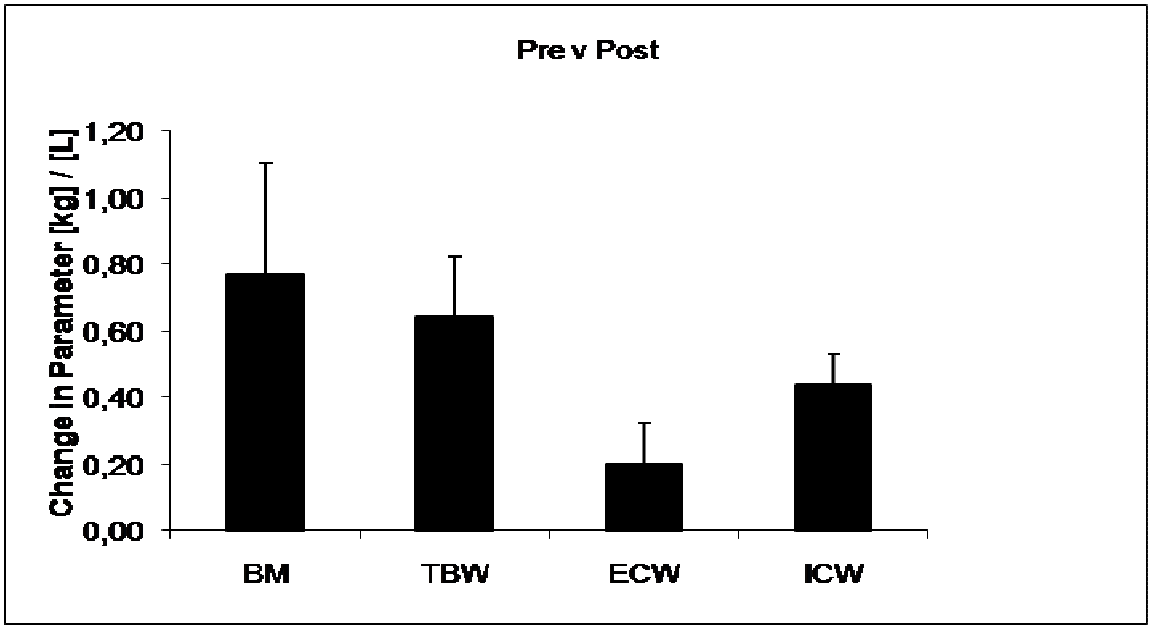


Figure 3.1 Changes in body mass (BM), total body water (TBW), extracellular water (ECW) and intracellular water (ICW) pre- vs post-supplementation.

methods section. The increase in total body water (TBW) was 0.64 ± 0.18 L, of which 0.20 ± 0.12 L could be assigned to an increase in extracellular water (ECW), and 0.44 ± 0.09 L were due to an increase in intracellular water (ICW). These numbers are for n=6 as the measurement for one subject at one test was faulty for an unknown reason (result stated a 10% of BM fluid loss) and could therefore not be considered for further analysis.

### 3.2 Urine Osmolality

No significant changes were found in urine osmolality between the pre- (571 ± 255 mosmol) and post-supplementation phase (568 ± 331 mosmol).

### 3.3 Sweat Rates and Total Sweat Loss during Exercise

At 10℃, no significant difference in total sweat loss and sweat rate was observed. The total sweat loss in the pre-supplementation trial was 0.3 ± 0.1 L and 0.3 ± 0.1 L for the post-supplementation trial. The sweat rate did not

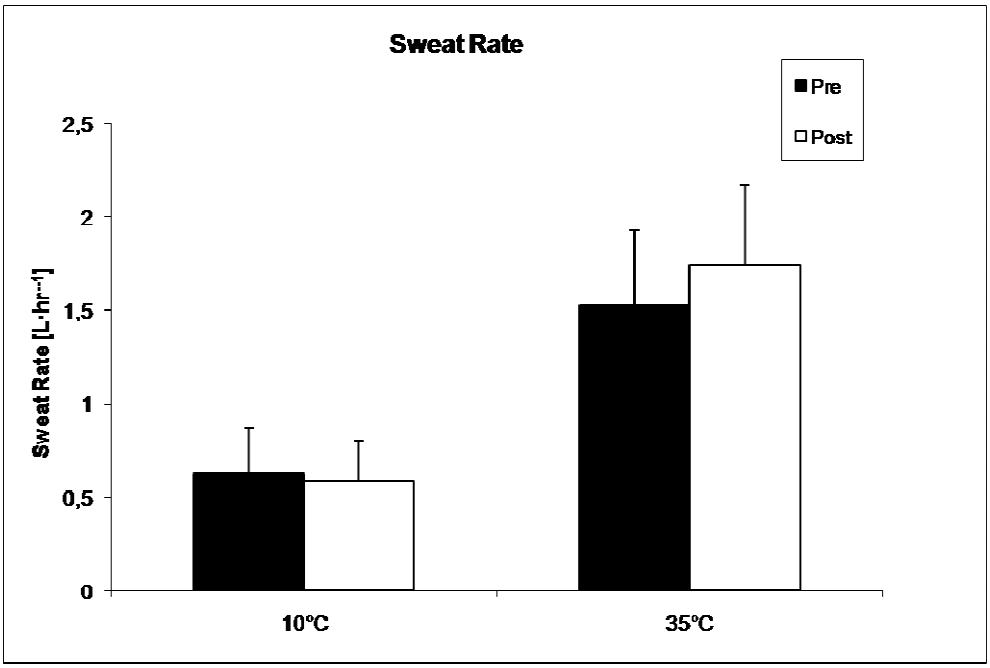


Figure 3.2 Changes in Sweat Rate for 10℃ and 35℃ pre- (white) and post- supplementation (black columns). Data presented as mean + s.d.

change significantly either, from 0.6 ± 0.2 L in the pre-supplementation trial to 0.6 ± 0.2 $L \cdot h^{-1}$ in the post-supplementation trial. Similarly, no significant changes were observed in the 35℃ trial. Here, the total sweat loss was 0.8 ± 0.2 L prior to supplementation and 0.9 ± 0.2 L after the regimen. Accordingly, there was a small, but non significant change in sweat rate from 1.5 ± 0.4 $L \cdot hr^{-1}$ to 1.7 ± 0.4 $L \cdot hr^{-1}$ in the pre- and post-supplementation test, respectively. Nevertheless, the total sweat loss and sweat rate increased when comparing the 10℃ to the 35℃ trial. This is true for all pr e- and post-supplementation trials.

### 3.4 Cardiopulmonary Variables

No change has been observed in $VO_2$, $VCO_2$, $V_E$ and respiratory exchange ratio (RER) during familiarization, pre- and post-supplementation testing. RER remained relatively constant throughout the trials with no significant increase over time. In comparison to this, $VO_2$, $VCO_2$ and $V_E$ increased significantly over time with increased duration of the exercise. Furthermore, RER showed great variability for the resting measurement at the beginning of the 10℃ trial. Within

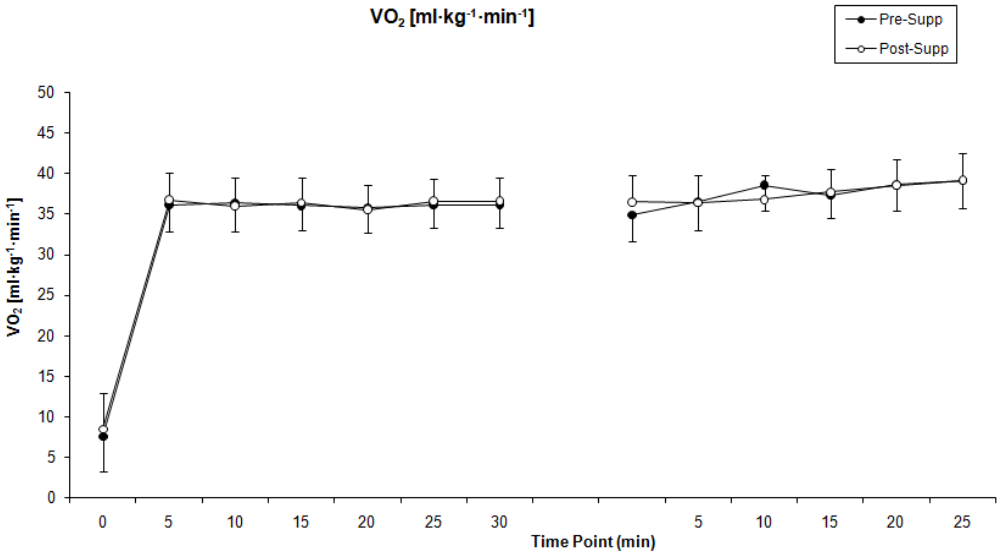


Figure 3.3 Mean $VO_2$ during exercise before (black circles) and after (white circles) supplementation. Data presented as mean ± s.d.

a single experiment the variables increased over time as one would expect considering the duration of the exercise. But there was no change within the variables at each time point, nor did the increase over time change. No differences in resting HR were found before or after supplementation. The HR increased within the duration of a single experimental trial. There was no difference between the HR of the pre- compared to post-supplementation at any time point. Furthermore, the ∆ HR pre- and post-supplementation did not differ either, i.e. no attenuation in the rise of HR was found.

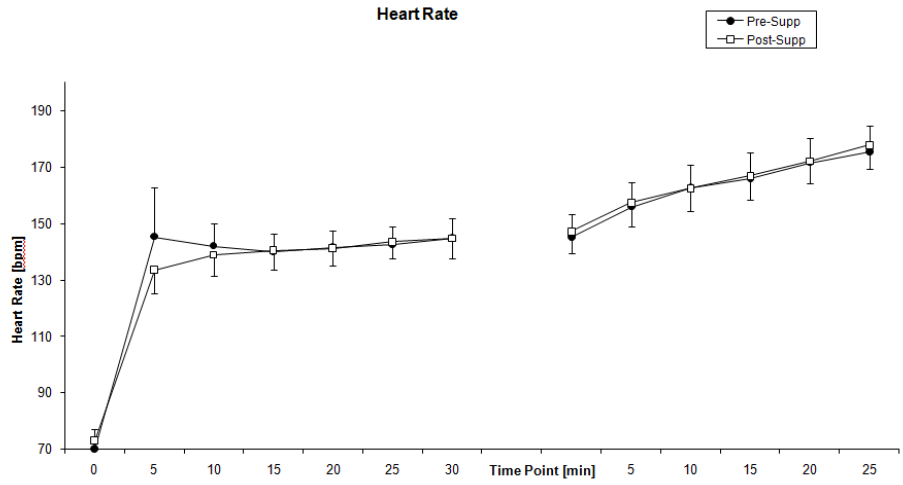


Figure 3.4 Mean heart rate during exercise before (black circles) and after (white circles) supplementation. Data presented as mean ± s.d.

