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THE EFFECTS OF COMBINED CREATINE AND
GLYCEROL HYPERHYDRATION ON
THERMOREGULATION, METABOLISM AND
EXERCISE PERFORMANCE IN THE HEAT IN
ENDURANCE TRAINED HUMANS

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Submitted for the degree of Doctor of Philosophy (PhD) in the
Faculty of Biomedical and Life Sciences, University of Glasgow.

June, 2007

“Quia quamdiu Centum ex nobis viui remanserint, nuncquam Anglorum dominio aliquatenus volumus subiugari. Non enim propter gloriam, diuicias aut honores pugnamus set propter libertatem solummodo quam Nemo bonus nisi simul cum vita amittit.”

Declaration of Arbroath 1320

Alba gu brath

Bas agus buaidh

Author's Declaration

I hereby declare that this thesis has been composed by myself, and that the work of which it is a record has been done by myself, except where specifically acknowledged. I also confirm that it has not been submitted in any previous application for a higher degree and that all sources of information have been specifically acknowledged by means of references.

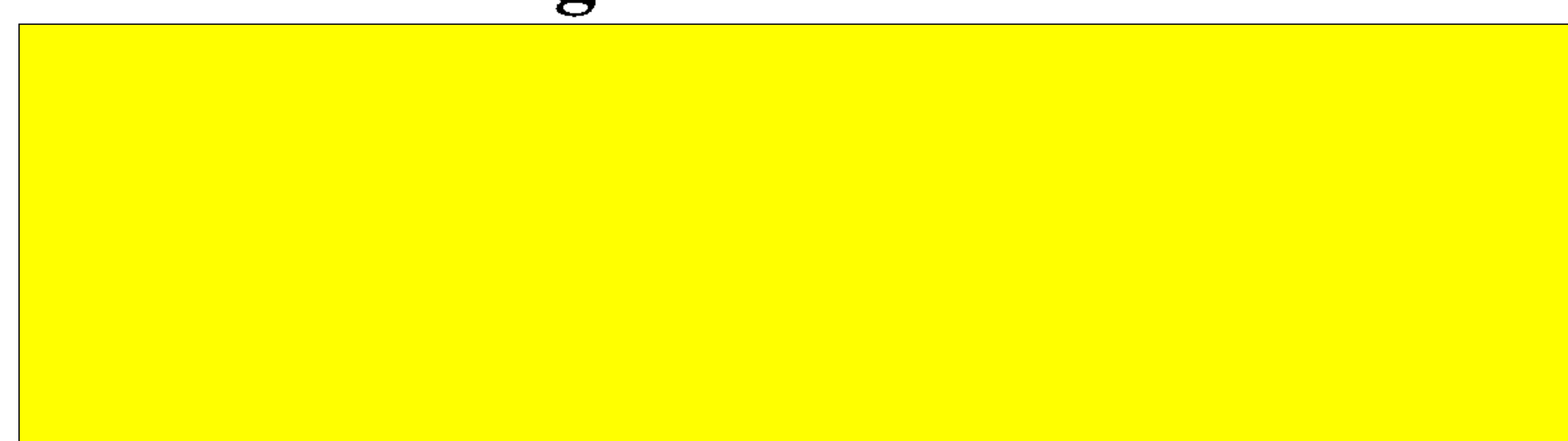
Some of the results contained in this thesis have been published in peer-reviewed journals as follows:

- i. **Easton C.**, Turner, S., and Pitsiladis Y.P. (2007) Creatine and glycerol hyperhydration in trained subjects prior to exercise in the heat. *Int. J. Sports Nutr. Ex. Metabol.* **17**, 70-91.
- ii. **Easton C.**, Calder A., Kingsmore D., and Pitsiladis Y.P. (2006) The effects of a novel 'fluid loading' strategy combining creatine and glycerol on fluid retention and distribution in humans. *Proceedings of the 57th meeting of the International Astronautical Congress.* 112-129.
- iii. **Easton, C.**, Fudge, B.W., and Pitsiladis Y.P. (2007) Rectal, telemetry pill and tympanic membrane thermometry during exercise heat stress. *J. Thermal Biol.* **32**, 78-86.

Some of the experiments presented in this thesis utilise a composition protected by the following patent:

Novel Hyperhydration solution (submitted by the University of Glasgow in Great Britain and USA, 23rd July 2004). Patent/GB2005/002913 - Hydrating Composition

Signed



Chris Easton

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Abstract

The primary objective of these series of experiments was to develop an optimal hyperhydration strategy for use during conditions of restricted water access or exercise-induced heat stress. This strategy was composed of two compounds, namely Cr and Gly which each targeted specific body water compartments in order to maximise the volume of retained water. Endurance-trained subjects were recruited to participate in the current series of three experiments, and following Cr/Gly supplementation, body water was estimated by multifrequency bioimpedance and the physiological responses to exercise in the heat (30°C, 70% relative humidity) recorded and compared to pre-supplementation values.

The aim of the first study presented in this thesis (Chapter 3 a) was to examine the effects of combined Cr and Gly supplementation on fluid retention and subsequently the effects on cardiovascular, thermoregulatory and metabolic responses and performance during exercise in the heat. Cr and Gly were delivered according to loading protocols previously established in the literature (20g of Cr for 6 days and 1 g Gly·kg⁻¹ body mass diluted in 500 ml of water 2 hours prior to the start of the experimental trial). Combined Cr and Gly supplementation increased body mass by 1.59 ± 0.41 kg with no change in TBW, ICW, ECW or RPE, heart rate and T_{re} during exercise in the heat compared to the pre-supplementation experimental trial. Given that previous Cr supplementation studies have consistently found significant increases in TBW it can be deduced that the Gly administered prior to exercise had in some way negated the Cr-induced increase in TBW. The results highlight the importance of the loading protocol design when attempting to fluid load prior to exercise.

The aim of Chapter 3 (b) was to examine the effects of a novel method of Cr and Gly delivery and ingestion on fluid retention and distribution. The novel loading protocol (ingestion of both Cr and Gly for 7 days) was designed to allow sufficient time for the retained fluid to be dispersed within body compartments. This regimen of Cr and Gly supplementation resulted in a significant increase in body mass of approximately 1.0 kg. Furthermore, TBW increased by 0.9 L and was dispersed equally between intra- and extra-cellular water compartments. Therefore, the large increase in TBW suggests that ingesting both Cr and Gly over

several days may be the most effective method of hyperhydration prior to exercise.

Chapter 4 (a) aimed to assess the effects of Cr and Gly supplementation ingested according to the loading protocol described in the previous chapter (6 days of Cr and Gly ingestion, with the final supplement consumed 3 hours prior to measurement) on cardiovascular, thermoregulatory and metabolic responses and performance during exercise in the heat. As before, combined Cr and Gly supplementation resulted in a significant increase in body mass (1.20 ± 0.57 kg). Yet despite ingesting both Cr and Gly over several days to allow sufficient time for the retained fluid to be dispersed within body compartments, there was no change in TBW, ICW, ECW or RPE, heart rate and T_{re} during exercise in the heat compared to pre-supplementation. It is probable that ingestion of a hypertonic solution such as the Cr and Gly mixture resulted in slowing of gastric emptying and an initial efflux of water from the plasma into the intestinal lumen. Therefore, the timing of ingestion is evidently critical, with the final supplement requiring to be consumed longer than 3 hours prior to the need for hyperhydration.

The aim of the study in Chapter 4 (b) was to examine the effects of extending the period of time between ingestion of the final Cr/Gly supplement on the retention and distribution of fluid. The overall aim was to develop an effective 'fluid-loading' strategy for use during exercise in the heat. 6 Subjects ingested both Cr and Gly for 6 days as before with half ingesting the final supplement 3 hours prior to body water measurement and the other half receiving their final supplement 5 hours prior to the experimental trial. Subjects in both groups experienced significant increases in body mass following supplementation (1.60 ± 0.34 kg and 1.21 ± 0.28 kg for 3 hour and 5 hour groups, respectively), but there was only a significant increase in TBW (1.1 ± 0.4 L) when the final supplement was ingested 5 hours prior to measurement. Therefore, consumption of both Cr and Gly over several days and ingestion of the final supplement 5 hours prior to exercise is the most effective method of fluid loading. This will allow sufficient time for the retained fluid to leave the stomach, pass across the intestinal lumen wall and be dispersed within body water compartments.

The experiment in Chapter 5 compared the effects of the novel Cr and Gly loading protocol established in Chapter 4 (b) on cardiovascular, thermoregulatory and metabolic responses and performance during exercise in the heat. Combined Cr

and Gly resulted in a significant increase in body mass (0.97 ± 0.28 L) and TBW (0.87 ± 0.21 L) and was associated with an attenuation in heart rate, T_{e} and perception of effort during prolonged exercise in the heat. The key finding of this study was that the increase in TBW after combined Cr and Gly supplementation was significantly greater than either Cr or Gly supplementation alone. Despite the increased hydration associated with combined Cr and Gly, there was no further attenuation in heart rate or T_{e} compared to Cr alone. Hyperhydrating prior to exercise through Cr, Gly or a combination of the two did not result in any significant improvement in 16.1 km time trial performance compared to euhydration. This may be because the time trial was too short to induce a degree of dehydration high enough to confer a significant reduction in exercise performance as a result of the altered hydration status. Alternatively, hyperhydration may not offer any significant advantage in terms of exercise performance compared to euhydration or indeed modest dehydration (i.e. loss of 2-3% body mass).

It has previously been reported that differences in wind speed and resistance between internal and external environments mean that it is of limited efficacy to extrapolate research findings from the laboratory to the field. Therefore, there is a need to determine the effects of combined Cr and Gly supplementation on thermal strain during exercise in the field. However, as yet no method of T_{e} measurement for use in field studies has been validated during periods of severe heat stress. The aim of Chapter 6 was to compare T_{e} measurements obtained using an ingestible telemetry pill and a tympanic membrane thermometer with those from a rectal thermistor during rest and high intensity exercise conducted in a hot and humid environment (30°C and 70% relative humidity) intended to raise T_{e} above 39°C. It was concluded that the ingestible telemetry pill system provides valid measurements of T_{e} during both rest and exercise-induced hyperthermia up to the limits of T_{e} measurement and therefore can be used in the field where T_{re} and esophageal temperatures cannot be taken. This will allow the effect of combined Cr and Gly supplementation on thermoregulatory responses during field studies to be precisely quantified.

List of Abbreviations

| | |
|--------------------|-------------------------------------|
| ACSM | American College of Sports Medicine |
| ADH | Anti-diuretic hormone |
| ANOVA | Analysis of variance |
| c.v. | Coefficient of variation |
| Cr | Creatine |
| ECW | Extra-cellular water |
| Gly | Glycerol |
| Hb | Haemoglobin |
| ICW | Intra-cellular water |
| KJ | Kilojoules |
| L | Litres |
| LOA | Limits of agreement |
| LP1 | Loading protocol 1 |
| LP2 | Loading protocol 2 |
| LP3 | Loading protocol 3 |
| LT | Lactate threshold |
| $\dot{V}CO_2$ | Carbon dioxide production |
| $\dot{V}E$ | Minute ventilation |
| $\dot{V}O_2$ | Oxygen uptake |
| $\dot{V}O_{2\max}$ | Maximal oxygen uptake |
| PCV | Packed cell volume |
| Pl | Placebo |
| RER | Respiratory exchange ratio |
| RPE | Rating of perceived exertion |
| s.d. | Standard deviation |
| TBW | Total body water |
| T_c | Core temperature |
| T_p | Telemetry pill temperature |
| T_{re} | Rectal temperature |
| T_{sk} | Skin temperature |
| WR_{\max} | Maximal work rate |

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Chapter 1

General Introduction

1.1 Exercise in the heat

As homeothermic organisms, humans must maintain their T_c within a relatively narrow range (35 to 42°C) in order to maintain physiological function (Noakes, 2001). Muscular contraction during exercise produces a significant amount of heat, which can potentially cause T_c to rise to the uppermost region of these tolerable limits unless heat is lost through either behavioural or homeostatic mechanisms. When exercise is performed under severe environmental conditions such as high heat and humidity, the amount of heat lost to the environment is reduced resulting in significant metabolic, cardiovascular and thermal strain and in extreme circumstances denaturing of essential proteins and enzymes with potentially fatal consequence (Blatteis, 2001). Some examples of the dangers of exercising in the heat include the Italian marathon runner Dorando Pietri who lay in a coma for two days after completing the 1908 London Olympics, and Francisco Lazaro the Portuguese runner who collapsed with heat stroke and sadly died after 19 miles of the marathon at the so called ‘Sunshine Olympics’ in Stockholm, 1912, a race ran in an ambient temperature of 39°C (Noakes, 2001). Furthermore, the incidents of deaths related to heat-induced injury are not wholly restricted to the athletics arena. The military is a prime example, with the official statistics reporting more soldiers treated for heat stroke than gun shot wounds in the recent Gulf War conflict (Wyatt, 2004). Firemen, rescue workers and astronauts are groups who also remain at significant risk of heat injury during their day-to-day vocation. Until our knowledge of human physiology and sports science improves to such an extent that adequate preventative measures can be developed, these unfortunate and unnecessary injuries will continue to happen. In addition, integration of preventative medical research into the sporting arena will ultimately lead to significant improvements in exercise performance as athletes become better equipped to tolerate extreme environmental conditions. Therefore just as humans have evolved to run long distances (Liebenberg, 2006), so must exercise physiology evolve to enable athletes to run faster.

Humans regulate T_c during exercise through two main avenues: non-evaporative (or ‘dry’) heat loss and evaporative heat loss. Thermoregulation through non-evaporative heat loss is the sum of the flux of heat loss through convection, conduction and radiation from the body to the surrounding environment. Evaporative heat loss is the process by which water (predominantly sweat) evaporates from the surface of the body, causing the loss of between 1092 and 2520 kJ of heat·L⁻¹ evaporated fluid and as a consequence, significant cooling of the skin (Sawka & Wenger, 1998). In environments where the ambient temperature exceeds T_{sk} , evaporative cooling is the only mechanism by which the body can

dissipate heat as cooling via conduction and convection cannot take place due to the reversal of the direction of heat transfer (Sawka & Wenger, 1998). Sweating is stimulated by an increase in T_c and increased cutaneous vasodilation causing a movement of water from plasma into the 1.6-4 million eccrine glands located in the skin (Sato, 1977). This water is then secreted from the eccrine glands as sweat onto the body surface, whereupon evaporative cooling of the skin occurs. Using chlorine distribution analysis, Nose *et al.* (1988) reported a strong association between loss of free water (sweat/urine) and the decrease in ICW following 90-110 min of exercise at 36°C, implicating a significant role for ICW in the sweating response. Thus, the water secreted in sweat is obtained in varying proportions from both the intra- and the extra-cellular fluid compartments to ensure both maintenance of blood volume and efficient thermoregulation (Fig. 1.1). However, the capacity to lose heat through sweating is significantly reduced when the air humidity is high as the air absorbs less water from the surface of the skin (Sawka & Wenger, 1998). As a result, sweat does not evaporate from the skin and drips off without significantly contributing to the heat loss process. The volume of sweat lost during exercise can be significant during prolonged endurance races in the heat and may result in a loss of body water in excess of 5 L which is between 6-10% of body mass (Hubbard & Armstrong, 1988; Wyndham & Strydom, 1969). The highest sweat rate recorded in the literature is an impressive 3.7 L·hr⁻¹ by Alberto Salazar the winner of the 1984 Olympic marathon in Los Angeles; a race run in extreme heat and humidity (Armstrong *et al.*, 1986). Indeed, sweat loss can be high even in a cool climate, where sweat rates of between 1 and 2 L·hr⁻¹ have been recorded in soccer players during training in ambient temperatures of between 5-10°C (Maughan *et al.*, 2005).

Evolutionary biologists suggest that the ability to lose heat via sweat evaporation may have developed from the practise of hunting animals over long distances by early humans in Africa, the so called Bernd Heinrich hypothesis (Liebenberg, 2006). This ritual still performed in the present day by the San tribe (often referred to as 'The First People') who are widely acknowledged to be the oldest inhabitants of southern Africa, with an unbroken link to their ancestors who have lived in the same region for over 30,000 years (Liebenberg, 2006). Hunters aim to run down their prey (predominantly antelopes) by tracking them at high speed over difficult terrain and in the heat of the day (e.g. 46°C), until man or animal must collapse from sheer exhaustion. These races are often run at speeds of around 4 to 6 miles per hour, for anywhere from two to six and a half hours, and traverse up to 22 miles of terrain. These stats fall well within the performance range of the world's fastest competitive marathoners (Fig. 1.3), who set a pace of roughly 12 miles per hour to cover 26 miles, albeit under far less harsh conditions (Liebenberg, 2006). The San

tribe hunt in groups of three with two initially doing the hard work of tracking and pursuing over the arid grassland and woodland terrain, while the other holds back. Eventually, the leaders drop behind, leaving the third man to hound and spear the antelope when it reaches its limit. ‘The animal will either just completely collapse, or it will actually slow down to a point where it just stands there . . . with sort of glazed-over eyes.’ (Liebenberg, 2006). ‘Essentially, you’re pushing the animal to overheat’. The large antelopes that the men hunt soon become dehydrated during the chase which causes a decrease in sweating and the rate of cutaneous evaporation by 12-89% which can lead to a reduction in the thermoregulatory capacity of the animal, and eventually hyperthermia (Cain *et al.*, 2006). Perhaps through a process of natural selection only those hunters who were able to tolerate these severe environmental conditions would survive. Indeed, it is believed that persistence hunting is so effective that it may have helped select for the excellent thermoregulatory system, bipedal posture, and long strides that we all possess today (Liebenberg, 2006).

1.2 Dehydration

The traditional ‘cardiovascular model’ of dehydration provides a simple explanation for the effects of dehydration on exercise performance and is best understood by reference to Figure 1.2. This model contends that fluid lost through sweat during exercise will reduce plasma volume and consequently venous return to the heart (Fig. 1.2). As a direct result, stroke volume will be reduced and heart rate will increase (‘cardiovascular drift’) in order to maintain cardiac output (Ekelund, 1967). Ultimately the physiological limitation of a maximum heart rate will fail to accommodate the ongoing reduction in stroke volume resulting in a decrease in cardiac output and aerobic capacity (Rowell, 1986) which will have a profound negative effect on exercise performance (Fig. 1.2). Additionally, circulating blood volume may be further decreased by cutaneous vasodilatation to allow heat dissipation that increases the compliance of the cutaneous venous blood vessels, thereby reducing venous pressure. This reduced venous pressure results in pooling of the blood in the cutaneous venules resulting in decreased venous return, reduced stroke volume, reduced end-diastolic filling pressure and resultant further cardiovascular stress (MacDougall *et al.*, 1974; Sawka *et al.*, 2001). Paradoxically, oxygen delivery to the working muscles must also be maintained in order to sustain energy metabolism during exercise, presenting the body with two competitive cardiovascular demands. However, Gonzalez-Alonso *et al.* (1995) have determined that during cardiovascular drift there is an increase in systemic vascular resistance as the cardiovascular system attempts to deal with

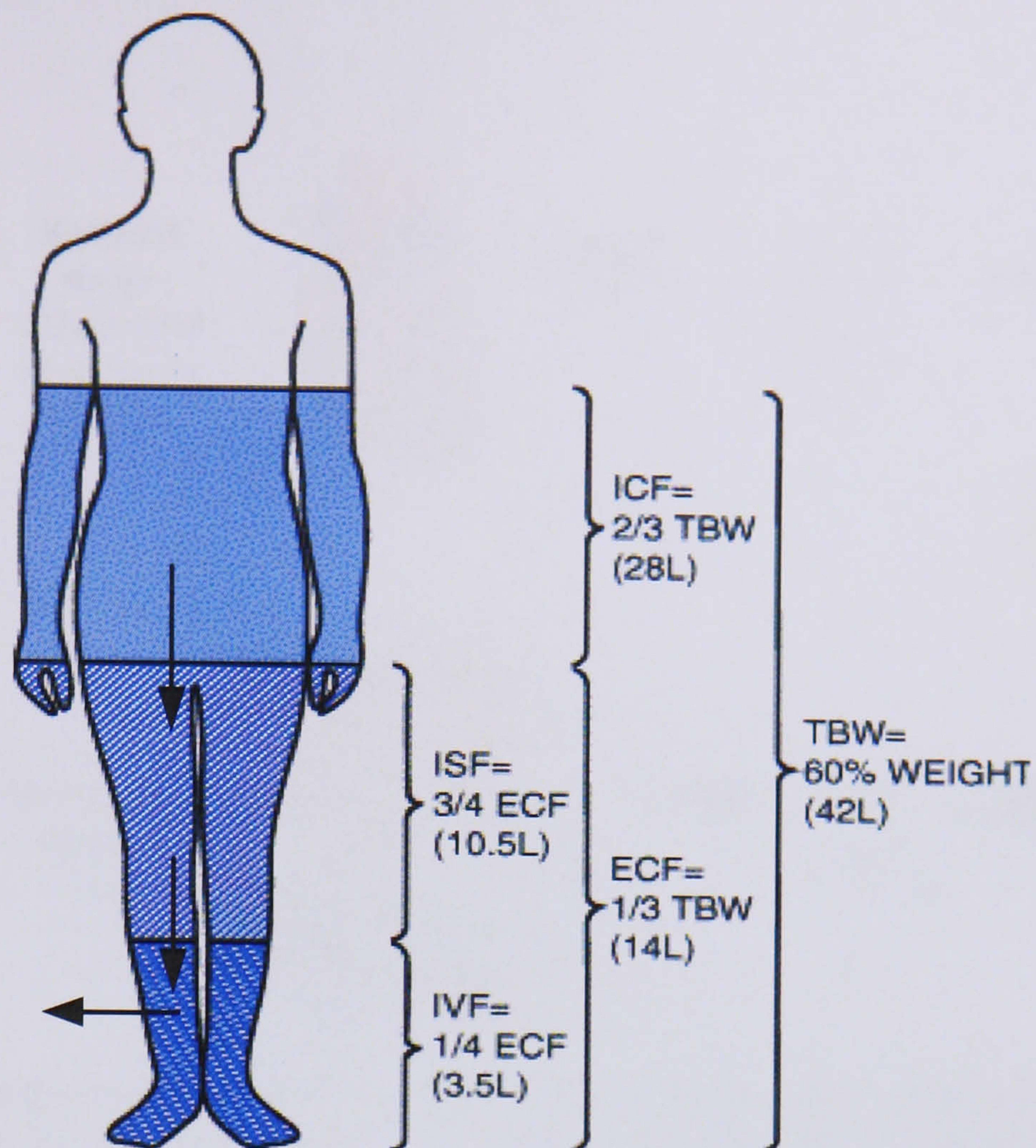


Figure 1.1 Schematic representation of body fluid compartments in man. The shaded areas depict the approximate size of each compartment as a function of body weight. The figures indicate the relative sizes of the various fluid compartments and the approximate absolute volumes of the compartments (in litres) in a 70-kg adult. TBW=total body water; ICF=intracellular fluid; ECF=extracellular fluid; ISF=interstitial fluid; IVF=intravascular fluid. The arrows indicated the direction of fluid shift during sweat loss. Adapted from Verbalis (2003).

the strain of a reduced cardiac output. Thus, the general vasoconstrictor response causes constriction in the cutaneous circulation resulting in a significant reduction in skin blood flow. Furthermore, Gonzalez-Alonso *et al.* (2000) found that stroke volume was similar whilst exercising at moderate intensity in hot (35 °C) vs. cold (8 °C) conditions, both in the euhydrated and dehydrated condition even though cutaneous blood flow varied. It follows therefore, that an increase in cutaneous blood flow does not explain the reduction in stroke volume, nor the progressive increase in heart rate (cardiac drift) observed during prolonged exercise, both of which are exacerbated in the heat. More recently, Coyle & Gonzalez-Alonso (2001) have suggested an alternative explanation for the cardiovascular drift phenomenon. These authors propose instead that an elevated heart rate caused by an increase in T_c and sympathetic nervous activity results in a reduction in diastolic filling time giving rise to a decline in stroke volume. This mechanism would account for approximately one half of the reduction in stroke volume, with the second half occurring as

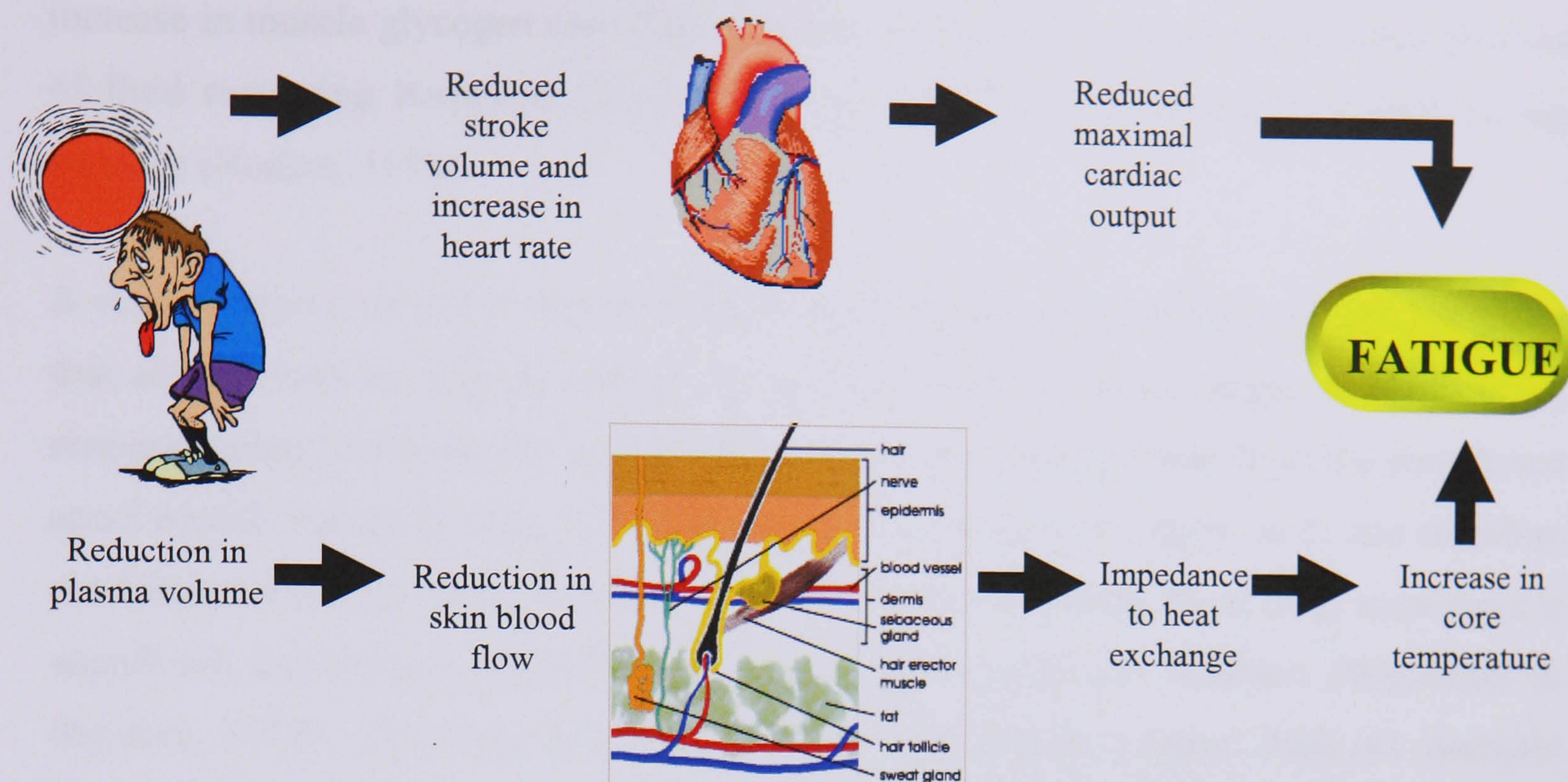


Figure 1.2 Schematic diagram of the cardiovascular model of dehydration. The arrows indicate the effects of sweat loss on the cardiovascular and thermoregulatory systems and the possible link to fatigue during exercise.

a result of hypovolemia (Coyle & Gonzalez-Alonso, 2001). According to the cardiovascular model of dehydration, a reduction in cutaneous blood flow can impede heat exchange and reduce the temperature regulating capacity of the body which will lead to an increase in T_c and resultant premature fatigue (Fortney *et al.*, 1984) (Fig. 1.2). Therefore it would be reasonable to assume that the extent of dehydration will be closely related to the elevation of T_c during exercise (Montain & Coyle, 1992a;1992b). However, Noakes *et al.* (1991) propose that the primary factor in determining T_c during prolonged exercise is not the extent of dehydration incurred but the metabolic rate. Early studies clearly demonstrated that relative exercise intensity (i.e. percentage $\dot{V}O_{2\max}$) correlates very well with T_{re} during exercise (Saltin & Hermansen, 1966). Furthermore, it is a common observation that the highest placed finishers in a marathon typically have the highest post race T_{re} (Maron *et al.*, 1975;Noakes *et al.*, 1991;Pugh *et al.*, 1967), a finding consistent with observations of elite athletes completing a marathon at a higher percentage $\dot{V}O_{2\max}$ than non-elite runners (Fox & Costill, 1972;Maughan & Leiper, 1983). Nevertheless, the notion that the increased heart rate and T_c during exercise in the heat and reductions in stroke volume and cardiac output all occur in proportion to the level of dehydration is well supported in the literature (e.g. Montain & Coyle, 1992a;1992b). Indeed Montain *et al.* (1998) have suggested that T_c rises by 0.1-0.2°C for every percent of body mass loss resulting from dehydration. Whether the dehydration is causative of the increase in T_c , or

whether there is merely a spurious relationship between the two variables remains to be determined (Noakes, 2005). Furthermore, dehydration has also been associated with an increase in muscle glycogen use (Hargreaves *et al.*, 1996), increased blood concentrations of fluid regulating hormones (McConell *et al.*, 1997) and increased discomfort during exercise (Noakes, 1993).

A comprehensive review of marathon literature by Cheuvront and Haymes (2001) suggests that athletes may be able to endure dehydration within a certain range. Analysis of all running studies involving active dehydration (process of losing water from the euhydrated state) reveals that body mass losses between 1.6-3.1% have no effect on T_c and therefore are within a tolerable range. However, when dehydration exceeds 3% of body mass there is significant impairment in cardiovascular and thermoregulatory function (Wyndham & Strydom, 1969). This may be due to the fact that plasma volume does not decrease considerably during running beyond the initial drop at the onset of exercise (Sawka & Coyle, 1999). This is providing dehydration remains lower than 4% body mass as when dehydration increases beyond this point there is a further loss in plasma volume and significant impairment of heat loss (Sawka & Coyle, 1999). The stability of plasma volume during running has also been reported during marathon and treadmill running despite even larger reductions (4-7%) in TBW (Kolka *et al.*, 1982; Sawka & Pandolf, 1990). However, although this may be the case for running, other sports such as cycling invoke a comparatively greater degree of haemoconcentration (Harrison, 1985). Therefore, the exercise modality may have a significant effect on the modification of cardiovascular and thermoregulatory responses during progressive dehydration. Montain and Coyle (1992b) have demonstrated that losses in plasma volume are significantly greater during 2 hours of cycling at 63-67% $\dot{V}O_{2\max}$ compared to marathon data. Furthermore, T_c responds to active dehydration (1-4%) in a linear fashion under controlled laboratory conditions (Montain & Coyle, 1992b).