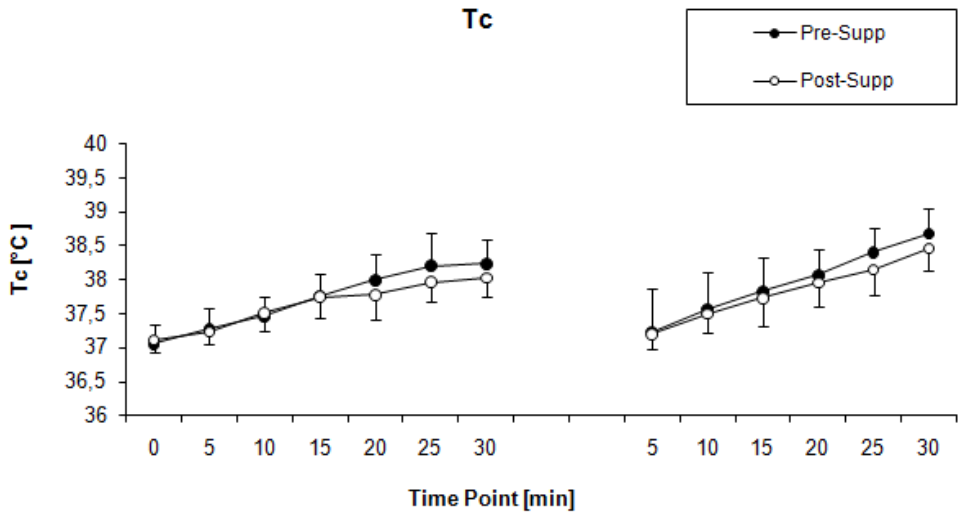


Figure 3.5 Mean core body temperature during exercise before (black circles) and after (white circles) supplementation. Data presented as mean ± s.d.

### 3.5 Core Body Temperature

In all trials core body temperature increased significantly with duration of exercise, where the increase was greater for the 35℃ trial. The differences in temperature increase were as follows: In the 10℃ t rial, before supplementation, the temperature increases by 1.18 ± 0.37 ℃, compar ed to 0.96 ± 0.21 ℃ after the loading regimen. A similar situation can be observed comparing the increase in $T_{core}$ during the 35℃ trial. The increase was attenuated from 1.70 ± 0.39 ℃ before the supplementation to 1.31 ± 0.27 ° C afterwards. A slight attenuation in the rise in $T_c$ could be observed in the graph in post-supplementation trial. However, this difference was not statistically significant (in 10℃: p = 0.6 and in 35℃: p = 0.20) and furthe r investigation is necessary. Possibly, increasing subject number to n = 16 as calculated by power analysis would give significant results.

### 3.6 Ratings of Perceived Exertion and Thermal Comfort

There was a significant progressive increase in RPE during the exercise over time. Nevertheless, no change was observed in the increase of RPE

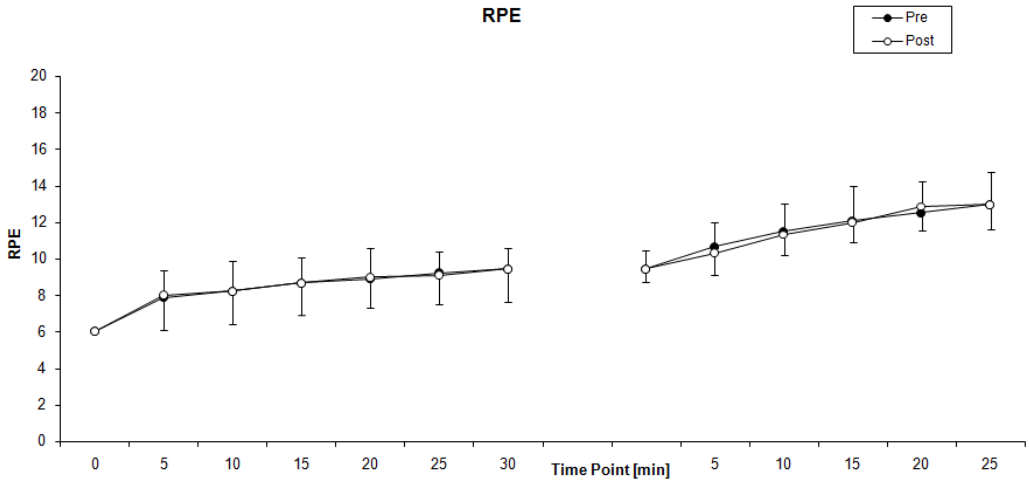


Figure 3.6 Mean Ratings of perceived exertion during exercise before (black circles) and after (white circles) supplementation. Data presented as mean ± s.d.

between the pre- and post-supplementation exercise. In contrast to this, the thermal comfort increased significantly over time during the exercise as well, but comparing the pre- to the post-supplementation trial the increase in the ratings of thermal comfort was attenuated. At time points 5 min and 10 min of the 35℃ part the trial the ratings were significantly lower. For time point 5 min, the ratings were 4.14 ± 1.77 pre- and 3.43 ± 1.81 post-supplementation (p= 0.047). For time point 10 min, the ratings were 5.14 ± 0.90 before and 4.29 ± 1.50 after the Creatine/glycerol regimen (p= 0.05).

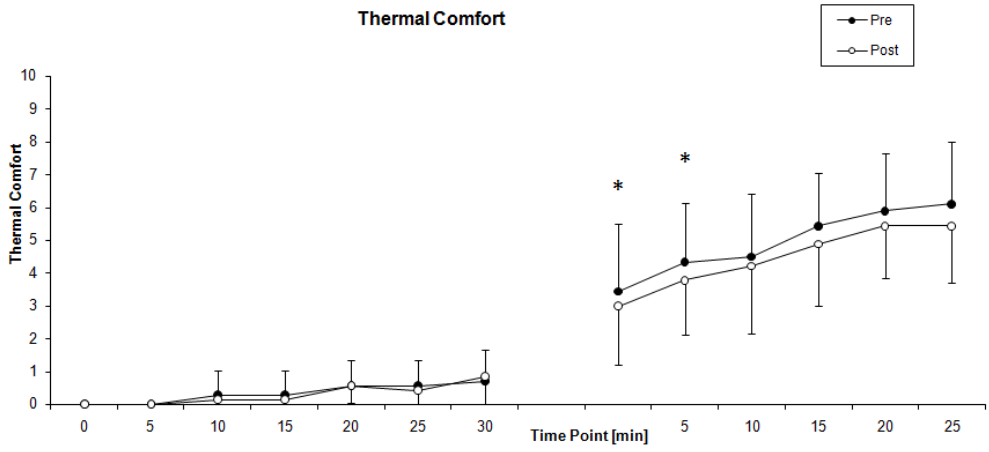


Figure 3.7 Mean Thermal Comfort during exercise before (black circles) and after (white circles) supplementation. *: indicates a statistically significant difference between pre- and post-supplementation. Data presented as mean ± s.d.

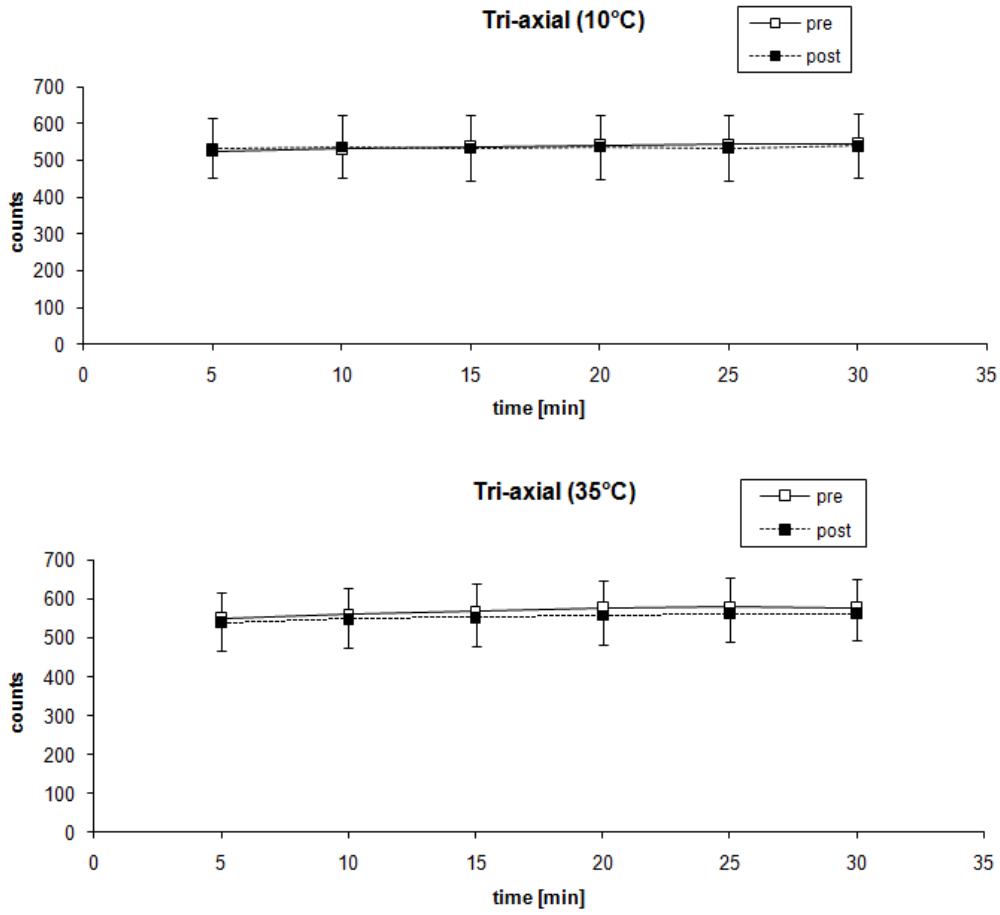


Figure 3.8 Triaxial count values of the accelerometry data before (white boxes and black line) and after (black boxes and dotted line) the supplementation period in 10℃ (top) and 35℃ (bottom). Data presented as mean ± s.d.