1.3 Exercise Performance

Despite the reported impact of dehydration on cardiovascular and thermoregulatory responses during exercise (Fig. 1.2) it remains unknown exactly how these physiological factors contribute to the fatigue process. Of course fatigue, defined as 'the failure to maintain an expected power output' (Hultman & Sjoholm, 1986), is an inevitable consequence of all prolonged physical exercise. Despite the well-documented negative effects of high ambient temperatures on exercise performance, the underlying

physiological mechanisms have been extensively debated without clear consensus. Early attempts to explain why fatigue occurs prematurely in the heat focused primarily on events occurring within skeletal muscle, usually termed peripheral fatigue. Peripheral fatigue is typically defined as any fatigue arising from the failure of mechanisms at or beyond the neuromuscular junction, including junctional transmission, electrical activity of muscle and its activation (Edwards, 1981). However, as several studies indicate, skeletal muscle ATP concentrations are never reduced to less than 50% of the resting value under all conditions of exercise (Gonzalez-Alonso & Calbet, 2003; Noakes, 2005). Furthermore, Pitsiladis & Maughan (1999) concluded that glycogen depletion was not the performance-limiting factor during exercise in the heat as exercise was terminated prior to the depletion of all carbohydrate stores. Similarly, Parkin *et al.* (1999) reported that after exercise to exhaustion in ambient temperatures of 3, 20 and 40°C, muscle [glycogen] was highest in the 40°C condition. Therefore, it is difficult to explain hyperthermia-induced fatigue by peripheral factors alone. There is now mounting evidence to suggest that fatigue during exercise may originate at a higher level than skeletal muscle, specifically within the central nervous system, a hypothesis first proposed by Newsholme *et al.* (1987). This hypothesis proposes that an increase in the concentration of tryptophan in the blood and hence the neurotransmitter 5-hydroxytryptamine in some neurons which are involved in control of motor activity in the brain, could lead to central fatigue (Newsholme *et al.*, 1987). Research in the early nineties by Nielsen *et al.* (1993) observed that humans always ceased exercising when T_{re} reached a certain limit (averaging 39.7°C) that was constant for each subject. However, at the point of exhaustion there was no reduction in cardiac output, muscle (leg) blood flow, no changes in substrate utilisation or availability, and no recognised accumulated ‘fatigue’ substances. This led the authors to propose that ‘...the high T_{re} per se, and not circulatory failure, is the critical factor for the exhaustion during exercise in heat stress’ (Nielsen *et al.*, 1993). In a follow up study comparing the effects of pre-heating and pre-cooling the body, these same researchers showed that high internal body temperature did indeed cause fatigue in trained subjects during prolonged exercise in hot environments as time to exhaustion was inversely related to the initial T_{re} and directly related to the rate of heat storage (Gonzalez-Alonso *et al.*, 1999). Furthermore, Nybo & Nielsen (2001) found that the ability to generate skeletal muscle force during a prolonged maximum voluntary contraction is attenuated with hyperthermia. Therefore, the premature fatigue that occurs during exercise in the heat could be caused by an increased T_{re} reducing the brain’s capacity to recruit skeletal muscle and not due to a peripheral impairment of skeletal muscle function. Conversely, Tucker *et al.* (2004) found that when comparing skeletal muscle recruitment during self paced exercise in both hot (35°C) and cool (15°C)

environments, power output and integrated electromyographic activity of the quadriceps muscle began to decrease early in the hot conditions when T_{c} , heart rate and RPE were similar in both conditions. These findings suggest that there may be an anticipatory response during self-paced exercise whereby the brain adjusts skeletal muscle recruitment and power output accordingly to reduced heat production and thereby ensuring maintenance of thermoregulatory and metabolic function (Tucker *et al.*, 2004). This has led to the development of the so called ‘central governor’ theory, which proposes that the brain reduces muscle fibre recruitment during prolonged exercise in the heat in order to maintain the integrity of the organism (Noakes, 2001). However, a subconscious central governor component of fatigue during exercise in the heat would fail to explain how humans can run to the point of excessive heat storage resulting in death, described in the first paragraph of this thesis. Indeed deaths during athletic pursuits have been reported since 490 BC when the Athenian messenger Phidippides collapsed and died after running the 26 miles from the Greek village of Marathon to Athens in what was likely to have been hot conditions, to report news of victory in battle. Until direct experimental evidence can be offered to support the existence of a central governor, this will remain a controversial and unproven theory. It has also been shown that cerebral blood flow is reduced by 18-20% during exercise in hyperthermia compared to normothermia (Nielsen & Nybo, 2003; Nybo *et al.*, 2002). These authors conclude that the reduction in cerebral blood flow is due to hyperthermia-induced hyperventilation causing a decrease in arterial CO_2 pressure and consequent cerebral vasoconstriction that may explain the pre-syncope symptoms occasionally observed during subjects exercising in the heat (Nielsen & Nybo, 2003).

Whatever the mechanism, previous studies have shown unequivocally that endurance exercise performance is impaired markedly when ambient temperature is high (Adams *et al.*, 1975; Galloway & Maughan, 1997; Kozlowski *et al.*, 1985; MacDougall *et al.*, 1974; Nielsen *et al.*, 1990; Saltin *et al.*, 1972; Suzuki, 1980) and increased when ambient temperature is low (Febbraio *et al.*, 1996; Galloway & Maughan, 1997). For example, Galloway & Maughan (1997) reported that when subjects were asked to complete exercise at 70% $\dot{V}\text{O}_{2\text{max}}$ to exhaustion at ambient temperatures of 4, 11, 21 and 31°C, exercise time was longest (93.5 ± 6.2 min) at 11°C and shortest (51.6 ± 3.7 min) at 31°C. Furthermore, the effect of ambient temperature on endurance performance is not solely limited to the laboratory setting. Figure 1.3 compares the ambient temperatures and completion time of the 10 fastest male marathon performances (left) and the Olympic marathons since 1972 (right) (Fudge *et al.*, 2005). Regardless of the Olympic games being the pinnacle of athletic

competition where world records are continually broken every four years, the Olympic marathon times since 1972 are vastly slower than the fastest 10 marathons ever completed. For example, in the recent Athens Olympic games in 2004, Stephan Baldini the Italian who won the race in 2:10:55 was 6 min slower than Kenyan Paul Tergat's world record of 2:04:55, set in Berlin in 2003. However, while Tergat competed in relatively cool conditions (10°C), Baldini had to battle sweltering conditions of 30°C and oppressively high humidity. The fact that all of the Olympic marathons since 1972 have been competed in ambient temperatures in excess of 20°C clearly emphasises the direct negative impact of environmental heat stress on endurance performance. Furthermore, this trend is likely to continue as athletes begin to prepare for what will likely be another hot Olympic games in 2008 in Beijing, China. The multitude of theories offered to explain the mechanism underlying the occurrence of premature fatigue during exercise in the heat clearly emphasises the need for continuing future research, especially in the field. Only when the interaction of the physiological processes that culminate in fatigue are determined will it be possible to provide definitive preventative measures that will reduce the adverse effects of heat stress on human performance. Yet despite this, development of strategies to improve exercise performance in the heat has been one of the corner stones of exercise physiology research for the last 40 years.

One of the vital practices currently used to prepare athletes for competition in high temperatures is heat acclimatisation. The negative impact of hot environments on the performance of an athlete can be greatly limited by a period of heat acclimatisation prior to competition (Terrados & Maughan, 1995). The major benefits of the acclimatisation process include an expansion of plasma volume, increased skin blood flow and sweating response leading to reduced heart rate, RPE, blood [lactate] and T_{re} during exercise (Armstrong & Maresh, 1991; Terrados & Maughan, 1995). Yet due to the increased sweat rate there is an associated increase in the volume of fluid required to minimise dehydration and any possible adverse effects on performance. Furthermore, pre-cooling the body prior to training or competition via cold air (5-10°C) or cold water immersion is another strategy utilised by athletes in recent years. This will increase the margin for metabolic heat production prolonging the time to reach the critical limiting temperature when a given exercise intensity can no longer be maintained (Marino, 2002; Nielsen *et al.*, 1993).

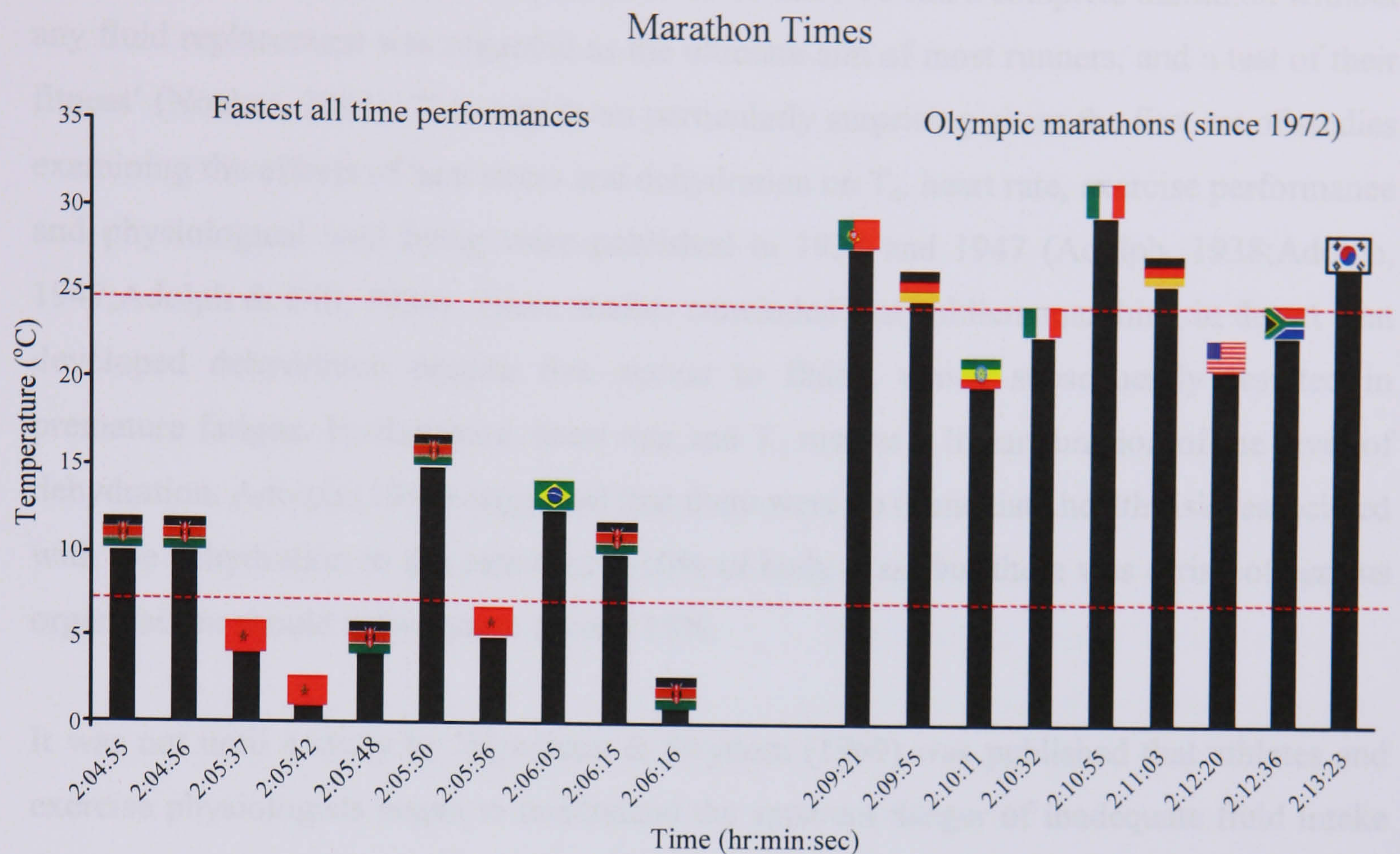


Figure 1.3 The impact of ambient temperature on elite male marathon performance time. Country's flag indicates nationality of the marathon winner. Red lines indicate average ambient temperature for both the fastest all time marathon performances (lower line - 7.3°C) and the Olympic marathons since 1972 (upper line - 24.3°C). Data correct 8/2006. Adapted from Fudge *et al.* (2005).

The rationale for fluid ingestion during exercise stems from the traditionally accepted 'cardiovascular model' of dehydration. Specifically, the fluid ingested would maintain plasma volume resulting in a reduction in cardiovascular strain during exercise in the heat. As a direct result, skin blood flow would be maintained allowing sufficient continuation of convective heat loss. Additionally, there would be sufficient body water to maintain adequate sweat production and optimum evaporative cooling, which overall would enhance thermoregulatory function. Thus, it has been proposed that the reduction in cardiovascular and thermal strain induced by fluid ingestion should ultimately improve exercise performance, especially in the heat (Convertino *et al.*, 1996). There has been vociferous debate between leading groups of researchers in the last 30 years regarding the most effective fluid ingestion strategy to improve exercise performance in the heat (Convertino *et al.*, 1996; Noakes, 2001). However, the idea that fluid should be ingested during exercise is a relatively recent phenomenon only coming to fruition in the second

half of the twentieth century. Indeed, Jackie Meckler who ran marathons and ultramarathons in a career spanning from 1945 to 1969 commented that, 'In those days it was quite fashionable not to drink, until one absolutely had to. After a race, runners would say with pride, 'I only had a drink after 30 or 40 km'. To run a complete marathon without any fluid replacement was regarded as the ultimate aim of most runners, and a test of their fitness' (Noakes, 2001). This may seem particularly surprising given the first set of studies examining the effects of heat stress and dehydration on T_{c} , heart rate, exercise performance and physiological well being were published in 1938 and 1947 (Adolph, 1938; Adolph, 1947; Adolph & Dill, 1938). These studies concluded that soldiers marching in desert heat developed dehydration despite free access to fluids, which subsequently resulted in premature fatigue. Furthermore, heart rate and T_{c} rose as a linear function of the level of dehydration. Adolph (1947) suggested that there were no immediate health risks associated with the dehydration to the extent of 7-10% of body mass but there was a risk of serious organ failure should dehydration exceed 15%.

It was not until a study by Wyndham & Strydom (1969) was published that athletes and exercise physiologists began to understand the apparent danger of inadequate fluid intake during exercise. These authors found that athletes competing in a marathon consumed significantly less fluid than was lost through sweating and hence a state of dehydration ensued. Furthermore, a linear relationship ($r=0.67$) between post race T_{c} and percent dehydration (greater than 3% body mass loss) was reported in the runners. This finding provoked the initial suggestion that those exercising for prolonged periods of time would need to consume fluids to prevent significant heat injury (Wyndham, 1977). These findings were the incentive for the International Amateur Athletics Federation to change their rules in 1977 to allow greater volumes of fluid at increased intervals to be available during distance races. In addition, the study by Wyndham & Strydom (1969) was cited in guidelines created by the influential body The American College of Sports Medicine (ACSM) for suggesting specific fluid intake (ACSM, 1975; 1987; Convertino *et al.*, 1996). These ACSM guidelines and Position Statements/Stands have been significantly revised and amended over the last three decades (ACSM, 1975; 1987; Convertino *et al.*, 1996). In the most recent of these (Convertino *et al.*, 1996), it is suggested that 'adequate fluid consumption before and during race can reduce the risk of heat illness, including disorientation and irrational behaviour, particularly in longer events such as the marathon, citing (Costill *et al.*, 1970; Gisolfi & Copping, 1974; Wyndham & Strydom, 1969). Secondly, 'dehydration can predispose the runner to heat exhaustion or the more dangerous hyperthermia and exertional heat stroke' (Hubbard & Armstrong, 1988; Pearlmuter, 1986). Finally, 'athletes should replace their sweat losses or consume 150-300 ml every 15 min'

(Hubbard & Armstrong, 1988; Nash, 1985). However, none of these studies were prospective intervention trials during which the variable in question (hydration status) was the only thing to change and therefore the extent of dehydration cannot be proved conclusively to be causative of hyperthermia. In 2000, the National Athletic Association of Trainers (NAAT) also published a position statement concluding that 'Fluid replacement should approximate sweat and urine losses and at least maintain hydration at less than 2% body mass reduction' (Casa *et al.*, 2000). Several studies have indicated that dehydration above 2% body mass results in a significant impairment in exercise performance exceeding 90 min in both a temperate (20-21°C) (Cheuvront *et al.*, 2003; Fallowfield *et al.*, 1996; McConell *et al.*, 1997) and a hot (31-32°C) environment (Below *et al.*, 1995; Walsh *et al.*, 1994). Furthermore, given that it takes 40-60 min for ingested fluid to be evenly distributed throughout the body after gastric emptying, intestinal absorption and osmotic flow (Schedl *et al.*, 1994), means athletes must begin drinking early to delay the onset of dehydration and prevent water loss exceeding 2% body mass. These facts contributed significantly to the ACSM and NAAT guidelines and have become the adopted dogma of exercise physiologists, race organisers and sports drinks companies alike.

However, Noakes (2001) argues that the ACSM and NAAT guidelines are 'not evidence-based, since neither refers to specific, prospective, interventional studies from which such definite conclusions can be drawn' (Noakes, 2005). Several studies confirm that the voluntary fluid intake of runners during distance races is approximately 500 ml each hour (Maughan, 1985; Noakes *et al.*, 1988; Noakes, 1993; Shephard & Kavanagh, 1978), which is lower than the 600-1200 ml suggested by (Convertino *et al.*, 1996). Indeed Noakes (2001) believes that elite athletes may drink as little as 200 to 400 ml each hour during races. So how can the exceptional performances of elite endurance athletes be explained despite an *ad libitum* fluid intake that is well below the established recommendations? The ACSM and NAAT guidelines are based on laboratory studies where the degree of heat stress encountered would be significantly greater than in the field (Saunders *et al.*, 2005), perhaps contributing to an overestimation in suggested fluid replacement guidelines. Studies comparing *ad libitum* fluid intake to the rates of fluid ingestion set out in the ACSM guidelines found no difference on endurance performance (Daries *et al.*, 2000; McConell *et al.*, 1999). Essentially the only measured difference between the fluid replacement strategies was an increased feeling of intestinal discomfort when the rate of fluid ingestion was high (Daries *et al.*, 2000; McConell *et al.*, 1999). Indeed, there is also theoretical opinion that dehydration within a tolerable range will not have a negative impact on exercise performance, but may even confer an advantage by preventing inevitable increases in body mass due to consumption of large volumes of fluid (Armstrong *et al.*,

1985;Noakes, 2001). Therefore, if body mass can be reduced while power output remains constant there will be a concomitant reduction in the energy cost of exercise, especially in weight bearing activities. There is also a possibility that over drinking during exercise may result in a progressive fluid overload, leading to dilution of blood [sodium] below $130 \text{ mmol}\cdot\text{L}^{-1}$ and ultimately hyponatraemic encephalopathy (brain swelling and dysfunction due to voluntary overdrinking either before, during or after exercise), and even death (Noakes, 2005). However, this is more likely to occur in slow runners who take longer to complete the race, and thus consume substantial volumes of fluid (Almond *et al.*, 2005). Thus, based on this evidence the United States of America Track and Field (USATF) announced that all future running races in the United States would be run according to new guidelines (Noakes, 2003a;2003b;Noakes & Martin, 2002) which advocate that athletes should drink according to the dictates of their thirst during exercise and not to the limits of their individual tolerance. Clearly, the debate surrounding fluid replacement strategies looks set to continue for many years to come until direct observational evidence can prove beyond all reasonable doubt how much athletes should drink during exercise in different environmental conditions.

1.4 Hyperhydration

Given the potentially deleterious effects of dehydration on T_c and exercise performance then logically, it would be beneficial to increase body water stores prior to exercise to provide a fluid reservoir. One approach has been to maintain plasma volume during exercise in the heat by infusion of isotonic saline. Using this method, Fortney *et al.* (1988) found an attenuated rise in T_c during exercise in the heat that they attributed to a maintenance of central blood volume resulting in an increase in skin blood flow and associated convective heat loss. Several other studies using acute plasma volume expansion with either saline or dextran infusions reported an attenuation in the rise in heart rate and T_c (Deschamps *et al.*, 1989). Indeed, Luetkemeier & Thomas (1994) reported that pre-exercise plasma volume expansion with intravenous dextran solution improved cycling performance by more than 10%. However, the medical expertise required to insert and maintain a venous infusion and the restricted mobility that a saline drip would incur means that this method of hyperhydration would be impossible during exercise in the field. Furthermore, the finding of similar forearm blood flow during hypervolemia in the study by Watt *et al.* (2000) led these authors to conclude that acute plasma volume expansion did not directly enhance thermoregulation. Other studies have also failed to show any effect of

plasma volume expansion on heart rate, T_c , skin blood flow or indeed performance during exercise in the heat (e.g. Grant *et al.*, 1997).

Hyperhydration prior to exercise by ingestion of water or carbohydrate/electrolyte solutions is less effective than infusion methods as most 'excess' fluid ingested is rapidly filtered and excreted by the kidneys (Freund *et al.*, 1995). On the other hand, hydrating agents such as Gly (1,2,3-propanetriol) have been shown to increase TBW and effectively minimise an exercise induced reduction in plasma volume (Murray *et al.*, 1991). Gly is a naturally occurring 3-carbon alcohol metabolite that is produced in the human body and distributed within and between all cells at low concentrations ($<0.1 \text{ mmol}\cdot\text{L}^{-1}$), with the exception of the cerebral spinal fluid and aqueous humor (Lin, 1977; Tourtellotte *et al.*, 1972). Seifert *et al.* (1995) reported a 701 ml increase in mean TBW after Gly ingestion, including a 385 ml increase in interstitial fluid and a 225 ml increase in ICW with the remainder distributed within the plasma. Some researchers propose this Gly-induced water retention is attributed to an increased concentration of ADH (Freund *et al.*, 1995). However, previously reported differences in [ADH] between Gly and water interventions were small and only approached statistical significance (Freund *et al.*, 1995). While an ADH mechanism cannot be ruled out, it is more likely that this Gly-induced water retention is mediated by the action of Gly on the kidneys. When blood [Gly] is at normal physiological levels, almost all filtered Gly is passively reabsorbed by the proximal and distal renal tubules of the kidneys (Sommer *et al.*, 1993). When blood [Gly] is increased with exogenous Gly ingestion, there is an increase in Gly and associated water reabsorption (Kruhoffer & Nissen, 1963). Several studies have now concluded that a Gly bolus delivered 2-3 hours prior to exercise reduces thermal and cardiovascular strain during exercise in the heat (Anderson *et al.*, 2001; Lyons *et al.*, 1990; Montner *et al.*, 1996) and argue these effects are due to a preservation of blood volume and cutaneous blood flow (Lyons *et al.*, 1990). For example, Montner *et al.* (1996) reported that time to exhaustion was increased by approximately 23% after Gly induced hyperhydration compared with a placebo (Pl). However, not all studies have shown such effects of Gly on thermoregulation during exercise in the heat (Inder *et al.*, 1998; Latzka *et al.*, 1998; Murray *et al.*, 1991). Methodological differences, including the amount of Gly and timing of ingestion prior to exercise, the exercise protocol, ambient conditions, methods used to assess hydration status and T_c are all likely to have contributed to the conflicting results.

Creatine (methyl guanidine acetic acid) (Cr) is a naturally synthesised compound, important in the energy metabolism process that has been used as an ergogenic aid for

several years in order to improve performance in short duration, high intensity exercise (Casey *et al.*, 1996; Kilduff *et al.*, 2002). Ingestion of Cr has also been shown to have substantial hydrating effects (Kern *et al.*, 2001; Kilduff *et al.*, 2004), although the exact mechanisms remain uncertain. The water retention may simply be due to osmotic effects, cell swelling and a consequent increase in protein synthesis (Haussinger *et al.*, 1993). Conversely, it may be an increase in protein synthesis that precedes the associated increase in water content (Kreider *et al.*, 1998). However, unlike the whole body hydrating effects of Gly, Cr retains fluid predominantly in the ICW compartments (Kilduff *et al.*, 2004). Like Gly, oral Cr supplementation has been shown to be effective in attenuating the rise in heart rate and T_c and improving performance during exercise in the heat (Kilduff *et al.*, 2004). These effects have been attributed to an increase in ICW resulting in an increased specific heat capacity of the body (Kern *et al.*, 2001; Kilduff *et al.*, 2004). Supplementation with hydrating agents such as Gly or Cr has consistently produced modest fluid retention of 400 to 800 ml (Kilduff *et al.*, 2004; Montner *et al.*, 1996). However, it seems plausible that a Gly-induced increase in extra-cellular water (ECW) coupled with a Cr-mediated increase in ICW could have synergistic effects resulting in a much larger increase in TBW than if either supplement was consumed alone.

To assess acute changes in hydration status, researchers must indirectly estimate TBW as it is not possible to measure this parameter directly in a live human being. For example, measurements of body water content in muscle biopsy samples are subject to error because of the assumptions involved and the rapid evaporation of water after biopsy (Proctor *et al.*, 1999). However, isotope dilution has been successfully used to measure TBW in animals and humans for 80 years. Deuterium oxide was first used to estimate TBW in 1934 when data was published using 2 rabbits and 1 human as volunteers (Hevesy & Hofer, 1934). A further study compared deuterium oxide predictions of TBW against direct measurements using desiccation of cadavers, and reported only minor differences between measurement techniques equating to approximately 0.7% of body weight (Moore, 1946). Tritiated water (isolated by Alvarez & Cornog (1939)) has distribution properties similar to those of deuterium oxide and has since become the preferred method of TBW measurement (Pinson & Langham, 1957). Indeed, isotope dilution is now the accepted 'gold standard' for determining TBW (O'Brien *et al.*, 2002) and is a method commonly utilised in research studies (e.g. Fudge *et al.*, 2006). However, repeat measurements of TBW using dilution techniques are difficult due to the necessity of a waiting period while the tracers are cleared from the body and therefore would not always be applicable to measure acute changes in TBW. Therefore, the majority of researchers to date investigating

hyperhydration have used the volume of urine production (and hence water retention) as an indirect measurement of TBW change following supplementation with either Cr or Gly (Freund *et al.*, 1995;Latzka *et al.*, 1997;1998). However, this method is extremely limited, as it does not provide a measurement of the distribution of fluid within the body compartments.

Bioelectrical impedance has gained much attention as a rapid, inexpensive and non-invasive method of estimating TBW. Bioelectrical impedance is based on the assumption that electricity is conducted poorly by fat and bone but well by tissues that contain predominantly water and electrolytes. Therefore, by passing a low level alternating current at a 50 kHz frequency between two parts of the body (e.g. leg to arm) the resistance to the current can be measured and used to predict TBW using the equation:

$$Z = \rho * L^2 * V^{-1}$$

where Z is the electrical impedance, ρ is the resistivity, L is the height and V is the volume of water contained within the body. This relationship relies on certain assumptions, firstly that ρ is known and a constant and secondly that V is evenly distributed within a cylinder of uniform cross-sectional area. However, when applying this to the human body, these assumptions are violated, thereby introducing a degree of error into the estimation of TBW. Nevertheless, bioelectrical impedance has been shown to provide a reasonable prediction of TBW ($r=0.86$, $P<0.01$) compared with isotope dilution techniques and has a coefficient of variation for repeated measures of 2-3.4% (Lukaski *et al.*, 1985). The recent development of multi-frequency bioelectrical impedance machines allows TBW measurements to distinguish between ECW and ICW as at low frequencies the current passes through ECW, but at higher frequencies it is able to penetrate the cell membrane. Multifrequency bioelectrical impedance has been consistently shown to provide reliable and repeatable estimations of TBW in euhydrated individuals if ambient temperature, electrode placement, subject posture and use of a non-conductive surface are standardised (Armstrong *et al.*, 1997;Kushner, 1992;Kushner *et al.*, 1992;1996). Furthermore, multifrequency bioelectrical impedance has been recently shown to provide accurate estimates of the change in TBW following both Gly (Koulmann *et al.*, 2000) and Cr (Powers *et al.*, 2003) hyperhydration compared to the isotope dilution technique. Therefore it can be expected that bioelectrical impedance will provide a valid and reliable estimate of TBW change following combined Cr/Gly supplementation and a measure of where the retained fluid is distributed within fluid compartments.

1.5 Aims and objectives

Given the extensive debate on the influence of hydration on exercise performance in the heat and the accumulating evidence of the benefits of hyperhydration, the main objectives of the following research were as follows:

- i. To investigate the effects of ingesting two different fluid retaining agents simultaneously on body fluid balance and in doing so determine whether combining Cr and Gly can induce a greater hyperhydration than either Cr or Gly alone. This was achieved by designing a series of studies that measured the effects of all combinations of supplements on TBW, ECW, ICW and plasma volume.
- ii. To develop the optimal hyperhydration strategy for use during conditions of restricted water access or exercise induced heat stress. This was achieved by comparing a Cr/Gly supplementation strategy based on previously established protocols from the literature with novel methodologies.
- iii. To assess the effects of these novel ‘water-loading’ strategies on metabolism, cardiovascular and thermoregulatory responses and performance during exercise in the heat. This was achieved by examining the effects of combined Cr and Gly hyperhydration on physiological responses during steady state exercise in hot and humid environment (30°C and 70% relative humidity) and performance in a 16.1 km time trial and in doing so provides further insight into the relationship between dehydration and performance.
- iv. To validate a new method of T_{e} measurement for use outwith the laboratory in training and competitive situations. This was achieved by comparing T_{e} measurements obtained from an ingestible telemetry pill and an infrared tympanic membrane thermometer with those from a rectal thermistor during rest and high intensity exercise conducted in a hot and humid environment (30°C and 70% relative humidity) intended to raise T_{e} above 39°C. This will allow future research examining the effects of combined Cr and Gly hyperhydration on thermal and cardiovascular strain and exercise performance to be completed in the field.

Chapter 2

General Methods

2.1 Subjects

All experiments described in this thesis involved human volunteers who were all endurance trained healthy males. All experiments were approved by the University of Glasgow Ethics Committee and were performed according to the code of ethics of the World Medical Association (Declaration of Helsinki). The ethics document for Chapter 5 is displayed in Appendix 1 and was adapted from the original ethics documents from Chapters 3 and 4. Subjects were questioned as to their training practices and it was determined that no subject was acclimatised to exercise in the heat at the time of study. This interview also confirmed that all subjects were Cr free for at least 8 weeks prior to the study. The investigators did not reveal prior to the interview that subjects would be excluded if they had supplemented with Cr in the previous 8 weeks. The subjects were fully informed of any risks and discomforts associated with the experiments and informed they could withdraw at any point without explanation before giving their written informed consent to participate (Appendix 1).

2.2 Determination of $\dot{V}O_{2\max}$ and test workloads

All subjects had their $\dot{V}O_{2\max}$, WR_{\max} and LT measured during an initial continuous incremental test to volitional exhaustion at standard room temperature (20-21°C). LT was estimated non-invasively as the $\dot{V}O_2$ at which: (a) the break point in the relationship between CO_2 production ($\dot{V}CO_2$) and $\dot{V}O_2$ ('V-slope' technique, ((Beaver *et al.*, 1986)) occurred and (b) the ventilatory equivalent for O_2 ($\dot{V}E/\dot{V}O_2$) started to increase systematically without a concomitant increase in ventilatory equivalent for CO_2 ($\dot{V}E/\dot{V}CO_2$). After a 5 min warm-up at 20 W the WR was gradually increased at a rate of $15\text{ W}\cdot\text{min}^{-1}$ using an electrically braked cycle ergometer (Excalibur Sport, Lode, The Netherlands) until cadence could no longer be maintained above $50\text{ revs}\cdot\text{min}^{-1}$. Respired volumes were measured with a bi-directional turbine transducer (VMM; Alpha Technologies, Laguna Niguel, CA, U.S.A.) calibrated with a 3 L syringe using a range of different flow profiles (Hans Rudolph, Kansas City, MO, U.S.A.). Respired gas concentrations were measured every 20 ms by a quadruple mass spectrometer (QP9000: Morgan Medical, Gillingham, Kent, U.K.), which was calibrated against precision-analysed gas mixtures. Barometric pressure was measured using a standard mercury

barometer. From these measurements 63% of each subject's WR_{\max} was calculated and used in all subsequent experimental trials.

2.3 Dietary analysis

Subjects followed their normal diet and weighed all food and drink consumed during each supplementation period using digital weighing scales readable to 1 g. The diet was analysed for energy intake and macronutrient content using a computerised version of McCance & Widdowson's food composition tables as revised by Holland *et al.* (1991). Subjects were asked to minimise caffeine intake to lessen any possible confounding effects of caffeine on muscle Cr loading (Vandenberghe *et al.*, 1996).

2.4 Experimental exercise trials (Chapters 3(a), 4(a) and 5)

Prior to the first experimental trial, familiarisation trials were completed until the variability of two consecutive trials was within 5%. Subjects reported to the laboratory on each of day of exercise testing following a 3 hour fast and having refrained from alcohol, caffeine and strenuous exercise the day before. Upon arrival at the laboratory, height and nude body mass were measured and body water compartments estimated using a Bodystat Multiscan 5000 Bioimpedance analyser (Bodystat Ltd, Isle of Man). This method allows TBW and ECW to be estimated; from these measurements ICW can also be deduced. The bioimpedance measurements were taken while the subjects lay comfortably in a supine position for 10 min on a non-conductive surface with their arms and legs slightly abducted. Two electrodes were attached to the right hand (one behind the knuckles and one on the wrist next to the ulnar head) and two attached to the right foot (one behind the toes and one between the lateral and medial malleoli). A current with alternating frequency was then passed between the electrodes on the hand and foot and the resistance calculated. The resistance to each current was then used to calculate TBW and ECW using the equation described previously (Chapter 1). There is good evidence to suggest that the estimation of TBW by bioimpedance is reliable and valid when subjects are euhydrated (O'Brien *et al.*, 2002). To date, several studies have successfully utilised this technique in order to estimate hyperhydration induced changes in TBW (Kern *et al.*, 2001; Kilduff *et al.*, 2002; Kilduff *et al.*, 2004). Furthermore, the change in body mass from pre- to post-supplementation was determined to provide a further indirect measurement of the volume of fluid retained. Following the bioimpedance measurement, a flexible rectal thermistor was inserted 10 cm beyond the anal sphincter to measure T_{re} , an index of T_c and a heart rate monitor (Polar

Sports Tester, Polar Electro Oy, Kempele, Finland) was attached. The subject's right hand and forearm were immersed in water at 42-44°C for 15 min in order to allow for arterialisation of the venous blood (Forster *et al.*, 1972). Following this, a 21G cannula was introduced into a superficial vein on the dorsal surface of the heated hand. The subject was transferred to the climatic chamber (ambient temperature $30 \pm 1^\circ\text{C}$ with a relative humidity of $70 \pm 3\%$ and air velocity of approximately $1.8 \text{ m}\cdot\text{s}^{-1}$) and seated on the cycle ergometer for 5 min. During this period, thermistors (C8600 10 channel microprocessor, Comark, Hertfordshire, U.K.) were attached to the subject's chest, tricep, thigh and calf for the determination of weighted mean T_{sk} (Ramanathan, 1964).

The subject remained seated on the cycle ergometer for a further 1 min while resting heart rate, T_{re} , T_{sk} were determined and a blood sample (10 ml) obtained (Figure 2.1). The venous cannula was kept patent by a 10 ml flush of isotonic saline between samples. Subjects were then instructed to begin 5 min of unloaded cycling before the WR was increased in a 'single step' to the predetermined 63% WR_{max} . Subjects were required to maintain a pedal cadence of 70-100 $\text{revs}\cdot\text{min}^{-1}$ for 40 min. Measurements of heart rate, T_{re} and T_{sk} were obtained at 5 min intervals throughout the 40 min period and the time trial. Blood samples (10 ml) were obtained every 10 min during the constant-load exercise and upon completion of the time trial. One min expired gas collections were made every 10 min of the constant-load exercise and analysed within 5 min for the determination of $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}E$. Subjects were required to consume $2.14 \text{ ml water}\cdot\text{kg}^{-1}$ body mass (e.g. 150 ml for 70 kg subject) every 10 min throughout the 40 min constant-load exercise (Convertino *et al.*, 1996). Ratings of perceived leg fatigue and dyspnoea were recorded every 5 min of the constant-load exercise and at the end of the time trial using the Borg category scale (Borg, 1982). On completion of the 40 min period, WR was decreased to 20 W and the subject asked to maintain cadence for 1 min. After a further 4 min rest period, the subject was instructed to complete a 16.1 km (10 mile) self-paced time trial on a road-mounted cycle (King Cycle Indoor Trainer, Buckinghamshire, U.K.). After exercise, nude body mass was measured and the difference before and after exercise was calculated and subsequently used to estimate sweat rate (change in body mass divided by the total exercise time) and sweat loss (total change in body mass), after correcting for respiratory water loss and substrate oxidation (Mitchell *et al.*, 1972). The time trial completion time was recorded but withheld from the subject until all exercise tests had been completed.

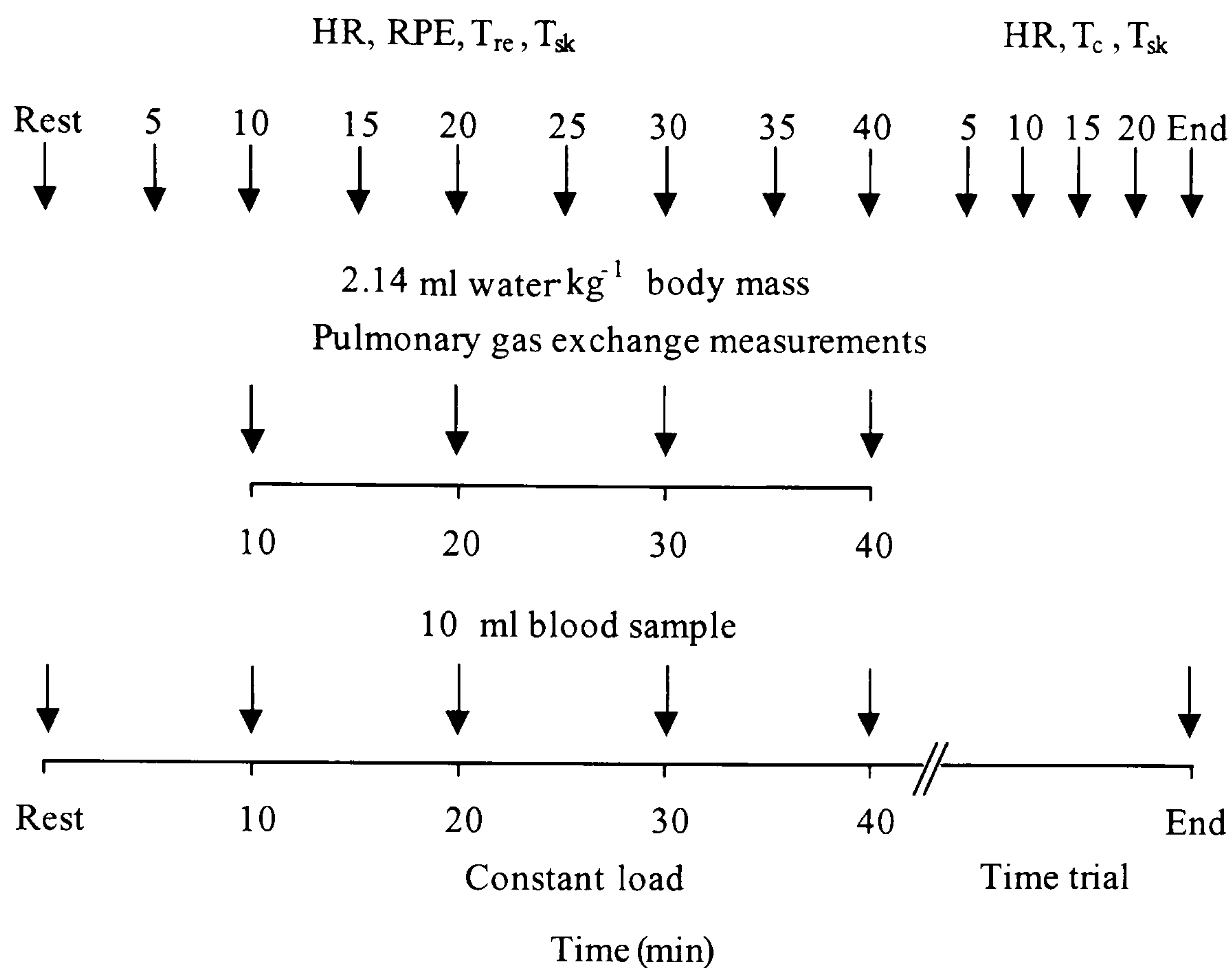


Figure 2.1. Schematic representation of the experimental protocol (Chapters 3(a), 4(a) and 5).

2.5 Blood treatment and analysis

Blood was drawn into dry syringes and 6 ml dispensed into a tube containing K_3EDTA and the remaining 4 ml dispensed into tubes without anticoagulant. Duplicate aliquots (400 μl) of whole blood from the K_3EDTA tube was rapidly deproteinised in 800 μL of ice cold 0.3 $\text{mol}\cdot\text{L}^{-1}$ perchloric acid, centrifuged and the supernatant used for the measurement of glucose and lactate using standard enzymatic methods with spectrophotometric detection (Mira Plus, ABX Diagnostics, Montpellier, France) (Maughan, 1982). A further aliquot of blood was centrifuged and the plasma obtained was separated and used for the measurement of Gly (Boobis & Maughan, 1983). The blood in tubes without anticoagulant was allowed to coagulate and then centrifuged; the serum collected was used for the measurement of osmolality by freezing point depression (Micro-osmometer 3300, Vitech Scientific, West Sussex, U.K.) (Chapters 4 and 5). The blood from the K_3EDTA tubes was also analysed for haemoglobin (Hb) (cyanmethaemoglobin method, Sigma, Chemical Company Ltd., Dorset, U.K.) and packed cell volume (PCV) (conventional microhematocrit method). All blood analyses were carried out in duplicate with the exception of PCV, which was carried out in triplicate. Plasma volume changes during exercise were calculated from changes in Hb and PCV relative to initial resting values as described by Dill & Costill (1974).

2.6 Statistical analysis

Data were expressed as the mean \pm standard deviation (s.d.) following a test for the normality of distribution. Statistical analysis was carried out using three factor mixed model ANOVA with repeated measures, followed by a simple main effects analysis for significant three way interactions (i.e. pre- vs. post-supplementation at each combination of time point and treatment) and simple main effects analysis for two way interactions (Chapters 3(a), 4(a) and 5). In addition, the magnitude of change (Δ) between experimental trials (Pl/Pl, Pl/Gly, Cr/Pl and Cr/Gly) was examined using either a two-sample or a paired *t*-test when significance was identified using the simple main effects analysis. Pearson's product moment correlation coefficient (*r*) was used to assess the relationship between methods of T_c measurement (Chapter 6). The limits of agreement (LOA) between T_c measurement methods were investigated by plotting the individual differences between methods against their respective means (Bland-Altman plots) (Bland & Altman, 1986). Heterocedasticity was examined by plotting the absolute (positive) differences against the individual means and calculating the correlation coefficient (Bland & Altman, 1986). If the heterocedasticity correlation was close to zero and the differences were normally distributed (Shapiro-Wilk test), the mean bias and 95% LOA were calculated as mean \pm 1.96 s.d. of the between method difference (Bland & Altman, 1986). Further analysis was carried out using a two factor ANOVA with repeated measures. In addition, the difference in T_c measurement at each time point was examined using paired-sample *t*-tests when significance was identified using the simple main effects analysis. Statistical power calculations (80% power) were carried out using the Δ TBW data obtained. Statistical significance was declared at $P \leq 0.05$.

The intra-assay coefficient of variation (C.V.) was calculated from the s.d. of the difference between double measurements of the sample expressed as a percentage of the total mean sample (Table 2.1).

Table 2.1 Coefficient of variation of blood and serum assays

| Assay | Method | n | C.V. |
|----------------|---------------------------|----|------|
| Blood glucose | Maughan 1982 | 50 | 1.3 |
| Blood lactate | Maughan 1982 | 50 | 2.3 |
| Blood glycerol | Boobis & Maugan 1983 | 50 | 4.9 |
| Osmolality | Freezing point depression | 50 | 0.2 |
| Blood Hb | Colorimetric method | 50 | 0.4 |
| PCV | Microhaematocrit method | 50 | 0.7 |

Chapter 3

(a) The effects of combined creatine and glycerol hyperhydration on metabolism, thermoregulation and exercise performance in the heat: Loading protocol 1.

3.1 Introduction

The possible advantages of hyperhydration over euhydration during exercise in the heat have been extensively debated without clear consensus (Anderson *et al.*, 2001; Kilduff *et al.*, 2004; Latzka *et al.*, 1998; Lyons *et al.*, 1990; Montner *et al.*, 1996; Murray *et al.*, 1991). The rationale for hyperhydration stems from the traditionally accepted ‘cardiovascular model’ of dehydration which contends that fluid loss during exercise reduces plasma volume and consequently stroke volume and increases heart rate (‘cardiac drift’) in order to maintain cardiac output (Ekelund, 1967). During strenuous exercise in the heat the increase in heart rate may at times be insufficient to compensate for the decrease in stroke volume and consequently maximal cardiac output is reduced (Gonzalez-Alonso *et al.*, 1995). A significant linear relationship has been reported between the reduction in skin blood flow and the level of dehydration (Montain & Coyle, 1992a). Therefore, according to the ‘cardiovascular model’ of dehydration, a reduction in cutaneous blood flow can impede heat exchange and reduce the temperature regulating capacity of the body (Fortney *et al.*, 1984). These physiological responses that occur in response to dehydration of over 2% of body mass have also been associated with a reduction in exercise performance (Cheuvront *et al.*, 2005). If the ‘cardiovascular model’ holds true, then maintenance of blood volume and/or expansion of plasma volume should result in the preservation of cardiovascular and thermoregulatory function and the improvement of exercise performance in the heat, a matter of much research interest (Watt *et al.*, 2000). One approach has been to maintain plasma volume during exercise in the heat by infusion of isotonic saline. Using this method, Fortney *et al.* (Fortney *et al.*, 1988) found an attenuated rise in T_{c} during exercise in the heat that they attributed to a maintenance of central blood volume resulting in an increase in skin blood flow and associated convective heat loss. Several other studies using acute plasma volume expansion with either saline or dextran infusions reported an attenuation in the rise in heart rate and T_{c} e.g. (Deschamps *et al.*, 1989). However, the finding of similar forearm blood flow during hypervolemia in the study by Watt *et al.* (2000) led these authors to conclude that acute plasma volume expansion did not directly enhance thermoregulation. Other studies have also failed to show any effect of plasma volume expansion on heart rate, T_{c} , skin blood flow or indeed performance during exercise in the heat (Grant *et al.*, 1997).

Hyperhydration prior to exercise by ingestion of water or carbohydrate/electrolyte solutions is less effective than infusion methods at acutely expanding plasma volume as most ‘excess’ fluid ingested is rapidly filtered and excreted by the kidneys (Freund *et al.*,

1995). On the other hand, hydrating agents such as Gly have been shown to effectively minimise the reduction in plasma volume that occurs during exercise in the heat (Murray *et al.*, 1991). Seifert *et al.* (1995) reported a 701 ml increase in mean TBW after Gly ingestion, including a 385 ml increase in interstitial fluid and a 225 ml increase in ICW with the remainder distributed within the plasma. Several studies have now concluded that Gly ingestion reduces T_c and heart rate during exercise in the heat (Anderson *et al.*, 2001; Lyons *et al.*, 1990; Montner *et al.*, 1996) and argue these effects are due to a preservation of blood volume and cutaneous blood flow (Lyons *et al.*, 1990). However, not all studies have shown such effects of Gly on thermoregulation during exercise in the heat (Latzka *et al.*, 1998; Murray *et al.*, 1991). Methodological differences, including the amount of Gly and timing of ingestion prior to exercise, the exercise protocol, ambient conditions, methods used to assess hydration status and T_c are all likely to have contributed to the conflicting results. Ingestion of Cr has also been shown to have substantial hydrating effects (Kern *et al.*, 2001; Kilduff *et al.*, 2002), although the exact mechanisms remain uncertain. However, unlike the whole body hydrating effects of Gly, Cr retains fluid predominantly in the ICW compartments (Kilduff *et al.*, 2002). Like Gly, oral Cr supplementation has been shown to be effective in attenuating the rise in heart rate and T_c during exercise in the heat (Kilduff *et al.*, 2002). These effects have been attributed to an increase in ICW resulting in an increased specific heat capacity of the body (Kern *et al.*, 2001; Kilduff *et al.*, 2002). Supplementation with hydrating agents such as Gly or Cr has consistently produced modest fluid retention of 400 to 800 ml (Kilduff *et al.*, 2002; Montner *et al.*, 1996). However, it seems plausible that a Gly-induced increase in ECW coupled with a Cr-mediated increase in ICW could have additive effects resulting in a much larger increase in TBW than if either supplement was consumed alone. Therefore, the aim of this study was to assess the effects of this novel ‘water-loading’ strategy on thermoregulation and performance during exercise in the heat.

3.2 Methods

3.2.1 Subjects

Six endurance-trained males gave their written informed consent to take part in the present study that was approved by the local Ethics Committee and was performed according to the code of ethics of the World Medical Association (Declaration of Helsinki). The physical characteristics of the six subjects were: age 29 ± 5 years; height 180 ± 6 cm; body mass 79.2 ± 13 kg; $\dot{V}O_{2\max}$ 58 ± 8 ml·kg⁻¹·min⁻¹; WR_{\max} 335 ± 32 W; LT 221 ± 26 W.

3.2.2 Experimental design

The study consisted of two supplementation regimens, each lasting 7 days and encompassing three cycle performance trials consisting of 40 min constant-load exercise at 63% WR_{max} followed by a 16.1 km (10 mile) time trial. The methodology for the exercise trials is described in the general methods section of this thesis. Prior to the first of these experimental trials, familiarisation trials were completed until the variability of two consecutive trials was within 5%; no subject had to perform a third familiarisation trial to achieve less than 5% variability. Following this familiarisation period, subjects performed a pre-supplementation exercise performance trial on experimental day 1 (control) (Fig. 3.1). The subjects were then randomly assigned into two groups (A and B). Subjects in group A received Pl supplementation in week 1 before crossing over and receiving Cr and Gly in the second week whereas subjects in group B supplemented in the opposite order (Fig. 3.1). However, due to the long wash out period associated with Cr supplementation (Vandenbergh *et al.*, 1996), subjects receiving Pl in the second week ($n=2$) were excluded from the analysis. Subjects in both groups performed an exercise trial post-supplementation during both supplementation regimens (on experimental days 8 and 15) (i.e. a total of 3 experimental exercise trials) (Fig. 3.1). The control trial was conducted at least 48 hours after each subject's final familiarisation trial. Each supplementation period started on the day after the first test and finished on the day of the second test.

Cr/Gly supplementation consisted of 11.4 g of $Cr \cdot H_2O$ (equivalent to 10 g Cr) and 70 g of glucose polymer made up in 1 L of warm water and consumed twice daily for 6 days and a bolus of 1 g $Gly \cdot kg^{-1}$ body mass diluted in 500 ml of water with 125 ml unsweetened diluting juice 2 hours prior to the start of the experimental trial. This Cr supplementation protocol has been shown to increase resting muscle phosphocreatine levels within 5 days (Harris *et al.*, 1992). Each supplement was made fresh prior to consumption in order to prevent any degradation of Cr to creatinine. The Pl supplement consisted of 85 g of glucose polymer made up in 1 L of warm water and consumed twice daily for 6 days and a bolus of 500 ml of water with 125 ml unsweetened diluting juice 2 hours prior to the assessment. All supplements had similar taste, texture and appearance and were placed in generic water bottles to ensure double blind administration. On each of the assessment days subjects ingested a further 500 ml of water 1 hour prior to the assessment in an attempt to ensure subjects were euhydrated (Convertino *et al.*, 1996).

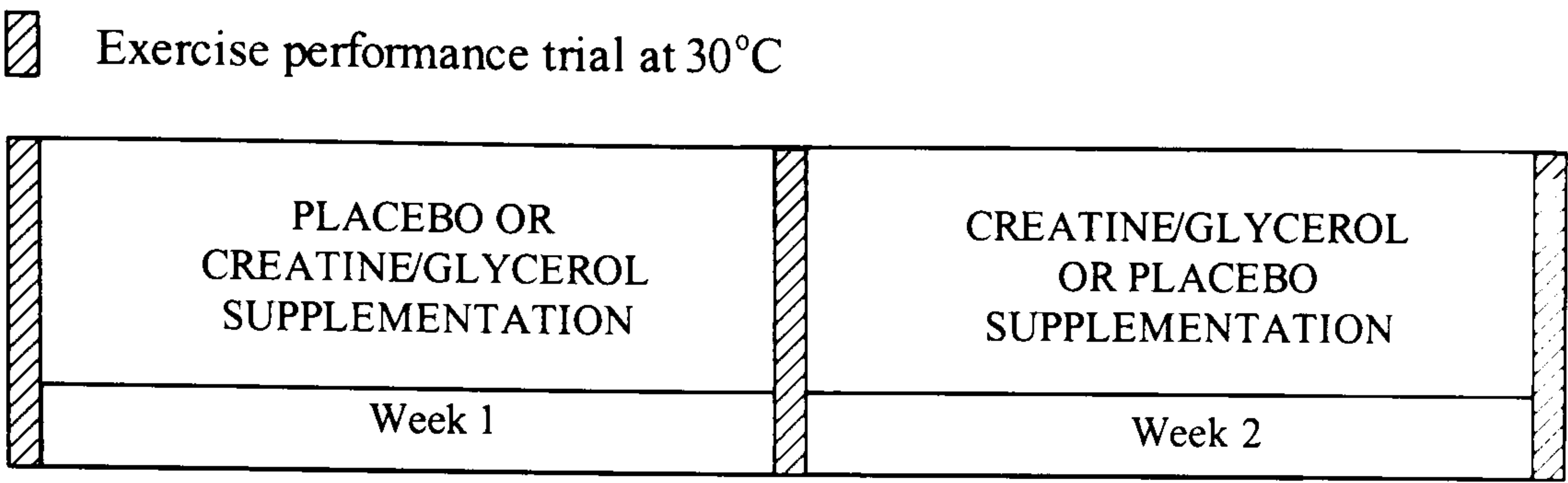


Figure 3.1 Schematic representation of the experimental design.

3.3 Results

3.3.1 Diet, Body mass and water compartments.

There was no significant change in body mass from pre- to post-supplementation following the Pl supplementation regimen (P=0.29). Following Cr/Gly supplementation, body mass increased significantly from pre-supplementation (P<0.01), which was significantly greater than the rise in the Pl group (P=0.04) (Fig. 3.2). There were no significant changes in TBW, ECW or ICW following either Pl or Cr/Gly supplementation (Fig. 3.2). There were no significant differences in the daily diet between the two supplementation regimens (Pl: 13.6 ± 4.1 MJ·day⁻¹, 59 ± 14% carbohydrate, 26 ± 10% fat, 15 ± 7% protein; Cr/Gly: 13.0 ± 2.6 MJ·day⁻¹, 63 ± 8% carbohydrate 25 ± 7% fat, 12 ± 5% protein.

3.3.2 Cardiopulmonary variables.

There was a steady increase in $\dot{V}O_2$, $\dot{V}CO_2$ and $\dot{V}E$ throughout the constant-load exercise with no differences between pre- and post-supplementation in either supplementation regimen (Table 3.1). Respiratory exchange ratio (RER) did not change throughout the constant-load exercise period with no differences between pre- and post-supplementation in either supplementation regimen (Table 3.1). There was no difference in resting heart rate between any of the exercise trials (Fig. 3.3). During exercise, heart rate increased during all trials. There were no differences in heart rate during exercise between pre-supplementation and post-Pl supplementation (P=0.21) or Cr/Gly supplementation (P=0.23) (Fig. 3.3).

3.3.3 Ratings of perceived exertion

During exercise, RPE for both dyspnoea and leg fatigue increased during all trials (Fig. 3.4). There were no differences in RPE for dyspnoea during exercise between pre-supplementation and post- Pl supplementation (P=0.42) or Cr/Gly supplementation (P=0.31) (Fig. 3.4). Similarly, there was no change in RPE for leg fatigue from pre- to post- Pl supplementation (P=0.12) or Cr/Gly supplementation (P=0.19).

Table 3.1 Cardiopulmonary responses during constant-load exercise.

| Regimen | Exercise time (min) | | | |
|---|---------------------|---------------|---------------|---------------|
| | 10 | 20 | 30 | 40 |
| VO₂ (L·min⁻¹) | | | | |
| Control | 3.03 ± 0.26 | 3.14 ± 0.21 | 3.23 ± 0.22 | 3.40 ± 0.31 |
| Pl | 3.04 ± 0.26 | 3.10 ± 0.17 | 3.20 ± 0.16 | 3.29 ± 0.15 |
| Creatine/Glycerol | 3.05 ± 0.22 | 3.20 ± 0.19 | 3.24 ± 0.14 | 3.30 ± 0.13 |
| VCO₂ (L·min⁻¹) | | | | |
| Control | 2.64 ± 0.20 | 2.74 ± 0.19 | 2.83 ± 0.18 | 2.96 ± 0.22 |
| Pl | 2.69 ± 0.15 | 2.77 ± 0.18 | 2.82 ± 0.22 | 2.85 ± 0.30 |
| Creatine/Glycerol | 2.66 ± 0.23 | 2.76 ± 0.23 | 2.78 ± 0.16 | 2.83 ± 0.19 |
| V_E (L·min-1) | | | | |
| Control | 63.73 ± 9.14 | 69.70 ± 11.34 | 73.07 ± 10.58 | 78.15 ± 11.96 |
| Pl | 62.83 ± 8.64 | 68.49 ± 11.47 | 69.40 ± 7.51 | 72.00 ± 4.25 |
| Creatine/Glycerol | 62.23 ± 5.31 | 65.01 ± 4.25 | 67.74 ± 4.99 | 71.73 ± 6.19 |
| RER | | | | |
| Control | 0.87 ± 0.06 | 0.87 ± 0.06 | 0.88 ± 0.05 | 0.87 ± 0.06 |
| Pl | 0.89 ± 0.02 | 0.89 ± 0.03 | 0.87 ± 0.01 | 0.85 ± 0.02 |
| Creatine/Glycerol | 0.88 ± 0.02 | 0.86 ± 0.03 | 0.87 ± 0.03 | 0.86 ± 0.03 |

Data presented as the mean ± s.d.

3.3.4 Rectal and skin temperature responses

There was no difference in resting T_{re} between the two groups or after supplementation (Fig. 3.5). Throughout the exercise period, T_{re} increased significantly during all trials (Fig. 3.5). There were no differences in T_{re} during exercise between pre-supplementation and post-Pl supplementation (P=0.32) or Cr/Gly supplementation (P=0.12) (Fig. 3.3). There

was a significant increase in mean T_{sk} from rest, with no significant differences following supplementation (Fig. 3.5).

3.3.5 Sweat rates and total sweat loss during exercise

There were no differences in sweat rate between trials (Control: $2.0 \pm 0.2 \text{ L}\cdot\text{hr}^{-1}$; Pl: $1.7 \pm 0.6 \text{ L}\cdot\text{hr}^{-1}$; Cr/Gly: $2.1 \pm 0.5 \text{ L}\cdot\text{hr}^{-1}$, $P=0.20$). Furthermore, total sweat loss was not different between trials (Control: $2.1 \pm 0.3 \text{ L}$; Pl: $1.8 \pm 0.6 \text{ L}$; Cr/Gly: $2.1 \pm 0.5 \text{ L}$, $P=0.31$).

3.3.6 Blood metabolite concentrations and plasma volume changes

Resting blood [glucose] and [lactate] were not different between experimental trials ($P=0.56$ and $P=0.32$ respectively) (Table 3.2). Briefly, blood [glucose] decreased significantly from rest to initiation of exercise before rising gradually throughout the constant-load exercise and peaking at the end of the time trial. There were no differences in blood [glucose] during exercise between experimental trials ($P=0.26$) (Table 3.2). The initial increase in blood [lactate] from rest to initiation of exercise was maintained until the end of the constant-load period. There was a further significant increase in blood [lactate] between the constant-load exercise and the end of the time trial. There were no differences in blood [lactate] during exercise between experimental trials ($P=0.28$) (Table 3.2). Resting plasma [Gly] was significantly higher post Cr/Gly supplementation compared to pre-supplementation ($P<0.01$) (Table 3.2). Plasma [Gly] remained significantly higher throughout exercise after Cr/Gly supplementation compared to the pre-supplementation trial. There was no difference in resting plasma [Gly] or during exercise between pre- and post-supplementation during the Pl supplementation regimen ($P=0.69$) (Table 3.2). Plasma volume was reduced by approximately 8% after 40 min of constant-load exercise and 13% after the 16.1 km time trial with no significant differences between experimental trials. Resting plasma volume changes following supplementation were also calculated using the control trial as a baseline, assuming no change in red cell mass during the 7 day supplementation regimen. Using this method of analysis, plasma volume was not significantly altered by either supplementation regimen, although there was a tendency ($P=0.07$) for plasma volume to be reduced ($\sim 3\%$) after Cr/Gly supplementation.

3.3.7 Time trial performance

Time trial performance did not differ significantly between experimental trials (Control: $22.40 \pm 1.1 \text{ min}$; Pl: $22.33 \pm 0.92 \text{ min}$; Cr/Gly: $22.13 \pm 0.71 \text{ min}$).

3.3.8 Side effects

In general, subjects tolerated the supplementation protocol well, with only one report of gastrointestinal distress and one report of muscle cramping (gastrocnemius) during the Cr/Gly supplementation week. Two subjects identified the supplementation they were receiving due to prior knowledge of the side effects while all other subjects were unsure of the treatment they received.

Table 3.2 Plasma [glucose], [lactate] and [glycerol] (mmol·L⁻¹) during exercise.

| Regimen | Exercise time (min) | | | | | End TT |
|---------------------------------------|---------------------|----------------|----------------|----------------|---------------|---------------|
| | Rest | 10 | 20 | 30 | 40 | |
| [Glucose] mmol·L⁻¹ | | | | | | |
| Control | 4.69 ± 0.42 | 4.18 ± 0.63 | 4.22 ± 0.60 | 4.37 ± 0.53 | 4.50 ± 0.59 | 4.70 ± 0.62 |
| PI | 4.93 ± 0.61 | 4.32 ± 0.92 | 4.48 ± 0.98 | 4.57 ± 0.86 | 4.83 ± 0.66 | 5.08 ± 0.78 |
| Creatine/Glycerol | 4.63 ± 0.57 | 4.27 ± 0.51 | 4.43 ± 0.53 | 4.57 ± 0.55 | 4.71 ± 0.65 | 5.04 ± 0.63 |
| [Lactate] mmol·L⁻¹ | | | | | | |
| Control | 0.77 ± 0.30 | 3.50 ± 0.96 | 4.19 ± 1.01 | 4.55 ± 1.11 | 4.76 ± 1.22 | 8.03 ± 1.85 |
| PI | 0.86 ± 0.48 | 3.86 ± 1.25 | 3.99 ± 1.13 | 4.15 ± 1.57 | 4.51 ± 1.51 | 8.22 ± 2.44 |
| Creatine/Glycerol | 0.91 ± 0.38 | 3.98 ± 1.21 | 3.99 ± 1.37 | 4.09 ± 1.76 | 4.40 ± 1.66 | 8.13 ± 2.43 |
| [Glycerol] mmol·L⁻¹ | | | | | | |
| Control | 0.05 ± 0.02 | 0.19 ± 0.09 | 0.22 ± 0.10 | 0.30 ± 0.11 | 0.31 ± 0.12 | 0.44 ± 0.18 |
| PI | 0.05 ± 0.02 | 0.18 ± 0.07 | 0.23 ± 0.15 | 0.33 ± 0.13 | 0.38 ± 0.17 | 0.50 ± 0.22 |
| Creatine/Glycerol | 11.04 ± 3.22 † | 10.56 ± 3.04 † | 10.22 ± 2.99 † | 10.08 ± 3.02 † | 9.97 ± 3.11 † | 9.01 ± 2.89 † |

Data presented as the mean ± s.d. †: indicates a significant difference pre- vs post-supplementation.