### 3.7 Accelerometry

No changes could be observed in the accelerometry data. There were no changes in the accelerations into the x-, y-, z-, dual-, or triaxial-axis before and after the supplementation period. All data is attached in the appendix to this thesis; figure 3.8 represents the tri-axial data of the accelerometer measurements.

### 3.8 Blood Metabolite concentrations and Plasma Volume changes

There is a small, non-significant difference in resting glucose levels before and after the supplementation period. Pre-supplementation resting glucose was 4.91

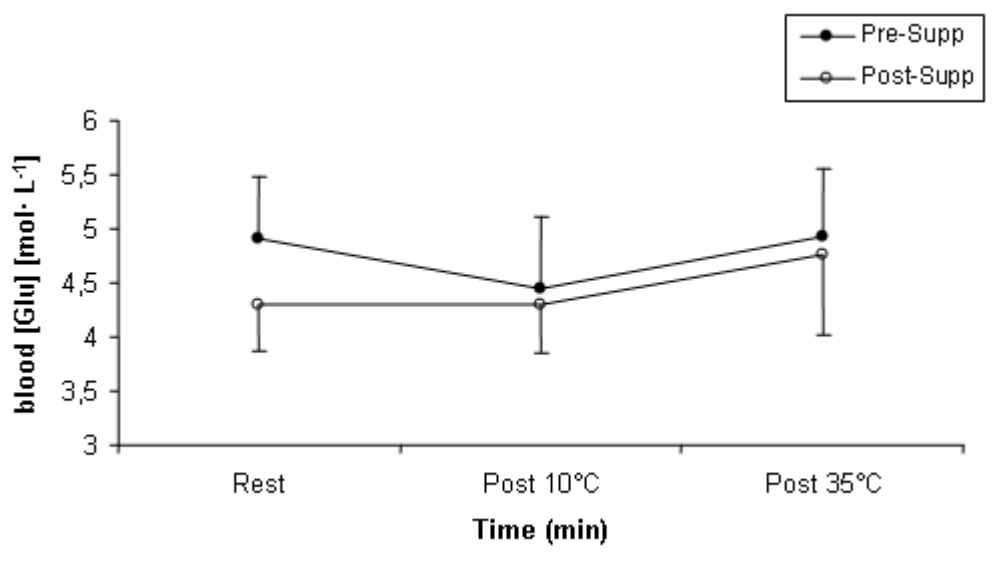


Figure 3.9 Mean blood glucose concentrations before (black circles) and after supplementation (white circles). Data are presented as mean ± s.d.

± 0.58 mmol·L$^{-1}$, whereas post-supplementation this value was 4.30 ± 0.43 mmol·L$^{-1}$. Comparing the other time points of blood analysis, there was no difference pre- and post-treatment to be observed. After the bout in 10℃ the glucose level is 4.45 ± 0.65 mmol·L$^{-1}$ pre- and 4.30 ± 0.44 mmol·L$^{-1}$ post-supplementation. After running in 35℃, glucose measurements were 4.94 ± 0.63 mmol·L$^{-1}$ pre- and 4.75 ± 0.73 mmol·L$^{-1}$ post-treatment with creatine and glycerol. These values are for n=6 as we could not draw blood from one subject at one trial and hence had to leave out his blood data for all his session altogether. Overall over time during the trial, there was a reduction in plasma volume. During the 10℃ trial, this change was at a magnitude of -1.68 ± 2.24 % pre- and -3.58 ± 1.68 % post-supplementation. An even further decrease in plasma volume was observed after running in 35℃, - 2.52 ± 3.95 % pre- and -5.48 ± 1.78 % post-supplementation (n=5). This increase from pre- to post-supplementation testing did not reach a significant level.

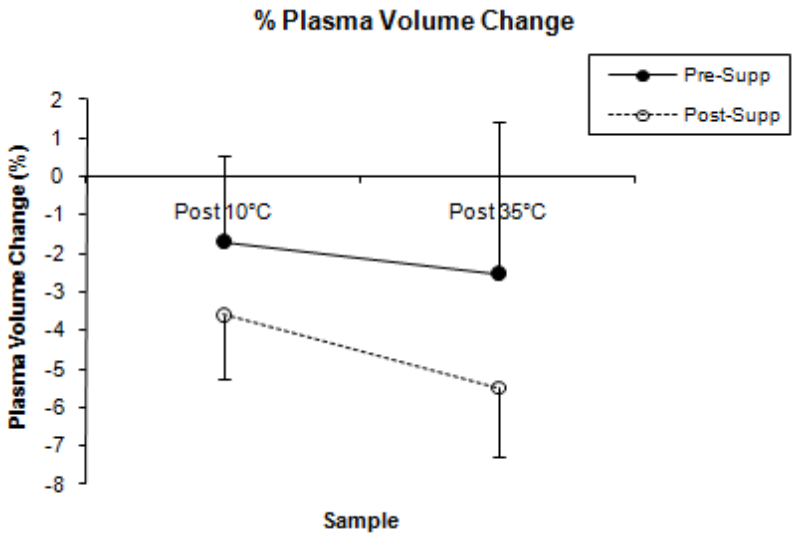


Figure 3.10 Plasma Volume Change during exercise before (black circles) and after (white circles) supplementation. Data presented as mean ± s.d.

### 3.9 Osmolality

The resting serum osmolality did not differ between pre- and post-treatment, with 285 ± 5 mosmol·kg$^{-1}$ and 288 ± 4 mosmol·kg$^{-1}$, respectively. Although the mean value for osmolality in the pre-trial was decreased after the 10℃ bout to 284 ± 4 mosmol·kg$^{-1}$, in the post-trial the serum osmolality increased to 290 ± 3 mosmol·kg$^{-1}$. Further, no difference was observed in the post 35℃ bout osmolality. Here, the value was 286 ± 4 mosmol·kg$^{-1}$ before the treatment with Cr/Gly and 290 ± 5 mosmol·kg$^{-1}$ afterwards. These values are also for n=6 for reasons stated under 3.8.

### 3.10 Side effects

No major side effects were observed during the supplementation regimen, although a small number of subjects (n=2) reported slight gastro-intestinal discomfort after taking the drinking supplement containing glycerol.

# 4. Discussion

The primary aims and objectives of this study were

(i) to evaluate the effect of an increase in BM on energy expenditure when no heat stress is asserted to the subject. This was achieved by collecting measurements for running economy while exercising in a cool (10℃) environment, hence enabling conclusion on energy cost of the exercise.

(ii) to evaluate the effects of hyperhydration induced by a Cr and Gly supplementation on cardiovascular and thermoregulatory parameters during physical exercise in the heat. This was achieved by repeating the trial in moderate conditions in a hot and humid environment.

(iii) to evaluate the new hyperhydrating protocol as amendments were made to current strategies in the literature. This was achieved by measuring TBW; ECW, ICW before and after the supplementation.

## 4.1 Effect of BM increase on Running Economy

The major aim of the present study was to determine the effects of hyperhydration on running economy during exercise. Coyle proposed that with a decrease in body mass (through dehydration) oxygen costs are lowered in marathon runners (Coyle, 2004). Hence, hyperhydration should theoretically increase running economy as with the increase in body mass the work load on the muscle is also increased and therefore oxygen demand is higher in the athlete. This in turn leads to impaired performance as an expected power output cannot be maintained for a prolonged period of time, i.e. an earlier onset of fatigue. We did not find increases in $VO_2$ over time during the trial at 10℃. This means that the intensity was chosen well and subjects were working steadily at the calculated individual exercise intensity in a cool environment. However, the oxygen uptake increased during the trial in the heat. This was an expected

effect whatsoever. While exercising in hot environmental conditions $T_c$ rises accordingly due to reasons extensively described in the introduction. It has been shown that with an increase in $T_c$, RE (and therefore $VO_2$) also increases (Brooks et al., 1971, Saltin and Stenberg, 1964, Macdougall et al., 1974). Nevertheless, we could not find a difference in $VO_2$ in the pre- and post-supplementation. Accordingly, no other changes in any of the respiratory variables could be observed in the pre- and post trial. The same results have been shown in several other studies where Cr has been the hyperhydrating agent (Kilduff et al., 2004, Volek et al., 2001) as well as for the constant-load trial in the Easton study where hyperhydration was induced by Cr/Gly (Easton et al., 2007). These results mean that an increase in body mass of about 1% (average of increase in BM in the present study) has no measurable effect on $VO_2$ and hence no increase in oxygen demand and consequently no decrease (theoretically) in exercise performance due to a higher body mass and therefore muscle work load. For this reason, it can be said that a hyperhydration has no negative effect on energy expenditure despite the increase in BM. This notion is also supported by the fact that no differences have been found in the accelerometer data. These, in conjunction with HR, have been demonstrated to be a good predictor of $VO_2$ (Fudge et al., 2007), and hence RE. This is a positive and very favourable outcome. One has to be cautious though and consider the limitations of the measurements of the methods we used. Many studies have been using the Douglas bag method and it is considered the gold standard method in the scientific community (Carter and Jeukendrup, 2002). But some articles have been published putting the accuracy of this method into question (Shephard, 1955). Diffusion through the bag has always been an issue, changing concentrations in the bag and leading to false measurements. Additionally, as Douglas bags are collected per hand, human error cannot be ruled out.