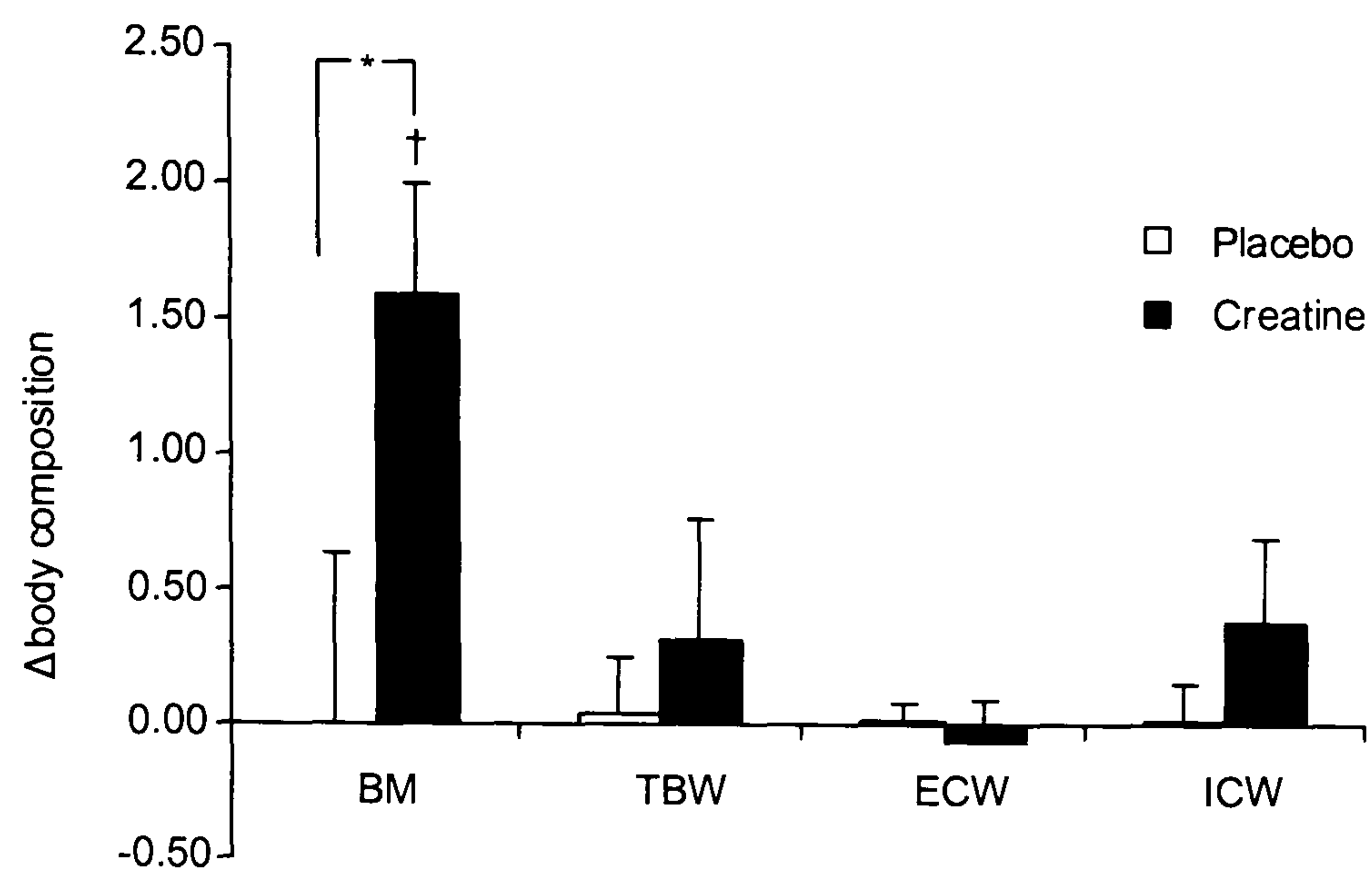


Figure 3.2 Changes in body mass (BM), total body water (TBW), intra-cellular water (ICW) and extra-cellular water (ECW) in the two groups. Data presented as the mean \pm s.d. †: indicates a significant difference pre- vs. post-supplementation. *: indicates a significant greater change (Δ) in the Cr/Gly supplementation regimen compared to the Pl supplementation regimen.

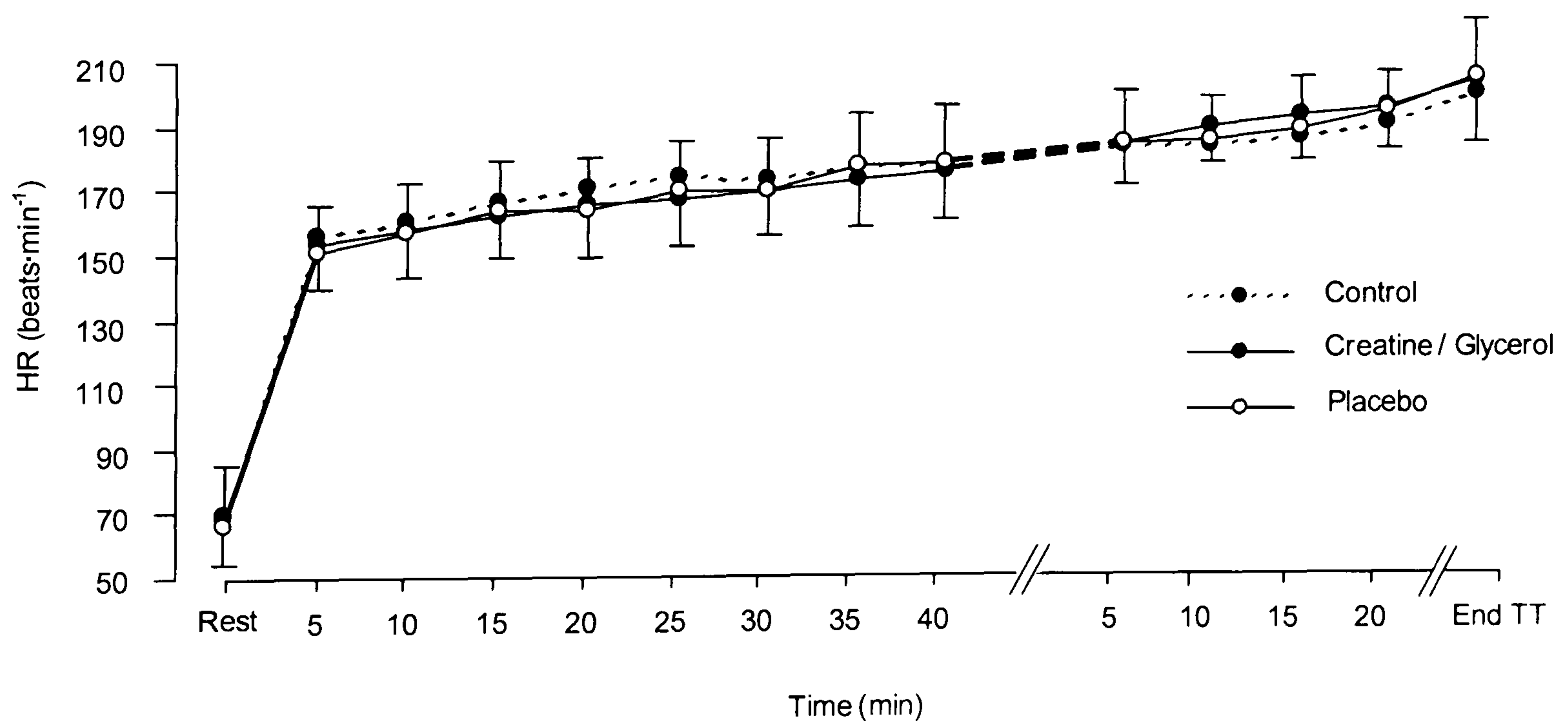


Figure 3.3 Heart rate during exercise. Data presented as the mean \pm s.d.

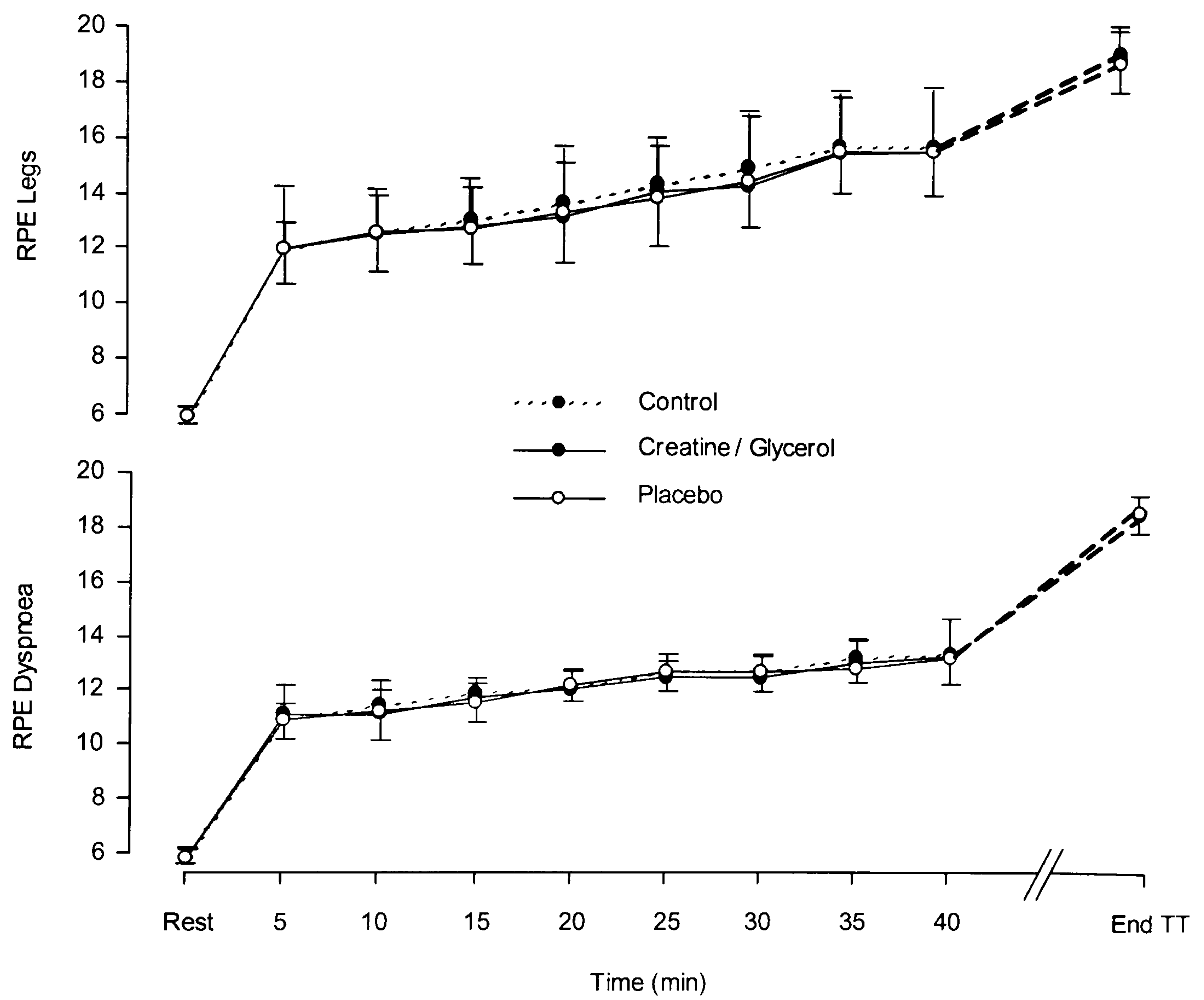


Figure 3.4 RPE for perceived leg fatigue and dyspnoea during exercise. Data presented as the mean \pm s.d.

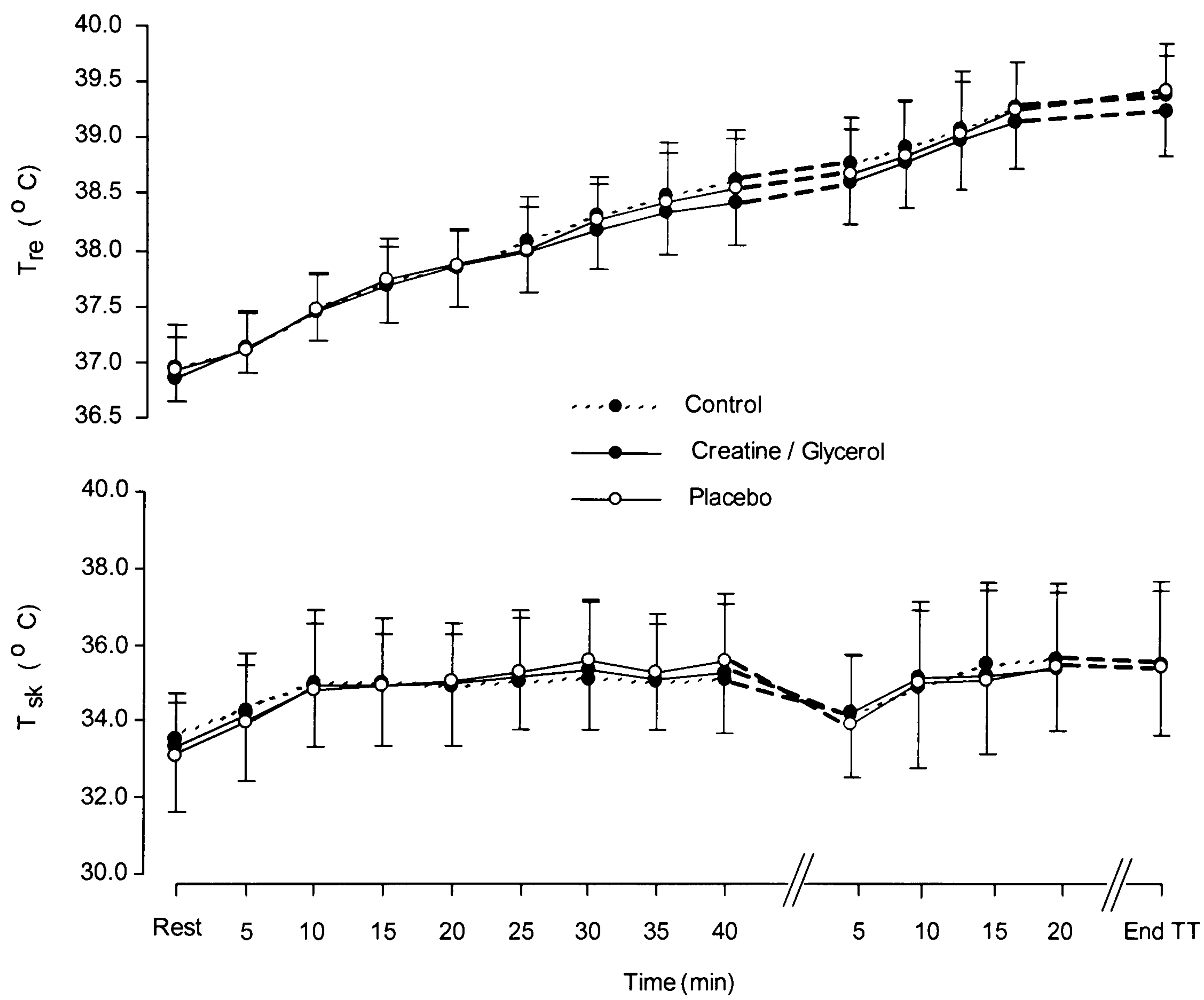


Figure 3.5 Rectal and mean skin temperature during exercise. Data presented as the mean \pm s.d..

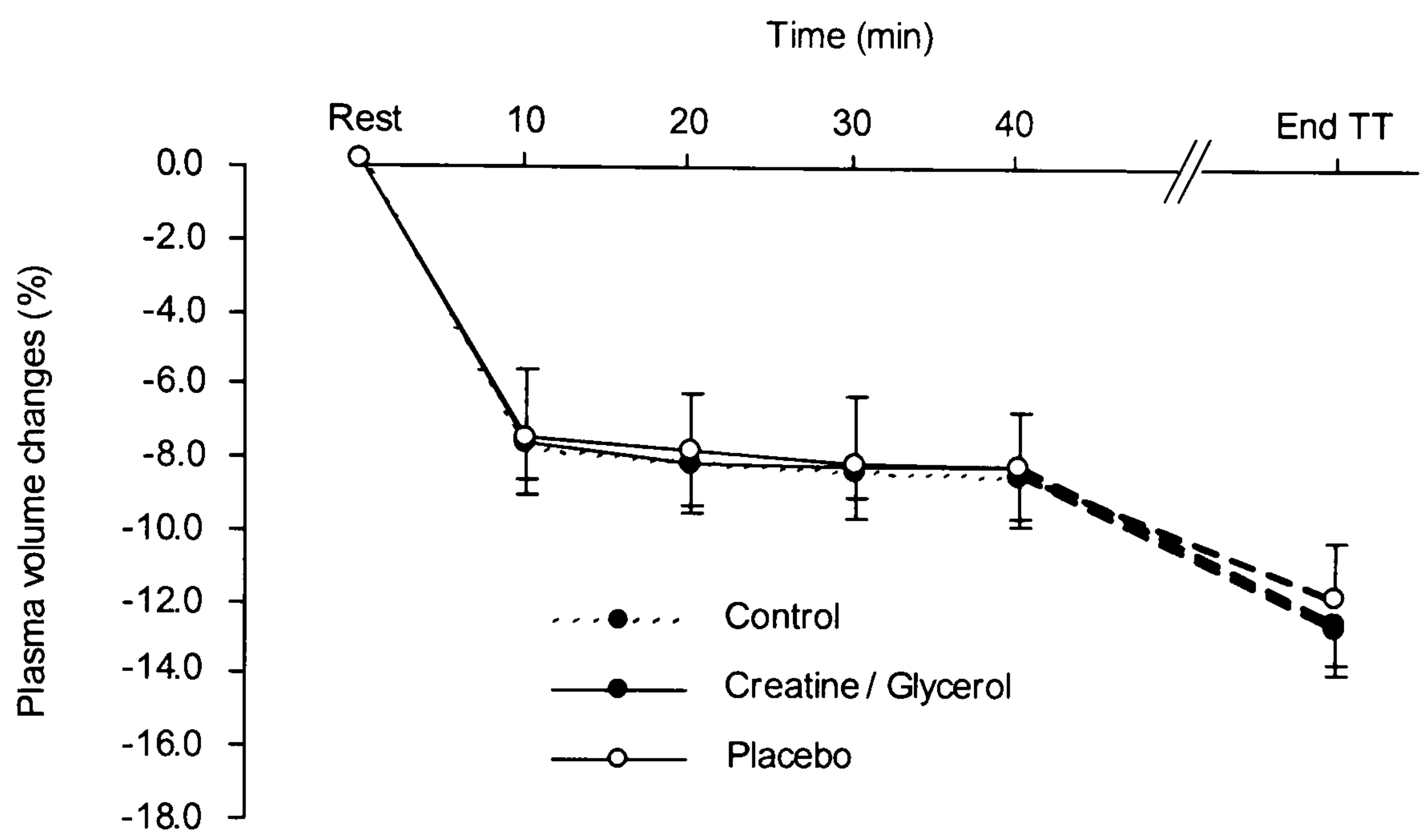


Figure 3.6 Percentage changes in plasma volume during exercise before. Data presented as the mean \pm s.d.

3.4 Discussion

This study has demonstrated that supplementation with a combination of a Cr and Gly resulted in a significant increase in body mass (Fig. 3.2). Despite this, neither Pl nor Cr/Gly supplementation resulted in any change in TBW, ECW, ICW (Fig. 3.2), perception of effort (Fig. 3.4), cardiovascular (Fig. 3.3) or thermoregulatory (Fig. 3.5) responses during exercise in the heat. Furthermore, neither supplementation protocol had any effect on exercise performance.

The mean body mass increase of 1.59 kg induced by combined Cr and Gly supplementation in the present study is among the highest reported in the literature to date (Anderson *et al.*, 2001; Kilduff *et al.*, 2002; 2004; Kreider *et al.*, 1998; Lyons *et al.*, 1990; Montner *et al.*, 1996; Ziegenfuss *et al.*, 1998). However, it is puzzling as to why Cr/Gly supplementation resulted in a significant increase in body mass without the expected concomitant increase in TBW as consistently observed in previous studies with either Cr or Gly supplementation (Kern *et al.*, 2001; Kilduff *et al.*, 2002; 2004; Lyons *et al.*, 1990; Montner *et al.*, 1996). A statistical power calculation using the mean change in TBW from pre- to post-Cr/Gly supplementation (0.32 L) revealed that 77 subjects would be required to identify a significant difference in TBW at 80% power (with 6 subjects a significant increase in TBW would only be observed if the mean increase in TBW exceeded 1.1 L). Therefore despite the small increase in TBW, the number of subjects utilised in the present study may be too low to find any significant change in body water parameters following hyperhydration. However, the increase in TBW observed in the present study is considerably lower than that reported in other hyperhydration studies (e.g. Freund *et al.*, 1995; Kern *et al.*, 2001; Kilduff *et al.*, 2004; Lyons *et al.*, 1990) and therefore there must be some other explanation for the observed lack of significant TBW increase. Considering the homogenous diet ingested during the two supplementation periods it is unlikely that the increase in body mass observed in the present study could be attributed to an increase in fat mass. In several previous studies conducted in this laboratory (Kilduff *et al.*, 2002; 2003; 2004), Cr induced body mass gains were attributed to TBW increases. For example Kilduff *et al.* (2004) observed a 0.8 kg increase in body mass following Cr supplementation was primarily accounted for by a 0.6 L increase in TBW. Similar findings have been observed in other Cr supplementation studies where an increase in body mass has been attributed to increases in TBW and in particular ICW compartments (Kreider *et al.*, 1998; Ziegenfuss *et al.*, 1998). Of note, it has been suggested that an increase in body mass of > 0.2 kg identifies a 'responder' to a Cr supplementation loading programme

(Kilduff *et al.*, 2003). The range of body mass increase after Cr/Gly supplementation in the present study was 1.00-1.98 kg suggesting that all 6 subjects were Cr responders. On the other hand, Gly supplementation has been previously shown to increase both ICW and ECW after ingestion owing to the free distribution of Gly in all body water compartments with the exception of cerebral spinal fluid and aqueous humor (Freund *et al.*, 1995; Lin, 1977; Seifert *et al.*, 1995; Tourtellotte *et al.*, 1972). The average peak [Gly] in the present study after Cr/Gly supplementation was $11.04 \text{ mmol}\cdot\text{L}^{-1}$ (range $9.89\text{-}12.92 \text{ mmol}\cdot\text{L}^{-1}$) (Table 3.2), which is similar to the peak concentration reported by Montner *et al.* (1996) ($11.4 \text{ mmol}\cdot\text{L}^{-1}$) and Freund *et al.* (1995) ($13.0 \text{ mmol}\cdot\text{L}^{-1}$) but higher than the concentration reported by Murray *et al.* (1991) ($2.8 \text{ mmol}\cdot\text{L}^{-1}$). Differences in the size of the Gly dose and time between ingestion and measurement are likely to account for the noted differences in [Gly]. Despite the increase in plasma [Gly] observed in the current study, there was no change in plasma volume from pre- to post-supplementation after either Pl or Cr/Gly supplementation and no differences in the exercise induced percentage reduction in plasma volume (Fig. 3.6). The results from the present study clearly indicate that combined Cr and Gly supplementation resulted in a significant increase in water retention that was not measured by bioimpedance analysis. Whether this was due to a limitation in the ability of multifrequency bioimpedance to accurately measure acute changes in TBW or whether the Gly administered prior to exercise had in some way negated any positive increases in TBW gained from Cr supplementation remains uncertain. However, based on the evidence of the present study it is hypothesised that when Gly and Cr are consumed in unison there is some unknown negative interaction on the immediate fluid retaining abilities of both hyperhydrating agents. Clearly, further research is required to investigate the mechanism behind this conflict and whether a supplementation protocol can be designed such that this negative interaction is bypassed.

Combined Cr and Gly supplementation in the present study was unsuccessful in attenuating the rise in perceived exertion and metabolic, cardiovascular and thermoregulatory responses during constant-load exercise in the heat (Figs. 3.3, 3.4, 3.5) (Table 3.2). Previous studies examining the effects of either Gly or Cr supplementation on cardiovascular and thermoregulatory responses during exercise in the heat have been equivocal, with some showing a reduction in heart rate (Anderson *et al.*, 2001; Kilduff *et al.*, 2004; Montner *et al.*, 1996) and T_{c} (Anderson *et al.*, 2001; Kern *et al.*, 2001; Kilduff *et al.*, 2004; Lyons *et al.*, 1990; Seifert *et al.*, 1995) and others finding no such effect (Latzka *et al.*, 1998; Murray *et al.*, 1991). It is well established that dehydration results in an increased heart rate T_{c} during exercise (Montain & Coyle, 1992b) and thus the beneficial effects of hyperhydration are perpetrated by preservation of blood volume resulting in

maintenance of stroke volume and skin blood flow. However, the fact that Cr/Gly supplementation did not result in any significant increase in TBW means there was no mechanism to significantly alter the physiological responses to exercise in the conditions of the present study. Similarly, in a series of studies carried out by Latzka and colleagues no differences were observed in the increase in TBW following either Gly or water ingestion and consequently T_{c} , T_{sk} , sweat rate, cardiac output, blood pressure and heart rate were not different during constant-load exercise in 35°C, 45% relative humidity (Latzka *et al.*, 1997;1998). These findings led the authors to conclude that hyperhydration provides no meaningful physiological advantage over euhydration.

Time trial performance in the present study was not affected by supplementation with either Pl or combined Cr and Gly. Several studies have indicated that the increased heart rate and T_{c} resulting from dehydration can have a negative impact on exercise performance (Below *et al.*, 1995;Cheuvront *et al.*, 2003;Cheuvront *et al.*, 2005;Fallowfield *et al.*, 1996;McConnell *et al.*, 1997;Walsh *et al.*, 1994). For example, Cheuvront *et al.* (2005) determined that hypohydration was associated with an increased T_{c} and heart rate and a significant reduction in work performed during a 30 min cycling time trial, even in a temperate (20°C) environment. Therefore, if dehydration could be minimised then there would potentially be less of an associated reduction in exercise performance. As such, several studies have concluded that hyperhydration is associated with a significant improvement in exercise performance in the heat (Anderson *et al.*, 2001;Hitchins *et al.*, 1999;Kilduff *et al.*, 2004;Montner *et al.*, 1996). Subjects in the studies by Kilduff *et al.* (2004) and Montner *et al.* (1996) were required to cycle submaximally until exhaustion, whereas the studies by Hitchins *et al.* (1999) and Anderson *et al.* (2001) utilised a self paced time trial for 30 and 15 min respectively, to quantify performance. The findings of Hitchins *et al.* (1999) seem particularly surprising given that cardiovascular and thermoregulatory responses during exercise were not different between Gly and water ingestion trials, meaning the authors could provide no explanation for the observed ergogenic effect of Gly. Conversely, other studies find no effect of hyperhydration on exercise performance when compared to euhydration (Latzka *et al.*, 1998;Marino *et al.*, 2003). For example, Marino *et al.* (2003) found Gly hyperhydration had no effect on a 60 min cycling time trial in hot and humid conditions compared to pre-exercise water ingestion. Latzka *et al.* (1998) produced similar findings when subjects were asked to complete treadmill exercise at 55% $\dot{V}O_{2\max}$ until exhaustion. However, these authors also reported that after either Gly or water ingestion, exercise time to exhaustion was significantly greater than if no water had been consumed prior to exercise. Therefore, it

would appear that commencing exercise in a hyperhydrated state may not confer any significant advantage in terms of exercise performance compared to euhydration or indeed modest dehydration (i.e. loss of 2-3% body mass). The results from the present study are compatible with such an idea although further research using a successful Cr/Gly hyperhydration strategy is required before any convincing conclusions can be made.

3.4.1 Conclusions

This study has demonstrated that although supplementation with a combination of a Cr and Gly resulted in a significant increase in body mass there was no change in TBW, ICW, ECW or RPE, heart rate and T_{c} during exercise in the heat compared to pre-supplementation. Given that previous Cr supplementation studies performed in this laboratory have consistently resulted in significant increases in TBW it can be hypothesised that the Gly administered prior to exercise had in some way negated the Cr induced increase in TBW. Further research is required to develop a Cr/Gly supplementation protocol that results in significant increases in TBW and to examine the consequent effects on cardiovascular and thermoregulatory responses and performance during exercise in the heat.

(b) The effects of a novel combined creatine and glycerol fluid loading strategy on fluid retention and distribution.

3.5 Introduction

The associated deleterious effects of dehydration on the thermoregulatory and cardiovascular systems and exercise performance in the heat have resulted in some athletes attempting to fluid-load prior to exercise to offset the development of dehydration. Fluid-loading with water or carbohydrate/electrolyte solutions is not effective as most 'excess' fluid ingested is rapidly filtered and excreted by the kidneys (Freund *et al.*, 1995), so hyperhydrating agents such as Cr or Gly which actively retain fluid, must also be ingested. Oral Cr supplementation has been consistently shown to result in significant increases in TBW, primarily in the ICW compartments (Kilduff *et al.*, 2002; Kilduff *et al.*, 2004; Ziegenfuss *et al.*, 1998) whereas Gly ingestion also retains water, with the fluid dispersed equally between ICW and ECW compartments (Lin, 1977; Seifert *et al.*, 1995). Given the potential of both Cr and Gly to retain fluid it seems plausible that a Gly-induced increase in ECW coupled with a Cr-mediated increase in ICW could have additive effects resulting in a much larger increase in TBW than if either supplement was consumed alone. However, when Cr and Gly were ingested simultaneously, subjects experienced a significant increase in body mass, with no significant change in TBW (Chapter 3 (a)). It was concluded that although combined Cr/Gly supplementation resulted in significant fluid retention, the fluid was not dispersed in body compartments, perhaps due to some unknown negative interaction on the immediate fluid retaining abilities of both hyperhydrating agents. While the vast majority of Gly supplementation studies and that described in Chapter 3 (a) utilised a single Gly bolus delivered 2-3 hour prior to exercise, Koenigsberg *et al.* (1995) have suggested that Gly hyperhydration may be most effective if consumed continually over several days. Indeed, these authors have demonstrated that Gly hyperhydration can be sustained for at least 49 hours when consumed continually (Koenigsberg *et al.*, 1995). Therefore, it is suggested that ingesting both Cr and Gly over several days may be the most effective method of fluid loading as there will be sufficient time for the retained fluid to be dispersed within body compartments. Thus, the aim of this study is to examine the effects of a novel method of Cr and Gly supplementation on fluid retention and distribution in healthy volunteers.

3.6 Methods

3.6.1 Subjects

2 healthy males gave their written informed consent to take part in the present study that was approved by the local Ethics Committee and was performed according to the code of ethics of the World Medical Association (Declaration of Helsinki). The physical characteristics of the two subjects were, subject 1: age 37 years; height 176 cm; body mass 71.5 kg, subject 2: age 21 years; height 171 cm; body mass 64.2 kg.

3.6.2 Experimental design

The study consisted of one Cr/Gly supplementation regimen lasting for 7 days during which physiological measurements were collected daily. The supplementation period started immediately after the first experimental trial (day 1) and finished on the day prior to the final trial (day 7). Cr/Gly supplementation consisted of 11.4 g of Cr·H₂O (equivalent to 10 g Cr), 1 g Gly·kg⁻¹ body mass and 70 g of glucose polymer made up in 1 L of warm water and consumed twice daily for 6 days. This Cr supplementation protocol has been shown to increase resting muscle phosphocreatine levels within 5 days (Harris *et al.*, 1992). Each supplement was made fresh prior to consumption in order to prevent any degradation of Cr to creatinine. At 9 am on each day the subjects reported to the laboratory having refrained from food or water in the previous 8 hours and alcohol and strenuous exercise in the previous 24 hours. Subjects were asked to void before nude body mass was recorded and body water compartments estimated using a Bodystat Multiscan 5000 Bioimpedance analyser (Bodystat Ltd, Isle of Man) (sample A). This method allows TBW and ECW to be estimated; from these measurements ICW can also be deduced. The bioimpedance measurements were taken while the subjects lay comfortably in a supine position for 10 min on a non-conductive surface with their arms and legs slightly abducted. The subject's right hand and forearm were immersed in water at 42-44°C for 15 min in order to allow for arterialisation of the venous blood (Forster *et al.*, 1972). Following this, a 21G cannula was introduced into a superficial vein on the dorsal surface of the heated hand and a baseline blood sample (10 ml) drawn. The line was kept patent with a 10 ml flush of isotonic saline after each blood sample was taken. Immediately after the first blood sample the subject was asked to consume the first litre of Cr/Gly solution. 1 hour and 30 min (when blood Gly concentration is expected to peak following ingestion) after the solution was finished a further 10 ml blood sample was collected and TBW again

estimated by bioimpedance (sample B). The subject was allowed to rest for 2 hours before ingesting the second solution. Again a final 10 ml blood sample was taken 1 hour and 30 min after the solution had been finished and both body mass and TBW measured (sample C). Subjects were asked to refrain from eating or drinking anything during the hours of the experiment each day with the exception of the Cr/Gly solution and from drinking alcohol at any point during the seven days. Blood samples were analysed for [glucose], [Gly], [Hb] and PCV as described in Chapter 2.