### 4.2 Cardiovascular, thermoregulatory and blood metabolite effects of hyperhydration

One factor from which runners could benefit from hyperhydration is the attenuation of the rise in HR. Many studies have shown an attenuation of the

HR increase mediated by hyperhydration (Anderson et al., 2001, Easton et al., 2007, Kilduff et al., 2004, Montner et al., 1996). As dehydration has been shown to increase HR and reduce SV and hence cardiac output (Gonzalez-Alonso et al., 1995), then if more water is available in the body through hyperhydration, the dehydration can be reduced and hence plasma volume can be maintained over a longer period of time during exercise in the heat. Hence, cardiac output can be maintained at a lower HR as SV is constant for a longer time as a lower fraction of TBW of fluid is lost through sweating (see cardiovascular model in introduction section). We did not observe this attenuation in HR. This is in line with the findings of other studies on hyperhydration (Murray et al., 1991, Latzka et al., 1998, Kern et al., 2001). Possibly, in the conditions of the present study, the induced hyperhydration is not was not large enough to alter any of the physiological responses by any means.

One parameter that needs to be discussed is the serum osmolality. No differences were found in this investigation in contrast to Easton et al. who found an increase in serum osmolality after Gly and Cr/Gly hyperhydration (Easton et al., 2007). Noticeably, this change did not occur in the Cr only group, suggesting that in our study the effects of Gly are lost due to the change in administration protocol and hence this difference cannot be observed. This is an indicator of the findings that are discussed in more detail in section 4.5.

During physical exercise heat is generated in the working skeletal muscle. The only way for the human body to effectively lose the produced heat is through the excretion and evaporation of water through the skin. This cools the blood in the subcutaneous vessels, i.e. the blood returning to the body core. Hence $T_c$ can be kept at a lower temperature for a longer period of time. As more water is stored in the intra- and extracellular compartments of the body after the supplementation, we would expect an increase in sweat rate as more water is readily available to increase evaporative heat loss. Hence, the associated thermoregulatory benefit, i.e. attenuation in the rise of $T_c$ should be observed. There has been a long debate in the literature about the reduction in $T_c$ during

exercise after hyperhydration. Cr has been shown repeatedly to effectively decrease $T_c$ (Kern et al., 2001, Kilduff et al., 2004, Easton et al., 2007), whereas for Gly there is a lively debate with some studies supporting a smaller increase in $T_c$ (Anderson et al., 2001, Lyons et al., 1990) or no change in sweat rate, and thus no change in the rise of $T_c$ (Latzka et al., 1998). In contrast to Easton the present study did not show any significant changes in sweat rate or sweat loss (Easton et al., 2007). As sweat rate and total sweat loss did not increase, it is not surprising that there was no attenuation in the increase in $T_c$ or any significant change in $\Delta T_c$. Sawka et al were able to show that in order to maintain blood volume during sweat loss, extra fluid is obtained in various proportions from intra- and extracellular compartments (Sawka et al., 2001). Nose et al. were even able to show a strong relation of fluid loss (from sweat/urine) during prolonged exercise and the reduction of fluid in intracellular water compartments during exercise in the heat (Nose et al., 1988). Possibly, as can be seen figure 3.4, a longer duration of the exercise would have shown a significant difference in $T_c$ as the increase in $T_c$ after the supplementation is slightly attenuated compared to before. We observed only a small increase in ECW after the supplementation. It might be due to the fact that most of the extra water is stored within the cells, and it takes some time for this to be released into the blood stream and lead to have a beneficial ergogenic effect. This remains to be investigated though. This idea is supported by the findings of Kilduff as the authors only got significant differences in $T_c$ and HR with Cr supplementation after 35 and 40 minutes of exercise (Kilduff et al., 2004). Easton et al. (2007) found significant differences after 25 min. In that study the workload was slightly higher than in the present study (63% $VO_{2max}$ cf. 60% $VO_{2max}$), suggesting that at a higher intensity with an associated quicker increase in $T_c$ than at a lower intensity beneficial effects can be seen earlier on during physical activity.

Although time points of measurement of glucose levels are not necessarily comparable, we cannot confirm the results of the progression of blood glucose levels as observed by Easton et al. (2007). Although one might conclude from the graph (see figure 3.9) that we also see an initial decrease in blood [Glu]

followed by an increase of the same, these changes are not significant by any means. This phenomenon might be due to the smaller number of subjects (n=6 cf. n=12). Possibly, we could observe the same progression if number of tested subjects increased.

### 4.3 Effect of hyperhydration on subjective measurements

The only significant difference found was in the subjective measurement of Thermal Comfort. Here, at 5 and 10 minutes time points of the run in 35℃, subjects found it more comfortable to run in the heat after the supplementation than before. This effect is lost after 15 minutes. This is not an acclimatization effect as there should be then a difference between the familiarization and pre-supplementation trial as well which is not the case (see figure 4.2). As this is the only significant change found during the present study it remains to be elucidated whether this is a real effect or whether subjects found the heat more bearable because they knew through the informed consent letter what was expected of them.

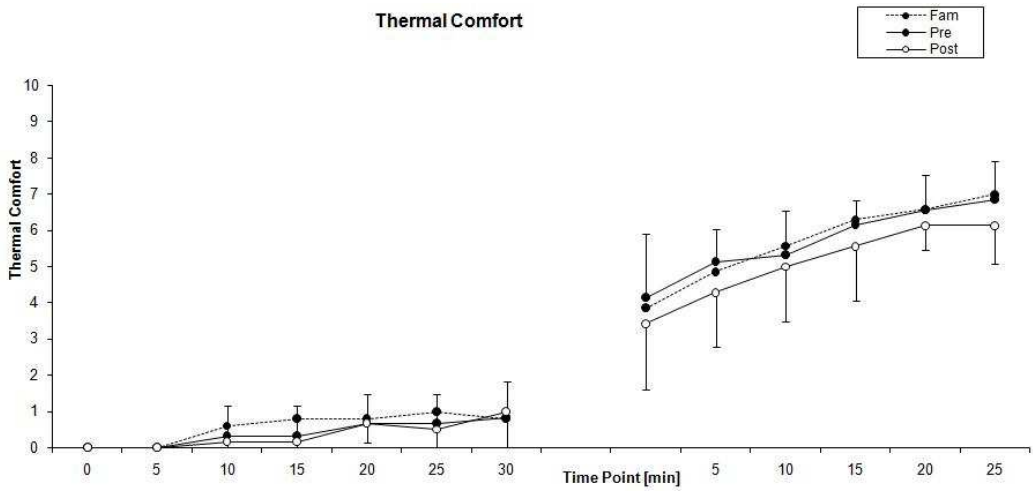


Figure 4.1 Mean Thermal Comfort during exercise before (black circles and solid line) and after (white circles and solid line) supplementation and in the familiarisation trial (black circles and dotted line). Data presented as mean ± s.d.

Easton et al. could demonstrate that after the supplementation RPE scores for leg fatigue are significantly decreased at some time points in the post-supplementation trial for both the Cr only as well as the Cr/Gly group (Easton et al., 2007). These results are in line with the findings of Kilduff et al. who found a reduction in leg RPE after Cr loading. On the other hand, both studies did not find significant differences in dyspnoe/breathing RPE (Easton et al., 2007, Kilduff et al., 2004). In the study conducted for this thesis, RPE for leg fatigue and breathing was not assessed separately. So, the result does not contradict the above mentioned findings, as the RPE scores if taken together as done in this study might come to the same result. Conversely, if RPE scores here had been collected disjointedly, a possible significant change might have been observed, just as it has been for the Thermal Comfort. This is speculation which remains to be investigated (by collecting the RPE scores for leg fatigue and dyspnoe separately) though.

### 4.4 Evaluation of the Hyperhydration Protocol

The previous study by Easton and colleagues (2007), has shown that a supplementation combination of creatine (22.8 g of $Cr{\cdot}H_20$ per day, equivalent to 10g of Cr) and glycerol (0.75 $mL{\cdot}kg^{-1}$ body weight per day) leads to a significant increase in TBW of 0.87 ± 0.21 L after 7 days of supplementation, where the increase in ECW was 0.46 ± 0.09 L and 0.42 ± 0.22 L for ICW. This demonstrated that a combined supplementation of the two hyperhydrating agents working in synergy leads to a significantly higher increase in TBW than each agent consumed alone. Easton et al. (Easton et al., 2007, Easton et al., 2009). therefore produced the highest hyperhydration reported to date (Kilduff et al., 2004, Montner et al., 1996). This study has demonstrated that the supplementation of Cr/Gly increased body weight in most, but not all subjects. With the amended protocol that excluded glucose and contained only one single dose of glycerol the average increase of TBW for the responders group was 0.64 ± 0.18 L which is almost a third less than previously reported by Easton et al. (2007) shown in figure 4.2.

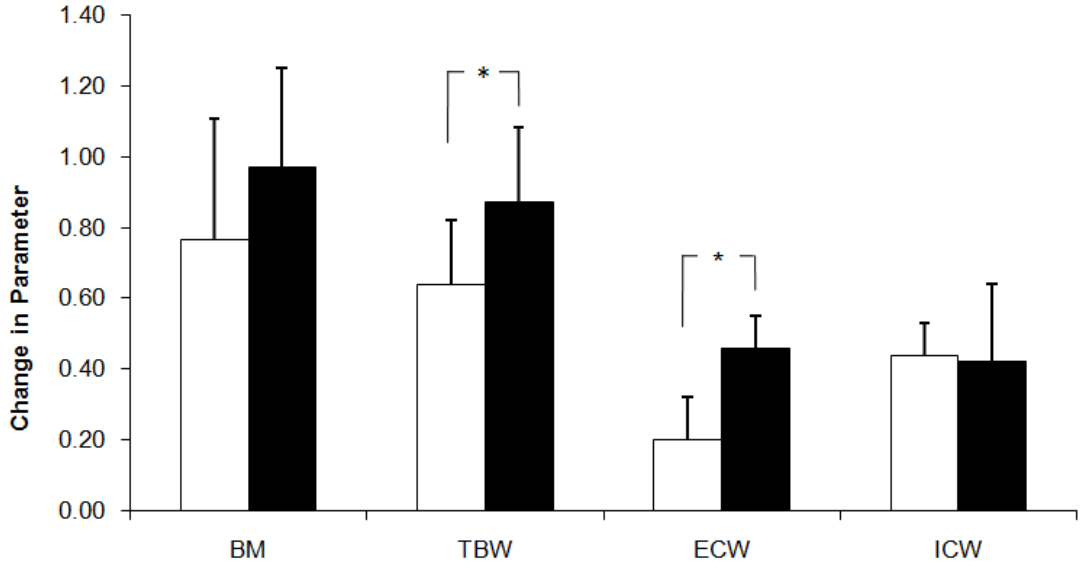


Figure 4.2 Changes in body mass (BM), total body water (TBW), extracellular water (ECW) and intracellular water (ICW) from the present (white columns) and Easton's study (black columns). *: indicates a significant difference ($p < 0.05$) in the increase between the two studies. Data presented as mean ± s.d.

The increase in intracellular water can be explained by an osmotic effect of creatine, creating a concentration gradient across the cell membrane that draws water into the cells, mainly skeletal muscle as it is the biggest organ of the human body (Kilduff et al., 2004, Kern et al., 2001, Ziegenfuss et al., 1998). It has been suggested in the literature that an increase in body mass of less than 0.2 kg is considered a non-responder of creatine supplementation (Kilduff et al., 2003). It was primarily aimed to investigate effects of hyperhydration on running economy and its thermoregulatory effects. Therefore, firstly, all subjects followed the supplementation, a control or placebo group was not included. The results from Easton et al. did not suggest any difficulties with the hyperhydration response as all subjects in the Cr/Gly group increased their BM by more than 0.2 kg (Easton et al., 2007). It cannot be ruled out that some of those subjects were non-responders as well, but given the results it can be concluded that the hyperhydration effect of glycerol has masked this phenomenon. Secondly, we left subjects with a weight gain of less than 0.2 kg out of consideration when looking at the changes in cardiovascular, thermoregulatory and respiratory

variables after supplementation. The exclusion criterion was chosen according to the literature (Kilduff et al., 2003). When comparing the data from only the responder group to that of the results from the Easton et al. study BM and ICW are not significantly different, This shows that within the group of responders the supplementation led to the same increase in ICW as previously demonstrated by Easton et al. (2007). We can conclude that the hydration with Cr worked for the responders.

But in contrast to this, the increase in TBW and ECW are significantly lower (about half) to that reported by Easton et al. (2007) with 0. 77 ± 0.34 L for TBW compared to 0.97 ± 0,28 L and 0.20 ± 0.12 L compared to 0.46 ± 0.09 L for ECW with $p = 0.04$ and $p = 2 \cdot 10^{-4}$, respectively (see Figure 4.2). As the increase in ECW has been associated with the Gly supplementation, it can be said that the new strategy for the administration of Gly has possibly not worked and needs to be revised. The problem that led to a consideration of change in the administration protocol from the Easton study was that many subjects reported side effects, especially gastrointestinal discomfort and diarrhoea (data not published). Many subjects had to drop out of the study which led to research on the mechanisms on fluid retention through glycerol in order to improve administration of the same. As published previously, there is small evidence that an increase in anti-diuretic hormone (ADH) concentration [ADH] caused by Gly ingestion is the cause of water retention and hence an increase in TBW (Freund et al., 1995). This was only approaching significance, hence not delivering strong evidence but nevertheless the report by Freud et al. cannot be neglected, especially as we cannot draw any conclusions as [ADH] was not measured here or in the Easton study (Easton et al., 2007). There is stronger evidence by Sommer and colleagues that the increase in TBW after Gly supplementation is mediated by the action of Gly on the kidneys (Sommer et al., 1993). When blood glycerol concentration [Gly] is at normal physiological levels, almost all filtered Gly is passively reabsorbed by the proximal and distal renal tubules of the kidneys. When blood Gly is increased with exogenous Gly ingestion, there is an increase in Gly and associated water retention (Easton et al., 2007). Unpublished data from our lab showed that once-daily Gly ingestion over a time period of 7 days led to an increase in blood [Gly] a few hours after ingestion which returned completely to basal levels after 24 hours (data not

shown). Hence, once the blood [Gly] levels return to normal physiological levels the beneficial effect of water retention is lost at the same time. Therefore the supplementation protocol was adjusted to giving Gly only once, a few hours before the post-supplementation test allowing enough time for blood [Gly] levels to rise so that water would be retained before the exercise. This would decrease the unwell being of the subjects significantly and reduce the drop-out rate. Furthermore, it decreases the cost of the supplements needed for an individual. Additionally, we used sugar-free juice in contrast to Easton et al. (2007). This was done in order to reduce the high dose of glucose contained in 150 mL of juice and hence lowering the insulin response to the glucose ingestion. This might also have implications on the uptake and effectiveness of the hyperhydrating agents Cr and Gly. Generally, exact mechanisms are unknown for the absorption of Cr and Gly. As described in more detail in the introduction, there is plenty of evidence to suggest that glucose plays an important role in the uptake of Cr via an insulin-related mechanism (Green et al., 1996, Steenge et al., 2000). The administration protocol has been changed to a non-glucose supplement in order to reduce insulin response. Hence, this study shows that there must be other ways for Cr uptake into the muscle which are not directly insulin related as we achieved the same increase in ICW as reported by Easton et al. who added glucose to their supplement (Easton et al., 2007). However, this study did not assess the subjects' diet. Hence, it cannot be ruled out that the subjects were consuming a high carbohydrate diet during the supplementation period and thus augmenting Cr uptake although no carbohydrates were given with the supplement. Less is known about on how glucose affects glycerol absorption. A study in rats showed that a combination of glycerol and glucose at a ratio of 3:1 enhances glycerol and water absorption in the intestines (Wapnir et al., 1996, Goulet, 2009). Hence, leaving out glucose in the supplementation drink might be one of the reasons why the hydration strategy has not worked as well as it did before in other experiments (Easton et al., 2007, Easton et al., 2009). This might very well be the case. It has been established that as early as 60 to 90 minutes after ingestion of glycerol hyperhydrating effects can be observed (Goulet, 2009). In this study, the glycerol containing supplement was consumed 300 minutes before the trial.

Hence, it might be worth considering reducing the time between the glycerol ingestion and start of exercise.

### 4.5 Future Perspective

The major focus of future investigations should be the administration protocol of the supplementation. Obviously, the changes made to the protocol did not lead to the desired thermoregulatory effects, suggesting that the induced hyperhydration was too small to benefit from. We conclude that this was due to an impairment of the hyperhydrating effect of glycerol as we could not observe the same increase in ECW as reported in Easton et al. (2007, 2009). Hence, future experiments should investigate the mechanisms which are underlying the hydration of glycerol but also of creatine. From this data, valuable information might be gained on the exact dose, duration, and time point of supplementation. Attempts to explain the effect have been made (Freund et al., 1995, Sommer et al., 1993) but the whole picture remains to be elucidated. Furthermore, the role of glucose in terms of Cr/Gly uptake has to be examined in more detail. This was another component changed in the supplementation regime and might explain further the smaller increases in TBW and ECW observed in this study as explained in more detail above. A close examination of different administration strategies considering time of the day, doses, length of supplementation, addition of glucose, and exact composition of the drink should be undertaken. Including measurements of Cr and Gly uptake and daily measurement of BM and TBW should enable investigators to find the most convenient and effective supplementation protocol leading to the desired thermoregulatory and cardiovascular responses improving exercise performance while not elevating energy expenditure of the athlete.

## 5. Conclusion

The present study showed that a supplementation with Cr and Gly had an increasing effect on TBW over a 7d Creatine and 1d glycerol period without affecting RE or any other cardiopulmonary and thermoregulatory parameters. The amendment in the administration protocol did lead to a smaller increase in TBW, and here especially ECW, than shown by Easton et al. (2007). Hence, the Gly supplementation protocol has to be revised to have the same effects as reported before. It seems essential to load with Cr/Gly in an appropriate manner in order to achieve the best results. One single load on the day of testing with Gly does not seem sufficient. These findings also show how crucial the timing of ingestion, i.e. time prior to exercise, before or after a meal, of the final supplement is. Furthermore, the contents of the supplement should be revised, especially in regards to re-introducing glucose. No conclusions can be drawn whatsoever on the effect of hyperhydration on exercise performance. No measures were taken that would enable exercise performance to be quantified in this study.

# 6. APPENDIX

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**UNIVERSITY OF GLASGOW**

**FACULTY OF BIOMEDICAL AND LIFE SCIENCES**

**ETHICS COMMITTEE FOR NON CLINICAL RESEARCH**

**INVOLVING HUMAN SUBJECTS, MATERIAL OR DATA**

# APPLICATION FORM FOR ETHICAL APPROVAL

**NOTES:**

**A submission to this Committee does not automatically result in approval. Investigators must wait for written approval before commencing data collection. Disciplinary measures will be taken if work commences without ethical approval being in place. The matter will be referred to the Dean for appropriate action.**

**THIS APPLICATION FORM SHOULD BE TYPED, NOT HAND WRITTEN.**

**ALL QUESTIONS MUST BE ANSWERED. "NOT APPLICABLE" IS A SATISFACTORY ANSWER WHERE APPROPRIATE.**

Project Title: **The effects of hyperhydration on running economy of endurance trained runners.**

Is this project from a commercial source? **No**

If yes, give details and ensure that this is stated on the Informed Consent form.

Date of submission: **October 2007**

Name of all person(s) submitting research proposal: **Dr Yannis Pitsiladis**

Position(s) held: **Reader in Exercise Physiology**

Division: **NABS**

Address for correspondence relating to this submission: **Lab 245, West Medical Building. Phone: 0141 330 3858, email: Y.Pitsiladis@bio.gla.ac.uk**

Name of Principal Researcher (if different from above e.g., Student's Supervisor): **N/A**

1. Describe the purposes of the research proposed.

The vast majority of the differences in endurance running performance in elite athletes are primarily accounted for by running economy with maximal aerobic capacity ($\dot{V}O_{2\,max}$) and $\%\dot{V}O_{2\,max}$ also having a significant impact (Bassett, Jr. & Howley, 2000;Conley & Krahenbuhl, 1980). Running economy is defined as the rate of oxygen utilization ($\dot{V}O_2$) required running at a submaximal given velocity and is expressed as the $\dot{V}O_2$ per unit mass per minute ($ml \cdot kg^{-1} \cdot min^{-1}$) (Bassett, Jr. & Howley, 2000). Those athletes who maintain a lower $\dot{V}O_2$ while running at a given velocity are said to have a better running economy. Therefore, if body mass can be reduced and power output maintained, athletes should theoretically experience an improvement in running economy. This physiological phenomenon may help explain why despite the reported negative impact of dehydration on thermoregulatory and cardiovascular parameters (Montain & Coyle, 1992), athletes consistently perform well during competition despite losing between 4 and 8% body mass (Coyle, 2004). Conversely, hyperhydrating prior to competing in weight bearing sports such as running via ingestion of osmotic agents such as glycerol or creatine (Easton *et al.*, 2007) may negatively impact performance due to the associated increase in body mass (Noakes, 2003).