3.7 Results

3.7.1 Body mass and water compartments.

There was a progressive rise in body mass from day 1 (baseline) to day 7 in both subjects following Cr/Gly supplementation (Figs. 3.7, 3.8, 3.9). There were also progressive increases in TBW, ECW and ICW in both subjects during the supplementation regimen (Figs. 3.7, 3.8, 3.9). On each individual experimental day, body mass increased between sample A to sample B and was maintained to sample C before decreasing again to sample A on the next experimental day (Figs. 3.7, 3.8). Both TBW and ICW increased progressively between samples A to C on the majority of experimental days before decreasing to sample A on the next day (Figs. 3.7, 3.8). There was a small reduction in ECW between samples A to B before an increase in ECW was observed by sample C (Figs. 3.7, 3.8).

3.7.2 Blood metabolite concentrations and plasma volume changes

Plasma volume changes were calculated using sample A on day 1 as a baseline, assuming no change in red cell mass during the 7 day supplementation regimen. Using this method of analysis, plasma volume increased by approximately 1% following 7 days of Cr/Gly supplementation (Fig. 3.10). On each individual experimental day, there was a small percentage reduction in plasma volume between samples A to B before a small increase between samples B and C and a larger increase to sample A on the next experimental day (Fig. 3.10). Following 7 days of Cr/Gly supplementation there was a small increase in blood [Gly] from 0.06 to 1.23 mmol·L⁻¹ (Fig. 3.10). Blood [Gly] increased to a large extent between samples A and B on each experimental day with a further increase observed by sample C. Blood [Gly] decreased significantly from sample C to sample A on the next

experimental day (Fig. 3.10). Blood [glucose] was not different between day 1 and day 7 of the experimental trial and remained relatively stable throughout each experimental day.

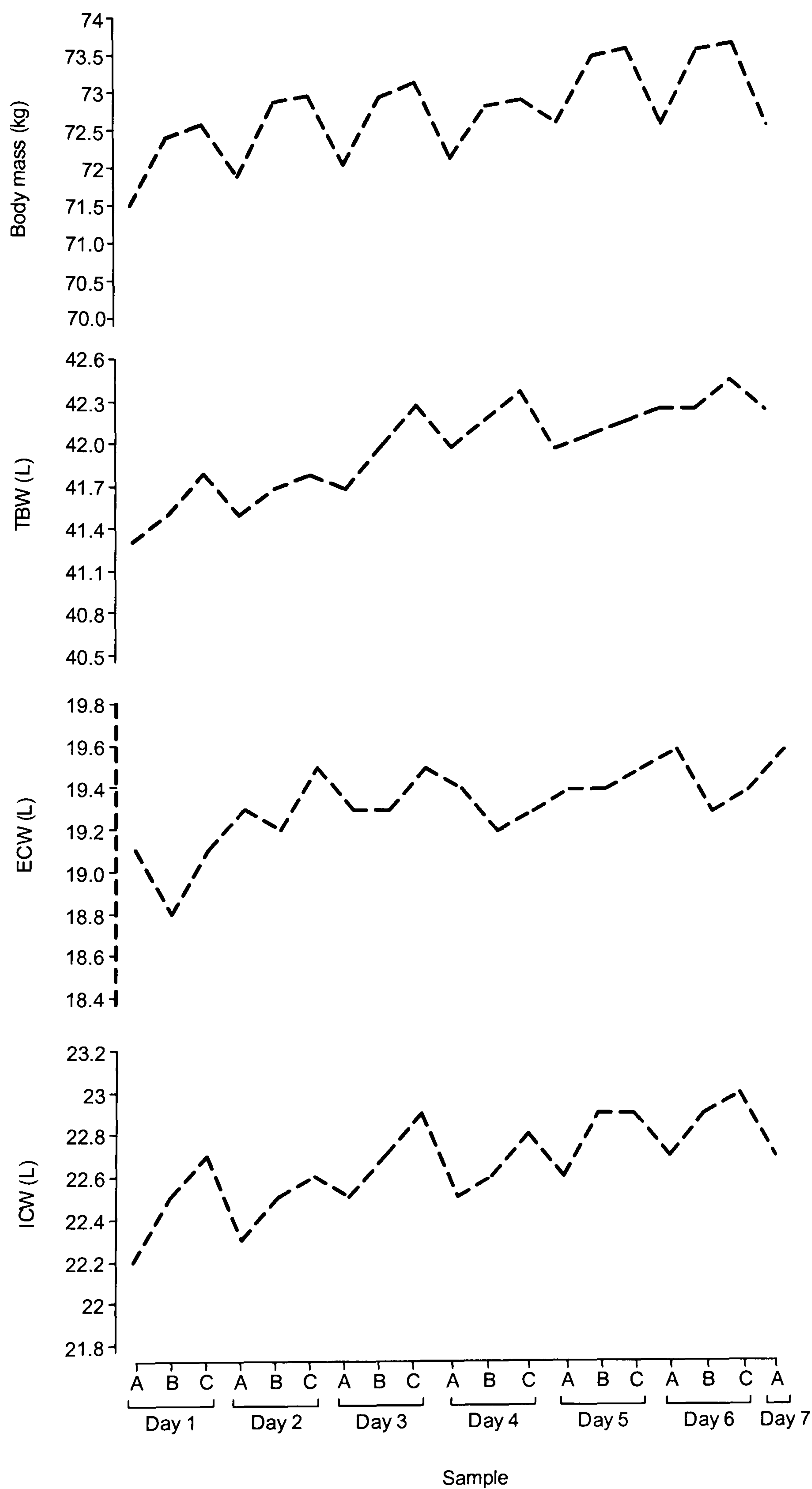


Figure 3.7 Subject 1: Daily changes in body mass, total body water (TBW), intra-cellular water (ICW) and extra-cellular water (ECW) during 7 days of Cr/Gly supplementation.

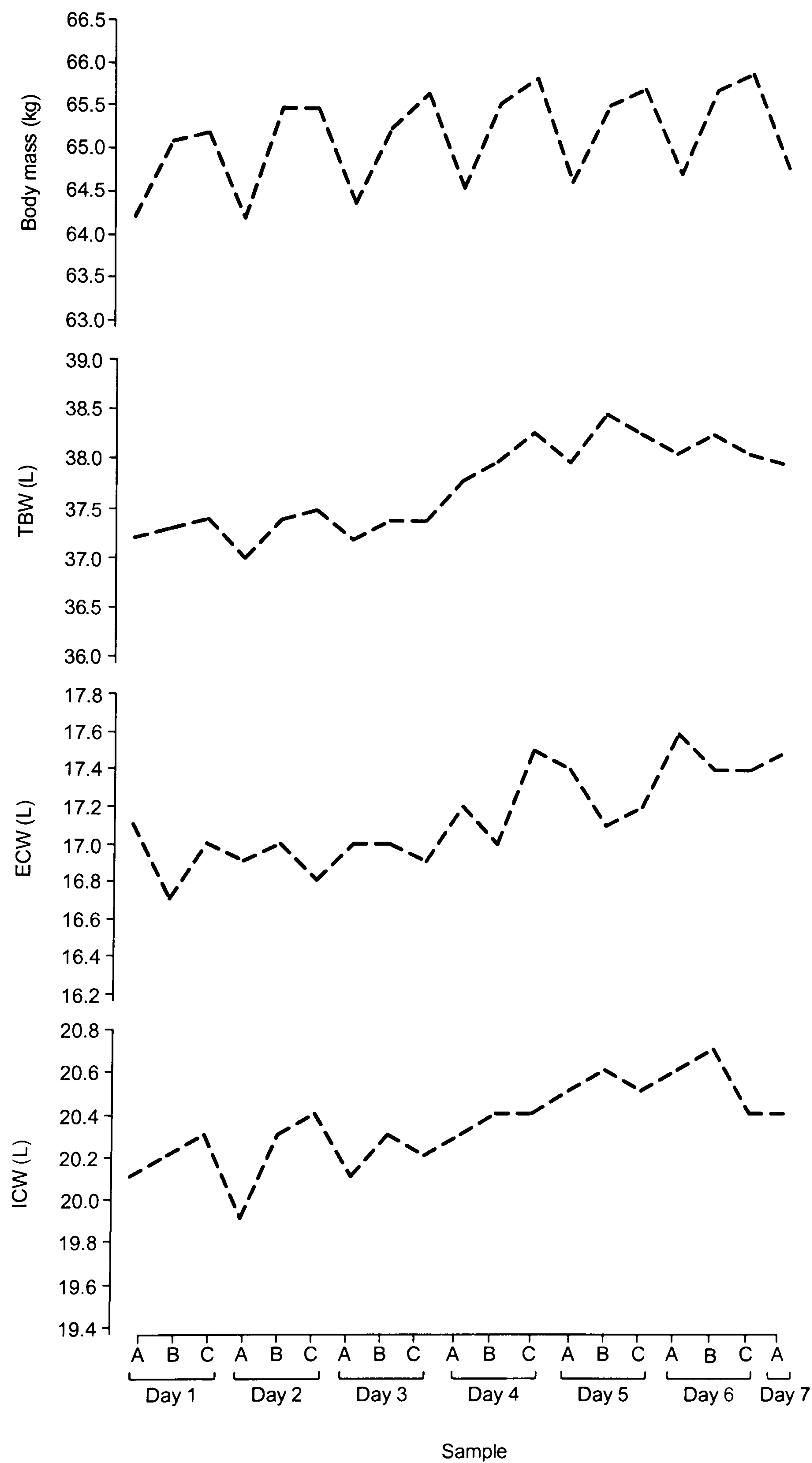


Figure 3.8 Subject 2: Daily changes in body mass, total body water (TBW), intra-cellular water (ICW) and extra-cellular water (ECW) during 7 days of Cr/Gly supplementation.

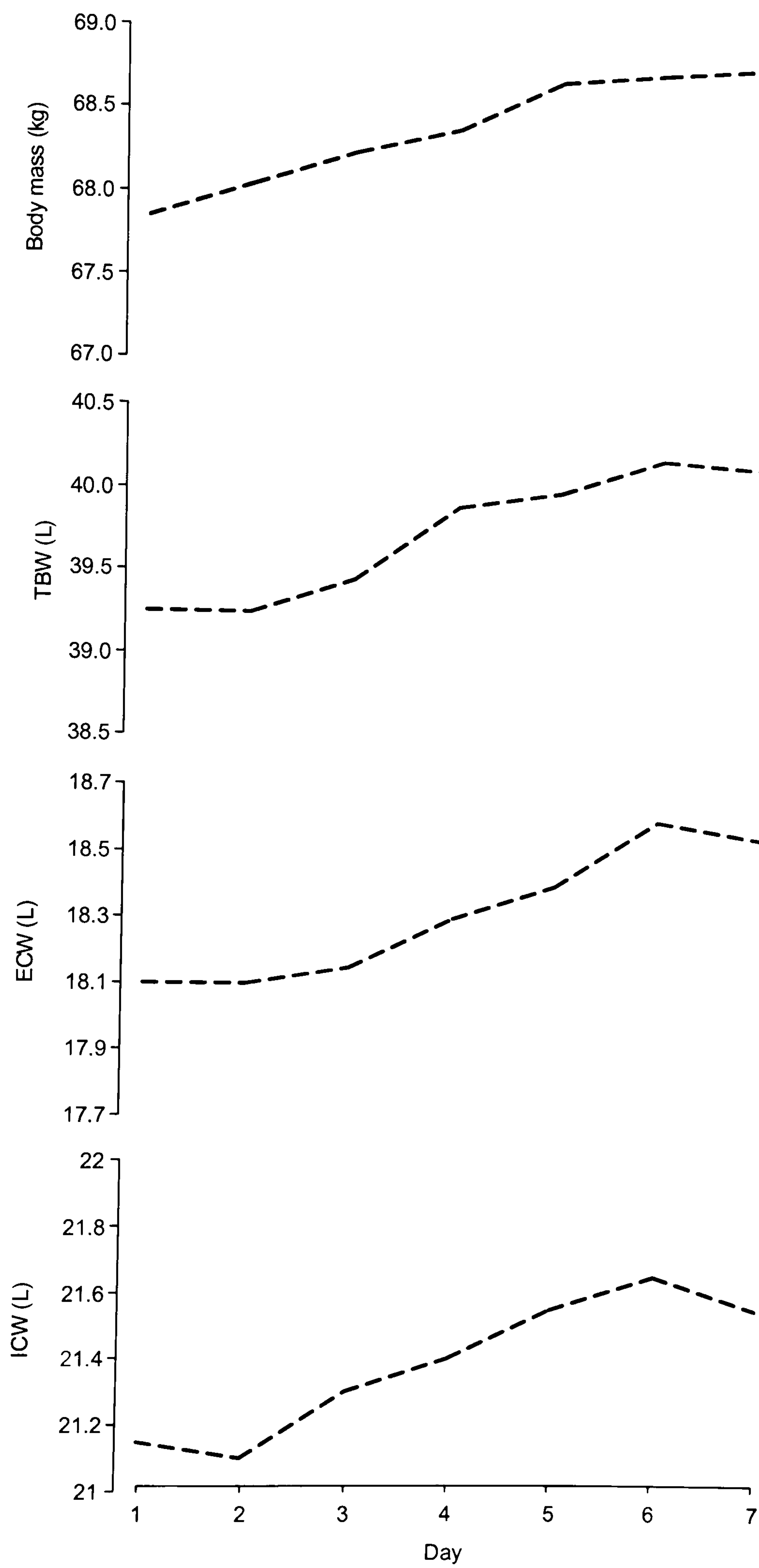


Figure 3.9 Mean changes in body mass, total body water (TBW), intra-cellular water (ICW) and extra-cellular water (ECW) during 7 days of Cr/Gly supplementation. Data is from 1st sample (sample A) on each experimental day.

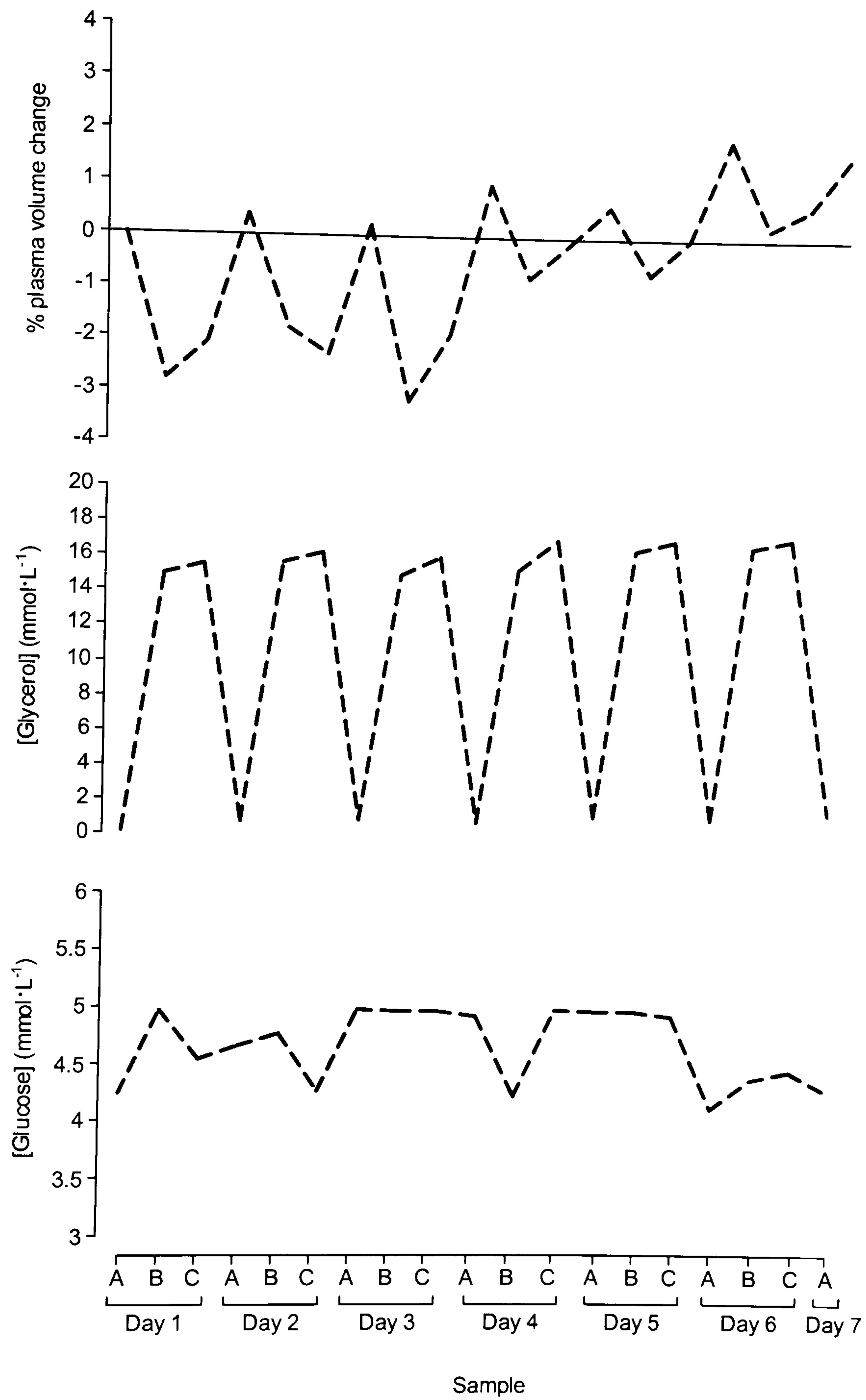


Figure 3.10 Mean blood [glucose], [Gly] and percentage changes in plasma volume during 7 days of Cr/Gly supplementation.

3.8 Discussion

This study is the first to demonstrate that ingestion of a combination of Cr and Gly utilising a novel supplementation protocol results in a significant increase in body mass, TBW, ECW and ICW (Fig. 3.9). Based on these findings it is rational to assume that supplementation with combined Cr and Gly will delay the onset of dehydration during exercise in the heat resulting in a reduced T_c and heart rate and improved exercise performance.

In the present study supplementation with combined Cr and Gly for 7 days resulted in a mean body mass increase of approximately 1.0 kg (Fig. 3.9), which is among the highest reported in the literature to date (Anderson *et al.*, 2001; Kilduff *et al.*, 2002;2004; Kreider *et al.*, 1998; Lyons *et al.*, 1990; Montner *et al.*, 1996; Ziegenfuss *et al.*, 1998) but significantly less than the 1.59 kg increase in body mass observed after Cr/Gly supplementation in Chapter 3 (a). However, subjects in the present study had a significantly lower mean body mass (67.9 kg) than those in Chapter 3 (a) (79.2 kg) which may have contributed to a smaller increase in body mass, as TBW is primarily dependent on body size. Alternatively, the pharmacokinetics of Gly metabolism following oral Gly ingestion may also explain the different increases in body mass observed in Chapter 3 (a) and (b). When exogenous Gly is ingested, appearance of Gly in the blood is relatively rapid with a distribution half life of just 23 min. (Sommer *et al.*, 1993). In the present study, plasma [Gly] increased from 0.06 mmol·L⁻¹ to 14.96 mmol·L⁻¹ on day 1 of the study, 1 hour and 30 min after ingestion of the Cr/Gly solution. Just as Gly appears quickly in the blood, the high activity of [Gly] kinase in the liver and kidney leads to rapid removal of [Gly] from the circulation and subsequent urinary excretion (Sommer *et al.*, 1993). Indeed plasma [Gly] in the present study decreased to approximately 1.0 mmol·L⁻¹ by the first blood sample of each experimental day (sample A) from approximately 16 mmol·L⁻¹ at sample C on the previous day. Therefore, given the significant reduction in blood [Gly] it is likely that some of the water retained by the osmotic action of Gly will be excreted and thus causing a reduction in body mass. Despite this, Cr/Gly supplementation resulted in a significant increase in TBW with the retained fluid dispersed equally between ICW and ECW compartments (Fig. 3.9). Therefore, it is suggested that ingesting both Cr and Gly over several days is the most effective method of fluid loading as there is sufficient time for the retained fluid to be dispersed within body compartments. Given that Cr supplementation has been consistently shown to increase ICW (Kilduff *et al.*, 2002;2004; Ziegenfuss *et al.*, 1998) whereas Gly retains fluid in all body compartments with the exception of the cerebral spinal fluid and

aqueous humor (Freund *et al.*, 1995; Lin, 1977; Seifert *et al.*, 1995; Tourtellotte *et al.*, 1972) it is reasonable to assume that the observed increases in ICW and ECW were mediated by Cr and Gly respectively. Although some of the fluid retained by Gly is likely to have been excreted by the time the final TBW measurement was recorded on day 7, the fact that ECW remains elevated suggests that some remains. Dill (1938) reported that 12 to 18 hours were required before subjects returned to euhydration following a period of dehydration and subsequent rehydration, and thus it is unsurprising that the large volume of water retained by Gly distributed throughout all body tissues should take over 15 hours to be lost (Koenigsberg *et al.*, 1995). However, it is tempting to suggest that had the final body water measurement been taken closer to the ingestion of the final supplement, the Cr/Gly induced increase in TBW and therefore body mass would have been greater.

It has previously been established that heart rate and T_c during exercise in the heat rise proportionally to the level of dehydration (Montain & Coyle, 1992a; 1992b) and that dehydration above 2% body mass results in a significant impairment in exercise performance (Cheuvront *et al.*, 2003; Fallowfield *et al.*, 1996; McConell *et al.*, 1997). Therefore, it is reasonable to assume that a 0.9 L increase in TBW such as that induced by Cr/Gly supplementation in the present study would be enough to offset dehydration and limit the increases in heart rate and T_c induced by exercise heat stress and prevent a decline in exercise performance. Indeed, several previous studies have concluded that hyperhydration is associated with a reduction in T_c and heart rate and a significant improvement in performance during exercise in the heat (Anderson *et al.*, 2001; Hitchins *et al.*, 1999; Kilduff *et al.*, 2004; Montner *et al.*, 1996). Nevertheless, when the effects of Cr/Gly supplementation on thermoregulatory and cardiovascular responses and exercise performance were examined in a previous investigation (Chapter 3 (a)) no ergogenic effect was found. However, in contrast to the current study there were no reported increases in TBW after Cr/Gly supplementation in this previous investigation so the lack of a difference in physiological responses compared to euhydration is unsurprising.

3.8.1 Conclusions

This study has demonstrated that supplementation with a combination of Cr and Gly, using a novel loading protocol, resulted in a significant increase in body mass TBW, ICW and ECW compared to pre-supplementation. Therefore, ingesting both Cr and Gly over several days may be the most effective method of fluid loading as there will be sufficient time for the retained fluid to be dispersed within body compartments. Further research is required to

examine the effects of Cr/Gly hyperhydration on cardiovascular and thermoregulatory responses and performance during and exercise in the heat.

Chapter 4

(a) The effects of combined creatine and glycerol hyperhydration on metabolism, thermoregulation and exercise performance in the heat – Loading protocol 2.

4.1 Introduction

The possibility that combined Cr/Gly supplementation may result in significant water retention and consequently reduce T_c and heart rate and improve performance during exercise in the heat has been previously examined and shown to be unsuccessful (Chapter 3 (a)). However, it appears as though the observed negative interaction on fluid retention of both hyperhydrating agents that occurs using previously established supplementation protocols can be effectively counteracted by ingesting both Cr and Gly over several days allowing sufficient time for the retained fluid to be dispersed within body compartments (Chapter 3 (b)). Intuitively, this increased fluid would maintain plasma volume resulting in a reduction in heart rate during exercise induced heat stress. As a direct result, skin blood flow would be maintained allowing sufficient continuation of convective heat loss. Additionally, there would be sufficient body water to maintain adequate sweat production and optimum evaporative cooling, which overall would enhance thermoregulatory function. Furthermore, it is possible that the Cr-induced increase in ICW may result in an increase in the specific heat capacity of the body, resulting in a greater capacity to store heat (Kilduff *et al.*, 2004). Thus, it is proposed that the predicted reduction in heart rate and T_c induced by Cr/Gly hyperhydration could ultimately improve exercise performance, especially in the heat (Convertino *et al.*, 1996). It seems plausible that a Gly-induced increase in ECW coupled with a Cr-mediated increase in ICW could have synergistic effects resulting in a much larger increase in TBW than if either supplement was consumed alone. Therefore, the primary aim of this study was to examine whether combining Cr and Gly can induce a greater hyperhydration than either Cr or Gly alone. If successful, a secondary aim of this study was to assess the effects of this novel ‘water-loading’ strategy on thermoregulation and performance during exercise in the heat.

4.2 Methods

4.2.1 Subjects

Six endurance-trained males (Table 4.1) gave their written informed consent to take part in the present study that was approved by the local Ethics Committee and was performed according to the code of ethics of the World Medical Association (Declaration of Helsinki).

Table 4.1. The physical characteristics, maximal oxygen uptake, lactate threshold and maximal work rate of the two groups of subjects. Data presented as the mean ± s.d.

| | Pl Group (n = 3) | Cr Group (n = 3) |
|--|---------------------|---------------------|
| Age (yr) | 23 ± 5 | 25 ± 9 |
| Height (cm) | 171 ± 14 | 174 ± 9 |
| Weight (kg) | 72.7 ± 12 | 74.1 ± 12 |
| $\dot{V}O_{2\text{ max}}$ (L·min ⁻¹) | 4.2 ± 0.1 | 4.3 ± 0.4 |
| LT (W) | 231 ± 17 | 243 ± 20 |
| WR _{max} (W) | 355 ± 10 | 382 ± 24 |

4.2.2 Experimental design

The experimental design by necessity is complicated and best understood by reference to Fig. 4.1. The study consisted of two supplementation regimens, each lasting 7 days and encompassing two cycle performance trials consisting of 40 min constant-load exercise at 63% WR_{max} followed by a 16.1 km (10 mile) time trial. The methodology for the exercise trials is described Chapter 2 of this thesis. Prior to the first of these experimental trials, familiarisation trials were completed until the variability of two consecutive trials was within 5%; no subject had to perform a third familiarisation trial to achieve less than 5% variability. Following this familiarisation period, subjects were matched for body mass and were randomised in a double blind fashion to either a Cr or a Pl group. Subjects were separated into two groups due to the long wash-out period associated with oral Cr supplementation (Vandenbergh *et al.*, 1996). Subjects in both groups performed an exercise trial pre- and post-supplementation during both supplementation regimens (i.e. a total of 4 experimental exercise trials) (see Fig. 4.1). The first test was conducted at least 48 hours after each subject’s final familiarisation trial. Each supplementation period started on the day after the first test and finished on the day of the second test.

Cr supplementation consisted of 11.4 g of Cr·H₂O (equivalent to 10 g Cr) and 70 g of glucose polymer made up in 1 L of warm water and consumed twice daily for 6 days and once more on the day of the experimental exercise trial. This protocol has been shown to

increase resting muscle phosphocreatine levels within 5 days (Harris *et al.*, 1992). Each supplement was made fresh prior to consumption in order to prevent any degradation of Cr to creatinine. The Pl supplement consisted of 85 g of glucose polymer made up in 1 L of warm water and consumed twice daily for 6 days and once more on the day of the experimental exercise trial. During the two supplementation regimens, subjects in both groups also received either 1 g Gly·kg⁻¹ body mass or an equivalent amount of Pl (i.e. sucrose) diluted in each 1 L supplement. Therefore, four possible combinations of supplements were administered: Pl group: Pl/Pl and Pl/Gly; Cr Group: Cr/Pl and Cr/Gly. For the two post-supplementation trials, subjects began consuming the final supplement 3 hours prior to the exercise performance trial. All supplements had similar taste, texture and appearance and were placed in generic water bottles to ensure double blind administration. On each of the experimental test days, subjects ingested 500 ml of water 2 hours prior to exercise and a further 500 ml of water 1 hour prior to exercise in an attempt to ensure subjects were euhydrated prior to all exercise trials (Convertino *et al.*, 1996).

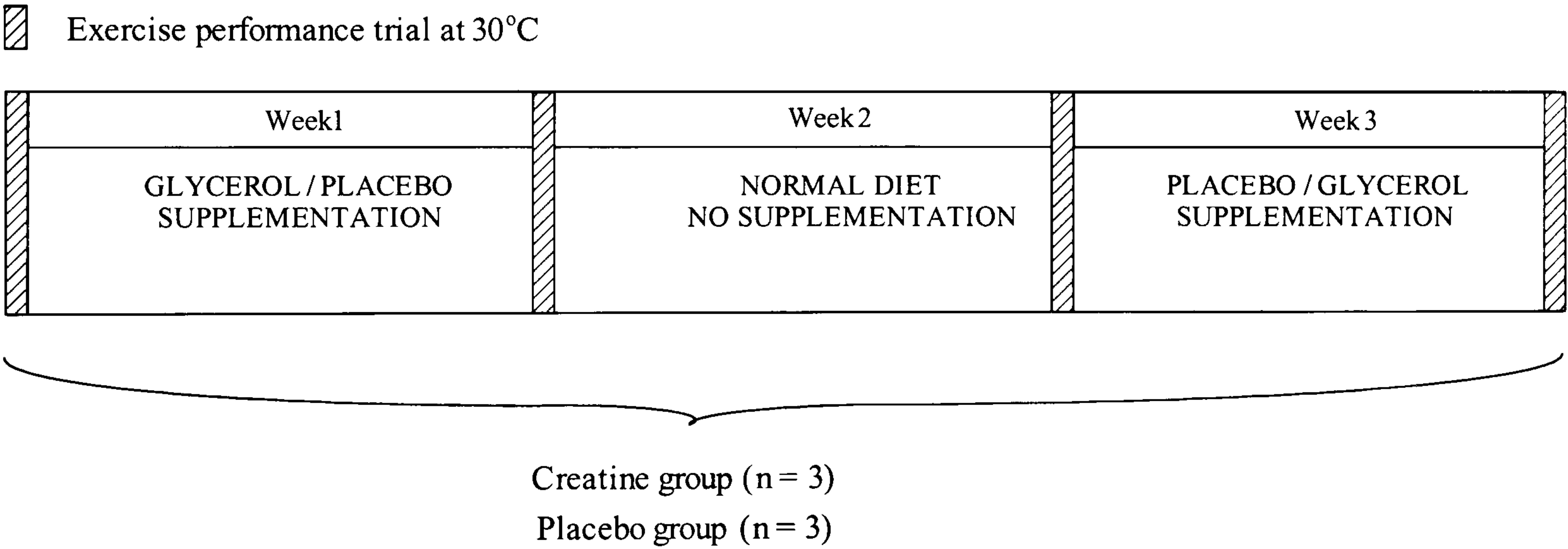


Figure 4.1 Schematic representation of the experimental design.