However, the only published study to date to examine the effects of hydration status on running economy found dehydration of up to 5% body mass had no effect on running economy and resulted in a significant increase in physiological strain during running in 23$^{o}$C (Armstrong *et al.*, 2006). Nevertheless, it is currently unknown whether hyperhydrating, and thus increasing body mass would negatively impact on running economy. Therefore, the primary aim of the present study is to examine the effects of combined creatine and glycerol hyperhydration on running economy, cardiovascular and metabolic responses to running exercise in endurance trained subjects. A secondary aim of this study is to determine the accuracy of a breath-by-breath portable gas analyser, K4, COSMED s.r.l., Rome, Italy) for measurement of gas exchange variables and running economy in the field.

2. Please give a summary of the design and methodology of the project. Please also include in this section details of the proposed sample size, giving indications of the calculations used to determine the required sample size, including any assumptions you may have made. (If in doubt, please obtain statistical advice).

**Methods/Design of investigation**

We propose to study 12 endurance-trained subjects (mostly runners aged 17-40 yrs). Subjects will be in good health at the time of testing and regularly take part in strenuous exercise. Eligibility will be assessed by subjects undergoing a medical examination (as previously approved by the University Ethics Committee). Subjects will also be required to read and sign the enclosed information sheet and a high intensity consent form.

Testing will take place in the laboratory of human physiology in the West Medical Building. A series of assessments will be carried out. These will include (see protocols): body composition using standard anthropometric methods; extra-cellular water and total body water measurement using multifrequency biolectrical impedance and five exercise tests. During the first laboratory visit, a health questionnaire and physical examination will be completed, the study and equipment will be explained to the subjects, and the subjects $\dot{V}O_{2\,max}$ will be measured. The subsequent 4 tests will involve 3 visits to the laboratory and 1 to the running track.

Following measurement of $\dot{V}O_{2\,max}$ and two baseline exercise trials, subjects will be ingest creatine and glycerol for a period of 7 days. Each supplement will consist of 11.4 g of Cr $H_2O$ (equivalent to 10 g Cr) and 1 g/kg BM glycerol made up in 1 litre of warm to hot water (x 2 times daily) and flavoured with sugar free fruit juice. Subjects will ingest the final creatine/glycerol drink 5 hrs before performing a post-supplementation exercise trial and a further 500 ml water 1 hour before the test. Subjects will be instructed to carry out a weighed intake of food and an activity diary in the 24 hours preceding each test.

**Protocols**

*Tests 1 and 2: Discontinuous Incremental Maximal Speed Test:* will take place both in the laboratory and in the field. Subjects will be instructed to run at 10, 11, 12, 13, 14, 15, 16, 17 and 18 $km \cdot hr^{-1}$ (or until volitional exhaustion) for 3 minutes at each speed, with a 3-5 minute rest interval between each bout walking at 4 $km \cdot hr^{-1}$. The laboratory test will be performed on a motorised treadmill, during which, the gradient shall remain at 1% throughout. Whereas the field test will take place on a 400m running track; an investigator will cycle alongside the subject to control the speed using a GPS system (GPSports SPI 10, GPSSports, Fyshwick ACT, Australia).
*Test 3, 4 and 5: Running Economy Tests:* will take place in the laboratory on a motorised treadmill. Subjects will be required to run at a constant pace of 60% $\dot{V}O_{2\,max}$ for 30 min.

Subjects will be given a warm-up before, and a warm-down after all four tests. The subject will wear a triaxial accelerometer (3dNX, Biotel, Bristol, U.K.) to record the activity counts per minute. Heart rate (Suunto t6, Suunto Oy, Vantaa, Finland) and gas exchange variables (Douglas bag collection) will be measured throughout exercise. Each subject will be required to swallow a CorTemp Ingestible Temperature Sensor the evening prior to each test. The pill is a small electronic device, which measures core temperature and transmits it through a radio wave signal to an external receiver (Rav-Acha et al, 2003). Expired gas values obtained from Douglas bag analysis in experiments 1 and 2 will be compared to data previously collected using a K4 metabolic analyser using similar experimental protocols. In exercise tests 3, 4 and 5 a venous cannula will be inserted and a 10ml blood sample taken pre- and post exercise.

*Biolectrical impedance:* Extra-cellular water and total body water will be prior to exercise tests 3, 4 and 5 using multifrequency biolectrical impedance (Bodystat Multiscan 500). This non-invasive method involves placing two current-inducing electrodes and two detector electrodes on the dorsal surfaces of the right hand and foot and a small (and imperceptible) electrical current (500 Micro-Amps) introduced between these.

3. Describe the research procedures as they affect the research subject and any other parties involved.

All experiments will take place in the Environmental Chamber in the West Medical Building. Dr Yannis Pitsiladis or a qualified (CPR-trained) and experienced colleague will be present at all tests. Dr Pitsiladis is a certified phlebotomist and trained in CPR and Advanced Life Support.

Some subjects may experience mild discomfort during the placement of and/or sampling of blood from a catheter placed in a vein on the dorsum of the heated hand. In our experience, this is minimal because: the catheter size is small (20 G); it is only placed when the hand has been heated to 44° C for at least 10 min, allowing a substantial local vasodilatation of the superficial blood vessels, which facilitates their cannulation; the catheter is indwelling, allowing for multiple sampling; while it is safely secured in place with adhesive tape, there is sufficient 'play' to allow sampling without 'pulling' on the vessel (i.e. the catheter can slide easily within the vessel); upon withdrawal of the catheter at the end of the experiment, firm pressure is maintained over the site to prevent any leakage from the vessel into the surrounding interstitium which could lead to local oedema and bruising. Importantly, if a vessel cannot be readily cannulated or if the subject is nonetheless not comfortable with proceeding, the experiment is halted. No more than 30 ml of blood will be sampled for each test.

Potential participants will be identified either by personal contact or by advertisement. They will be asked to meet with the investigators to discuss the project and whether they would be suitable as a subject. All subjects will be endurance trained individuals without a history of any significant medical problem(s). The good health of each subject will be established prior to the study by subjects undergoing a medical examination by a qualified medical practitioner (as previously approved by the University Ethics Committee), which is supported by written assurance from the subject in the form of a detailed medical questionnaire. Subjects with a history of cardiorespiratory or neurological disease will be excluded from participation, as will those having an acute upper respiratory tract infection. Subjects who take drugs (recreational or performance enhancing drugs) or who have consumed alcohol within 48 h of an experiment will be excluded.

*Exercise testing:* The risks associated with performing maximal exercise are minimal as long as the subject is appropriately instructed and familiarised with the device prior to participation and also is appropriately supervised during the experiment. All exercise bouts are both preceded by a 5 min "warm-up" and by a 5 min "warm-down". The latter is of particular importance during high-intensity exercise, when the local accumulation of exercise metabolites can cause an "expansion" (or vasodilatation) of the blood vessels in the lower limbs, which can impair the adequate return of blood to the heart – predisposing to fainting on dismounting from the ergometer. This risk is minimised by having the subject exercise at a mild level during recovery to "wash away" these metabolites and therefore to restore the capacity of the involved blood vessels to their resting levels.

Some subjects experience difficulty swallowing while breathing through a mouthpiece and wearing a noseclip, due to some transient build-up of pressure in the ears.

4. What in your opinion are the ethical considerations involved in this proposal? (You may wish for example to comment on issues to do with consent, confidentiality, risk to subjects, etc.)

All procedures described in this application have previously been approved by the University Ethics Committee and carried out without incident (i.e. three studies).

The subjects will complete a medical questionnaire and provide their written consent with the option to withdraw from participation at any point.

The insertion of a catheter into a vein may rarely cause irritation at the site of insertion, venospasm (or constriction of the cannulated vein which may lead to interference with blood flow through it) and phlebitis. These risks are minimized in this investigation by the short duration of the test and by the procedures described above.

Blood will be handled, stored and disposed of according to standard health and safety procedures.

Possible side-effects from the use of similar glycerol hyperhydration strategies include slight nausea, gastrointestinal distress and headaches. These problems have been reported among some subjects in the many published studies to date.

The only known 'side effect' of oral Cr supplementation that has been reported is a temporary increase in body weight.

5. Outline the reasons which lead you to be satisfied that the possible benefits to be gained from the project justify any risks or discomforts involved.

It is envisaged that this research will identify whether an increase in body mass induced by hyperhydration will negatively affect running economy. The minimal risk and discomfort associated with the above procedures are considered to be worthwhile to gain the information required.

6. Who are the investigators (including assistants) who will conduct the research and what are their qualifications and experience?

Dr Yannis Pitsiladis PhD MMedSci BA, Dr Chris Easton BSc PhD, Mr John Wilson, (Senior Technician), and BSc Honours Project and MRes Students. The principal investigators have wide ranging experience of physiological testing over periods of up to 10 years without incident. The principal researchers have carried out Cr/Gly supplementation studies and exercise to exhaustion studies in relatively extreme environmental conditions in the past.

7. Are arrangements for the provision of clinical facilities to handle emergencies necessary? If so, briefly describe the arrangements made.

In the event of an emergency, guidelines recently approved by the ethics committee will be followed.

In the event of an untoward incident that is not an emergency, the supervising Principal Investigator will administer appropriate first aid, if necessary. The subject will not be permitted to leave the laboratory until he has fully recovered. The subject will be encouraged to contact his local GP. The subject will be told that one of the Principal Investigators will conduct a follow-up by telephone at the end of the same day. The subject will also be provided with 24-hour contact numbers for both Principal Investigators.

8. In cases where subjects will be identified from information held by another party (for example, a doctor or hospital) describe the arrangements you intend to make to gain access to this information including, where appropriate, which Multi Centre Research Ethics Committee or Local Research Ethics Committee will be applied to.

N/A

9. Specify whether subjects will include students or others in a dependent relationship.

Some students may be recruited but will be under no pressure from staff to participate in the study. Steps will be taken to avoid the recruitment of students in a dependent relationship with the academic involved in the study.

10. Specify whether the research will include children or people with mental illness, disability or handicap. If so, please explain the necessity of involving these individuals as research subjects.

N/A

11. Will payment or any other incentive, such as a gift or free services, be made to any research subject? If so, please specify and state the level of payment to be made and/or the source of the funds/gift/free service to be used. Please explain the justification for offering payment or other incentive.

No

12. Please give details of how consent is to be obtained. A copy of the proposed consent form, along with a separate information sheet, written in simple, non-technical language MUST ACCOMPANY THIS PROPOSAL FORM.

Each subject will be provided with a consent form outlining the testing procedures, which asks them for their written consent to participate in the project with the option to withdraw at any time (see enclosed copy). A verbal explanation will also be given and any queries answered. If there is some doubt of the subject's eligibility for the study, the subject will be excluded. Information on creatine and glycerol supplementation will be given in the Information Sheet.

13. Comment on any cultural, social or gender-based characteristics of the subject which have affected the design of the project or which may affect its conduct.

All subjects are male. This constraint is imposed for standardisation purposes.

14. Please state who will have access to the data and what measures which will be adopted to maintain the confidentiality of the research subject and to comply with data protection requirements e.g. will the data be anonymised?

The information obtained will be anonymised and individual information will not be passed on to anyone outside the study group. The results of the tests will not be used for selection purposes.

15. Will the intended group of research subjects, to your knowledge, be involved in other research? If so, please justify.

No

16. Date on which the project will begin **November 2007** and end **May 2008**

17. Please state location(s) where the project will be carried out.

Laboratory of Human Physiology, West Medical Building.

Outdoor track (Scotstoun stadium and Bellahouston Park).

18. Please state briefly any precautions being taken to protect the health and safety of researchers and others associated with the project (as distinct from the research subjects) e.g. where blood samples are being taken.

All experiments will be conducted according to the code of practice for conducting experiments in non-patient human volunteers (including handling and disposal of human blood, urine and sputum) previously accepted by the University Ethics Committee.

Reference List

Armstrong, L. E., Whittlesey, M. J., Casa, D. J., Elliott, T. A., Kavouras, S. A., Keith, N. R., & Maresh, C. M. (2006). No effect of 5% hypohydration on running economy of competitive runners at 23 degrees C. *Medicine and Science in Sports and Exercise* **38**, 1762-1769.

Bassett, D. R., Jr. & Howley, E. T. (2000). Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med.Sci.Sports Exerc.* **32**, 70-84.

Conley, D. L. & Krahenbuhl, G. S. (1980). Running economy and distance running performance of highly trained athletes. *Med.Sci.Sports Exerc.* **12**, 357-360.

Coyle, E. F. (2004). Fluid and fuel intake during exercise. *Journal of Sports Sciences* **22**, 39-55.

Easton, C., Turner, S., & Pitsiladis, Y. P. (2007). Creatine and glycerol hyperhydration in trained subjects before exercise in the heat. *International Journal of Sport Nutrition and Exercise Metabolism* **17**, 70-91.

Montain, S. J. & Coyle, E. F. (1992). Influence of Graded Dehydration on Hyperthermia and Cardiovascular Drift During Exercise. *Journal of Applied Physiology* **73**, 1340-1350.

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# University of Glasgow

# Faculty of Biomedical & Life Sciences

## INFORMATION SHEET

**Study Title:** The effects of hyperhydration on running economy of endurance trained runners.

You are being invited to take part in a research study. Before you decide whether to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

**What is the purpose of the study?** We wish to find out whether taking certain substances (previously used by athletes) which increase the volume of water in your body may increase the amount of energy you use when running. We will measure your body water, blood volume and weight before and after exercise and your heart rate and expired gas while running. The substances you will be required to ingest orally are **creatine** (a food element found in high abundance in meat and fish but also made by the body), **glycerol** (another substance found naturally in the body, which contributes to making energy for exercise). Creatine in its pure form is commercially available over the counter from most health shops. This supplement is popular amongst athletes as it is thought to improve high intensity exercise performance especially when repeated bouts of exercise are required (e.g. football, rugby).

**Why have I been chosen?** You have been selected as a possible participant in this investigation because you regularly take part in endurance activity and you are in good health. Twelve volunteers are being sought.

**Do I have to take part?** It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form and fill in a lifestyle questionnaire. If you decide to take part you are still free to withdraw at any time and without giving reason.

**What will happen to me if I take part?** You will be asked to visit the laboratory on four occasions and a running track once, where a series of assessments will be carried out. All experimental trials should last no longer than 1 hour. On your initial visit to the

laboratory you will be medically examined by a qualified doctor. The first test will take place in the laboratory on a motorised treadmill at a fixed 1% gradient. During this test you will be instructed to run at 10, 11, 12, 13, 14, 15, 16, 17 and 18 $km \cdot hr^{-1}$ (or until volitional exhaustion) for 3 minutes at each speed, with a 3-5 minute rest interval between each bout walking at 4 $km \cdot hr^{-1}$. The second test is exactly the same, except it is conducted outdoors on a 400m running track; an investigator will cycle next to you to monitor and control the running speed. The final three tests will take pace in the laboratory on a motorised treadmill; you will be required to run at a constant pace equating to 60% of your maximal capacity measured in the first test, for 30 minutes. After the first 4 tests you will consume 2 litres of experimental drink per day for 6 days and 1 litre 5 hours before your final test. The drinks will contain 10 g of creatine and glycerol (0.75 g per kg of body mass) dissolved in one litre of warm to hot water and flavoured with sugar free fruit juice.

Heart rate and expired gas will be recorded throughout all tests via a heart rate monitor and a flexible rubber mouthpiece, respectively. You will also wear an accelerometer whilst running. In addition, you will be required to swallow a harmless recording device (a small pill like object) the evening prior to your test. This will enable the research team to monitor body temperature; there is no risk involved in swallowing the pill. All tests must be conducted at approximately the same time of day, and you will be required to wear the same athletic clothing/footwear for each test. During the final three tests we would like to take a small amount of blood from an intravenous line in the back of your hand. Your height, weight and percentage body fat will also be measured on each visit to the lab. Your percentage body fat will be estimated by a bioelectrical impedance technique, which involves placing slightly adhesive small patches ("electrodes") on your right hand and foot and introducing a very small and imperceptible electrical current between these.

In order to estimate your nutritional intake and energy expenditure, we may ask you to record your normal food and drink intake for 24 hour prior to each test and to keep a diary of your physical activity.

Finally, you will not be able to perform any exercise 24 hours prior to testing or consume any alcohol 48 hours prior to each lab visit. You will be excluded from participating in this study if you take drugs (recreational or performance enhancing drugs).

**What are the side effects of taking part?** Some subjects have previously reported slight nausea, gastrointestinal distress (i.e. diarrhoea) and headaches. The only known 'side effect' of oral creatine supplementation that has been reported is a temporary increase in body weight. This increase in body weight is mostly due to an increase in water retention, not fat.

**What are the possible disadvantages and risks of taking part?** Exercise has a negligible risk in healthy adults, although maximal exercise has a small risk of myocardial infarction ("heart attack"). The primary symptom of myocardial infarction is chest pain on exertion. If you experience any unusual sensations in your chest during the experiment, you should cease exercising immediately. Intravenous lines through which blood is collected, may cause some bruising and subsequent soreness over the

site of puncture and, rarely, a small wound (1-2 mm at most), which takes a few days to heal.

You will breathe through a rubber mouthpiece during the tests, in order for us to collect the air you breathe out. This is similar to the equipment used for snorkelling. You will also wear a noseclip. You may experience difficulty swallowing while breathing through a mouthpiece and wearing a noseclip, due to some pressure in the ears. In addition some subjects experience increased salvation when breathing through a mouthpiece.

**What are the possible benefits of taking part?** We hope to find out more about how your body responds to physical exercise after hyperhydration. This information will help us to decide whether hyperhydration should be recommended for those participating in running training or competition in a hot environment.

**What if something goes wrong?** If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. The principal investigators, although not medically qualified are fully trained in Advanced Life Support. In the event of an untoward incident, the principal investigator(s) will provide basic life support including chest compressions and ventilation until emergency medical staff are on hand. You may want to consult your GP if you are experiencing any side effects from taking part in the study and should also inform the principal investigator.

**Will my taking part in this study be kept confidential?** All information about you that is collected during the course of the research will be kept strictly confidential.

**What will happen to the results of the research study?** Results will be published in a peer-reviewed scientific journal once the study is completed. You will automatically be sent a copy of the full publication. You will not be identified in any publication.

If you wish to find out more about this investigation, you can contact:

Dr Yannis P. Pitsiladis
Institute of Biomedical & Life Sciences
West Medical Building
University of Glasgow
Glasgow, G12 8QQ

Phone: 0141-330-3858
Fax: 0141-330-2915
E-mail: Y.Pitsiladis@bio.gla.ac.uk

**CONSENT**

**Title of Investigation:** The effects of hyperhydration on running economy of endurance trained runners.

**I ……..**

**Give my consent to the research procedures which are outlined above, the aim, procedures and possible consequences of which have been outlined to me.**

**Signature** **…….....**

**Date** **…….....**

**UNIVERSITY OF GLASGOW**

**INSTITUTE OF BIOMEDICAL AND LIFE SCIENCES**

**SUBJECT'S QUESTIONNAIRE AND ASSENT FORM FOR**

# HIGH-INTENSITY EXERCISE TESTING

**If you feel unwell on the day of a proposed test, or have been feeling poorly over the preceding day or two, DO NOT TAKE PART in a high-intensity exercise test.**

The considerations which follow apply to people who are feeling well at the time.