4.3 Results

4.3.1 Diet, body mass and water compartments.

The physical characteristics of the Pl and Cr groups were similar before supplementation (Table 4.1). In the Pl group, body mass increased significantly following Gly supplementation ($P<0.01$), with no change following Pl ($P=0.14$) (Δ body mass was greater following Gly supplementation; Fig. 4.2). In the Cr group, body mass increased significantly during both the Pl ($P<0.01$) and Gly ($P<0.01$) regimens (Fig. 4.2) with no difference in Δ body mass between regimens. TBW, ICW and ECW did not differ significantly pre- to post-supplementation in either the Pl or Cr groups in either supplementation regimen (Fig. 4.2). There were no significant differences in the daily diet between the two groups or between Pl and Gly regimens (Pl/Pl: $11.2 \pm 3.6 \text{ MJ}\cdot\text{day}^{-1}$, $65 \pm 9\%$ carbohydrate, $23 \pm 4\%$ fat, $12 \pm 4\%$ protein; Pl/Gly: $11.6 \pm 3.2 \text{ MJ}\cdot\text{day}^{-1}$, $66 \pm 5\%$ carbohydrate, $22 \pm 5\%$ fat, $12 \pm 4\%$ protein; Cr/Pl: $12.2 \pm 3.8 \text{ MJ}\cdot\text{day}^{-1}$, $67 \pm 4\%$ carbohydrate, $20 \pm 6\%$ fat, $13 \pm 2\%$ protein, Cr/Gly: $12.4 \pm 3.1 \text{ MJ}\cdot\text{day}^{-1}$, $67 \pm 5\%$ carbohydrate, $21 \pm 8\%$ fat, $12 \pm 6\%$ protein).

4.3.2 Cardiopulmonary variables.

There was a steady increase in $\dot{V}O_2$ (Table 4.2), $\dot{V}CO_2$ and $\dot{V}E$ (data not presented) throughout the constant-load exercise with no difference between groups before or as a result of supplementation. There was a steady decline in RER throughout the constant-load exercise period with no differences between groups before or as a result of supplementation (data not presented). There was no difference in resting heart rate between the two groups or after supplementation (Fig. 4.3). During exercise, heart rate increased during all trials. There were no differences in heart rate during exercise between pre- and post-supplementation in any of the supplementation regimens (Fig. 4.3).

Table 4.2. VO_2 ($\text{L}\cdot\text{min}^{-1}$) during constant-load exercise

| Group | Regimen | Trial | Exercise time (min) | | | | |
|-------|---------|-------|---------------------|-----------|-----------|-----------|-----------|
| | | | Rest | 10 | 20 | 30 | 40 |
| Pl | Pl | Pre | 0.5 ± 0.2 | 2.6 ± 0.1 | 2.9 ± 0.4 | 2.9 ± 0.3 | 2.9 ± 0.4 |
| Pl | Pl | Post | 0.4 ± 0.3 | 2.7 ± 0.2 | 3.0 ± 0.4 | 2.9 ± 0.5 | 2.9 ± 0.6 |
| Pl | Gly | Pre | 0.5 ± 0.2 | 2.9 ± 0.2 | 2.9 ± 0.2 | 2.9 ± 0.2 | 2.9 ± 0.2 |
| Pl | Gly | Post | 0.5 ± 0.2 | 2.8 ± 0.2 | 2.9 ± 0.2 | 2.8 ± 0.3 | 3.0 ± 0.3 |
| Cr | Pl | Pre | 0.4 ± 0.2 | 3.0 ± 0.4 | 3.1 ± 0.3 | 3.2 ± 0.3 | 3.3 ± 0.4 |
| Cr | Pl | Post | 0.4 ± 0.1 | 3.0 ± 0.3 | 3.1 ± 0.4 | 3.2 ± 0.3 | 3.3 ± 0.4 |
| Cr | Gly | Pre | 0.4 ± 0.2 | 3.2 ± 0.2 | 3.2 ± 0.3 | 3.3 ± 0.3 | 3.2 ± 0.4 |
| Cr | Gly | Post | 0.5 ± 0.3 | 3.2 ± 0.3 | 3.3 ± 0.2 | 3.3 ± 0.3 | 3.3 ± 0.3 |

Data presented as the mean \pm s.d.

4.3.3 Ratings of perceived exertion

There was a progressive increase in RPE both for dyspnoea and perceived leg fatigue during exercise reaching near maximum ratings at the end of the time trial (Fig. 4.4). There were no differences in RPE either for dyspnoea or perceived leg fatigue during exercise between pre- and post-supplementation in any of the supplementation regimens (Fig. 4.4)

4.3.4 Rectal and skin temperature responses

There was no difference in resting T_{re} between the two groups or after supplementation (Fig. 4.5). Throughout the exercise period, T_{re} increased significantly during all trials (Fig. 4.5). There were no differences in T_{re} during exercise between pre-supplementation and post supplementation in any of the supplementation regimens (Fig. 4.5). There was a significant increase in mean T_{sk} from rest, with no significant differences following supplementation in any supplementation regimen (Fig. 4.5).

4.3.5 Sweat rates and total sweat loss during exercise

There were no changes in sweat rate from pre- to post supplementation in either the Pl group (Pl/Pl: pre $1.3 \pm 0.6 \text{ L}\cdot\text{hr}^{-1}$ vs. post $1.2 \pm 0.3 \text{ L}\cdot\text{hr}^{-1}$; $P=0.18$, Pl/Gly: pre $1.2 \pm 0.3 \text{ L}\cdot\text{hr}^{-1}$ vs. post $1.4 \pm 0.5 \text{ L}\cdot\text{hr}^{-1}$; $P=0.09$) or the Cr group (Cr/Pl: pre $1.4 \pm 0.2 \text{ L}\cdot\text{hr}^{-1}$ vs. post $1.4 \pm 0.2 \text{ L}\cdot\text{hr}^{-1}$; $P=0.29$, Cr/Gly: pre $1.4 \pm 0.2 \text{ L}\cdot\text{hr}^{-1}$ vs. post $1.5 \pm 0.1 \text{ L}\cdot\text{hr}^{-1}$; $P=0.11$). Furthermore, total sweat loss was no different pre- compared to post-supplementation in either the Pl group (Pl/Pl: pre $1.4 \pm 0.6 \text{ L}$ vs. post $1.4 \pm 0.3 \text{ L}$; $P=0.24$, Pl/Gly: pre $1.4 \pm 0.3 \text{ L}$ vs. post $1.6 \pm 0.5 \text{ L}$; $P=0.10$) or the Cr group (Pl/Pl: pre $1.5 \pm 0.2 \text{ L}$ vs. post $1.6 \pm 0.2 \text{ L}$; $P=0.12$, Cr/Gly: pre $1.4 \pm 0.2 \text{ L}$ vs. post $1.6 \pm 0.1 \text{ L}$; $P=0.08$).

4.3.6 Blood metabolite concentrations and plasma volume changes

Resting blood [glucose] and [lactate] were not different between groups or following Gly supplementation (Table 4.3, 4.4). Briefly, blood [glucose] decreased significantly from rest to initiation of exercise before rising gradually throughout the constant-load exercise and peaking at the end of the time trial (Table 4.3). Blood [glucose] during exercise was not different between groups or following Gly supplementation (Table 4.3). The initial increase in blood [lactate] from rest to initiation of exercise was maintained until the end of the constant-load period (Table 4.4). There was a further significant increase in blood [lactate] between the constant-load exercise and the end of the time trial (Table 4.4). There

were no differences in blood [lactate] during exercise between groups or following Gly supplementation (Table 4.4). Resting plasma [Gly] was significantly higher post Gly supplementation compared to pre-supplementation in both the Pl ($P<0.01$) and Cr ($P<0.01$) groups (Table 4.5). Plasma [Gly] remained significantly higher throughout exercise after Gly supplementation compared to the pre-supplementation trial in both the Cr and the Pl group. There was no difference in resting plasma [Gly] or during exercise between pre- and post-supplementation during the Pl supplementation regimen in either the Pl or the Cr group (Table 4.5). Plasma volume was reduced by approximately 8% after 40 min of constant-load exercise and 12% after the 16.1 km time trial with no significant differences between groups or following supplementation (Fig. 4.6). Resting plasma volume changes following supplementation were also calculated using the control trial as a baseline, assuming no change in red cell mass during the 7 day supplementation regimen. Using this method of analysis, plasma volume was not significantly altered following either Pl/Pl or Cr/Pl supplementation, whereas both Pl/Gly and Cr/Gly supplementation resulted in a small (2-3%) non-significant reduction in plasma volume ($P=0.11$ and $P=0.09$ respectively).

4.3.7 Osmolality.

Resting serum osmolality was significantly higher post Gly supplementation compared to pre-supplementation in both the Pl ($P<0.01$) and the Cr ($P<0.01$) groups (Table 4.6). Serum osmolality remained significantly higher throughout exercise after Gly supplementation compared to the pre-supplementation trial in both the Cr and the Pl group. There were no other differences in serum osmolality (Table 4.6).

4.3.8 Time trial performance

Time trial performance was not significantly different between the groups prior to supplementation ($P=0.22$). Time trial performance did not significantly differ pre- to post-supplementation in either the Pl group (Pl regimen, pre vs. post: 24.7 ± 2.0 min vs. 24.8 ± 2.2 min; Gly regimen, pre vs. post: 25.1 ± 1.9 min vs. 24.8 ± 2.4 min) or Cr group (Pl regimen, pre vs. post: 24.3 ± 1.8 min vs. 24.5 ± 1.8 min; Gly regimen, pre vs. post: 24.3 ± 2.2 min vs. 24.3 ± 2.3 min) in either supplementation week.

4.3.8 Side effects

In general, subjects tolerated the supplementation protocol well although there was one isolated incident of headache in a subject supplementing with Cr/Gly. However, symptoms soon disappeared and no further complaints were reported. One subject from the Cr group correctly identified the subject group and 3 subjects correctly identified the Gly supplementation regimen, while all other subjects were unsure of the treatment they received.

Table 4.3. Plasma [Glucose] (mmol·L⁻¹) during exercise before and after supplementation in the two groups

| Group | Regimen | Trial | Exercise time (min) | | | | | End TT |
|-------|---------|-------|---------------------|-------------|-------------|-------------|-------------|-------------|
| | | | Rest | 10 | 20 | 30 | 40 | |
| Pl | Pl | Pre | 4.87 ± 0.15 | 4.54 ± 0.33 | 4.63 ± 0.28 | 4.82 ± 0.39 | 4.92 ± 0.19 | 4.98 ± 0.68 |
| Pl | Pl | Post | 4.98 ± 0.13 | 4.73 ± 0.11 | 4.80 ± 0.14 | 4.95 ± 0.26 | 5.08 ± 0.39 | 5.15 ± 0.66 |
| Pl | Gly | Pre | 4.83 ± 0.21 | 4.68 ± 0.28 | 4.75 ± 0.38 | 4.98 ± 0.39 | 5.11 ± 0.28 | 5.24 ± 0.79 |
| Pl | Gly | Post | 5.01 ± 0.24 | 4.77 ± 0.22 | 4.84 ± 0.31 | 4.99 ± 0.33 | 5.17 ± 0.49 | 5.28 ± 0.89 |
| Cr | Pl | Pre | 4.63 ± 0.13 | 4.54 ± 0.24 | 4.80 ± 0.11 | 4.95 ± 0.47 | 5.19 ± 0.61 | 5.31 ± 0.76 |
| Cr | Pl | Post | 4.73 ± 0.49 | 4.46 ± 0.31 | 4.60 ± 0.25 | 4.72 ± 0.37 | 4.89 ± 0.56 | 4.99 ± 0.64 |
| Cr | Gly | Pre | 4.42 ± 0.32 | 4.45 ± 0.24 | 4.63 ± 0.36 | 4.79 ± 0.46 | 4.82 ± 0.61 | 4.94 ± 0.12 |
| Cr | Gly | Post | 4.62 ± 0.41 | 4.55 ± 0.33 | 4.71 ± 0.39 | 4.88 ± 0.51 | 4.92 ± 0.47 | 5.09 ± 0.51 |

Data presented as the mean ± s.d.

Table 4.4. Plasma [Lactate] (mmol·L⁻¹) during exercise before and after supplementation in the two groups

| Group | Regimen | Trial | Exercise time (min) | | | | | End TT |
|-------|---------|-------|---------------------|-------------|-------------|-------------|-------------|-------------|
| | | | Rest | 10 | 20 | 30 | 40 | |
| P1 | P1 | Pre | 0.88 ± 0.11 | 4.18 ± 1.26 | 4.45 ± 1.41 | 4.75 ± 1.56 | 4.91 ± 1.66 | 8.79 ± 2.99 |
| P1 | P1 | Post | 1.01 ± 0.28 | 4.24 ± 1.43 | 4.67 ± 1.47 | 4.89 ± 1.62 | 5.06 ± 1.71 | 8.96 ± 2.67 |
| P1 | Gly | Pre | 0.92 ± 0.24 | 4.26 ± 1.21 | 4.58 ± 1.37 | 4.81 ± 1.39 | 5.00 ± 1.52 | 8.58 ± 2.58 |
| P1 | Gly | Post | 0.96 ± 0.18 | 4.16 ± 1.11 | 4.49 ± 1.21 | 4.78 ± 1.28 | 4.96 ± 1.34 | 8.29 ± 2.46 |
| Cr | P1 | Pre | 0.79 ± 0.15 | 3.91 ± 0.89 | 4.12 ± 1.13 | 4.42 ± 1.20 | 4.79 ± 1.22 | 8.02 ± 1.99 |
| Cr | P1 | Post | 0.89 ± 0.22 | 3.78 ± 0.76 | 4.02 ± 0.95 | 4.30 ± 1.19 | 4.64 ± 1.25 | 7.79 ± 2.11 |
| Cr | Gly | Pre | 1.13 ± 0.16 | 3.79 ± 0.74 | 4.09 ± 0.99 | 4.29 ± 1.22 | 4.58 ± 1.27 | 7.83 ± 2.22 |
| Cr | Gly | Post | 0.96 ± 0.18 | 4.01 ± 0.91 | 4.26 ± 1.17 | 4.49 ± 1.21 | 4.80 ± 1.28 | 8.06 ± 2.16 |

Data presented as the mean ± s.d.

Table 4.5. Plasma [Gly] (mmol·L⁻¹) during exercise before and after supplementation in the two groups

| Group | Regimen | Trial | Exercise time (min) | | | | | End TT |
|-------|---------|-------|---------------------|-----------------|----------------|----------------|----------------|----------------|
| | | | Rest | 10 | 20 | 30 | 40 | |
| P1 | P1 | Pre | 0.06 ± 0.03 | 0.18 ± 0.03 | 0.23 ± 0.06 | 0.29 ± 0.05 | 0.32 ± 0.03 | 0.44 ± 0.09 |
| P1 | P1 | Post | 0.05 ± 0.03 | 0.17 ± 0.04 | 0.23 ± 0.06 | 0.28 ± 0.07 | 0.34 ± 0.06 | 0.46 ± 0.10 |
| P1 | Gly | Pre | 0.05 ± 0.02 | 0.19 ± 0.04 | 0.26 ± 0.05 | 0.30 ± 0.06 | 0.35 ± 0.08 | 0.47 ± 0.13 |
| P1 | Gly | Post | 10.92 ± 2.14 †* | 10.09 ± 2.13 †* | 9.86 ± 2.25 †* | 9.60 ± 2.19 †* | 9.36 ± 1.99 †* | 9.17 ± 1.97 †* |
| Cr | P1 | Pre | 0.03 ± 0.03 | 0.14 ± 0.03 | 0.19 ± 0.05 | 0.26 ± 0.06 | 0.30 ± 0.06 | 0.43 ± 0.10 |
| Cr | P1 | Post | 0.04 ± 0.03 | 0.17 ± 0.04 | 0.22 ± 0.07 | 0.25 ± 0.07 | 0.31 ± 0.09 | 0.42 ± 0.12 |
| Cr | Gly | Pre | 0.04 ± 0.02 | 0.16 ± 0.03 | 0.21 ± 0.06 | 0.26 ± 0.06 | 0.30 ± 0.07 | 0.44 ± 0.14 |
| Cr | Gly | Post | 10.18 ± 2.67 †* | 9.67 ± 2.43 †* | 9.39 ± 2.36 †* | 9.22 ± 2.18 †* | 9.01 ± 2.17 †* | 8.73 ± 2.00 †* |

Data presented as the mean ± s.d. †: indicates a significant difference pre- vs post-supplementation. *: indicates a significant greater change (Δ) in the Gly supplementation regimen compared to the P1 supplementation regimen.

Table 4.6. Serum osmolality (mosmol·kg⁻¹) during exercise before and after supplementation in the two groups

| Group | Regimen | Trial | Exercise time (min) | | | | | End TT |
|-------|---------|-------|---------------------|------------|------------|------------|------------|------------|
| | | | Rest | 10 | 20 | 30 | 40 | |
| Pl | Pl | Pre | 282 ± 5 | 286 ± 6 | 290 ± 5 | 290 ± 6 | 290 ± 6 | 294 ± 7 |
| Pl | Pl | Post | 281 ± 4 | 287 ± 5 | 290 ± 6 | 290 ± 6 | 290 ± 7 | 295 ± 6 |
| Pl | Gly | Pre | 282 ± 5 | 287 ± 6 | 289 ± 6 | 289 ± 6 | 290 ± 6 | 294 ± 6 |
| Pl | Gly | Post | 293 ± 7 †* | 296 ± 7 †* | 297 ± 7 †* | 298 ± 7 †* | 297 ± 8 †* | 300 ± 8 †* |
| Cr | Pl | Pre | 282 ± 5 | 289 ± 6 | 290 ± 6 | 289 ± 6 | 290 ± 6 | 294 ± 7 |
| Cr | Pl | Post | 282 ± 5 | 286 ± 5 | 288 ± 6 | 288 ± 7 | 289 ± 6 | 292 ± 6 |
| Cr | Gly | Pre | 283 ± 5 | 288 ± 5 | 289 ± 6 | 288 ± 7 | 289 ± 7 | 293 ± 6 |
| Cr | Gly | Post | 292 ± 5 †* | 295 ± 6 †* | 295 ± 6 †* | 297 ± 2 †* | 296 ± 2 | 299 ± 8 †* |

Data presented as the mean ± s.d. †: indicates a significant difference pre- vs post-supplementation. *: indicates a significant greater change (Δ) in the Gly supplementation regimen compared to the Pl supplementation regimen

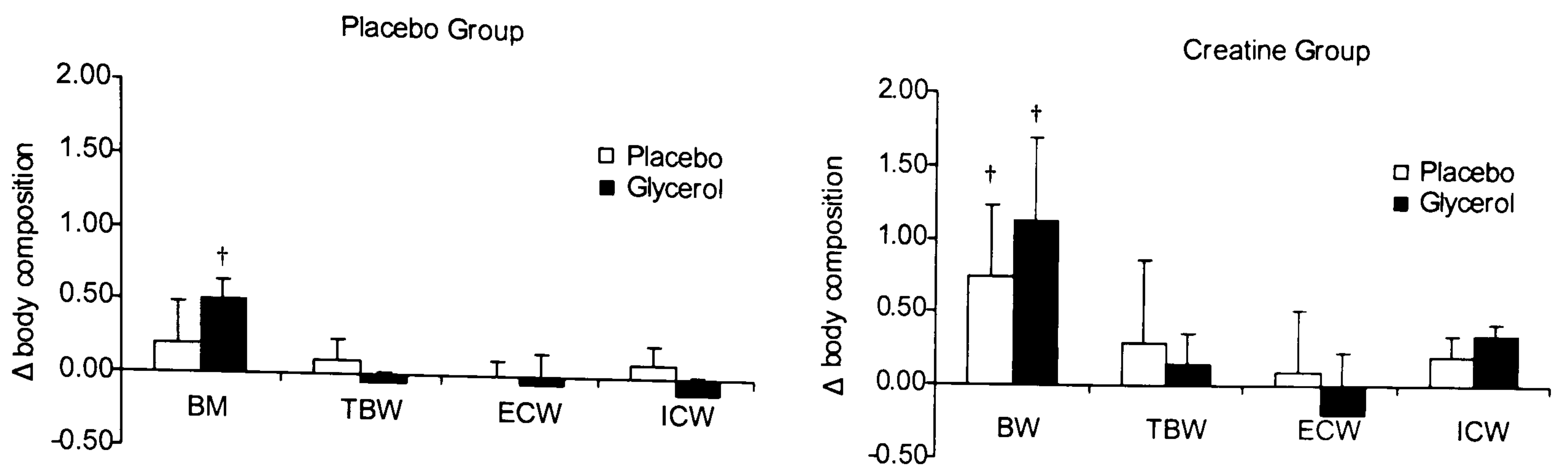


Figure 4.2 Changes in body mass (BM), total body water (TBW), intra-cellular water (ICW) and extra-cellular water (ECW) in the two groups. Data presented as the mean \pm s.d. †: indicates a significant difference pre- vs. post-supplementation.

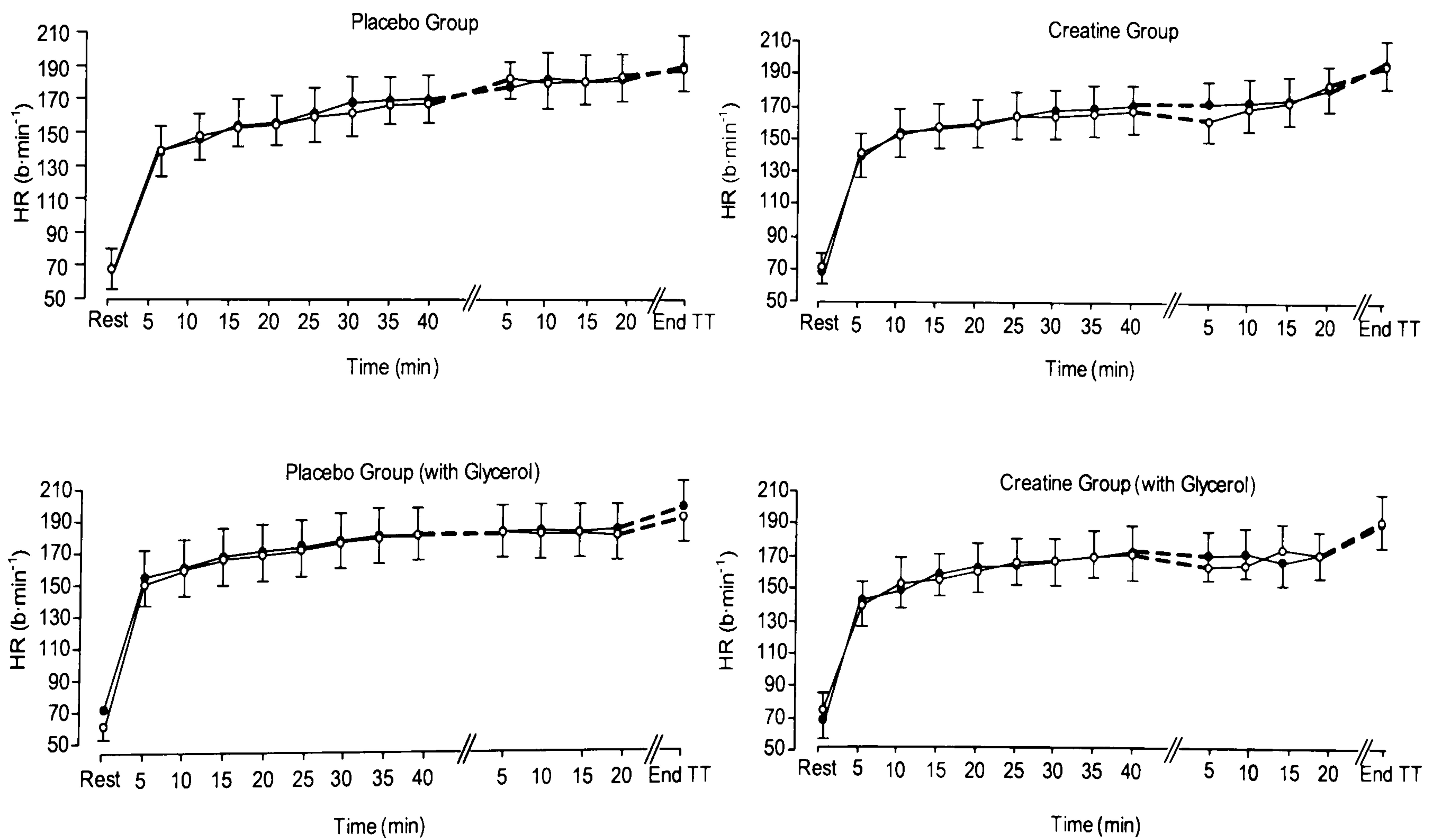


Figure 4.3. Heart rate during exercise before (black circles) and after (white circles) supplementation in the two groups. Data presented as the mean \pm s.d.

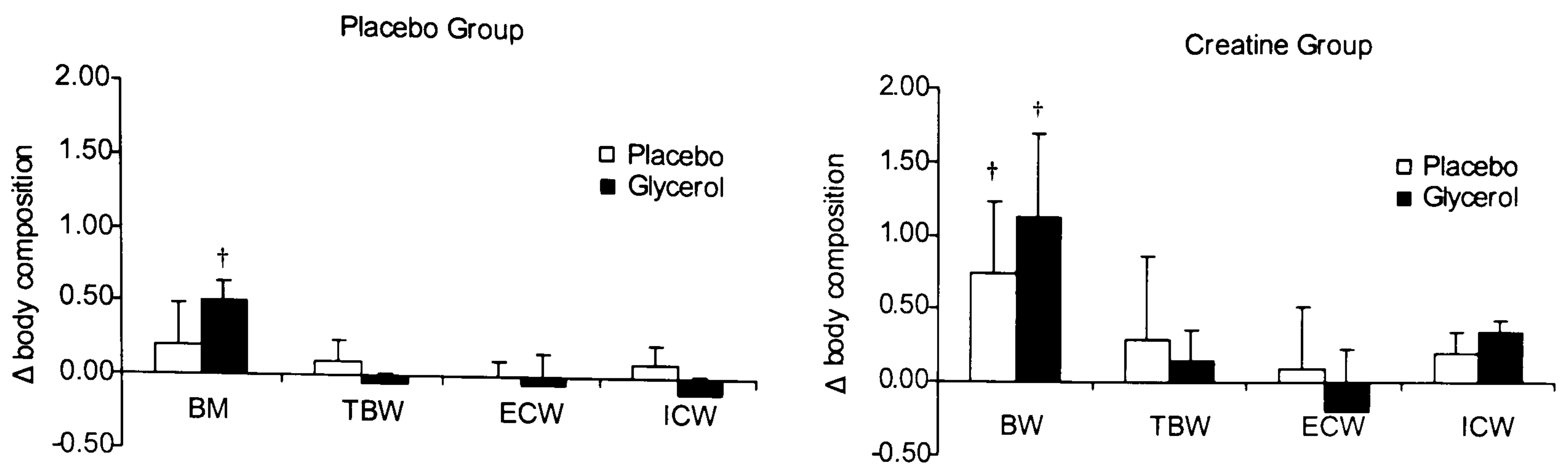


Figure 4.2 Changes in body mass (BM), total body water (TBW), intra-cellular water (ICW) and extra-cellular water (ECW) in the two groups. Data presented as the mean \pm s.d. [†]: indicates a significant difference pre- vs. post-supplementation.

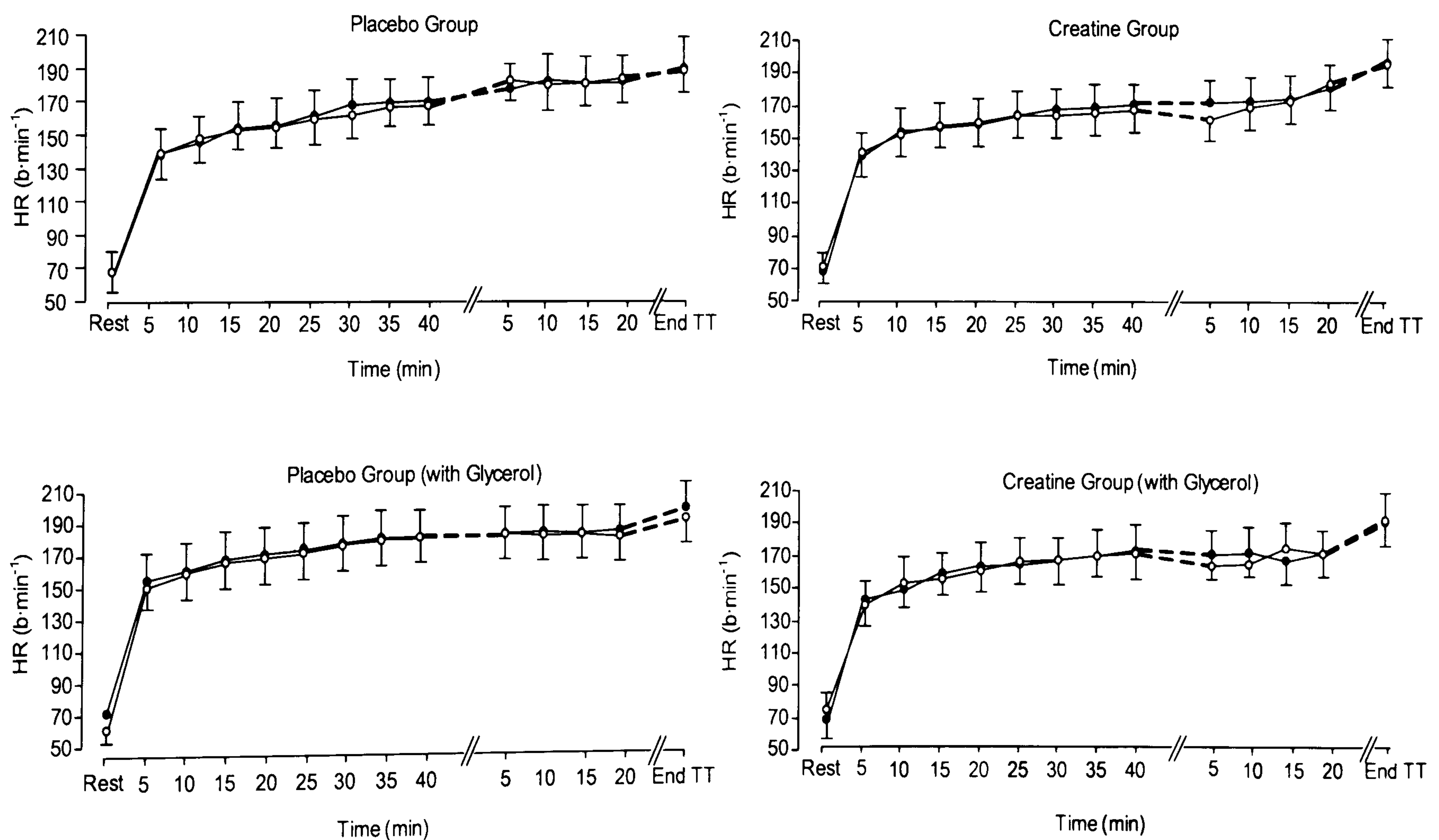


Figure 4.3. Heart rate during exercise before (black circles) and after (white circles) supplementation in the two groups. Data presented as the mean \pm s.d.

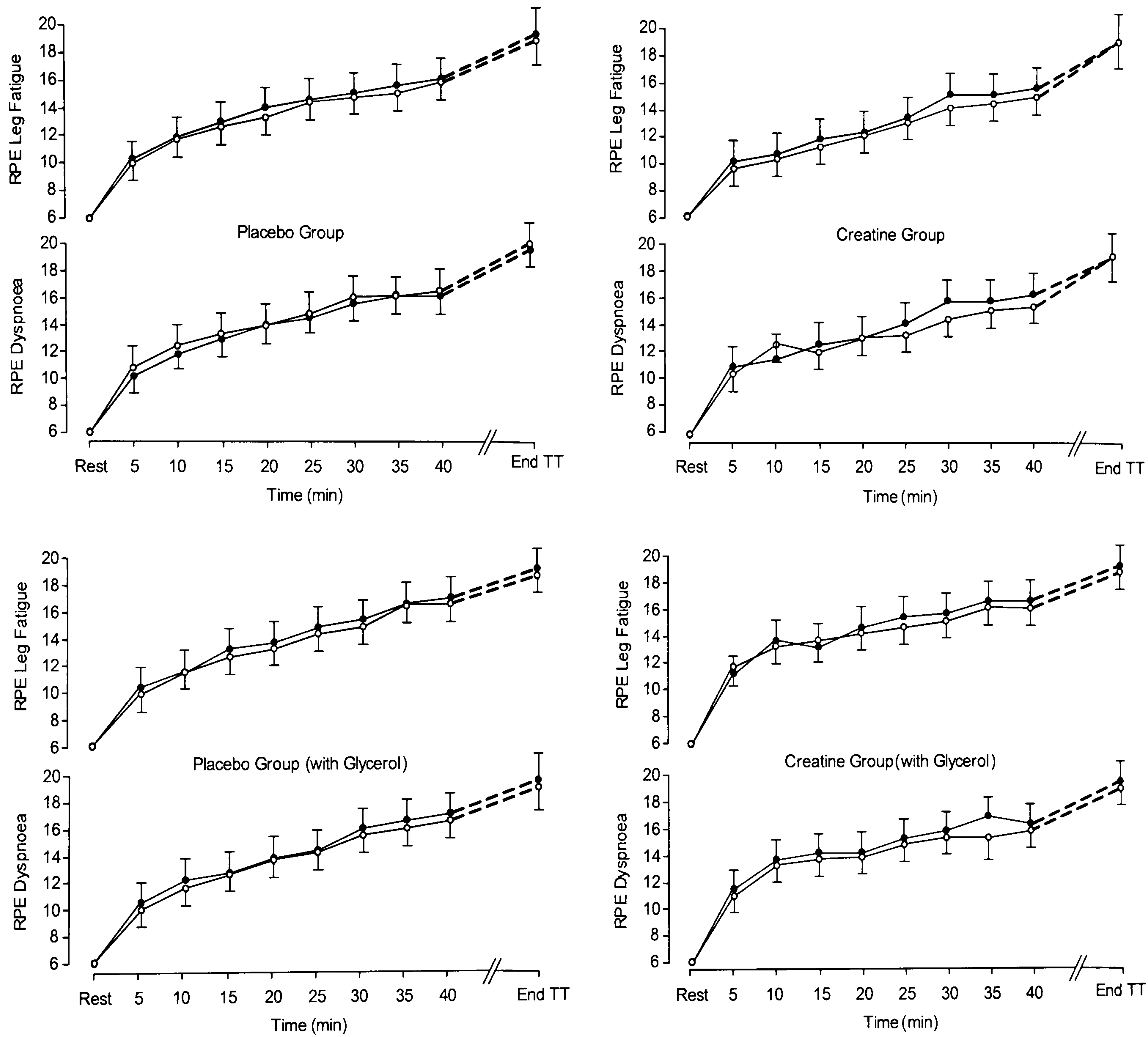


Figure 4.4. RPE for perceived leg fatigue and dyspnoea during exercise before (black circles) and after (white circles) supplementation in the two groups. Data presented as the mean ± s.d.

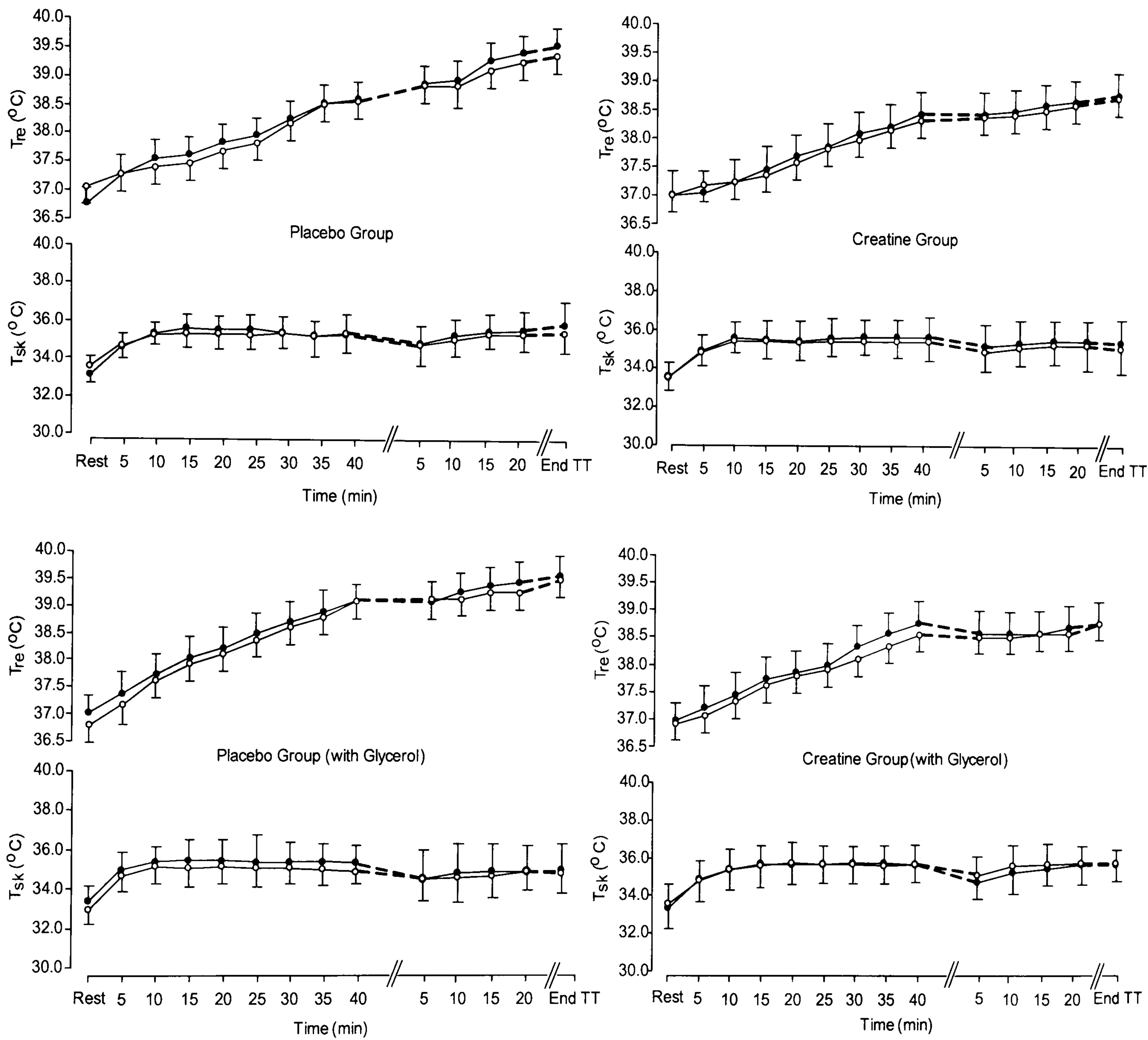


Figure 4.5. Rectal and mean skin temperature during exercise before (black circles) and after (white circles) supplementation in the two groups. Data presented as the mean \pm s.d.

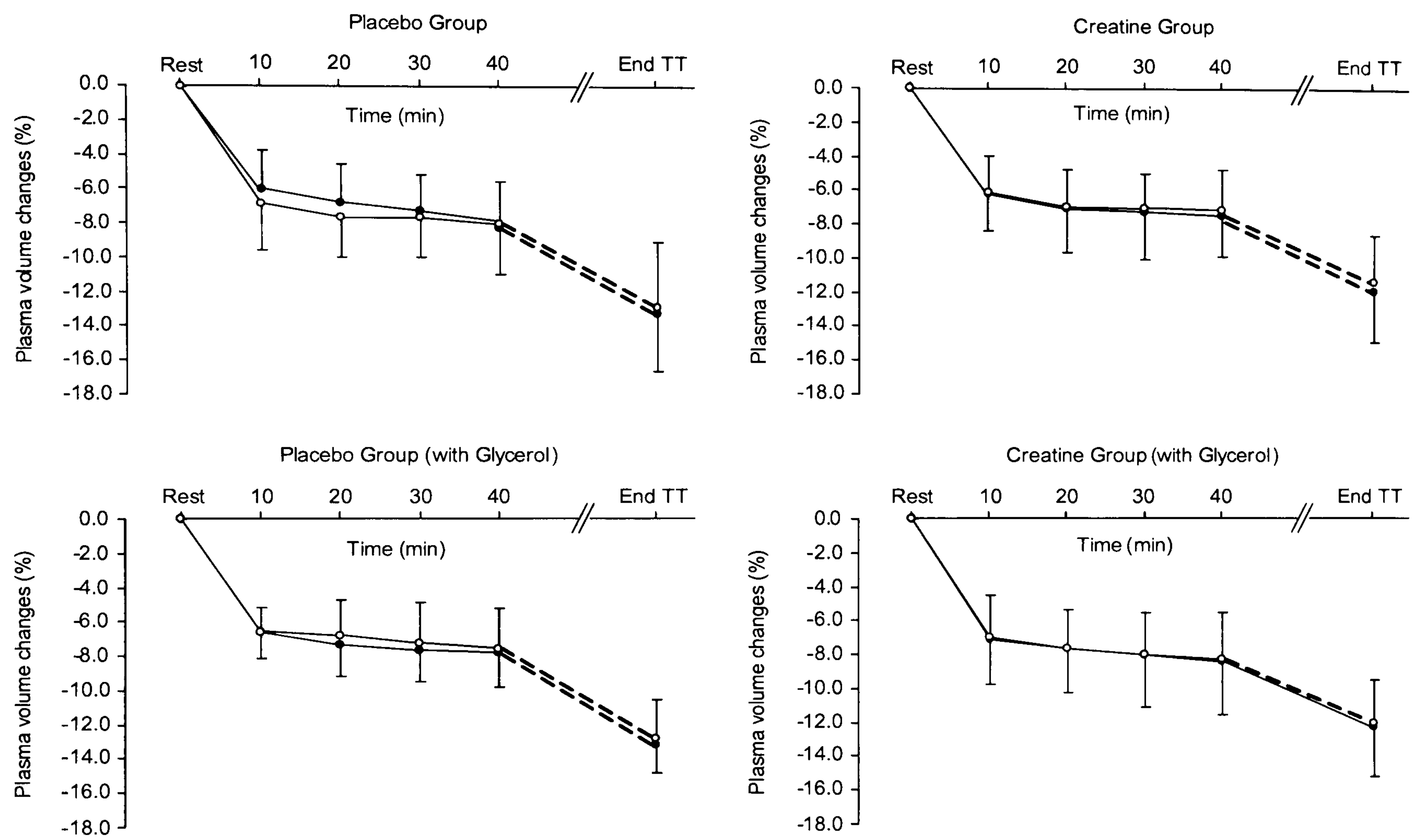


Figure 4.6. Percentage changes in plasma volume during exercise before (black circles) and after (white circles) supplementation in the two groups. Data presented as the mean \pm s.d.

4.4 Discussion

This study has demonstrated that supplementation with Cr, Gly and a combination of a Cr and Gly resulted in a significant increase in body mass (Fig. 4.2). Yet despite ingesting both Cr and Gly over several days to allow sufficient time for the retained fluid to be dispersed within body compartments neither Cr, Gly nor Cr/Gly supplementation resulted in any change in TBW, ECW, or ICW (Fig. 4.2). Furthermore, no supplementation protocol had any effect on perception of effort (Fig. 4.4), cardiovascular (Fig. 4.3) and thermoregulatory (Fig. 4.5) responses or performance during exercise in the heat.

The mean body mass increase of 1.20 kg induced by combined Cr and Gly supplementation in the present study is among the highest reported in the literature to date (Anderson *et al.*, 2001; Kilduff *et al.*, 2002; Kilduff *et al.*, 2004; Lyons *et al.*, 1990). However, as in Chapter 3 (a), the Cr/Gly induced increase in body mass was not accompanied by an accompanying increase in TBW. A statistical power calculation using the mean change in TBW from pre- to post-Cr/Gly supplementation (0.15 L) revealed that 349 subjects would be required to identify a significant difference in TBW at 80% power (with 6 subjects a significant increase in TBW would only be observed if the mean increase in TBW exceeded 1.1 L). Therefore despite the small increase in TBW, the number of subjects utilised in the present study may be too low to find any significant change in body water parameters following hyperhydration. However, the increase in TBW observed in the present study is considerably lower than that reported in other hyperhydration studies (e.g. Freund *et al.*, 1995; Kern *et al.*, 2001; Kilduff *et al.*, 2004; Lyons *et al.*, 1990) and therefore there must be some other explanation for the observed lack of TBW increase. Given that diet was homogenous throughout the supplementation period, it seems likely that Cr/Gly supplementation did increase water retention that was not measured by bioimpedance analysis. These results are in contrast with other hyperhydration studies that demonstrated an increase in fluid retention as reflected by a significant increase in TBW (Kilduff *et al.*, 2002; Kilduff *et al.*, 2004; Lyons *et al.*, 1990). For example, subjects in the study by Kilduff *et al.* (2004) experienced a 0.8 kg increase in body mass accompanied by a 0.6 L increase in TBW after 7 days of Cr supplementation. Therefore, it was concluded that some unknown negative interaction of the immediate fluid retaining abilities of both hyperhydrating agents resulted in an increase in body water that was not dispersed within body water compartments.

In a follow up study (Chapter 3 (b)) the limitations of the Cr and Gly loading protocol were addressed by ingesting both Cr and Gly over several days allowing sufficient time for the retained fluid to be dispersed within body compartments. In this study, supplementation with combined Cr and Gly for 7 days resulted in a mean body mass increase of approximately 1.0 kg and an increase in TBW of 0.9 L split evenly between the ICW and ECW compartments. The only difference between the Cr and Gly loading protocols utilised in Chapter 3 (b) and the present study was the length of time between the ingestion of the final supplement and testing. Thus, it would seem apparent that a period of time in excess of 3 hours is required for the retained water to be distributed within body water compartments. Previous studies examining the effectiveness of Gly supplementation as a hyperhydration method have consistently utilised a single Gly bolus mixed with water and ingested between 2-3 hours prior to analysis (Lyons *et al.*, 1990; Montner *et al.*, 1996). However, when Gly was delivered in a similar fashion alongside a Cr hyperhydration protocol previously shown to be successful in our lab (Kilduff *et al.*, 2004), no increase in hydration was measured (Fig. 4.2). Previous Gly hyperhydration studies have quantified water retention by the volume of urine produced (Anderson *et al.*, 2001; Freund *et al.*, 1995), which provides no information as to where the retained water was distributed. Body compartment analysis by multifrequency bioimpedance combined with changes in body mass used in the present study, provides data indicating fluid changes in both the intra- and extra-cellular water compartments. Furthermore, the mechanism by which bioimpedance estimates body water provides insight into the confounding data from Chapter 3 (b) and the present study. Since hypertonic solutions such as the Cr/Gly combination (965 ± 61 mosmol \cdot kg $^{-1}$) cause an initial net movement of fluid into the intestinal lumen (Gisolfi *et al.*, 1990), there is a loss of ECW and thus TBW, which ultimately leads to dehydration, albeit temporarily. This is confirmed by the small percentage reductions in plasma volume that occurred after supplementation with both Pl/Gly and Cr/Gly in both this study and Chapter 3 (a). Interestingly, fluid changes in the trunk have little effect on bioimpedance measurements as the trunk only accounts for 5-12% of total body impedance (Kushner, 1992). This is confirmed by the relatively small impact on bioimpedance measurements of up to 2 L of fluid within the abdominal cavity (Kushner *et al.*, 1996). Additionally, the profoundly high osmolality of the Cr/Gly solution may have inhibited gastric emptying (Costill & Saltin, 1974), further contributing to the lack of increase in TBW 2-3 hours after Cr/Gly ingestion as demonstrated in Chapter 3 (a) and the present study. Although Cr/Gly supplementation results in significant water retention, a period of time greater than 3 hours is required after ingestion of the final Cr/Gly supplement before significant hydrating effects are discerned throughout the body water compartments. Furthermore, it is possible

that a similar effect would have been observed after Cr/Gly supplementation in Chapter 3 (a) and the present study had a longer period of time been left between ingestion of the final supplement and testing.

Combined Cr and Gly supplementation in the present study was unsuccessful in attenuating the rise in perceived exertion and metabolic, cardiovascular and thermal responses during constant-load exercise in the heat (Figs. 4.3, 4.4, 4.5) (Table 4.2, 4.3). These findings are consistent with the only other study to examine the effects of combined Cr and Gly supplementation on metabolic, cardiovascular and thermoregulatory responses to exercise in the heat (Chapter 3 (a)). Given that heart rate and T_{c} rise in proportion to the level of dehydration during exercise in the heat (Montain & Coyle, 1992a;1992b) it is not surprising that physiological responses were the same following Cr/Gly supplementation as hydration status was not significantly altered. Previous studies investigating the effects of either Cr or Gly supplementation have reported reductions in heart rate and T_{c} during exercise in the heat, but only when TBW is significantly increased prior to commencement of exercise (Anderson *et al.*, 2001;Kilduff *et al.*, 2004). However, other studies have reported no difference in water retention between Gly and Pl supplementation and consequently no differences were observed in T_{c} , T_{sk} , sweat rate, cardiac output, blood pressure or heart rate during constant-load exercise in the heat (Latzka *et al.*, 1997;1998).

Exercise performance in the present study was not significantly altered following any of the supplementation regimens, which would be predictable given the lack of an increase in TBW from pre- to post-supplementation. Several studies have indicated that the increased heart rate and T_{c} resulting from dehydration can have a negative impact on exercise performance (Below *et al.*, 1995;Cheuvront *et al.*, 2003;Cheuvront *et al.*, 2005;Fallowfield *et al.*, 1996;McConnell *et al.*, 1997;Walsh *et al.*, 1994). For example, Cheuvront *et al.* (2005) determined that hypohydration was associated with an increased T_{c} and heart rate and a significant reduction in work performed during a 30 min cycling time trial, even in a temperate (20°C) environment. Conversely, other studies find no effect of hyperhydration on exercise performance when compared to euhydration (Latzka *et al.*, 1998;Marino *et al.*, 2003). For example, Marino *et al.* (2003) found Gly hyperhydration had no effect on a 60 min cycling time trial in hot and humid conditions compared to pre-exercise water ingestion. Therefore, it is presently unknown whether the lack of performance improvement observed in the present study is simply due to a similar hydration status between experimental trials or indeed whether commencing exercise in a hyperhydrated state may not confer any significant advantage in terms of exercise performance compared to euhydration.

4.4.1 Conclusions

This study has demonstrated that supplementation with Cr, Gly or a combination of Cr and Gly resulted in a significant increase in body mass. Yet despite ingesting both Cr and Gly over several days to allow sufficient time for the retained fluid to be dispersed within body compartments there was no change in TBW, ICW, ECW or RPE, heart rate and T_c during exercise in the heat compared to pre-supplementation. It is probable that ingestion of a hypertonic solution such as the Cr and Gly mixture resulted in slowing of gastric emptying and an initial efflux of water from the plasma into the intestinal lumen. Therefore, the timing of ingestion is evidently critical, with the final supplement requiring to be consumed longer than 3 hours prior to the need for hyperhydration.

(b) A comparison of two different combined creatine and glycerol fluid loading strategies on fluid retention and distribution

4.5 Introduction

The hypothesis that simultaneous ingestion of the hyperhydrating agents Cr and Gly would result in a greater retention of fluid than either Cr or Gly alone and therefore result in a significant reduction of T_c and heart rate during exercise in the heat has been previously examined (Chapter 3 (a), Chapter 4 (a)) and found to be unsuccessful. Initially, it was concluded that although combined Cr/Gly supplementation resulted in significant fluid retention, the fluid was not dispersed in body compartments, perhaps due to some unknown negative interaction on the immediate fluid retaining abilities of both hyperhydrating agents (Chapter 3 (a)). However, ingesting both Cr and Gly over several days allowing sufficient time for the retained fluid to be dispersed within body compartments still did not cause a significant increase in TBW (Chapter 4 (a)). Since hypertonic solutions such as the Cr/Gly combination ($965 \pm 61 \text{ mosmol} \cdot \text{kg}^{-1}$) cause an initial net movement of fluid into the intestinal lumen (Gisolfi *et al.*, 1990), there is a loss of ECW and thus TBW, which ultimately leads to dehydration, albeit temporarily. Additionally, the profoundly high osmolality of the Cr/Gly solution may have inhibited gastric emptying (Costill & Saltin, 1974), further contributing to the lack of increase in TBW 2-3 hours after Cr/Gly ingestion as demonstrated in Chapter 3 (a) and 4 (a). Thus, it would seem apparent that a period of time in excess of 3 hours is required for the retained water to be distributed within body water compartments. Therefore, the aim of this study to examine the effects of extending the period of time between ingestion of the final Cr/Gly supplement on the retention and distribution of fluid. The overall aim was to develop an effective ‘fluid-loading’ strategy for use during exercise in the heat.

4.6 Methods

4.6.1 Subjects

4 healthy males gave their written informed consent to take part in the present study that was approved by the local Ethics Committee and was performed according to the code of ethics of the World Medical Association (Declaration of Helsinki). The physical characteristics of the four subjects were, age 29 ± 8 years; height 174 ± 8 cm; body mass 67.9 ± 9.8 kg.

4.6.2 Experimental design

The study consisted of one Cr/Gly supplementation regimen lasting for 7 days with physiological measurements recorded pre- and post-supplementation. Subjects were matched for body mass and were randomised to either a '3 hour group' or a '5 hour group'. The supplementation period started immediately after the pre-supplementation measurements (day 1) and finished on the day of the post-supplementation experimental trial (day 7). Cr/Gly supplementation consisted of 11.4 g of Cr·H₂O (equivalent to 10 g Cr), 1 g Gly·kg⁻¹ body mass and 70 g of glucose polymer made up in 1 L of warm water and consumed twice daily for 6 days and once more on the day of the post-supplementation trial. Subjects in the '3 hour group' ingested their final Cr/Gly supplement 3 hours prior to the post-supplementation experimental trial and those in the '5 hour group' ingested the final supplement 5 hours prior to the experimental trial. Each supplement was made fresh prior to consumption in order to prevent any degradation of Cr to creatinine. On each of the experimental test days, subjects ingested 500 ml of water 2 hours prior to measurement and a further 500 ml of water 1 hour prior to measurement in an attempt to ensure subjects were euhydrated prior to all exercise trials (Convertino *et al.*, 1996).

On the day of each experimental trial subjects reported to the laboratory having refrained from alcohol and strenuous exercise in the previous 24 hours. Subjects were asked to void before nude body mass was recorded and body water compartments estimated using a Bodystat Multiscan 5000 Bioimpedance analyser (Bodystat Ltd, Isle of Man). This method allows TBW and ECW to be estimated; from these measurements ICW can also be deduced. The bioimpedance measurements were taken while the subjects lay comfortably in a supine position for 10 min on a non-conductive surface with their arms and legs slightly abducted.

4.7 Results

4.7.1 Body mass and water compartments.

Following Cr/Gly supplementation, there was a large increase in body mass from pre- to post-supplementation in both the 3 hour and the 5 hour group (Fig. 4.7). TBW increased by 0.4 ± 0.4 L from pre- to post-supplementation in the 3-hour group but increased by a greater extent in the 5 hour group (1.1 ± 0.4 L). ICW increased to a similar extent in both

groups (3 hour group: 0.4 ± 0.2 L, 5 hour group: 0.5 ± 0.3 L) whereas ECW increased in the 5 hour group (0.6 ± 0.3 L) but was unchanged in the 3 hour group.

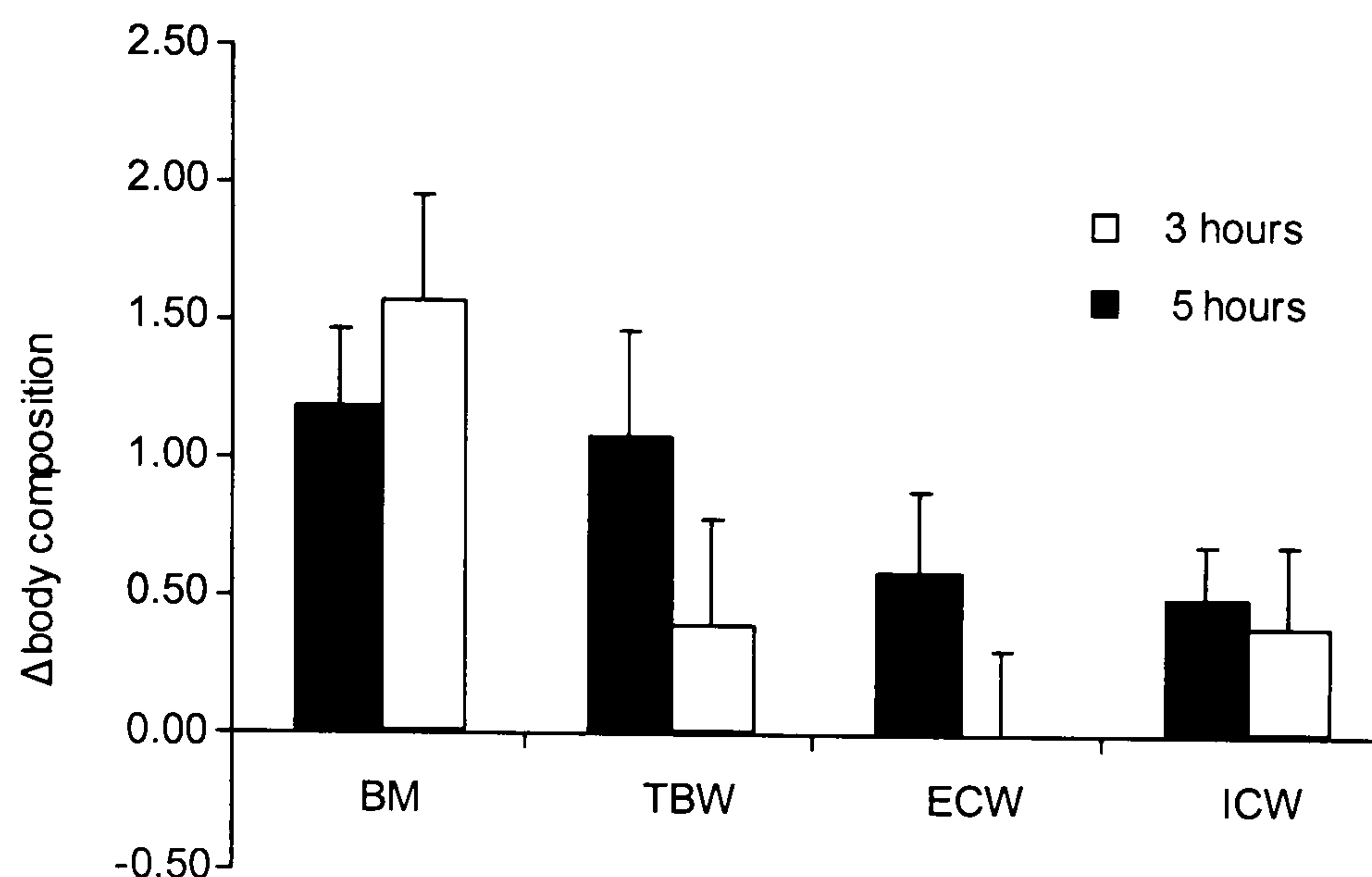


Figure 4.7 Changes in body mass (BM), total body water (TBW), intra-cellular water (ICW) and extra-cellular water (ECW) in the two groups. Data presented as the mean \pm s.d.

4.8 Discussion

This study has demonstrated that supplementation with combined Cr and Gly results in a significant increase in both body mass and TBW only when the final supplement is consumed 5 hours prior to measurement (Fig. 4.7). These findings highlight the importance of the timing of ingestion of the final supplement prior to exercise. Based on these results it is rational to assume that supplementation with combined Cr and Gly will delay the onset of dehydration during exercise in the heat resulting in a reduced T_c and heart rate and improved exercise performance.

In the present study supplementation with combined Cr and Gly for 7 days resulted in a mean body mass increase of 1.6 ± 0.3 kg (Fig. 4.7) in the 3 hour group and 1.2 ± 0.4 kg in the 5 hour group, which are among the highest reported in the literature to date (Anderson *et al.*, 2001; Kilduff *et al.*, 2002; 2004; Kreider *et al.*, 1998; Lyons *et al.*, 1990; Montner *et al.*, 1996; Ziegenfuss *et al.*, 1998) and similar to those reported in Chapter 3 (a), (b) and Chapter 4 (a). Cr/Gly supplementation resulted in a significant increase in TBW with the retained fluid dispersed equally between ICW and ECW compartments when the final supplement was consumed 5 hours prior to measurement but did not change when the final supplement was consumed 3 hours prior to measurement (Fig. 4.7). Given that Cr supplementation has been consistently shown to increase ICW (Kilduff *et al.*,

2002;2004;Ziegenfuss *et al.*, 1998) whereas Gly retains fluid in all body compartments with the exception of the cerebral spinal fluid and aqueous humor (Freund *et al.*, 1995;Lin, 1977;Seifert *et al.*, 1995;Tourtellotte *et al.*, 1972) it is reasonable to assume that the observed increases in ICW and ECW were mediated by Cr and Gly respectively.

4.8.3 Conclusion

The results of this study indicate that consuming both Cr and Gly over several days and ingesting the final supplement 5 hours prior to exercise is the most effective method of fluid loading. This will allow sufficient time for the retained fluid to leave the stomach, pass across the intestinal lumen wall and be dispersed within body compartments.

Chapter 5

The effects of combined creatine and glycerol hyperhydration on metabolism, thermoregulation and exercise performance in the heat: Loading protocol 3.

5.1 Introduction

Previous attempts to ‘fluid load’ prior to exercise using a combination of Cr and Gly have been unsuccessful due to the complex interaction between the hyperhydrating agents and body water compartments. Since hypertonic solutions such as the Cr/Gly combination ($965 \pm 61 \text{ mosmol}\cdot\text{kg}^{-1}$) cause an initial net movement of fluid into the intestinal lumen (Gisolfi *et al.*, 1990), there is a loss of ECW and thus TBW, which ultimately leads to dehydration, albeit temporarily. Additionally, the profoundly high osmolality of the Cr/Gly solution may have inhibited gastric emptying (Costill & Saltin, 1974), further contributing to the lack of increase in TBW 2-3 hours after Cr/Gly ingestion as demonstrated in Chapter 3 (a) and 4 (a). Results from Chapter 4 (b) confirm that a period of at least 5 hours is required for the retained water to be distributed within body water compartments. Intuitively, this increased fluid would maintain plasma volume resulting in a reduction in heart rate during exercise induced heat stress. As a direct result, skin blood flow would be maintained allowing sufficient continuation of convective heat loss. Additionally, there would be sufficient body water to maintain adequate sweat production and optimum evaporative cooling, which overall would enhance thermoregulatory function. Furthermore, it is possible that the Cr-induced increase in ICW may result in an increase in the specific heat capacity of the body, resulting in a greater capacity to store heat (Kilduff *et al.*, 2004). Thus, it is proposed that the reduction in heart rate and T_{e} induced by Cr/Gly hyperhydration should ultimately improve exercise performance, especially in the heat (Convertino *et al.*, 1996). It seems plausible that a Gly-induced increase in ECW coupled with a Cr-mediated increase in ICW could have synergistic effects resulting in a much larger increase in TBW than if either supplement was consumed alone. Therefore, the primary aim of this study was to examine whether combining Cr and Gly can induce a greater hyperhydration than either Cr or Gly alone. If successful, a secondary aim of this study was to assess the effects of this novel ‘water-loading’ strategy on thermoregulation and performance during exercise in the heat.

5.2 Methods

5.2.1 Subjects

24 endurance-trained males (Table 5.1) gave their written informed consent to take part in the present study which was approved by the local Ethics Committee and was performed

according to the code of ethics of the World Medical Association (Declaration of Helsinki); one subject withdrew from the study due to injury unrelated to this project.