**NAME _____**

Sex (M/F) _____ Age _____ (yrs) Height _____(m) Weight _____ (kg)

## Exercise Lifestyle

a) What kind(s) of exercise do you regularly do (20+ min/session)? (*Please circle*)

| | Number of times per average week | | | | |
|---|---|---|---|---|---|
| Walking | 1 | 2 | 3 | 4 | 5 |
| Running | 1 | 2 | 3 | 4 | 5 |
| Cycling | 1 | 2 | 3 | 4 | 5 |
| Swimming | 1 | 2 | 3 | 4 | 5 |
| Skiing | 1 | 2 | 3 | 4 | 5 |
| Rowing | 1 | 2 | 3 | 4 | 5 |
| Gymnastics | 1 | 2 | 3 | 4 | 5 |
| Martial arts | 1 | 2 | 3 | 4 | 5 |
| Tune Up | 1 | 2 | 3 | 4 | 5 |
| Popmobility | 1 | 2 | 3 | 4 | 5 |
| Sweat Session | 1 | 2 | 3 | 4 | 5 |
| Weight training | 1 | 2 | 3 | 4 | 5 |
| Field athletics | 1 | 2 | 3 | 4 | 5 |
| Racquet sports | 1 | 2 | 3 | 4 | 5 |
| Rugby/soccer/hockey | 1 | 2 | 3 | 4 | 5 |
| Other(s) * | 1 | 2 | 3 | 4 | 5 |

* (*Please specify)* _____

b) How long have you been exercising at least twice/week for at least 20 min/session

***Continued Over***

**Smoking**

(*Please tick one)*

| | |
|---|---|
| Never smoked | _____ |
| Not for > 6 months | _____ |
| Smoke <10 per day | _____ |
| Smoke > 10 per day | _____ |

**Illnesses**

Have you ever had …? (*Please circle Yes or No*)

| | | |
|---|---|---|
| Asthma | YES | NO |
| Diabetes | YES | NO |
| Epilepsy | YES | NO |
| Heart Disease | YES | NO |
| High Blood Pressure | YES | NO |

Any other illness that could affect your safety in performing maximal exercise

YES NO

*(If YES, please specify)* _____

**Symptoms**

Have you ever had any of the following symptoms to a significant degree?

i.e. have you had to consult a physician relating to any of the following?

(*Please circle Yes or No*)

| | | |
|---|---|---|
| Breathlessness | YES | NO |
| Chest Pain | YES | NO |
| Dizzy fits / Fainting | YES | NO |
| Heart Murmurs | YES | NO |
| Palpitations | YES | NO |

**Muscle or joint injury**
***Do you have / or have had any muscle or joint injury which could affect your safety in performing maximal exercise or strength testing or strength training?***

YES NO

**Medication**
Are you currently taking any medication? YES NO

(*Please circle Yes or No*)

(*If Yes, please specify*) _____

**Signature** _____

**Date** _____

Signed _____ Date _____

(Proposer of research)

Where the proposal is from a student, the Supervisor is asked to certify the accuracy of the above account.

Signed _____ Date _____

(Supervisor of student)

Email the completed form to: S.Morrison@bio.gla.ac.uk

And send the signed hard copy to:

Stuart Morrison

Faculty Research Office

Faculty of Biomedical & Life Sciences

West Medical Building

University of Glasgow

Gilmorehill

Glasgow

G12 8QQ

**Scale Thermal Comfort**

| | |
|---|---|
| 0 | Comfortable |
| 1 | |
| 2 | warm |
| 3 | |
| 4 | very warm |
| 5 | |
| 6 | hot |
| 7 | |
| 8 | very hot |
| 9 | |
| 10 | extremely hot |

## Accelerometer Data 10℃ trial

### 10℃

**x**

| | | | Stdev | |
|---|---|---|---|---|
| **time [min]** | **pre** | **post** | **pre** | **post** |
| 5 | 67.16300366 | 69.31868132 | 18.97420985 | 20.76112988 |
| 10 | 71.89010989 | 71.82417582 | 19.10527552 | 20.71139689 |
| 15 | 72.14652015 | 72.8021978 | 18.95542318 | 18.93713555 |
| 20 | 73.45054945 | 75.18589744 | 19.01372019 | 18.37913299 |
| 25 | 73.2967033 | 75.58241758 | 18.25933219 | 18.70843755 |
| 30 | 77.08791209 | 77.56043956 | 17.73292042 | 19.61371105 |

**y**

| | | | Stdev | |
|---|---|---|---|---|
| **time [min]** | **pre** | **post** | **pre** | **post** |
| 5 | 116.4551282 | 114.967033 | 22.33139674 | 20.73191601 |
| 10 | 120.7692308 | 116.010989 | 23.00021439 | 23.00021439 |
| 15 | 121.2032967 | 117.4505495 | 25.67657197 | 25.67657197 |
| 20 | 122.3296703 | 120.1245421 | 25.89813585 | 25.89813585 |
| 25 | 124.8351648 | 120.5824176 | 25.38680754 | 25.38680754 |
| 30 | 124.2417582 | 120.967033 | 25.28540943 | 25.28540943 |

**z**

| | | | Stdev | |
|---|---|---|---|---|
| **time [min]** | **pre** | **post** | **pre** | **post** |
| 5 | 333.6144689 | 346.7802198 | 46.0905817 | 38.56149648 |
| 10 | 331.2637363 | 347.967033 | 52.6419909 | 41.92540236 |
| 15 | 337.3928571 | 341.6703297 | 41.91530723 | 45.01754456 |
| 20 | 338.7142857 | 341.496337 | 40.27667417 | 43.65308289 |
| 25 | 338.5164835 | 337.2087912 | 38.26038976 | 47.44792046 |
| 30 | 336.5934066 | 340 | 41.32255652 | 42.96062598 |

**dual axis**

| | | | Stdev | |
|---|---|---|---|---|
| **time [min]** | **pre** | **post** | **pre** | **post** |
| 5 | 183.6181319 | 192.8901099 | 39.9907232 | 41.88993885 |
| 10 | 192.3956044 | 197.4505495 | 40.57139781 | 45.80309948 |
| 15 | 192.2289377 | 200.2857143 | 43.47984665 | 45.63169844 |
| 20 | 193.6593407 | 206.1565934 | 43.82599305 | 48.38968578 |
| 25 | 195.5714286 | 206.978022 | 42.86062585 | 49.94244644 |
| 30 | 198.7472527 | 209.6373626 | 41.80099171 | 49.66391414 |

**tri-axial**

| | | | Stdev | |
|---|---|---|---|---|
| **time [min]** | **pre** | **post** | **pre** | **post** |
| 5 | 525.8369963 | 531.0659341 | 87.69112082 | 76.02857384 |
| 10 | 530.967033 | 535.8021978 | 91.60692179 | 82.23904227 |
| 15 | 538.0943223 | 531.9230769 | 83.53785936 | 85.05515092 |
| 20 | 541.4945055 | 536.8067766 | 82.25714788 | 86.11230687 |
| 25 | 544.2197802 | 533.3736264 | 79.66520711 | 89.89465047 |
| 30 | 545.1208791 | 538.5274725 | 81.13345056 | 86.29735562 |

## Accelerometer Data 35℃ trial

35℃

x

| | | | Stdev | |
|---|---|---|---|---|
| time [min] | pre | post | pre | post |
| 5 | 68,63736264 | 73,1978022 | 16,08984356 | 19,2489072 |
| 10 | 73,52747253 | 74,92307692 | 15,6920204 | 21,18543097 |
| 15 | 77,23076923 | 75,61538462 | 15,92351046 | 20,89930777 |
| 20 | 78,64835165 | 77,01098901 | 14,70551542 | 21,91355747 |
| 25 | 79,74908425 | 78,9010989 | 15,5568103 | 22,25873116 |
| 30 | 80,3489011 | 79,07692308 | 15,90551725 | 21,02890844 |

y

| time [min] | Stdev | | | |
|---|---|---|---|---|
| | pre | post | pre | post |
| 5 | 127,6043956 | 123,7252747 | 26,40327622 | 22,00138319 |
| 10 | 131,2197802 | 128,7362637 | 27,51683826 | 22,94388391 |
| 15 | 136,2197802 | 131,0659341 | 29,04310299 | 25,38129071 |
| 20 | 140,7032967 | 133,1428571 | 29,82683186 | 27,97940312 |
| 25 | 143,0137363 | 135,9450549 | 32,89367605 | 28,64702945 |
| 30 | 142,7600733 | 134,4395604 | 33,14118804 | 26,67836458 |

z

| time [min] | Stdev | | | |
|---|---|---|---|---|
| | pre | post | pre | post |
| 5 | 346,8791209 | 342,2637363 | 37,54738905 | 41,63947493 |
| 10 | 346,8461538 | 344,8021978 | 42,04555152 | 41,13265347 |
| 15 | 347,8901099 | 344,9450549 | 44,08913459 | 40,66939189 |
| 20 | 350,4945055 | 347,1538462 | 41,62547527 | 38,62391366 |
| 25 | 349,246337 | 348,010989 | 43,00827543 | 38,05610487 |
| 30 | 346,7225275 | 349,2747253 | 40,19289192 | 37,41664917 |

dual axis

| time [min] | Stdev | | | |
|---|---|---|---|---|
| | pre | post | pre | post |
| 5 | 185,4285714 | 194,0989033 | 37,41464598 | 38,46101595 |
| 10 | 192,4065934 | 199,7582385 | 39,21426788 | 43,85390419 |
| 15 | 200,2637363 | 202,7472462 | 42,95545736 | 45,7803923 |
| 20 | 205,8461539 | 206,5164879 | 42,47361403 | 47,84381338 |
| 25 | 208,6858974 | 210,4835198 | 46,77031802 | 49,29498058 |
| 30 | 208,5265568 | 208,6483549 | 48,56018574 | 46,86355381 |

tri-axial

| | | | Stdev | |
|---|---|---|---|---|
| time [min] | pre | post | pre | post |
| 5 | 551,2197802 | 539,1868132 | 66,53267247 | 70,79880732 |
| 10 | 559,4615385 | 548,4615385 | 68,79294606 | 72,75062103 |
| 15 | 568,7582418 | 551,6263736 | 70,96738463 | 73,80901081 |
| 20 | 578,2527473 | 557,3076923 | 69,55896161 | 74,47944265 |
| 25 | 580,3058608 | 562,8571429 | 76,1089764 | 73,44689649 |
| 30 | 577,7106227 | 562,7912088 | 72,86497157 | 68,03767831 |

# Declaration of Authorship

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Name: Lena Willkomm

Signature: Date: 06/01/2009