Table 5.1. The physical characteristics, maximal oxygen uptake and maximal work rate of the two groups of subjects. Data presented as the mean \pm s.d.

| | Pl Group (n=11) | Cr Group (n=12) |
|--|--------------------|--------------------|
| Age (yr) | 31 \pm 8 | 31 \pm 6 |
| Height (cm) | 177 \pm 6 | 177 \pm 5 |
| Weight (kg) | 75.2 \pm 7 | 75.0 \pm 6 |
| $\dot{V}O_{2\text{ max}}$ (L \cdot min ⁻¹) | 4.3 \pm 0.5 | 4.2 \pm 0.4 |
| WR _{max} (W) | 361 \pm 28 | 357 \pm 28 |

5.2.2 Experimental design

The experimental design by necessity is complicated and best understood by reference to Fig. 5.1. The study consisted of two supplementation regimens, each lasting 7 days and encompassing two cycle performance trials consisting of 40 min constant-load exercise at 63% WR_{max} followed by a 16.1 km (10 mile) time trial. The methodology for the exercise trials is described in Chapter 2 of this thesis. Prior to the first of these experimental trials, familiarisation trials were completed until the variability of two consecutive trials was within 5%; no subject had to perform a third familiarisation trial to achieve less than 5% variability. Following this familiarisation period, subjects were matched for body mass and were randomised in a double blind fashion to either a Cr or a Pl group. Subjects were separated into two groups due to the long wash-out period associated with oral Cr supplementation (Vandenbergh *et al.*, 1996). Subjects in both groups performed an exercise trial pre- and post-supplementation during both supplementation regimens (i.e. a total of 4 experimental exercise trials) (see Fig. 5.1). The first test was conducted at least 48 hours after each subject's final familiarisation trial. Each supplementation period started on the day after the first test and finished on the day of the second test.

Cr supplementation consisted of 11.4 g of Cr·H₂O (equivalent to 10 g Cr) and 70 g of glucose polymer made up in 1 L of warm water and consumed twice daily for 6 days and once more on the day of the experimental exercise trial. This protocol has been shown to increase resting muscle phosphocreatine levels within 5 days (Harris *et al.*, 1992). Each supplement was made fresh prior to consumption in order to prevent any degradation of Cr to creatinine. The Pl supplement consisted of 85 g of glucose polymer made up in 1 L of warm water and consumed twice daily for 6 days and once more on the day of the experimental exercise trial. During the two supplementation regimens, subjects in both groups also received either 1 g Gly·kg⁻¹ body mass or an equivalent amount of Pl (i.e. sucrose) diluted in each 1 L supplement. Therefore, four possible combinations of supplements were administered: Pl group: Pl/Pl and Pl/Gly; Cr Group: Cr/Pl and Cr/Gly. For the two post-supplementation trials, subjects began consuming the final supplement 5 hours prior to the exercise performance trial. All supplements had similar taste, texture and appearance and were placed in generic water bottles to ensure double blind administration. On each of the experimental test days, subjects ingested 500 ml of water 3 hours prior to exercise and a further 500 ml of water 1 hour prior to exercise in an attempt to ensure subjects were euhydrated prior to all exercise trials (Convertino *et al.*, 1996).

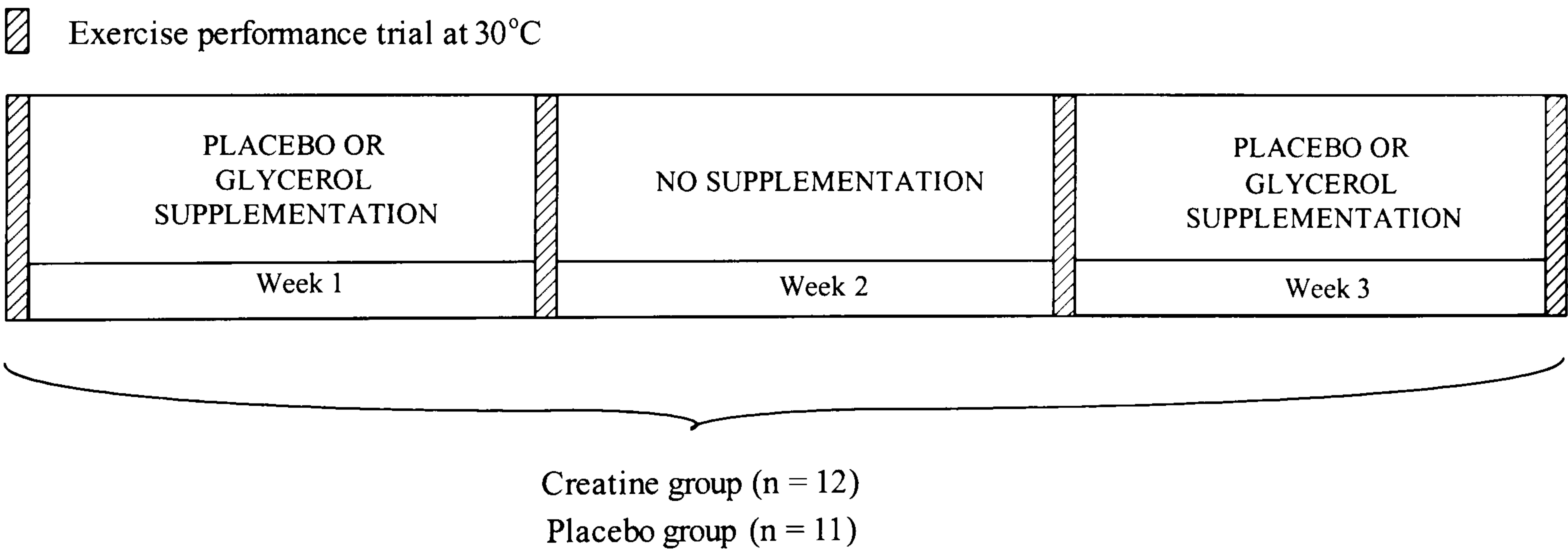


Figure 5.1 Schematic representation of the experimental design.

5.3 Results

5.3.1 Diet, body mass and water compartments.

The physical characteristics of the two groups were similar before supplementation (Table 5.1). In the Pl group, body mass increased significantly following Gly supplementation, with no change during the Pl regimen (Δ body mass was greater following Gly supplementation; Table 5.2, Fig. 5.2). In the Cr group, body mass increased significantly during both the Pl and Gly regimens (Table 5.2, Fig. 5.2). Furthermore, the increase in body mass was significantly greater when Gly was consumed in combination with Cr than when Cr was consumed alone ($P=0.02$) (Table 5.2, Fig. 5.2). There was no difference pre-supplementation in TBW, ICW and ECW between groups. In the Pl group, TBW and ECW increased significantly following Gly supplementation, whereas TBW and ECW were unaltered in the Pl regimen (Fig. 5.2). There was a significant increase in ICW in the Pl group following Gly supplementation ($P=0.01$) but not during the Pl regimen ($P=0.10$). In the Cr group, TBW and ICW increased significantly during both supplementation regimens (Fig. 5.2), and a significant increase in ECW observed only following the Gly regimen (Fig. 5.2). Additionally, the increase in TBW and ECW in the Cr group was significantly greater following Gly supplementation than Pl ($P=0.02$ and $P<0.01$, respectively) (Fig. 5.2). There were no significant differences in the daily diet between the two groups or between Pl and Gly regimens (Table 5.3).

5.3.2 Cardiopulmonary variables.

There was no significant change in $\dot{V}O_2$ (Table 5.4), $\dot{V}CO_2$ or $\dot{V}E$ (data not shown) during constant-load exercise and no differences were found between groups before or as a result of supplementation. There was no difference in resting heart rate between the two groups or after supplementation (Fig. 5.3). During exercise, heart rate increased during all trials. In the Pl group, heart rate during exercise was significantly lower following Gly supplementation compared to pre-supplementation ($P<0.01$) (Fig. 5.3). No such difference was found in the Pl trial (Fig. 5.3). In the Cr group, heart rate was significantly lower after both Cr/Pl and Cr/Gly supplementation regimens compared to pre-supplementation (Fig. 5.3). There was no difference in the Δ heart rate pre- and post-supplementation between the Pl and Gly supplementation regimens in the Cr group ($P=0.65$).

Table 5.2. Change in body mass from pre- to post-supplementation in each supplementation regimen

| Subject | Pl/Pl | Pl/Gly | Subject | Cr/Pl | Cr Gly |
|-------------|-------------|---------------|---------|--------------|---------------|
| 1 | 0.10 | 0.34 | 12 | 0.51 | 1.10 |
| 2 | -0.09 | 0.82 | 13 | 0.85 | 1.03 |
| 3 | -0.23 | 1.00 | 14 | 1.03 | 1.37 |
| 4 | 0.11 | 0.03 | 15 | 0.06 | 0.37 |
| 5 | -0.16 | 0.80 | 16 | 0.90 | 1.00 |
| 6 | 0.03 | 0.45 | 17 | 1.37 | 1.14 |
| 7 | 0.45 | 0.85 | 18 | 0.35 | 0.84 |
| 8 | 0.45 | 0.40 | 19 | 0.88 | 0.95 |
| 9 | 0.42 | 0.63 | 20 | 1.35 | 1.42 |
| 10 | 0.00 | 0.52 | 21 | 0.39 | 0.87 |
| 11 | 0.00 | 0.45 | 22 | 0.97 | 0.75 |
| | | | 23 | 0.13 | 0.79 |
| Mean ± s.d. | 0.10 ± 0.24 | 0.57 ± 0.28†* | | 0.73 ± 0.44† | 0.97 ± 0.28†* |

†: indicates a significant difference pre- vs post-supplementation. *: indicates a significant greater change (Δ) in the Gly supplementation regimen compared to the Pl supplementation regimen.

Table 5.3. Composition of the average daily diet in each supplementation regimen

| Group | Regimen | Energy (MJ·day ⁻¹) | Carbohydrate (%) | Fat (%) | Protein (%) |
|-------|---------|--------------------------------|------------------|---------|-------------|
| Pl | Pl | 12.3 ± 2.7 | 64 ± 8 | 24 ± 5 | 12 ± 3 |
| Pl | Gly | 12.9 ± 3.2 | 65 ± 6 | 22 ± 4 | 13 ± 3 |
| Cr | Pl | 13.5 ± 2.6 | 67 ± 4 | 21 ± 5 | 12 ± 2 |
| Cr | Gly | 13.6 ± 2.8 | 67 ± 5 | 22 ± 5 | 11 ± 3 |

Data presented as the mean ± s.d.

Table 5.4. VO_2 ($\text{L}\cdot\text{min}^{-1}$) during constant-load exercise

| Group | Regimen | Trial | Exercise time (min) | | | | |
|-------|---------|-------|---------------------|-----------|-----------|-----------|-----------|
| | | | Rest | 10 | 20 | 30 | 40 |
| P1 | P1 | Pre | 0.4 ± 0.1 | 2.8 ± 0.4 | 3.0 ± 0.4 | 3.0 ± 0.5 | 3.0 ± 0.4 |
| P1 | P1 | Post | 0.4 ± 0.1 | 2.9 ± 0.5 | 3.0 ± 0.5 | 3.0 ± 0.5 | 3.0 ± 0.5 |
| P1 | Gly | Pre | 0.3 ± 0.1 | 2.9 ± 0.3 | 3.0 ± 0.4 | 3.0 ± 0.4 | 3.0 ± 0.4 |
| P1 | Gly | Post | 0.4 ± 0.1 | 3.0 ± 0.4 | 3.0 ± 0.4 | 3.0 ± 0.4 | 3.0 ± 0.5 |
| Cr | P1 | Pre | 0.3 ± 0.1 | 3.0 ± 0.5 | 3.1 ± 0.5 | 3.1 ± 0.5 | 3.1 ± 0.4 |
| Cr | P1 | Post | 0.3 ± 0.1 | 3.0 ± 0.3 | 3.1 ± 0.3 | 3.2 ± 0.4 | 3.1 ± 0.3 |
| Cr | Gly | Pre | 0.3 ± 0.1 | 2.9 ± 0.4 | 3.0 ± 0.4 | 3.1 ± 0.4 | 3.1 ± 0.4 |
| Cr | Gly | Post | 0.3 ± 0.1 | 2.9 ± 0.4 | 3.0 ± 0.4 | 3.0 ± 0.4 | 3.0 ± 0.4 |

Data presented as the mean ± s.d.

5.3.3 Ratings of perceived exertion during exercise.

There was a progressive increase in RPE both for perceived leg fatigue (Fig. 5.4) and dyspnoea (Fig. 5.5) during exercise reaching near maximum ratings at the end of the time trial. A significant three-way interaction ($P=0.04$) was observed in RPE for perceived leg fatigue (Fig. 5.4). Significantly lower ratings of perceived leg fatigue were found in the Cr group following both supplementation regimens; no such effect was found in the Pl group (Fig. 5.4). There was also a significant three-way interaction in RPE for dyspnoea ($P=0.05$) (Fig. 5.5). Significantly lower ratings of dyspnoea were found after Cr/Gly supplementation ($P=0.02$) but not after Cr/Pl ($P=0.10$); no such effect was found in the Pl group in either supplementation regimen (Fig. 5.5). There was no difference in Δ RPE for perceived leg fatigue or dyspnoea pre- and post-supplementation between Pl and Gly supplementation regimens in either group.

5.3.4. Rectal and skin temperature responses.

There was a significant increase in mean T_{sk} from rest, with no significant differences between groups or following supplementation (Fig. 5.6). Throughout the exercise period, T_{re} increased significantly during all trials (Fig. 5.7). A simple main effects analysis revealed that T_{re} during exercise was not significantly different in the Pl group during either the Pl ($P=0.71$) or the Gly ($P=0.10$) supplementation regimen (Fig. 5.7). In the Cr group, T_{re} was significantly lower following both Pl ($P<0.01$) and Gly ($P<0.01$) supplementation regimens compared to pre-supplementation (Fig. 5.7). However, there was no difference in the ΔT_{re} pre- and post-supplementation between the Pl and Gly supplementation regimens in the Cr group ($P=0.29$).

5.3.5. Sweat rates and total sweat loss during exercise.

There was a significant increase in sweat rate following Gly supplementation in both the Pl ($1.4 \pm 0.3 \text{ L}\cdot\text{hr}^{-1}$ vs. $1.6 \pm 0.4 \text{ L}\cdot\text{hr}^{-1}$; $P=0.02$) and the Cr ($1.3 \pm 0.4 \text{ L}\cdot\text{hr}^{-1}$ vs. $1.5 \pm 0.4 \text{ L}\cdot\text{hr}^{-1}$; $P<0.01$) groups. No such increase was observed during the Pl supplementation regimen in either the Pl or the Cr group. Furthermore, total sweat loss increased significantly following Gly supplementation in both the Pl ($1.5 \pm 0.3 \text{ L}$ vs. $1.7 \pm 0.4 \text{ L}$; $P=0.02$) and the Cr ($1.4 \pm 0.4 \text{ L}$ vs. $1.5 \pm 0.4 \text{ L}$; $P=0.02$) groups. No such increase was observed during the Pl supplementation regimen in either the Pl or the Cr group.

5.3.6 Blood metabolite concentrations and plasma volume changes.

Blood [glucose] and [lactate] at rest and during exercise were not different between groups or following Gly supplementation (Table 5.5 and 5.6). Resting plasma [Gly] was significantly higher post Gly supplementation compared to pre-supplementation in both the Pl ($P<0.01$) and Cr ($P<0.01$) groups (Table 5.7). Plasma [Gly] remained significantly higher throughout exercise after Gly supplementation compared to the pre-supplementation trial in both the Cr and the Pl group. There was no difference in resting plasma [Gly] or during exercise between pre- and post-supplementation during the Pl supplementation regimen in either the Pl or the Cr group (Table 5.7). Plasma [Gly] was not correlated to the increase in TBW after either Pl/Gly ($r=0.37$, $P=0.48$) or Cr/Gly ($r=0.51$, $P=0.23$) supplementation. Plasma volume was reduced by approximately 8% after 40 min of constant-load exercise and 12% after the 16.1 km time trial with no significant differences between groups or following supplementation (Fig. 5.8). Resting plasma volume changes following supplementation were also calculated using the control trial as a baseline, assuming no change in red cell mass during the 7 day supplementation regimen. Using this method of analysis, plasma volume was not significantly altered following either Pl/Pl or Cr/Pl supplementation. Both Pl/Gly and Cr/Gly supplementation resulted in a percentage increase in plasma volume, although only after the Cr/Gly supplementation did this increase reach statistical significance ($P=0.06$ and $P=0.01$, respectively).

5.3.7 Osmolality.

Resting serum osmolality was significantly higher post Gly supplementation compared to pre-supplementation in both the Pl ($P=0.02$) and the Cr ($P<0.01$) groups (Table 5.8). Serum osmolality remained significantly higher throughout exercise after Gly supplementation compared to the pre-supplementation trial in both the Cr and the Pl group. There were no other differences in serum osmolality (Table 5.8).

5.3.8 Time Trial Performance.

Time trial performance was not significantly different between the groups prior to supplementation ($P=0.62$). Time trial performance did not differ significantly pre- to post-supplementation in either the Pl group (Pl/Pl, pre vs. post: 23.1 ± 1.0 min vs. 22.9 ± 1.1 min; Pl/Gly, pre vs. post: 23.1 ± 1.3 min vs. 22.9 ± 1.0 min) or Cr group (Cr/Pl, pre vs. post: 23.4 ± 1.5 min vs. 23.2 ± 1.2 min; Cr/Gly, pre vs. post: 23.4 ± 1.3 min vs. 23.0 ± 1.2 min) in either supplementation regimen.

5.3.9 Side effects.

In general, subjects tolerated the supplementation protocol well with only one report of gastrointestinal distress after Gly supplementation. Three subjects from each group correctly identified the subject group and 7 subjects correctly identified the Gly supplementation regimen, while all other subjects were unsure of the treatment they received.

Table 5.5. Plasma [Glucose] (mmol·L⁻¹) during exercise before and after supplementation in the two groups

| Group | Regimen | Trial | Exercise time (min) | | | | | End TT |
|-------|---------|-------|---------------------|-------------|-------------|-------------|-------------|-------------|
| | | | Rest | 10 | 20 | 30 | 40 | |
| P1 | P1 | Pre | 4.68 ± 0.48 | 4.53 ± 0.49 | 4.73 ± 0.47 | 5.01 ± 0.60 | 5.10 ± 0.82 | 5.27 ± 0.26 |
| P1 | P1 | Post | 4.88 ± 0.55 | 4.39 ± 0.52 | 4.62 ± 0.40 | 4.75 ± 0.25 | 4.93 ± 0.89 | 5.15 ± 0.50 |
| P1 | Gly | Pre | 4.74 ± 0.36 | 4.51 ± 0.35 | 4.67 ± 0.35 | 4.83 ± 0.59 | 4.79 ± 0.87 | 4.96 ± 1.18 |
| P1 | Gly | Post | 4.83 ± 0.35 | 4.46 ± 0.34 | 4.64 ± 0.46 | 4.86 ± 0.57 | 4.95 ± 0.74 | 5.29 ± 1.18 |
| Cr | P1 | Pre | 4.79 ± 0.45 | 4.35 ± 0.33 | 4.41 ± 0.66 | 4.49 ± 0.57 | 4.62 ± 0.80 | 5.17 ± 1.39 |
| Cr | P1 | Post | 4.74 ± 0.45 | 4.17 ± 0.38 | 4.25 ± 0.53 | 4.46 ± 0.62 | 4.59 ± 0.59 | 4.97 ± 0.80 |
| Cr | Gly | Pre | 4.77 ± 0.49 | 4.31 ± 0.42 | 4.27 ± 0.59 | 4.42 ± 0.55 | 4.62 ± 0.69 | 5.48 ± 1.15 |
| Cr | Gly | Post | 4.72 ± 0.35 | 4.40 ± 0.39 | 4.34 ± 0.50 | 4.50 ± 0.60 | 4.68 ± 0.71 | 5.58 ± 1.09 |

Data presented as the mean ± s.d.

Table 5.6. Plasma [Lactate] (mmol·L⁻¹) during exercise before and after supplementation in the two groups

| Group | Regimen | Trial | Exercise time (min) | | | | | End TT |
|-------|---------|-------|---------------------|-------------|-------------|-------------|-------------|-------------|
| | | | Rest | 10 | 20 | 30 | 40 | |
| Pl | Pl | Pre | 0.66 ± 0.09 | 2.00 ± 1.11 | 2.03 ± 1.18 | 2.02 ± 1.31 | 2.32 ± 1.49 | 6.65 ± 2.51 |
| Pl | Pl | Post | 0.71 ± 0.25 | 1.85 ± 1.04 | 1.80 ± 1.10 | 2.03 ± 1.24 | 2.10 ± 1.35 | 6.59 ± 2.34 |
| Pl | Gly | Pre | 0.67 ± 0.27 | 1.63 ± 0.90 | 1.51 ± 0.90 | 1.64 ± 1.13 | 1.68 ± 1.02 | 6.55 ± 2.27 |
| Pl | Gly | Post | 0.71 ± 0.18 | 1.83 ± 1.11 | 2.02 ± 1.50 | 2.18 ± 1.74 | 2.22 ± 1.87 | 6.86 ± 2.17 |
| Cr | Pl | Pre | 0.75 ± 0.20 | 2.35 ± 1.08 | 2.49 ± 1.59 | 2.58 ± 1.78 | 2.63 ± 1.85 | 6.77 ± 2.19 |
| Cr | Pl | Post | 0.84 ± 0.16 | 2.04 ± 1.16 | 2.31 ± 1.37 | 2.39 ± 1.56 | 2.52 ± 1.78 | 7.61 ± 1.80 |
| Cr | Gly | Pre | 0.68 ± 0.14 | 1.85 ± 0.74 | 1.86 ± 0.87 | 1.83 ± 0.81 | 2.03 ± 0.82 | 7.07 ± 2.23 |
| Cr | Gly | Post | 0.72 ± 0.26 | 1.94 ± 0.71 | 2.09 ± 0.90 | 2.29 ± 1.05 | 2.37 ± 0.89 | 8.10± 2.82 |

Data presented as the mean ± s.d.

Table 5.7. Plasma [Gly] (mmol·L⁻¹) during exercise before and after supplementation in the two groups

| Group | Regimen | Trial | Exercise time (min) | | | | | End TT |
|-------|---------|-------|---------------------|----------------|----------------|----------------|----------------|----------------|
| | | | Rest | 10 | 20 | 30 | 40 | |
| PI | PI | Pre | 0.05 ± 0.02 | 0.14 ± 0.04 | 0.19 ± 0.05 | 0.23 ± 0.04 | 0.26 ± 0.06 | 0.38 ± 0.09 |
| PI | PI | Post | 0.06 ± 0.03 | 0.16 ± 0.02 | 0.21 ± 0.05 | 0.25 ± 0.06 | 0.28 ± 0.06 | 0.40 ± 0.10 |
| PI | Gly | Pre | 0.04 ± 0.02 | 0.16 ± 0.04 | 0.19 ± 0.05 | 0.25 ± 0.06 | 0.28 ± 0.08 | 0.37 ± 0.11 |
| PI | Gly | Post | 7.60 ± 1.36 †* | 6.92 ± 1.38 †* | 6.62 ± 1.35 †* | 6.28 ± 1.39 †* | 6.06 ± 1.33 †* | 5.26 ± 1.22 †* |
| Cr | PI | Pre | 0.06 ± 0.02 | 0.15 ± 0.04 | 0.20 ± 0.05 | 0.27 ± 0.06 | 0.29 ± 0.09 | 0.40 ± 0.10 |
| Cr | PI | Post | 0.03 ± 0.02 | 0.13 ± 0.04 | 0.18 ± 0.05 | 0.22 ± 0.07 | 0.25 ± 0.06 | 0.36 ± 0.04 |
| Cr | Gly | Pre | 0.04 ± 0.02 | 0.16 ± 0.04 | 0.21 ± 0.05 | 0.25 ± 0.06 | 0.28 ± 0.07 | 0.42 ± 0.12 |
| Cr | Gly | Post | 7.12 ± 1.41 †* | 6.55 ± 1.43 †* | 6.32 ± 1.39 †* | 5.98 ± 1.38 †* | 5.76 ± 1.37 †* | 4.89 ± 1.40 †* |

Data presented as the mean ± s.d. †: indicates a significant difference pre- vs post-supplementation. *: indicates a significant greater change (Δ) in the Gly supplementation regimen compared to the PI supplementation regimen

Table 5.8. Serum osmolality (mosmol·kg⁻¹) during exercise before and after supplementation in the two groups

| Group | Regimen | Trial | Exercise time (min) | | | | | End TT |
|-------|---------|-------|---------------------|------------|------------|------------|------------|------------|
| | | | Rest | 10 | 20 | 30 | 40 | |
| Pl | Pl | Pre | 283 ± 2 | 286 ± 3 | 290 ± 2 | 290 ± 3 | 290 ± 3 | 294 ± 3 |
| Pl | Pl | Post | 283 ± 2 | 287 ± 2 | 290 ± 2 | 290 ± 3 | 290 ± 3 | 295 ± 3 |
| Pl | Gly | Pre | 284 ± 3 | 287 ± 3 | 289 ± 2 | 289 ± 2 | 290 ± 2 | 294 ± 3 |
| Pl | Gly | Post | 291 ± 3 †* | 294 ± 2 †* | 295 ± 3 †* | 296 ± 2 †* | 295 ± 2 †* | 299 ± 4 †* |
| Cr | Pl | Pre | 285 ± 2 | 289 ± 3 | 290 ± 3 | 289 ± 2 | 290 ± 2 | 294 ± 2 |
| Cr | Pl | Post | 281 ± 2 | 286 ± 3 | 288 ± 2 | 288 ± 2 | 289 ± 3 | 292 ± 2 |
| Cr | Gly | Pre | 284 ± 3 | 288 ± 4 | 289 ± 3 | 288 ± 3 | 289 ± 3 | 293 ± 2 |
| Cr | Gly | Post | 292 ± 2 †* | 294 ± 2 †* | 294 ± 3 †* | 293 ± 2 †* | 293 ± 2 | 298 ± 2 †* |

Data presented as the mean ± s.d. †: indicates a significant difference pre- vs post-supplementation. *: indicates a significant greater change (Δ) in the Gly supplementation regimen compared to the Pl supplementation regimen

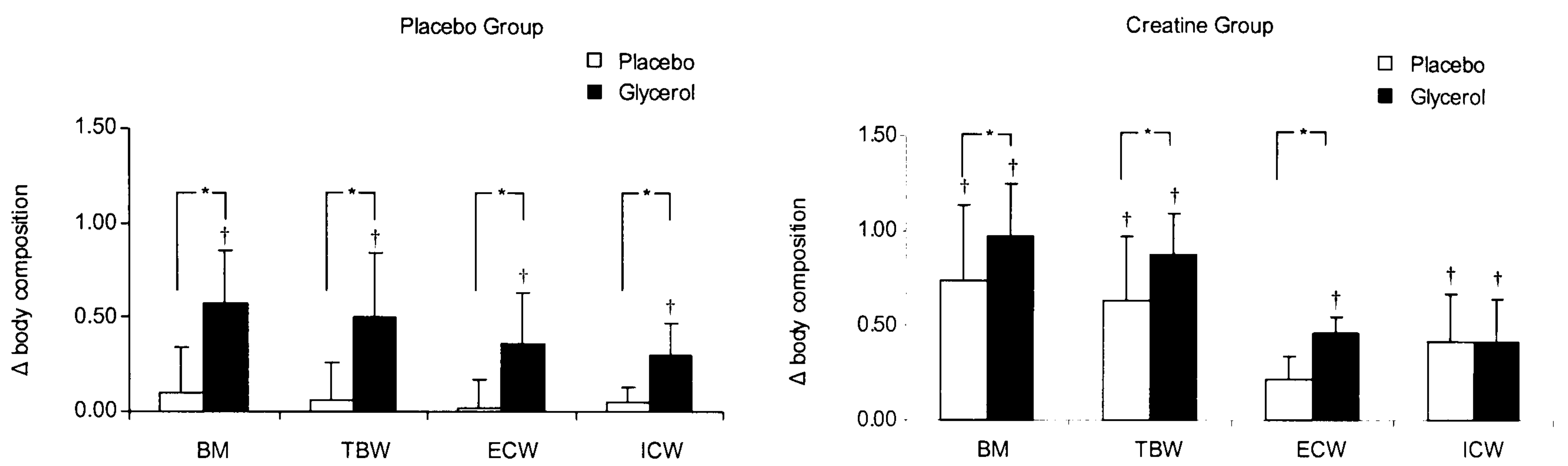


Figure 5.2 Changes in body mass (BM), total body water (TBW), intra-cellular water (ICW) and extra-cellular water (ECW) in the two groups. Data presented as the mean \pm s.d. †: indicates a significant difference pre- vs. post-supplementation. *: indicates a significant greater change (Δ) in the Gly supplementation regimen compared to the PI supplementation regimen.

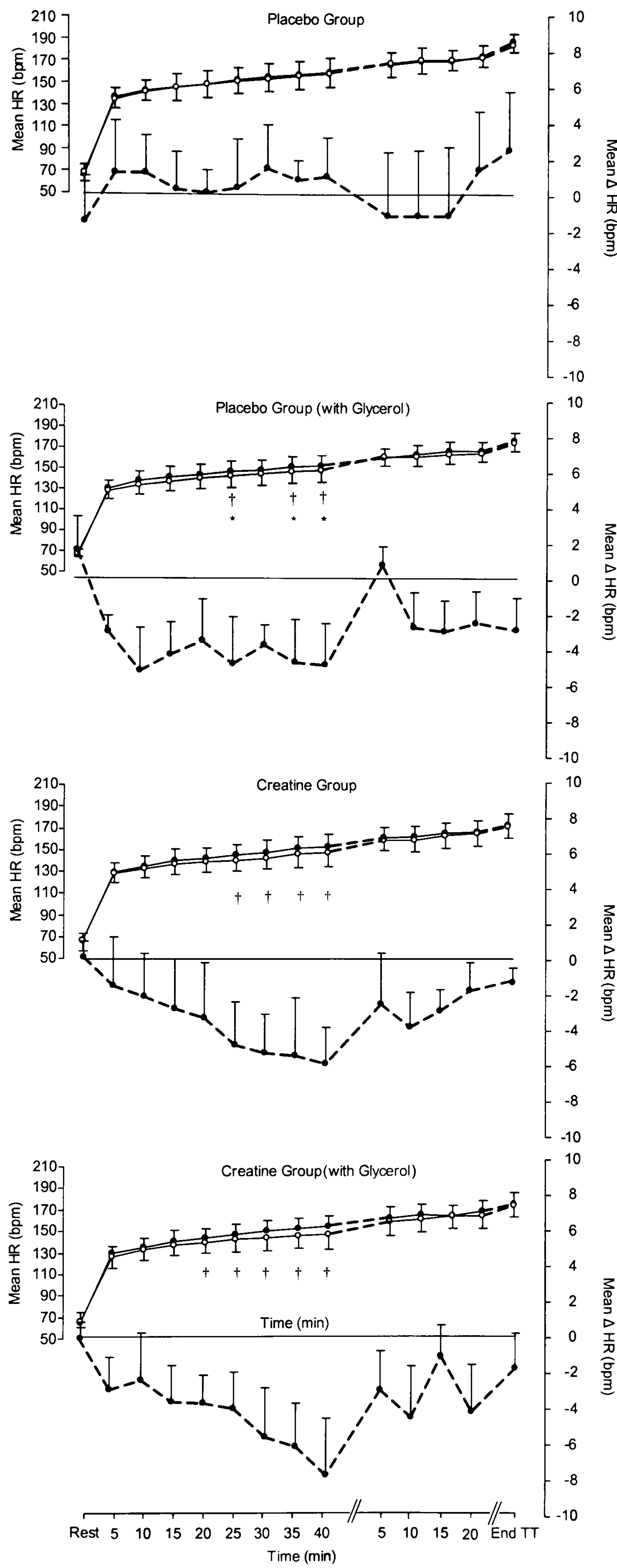


Figure 5.3 Mean heart rate (solid lines) during exercise before (black circles) and after (white circles) supplementation in the two groups and mean within-individual changes (treatment-control) (dashed line). Data presented as the mean ± s.d. †: indicates a significant difference pre- vs. post-supplementation. *: indicates a significant greater change (Δ) in the Gly supplementation regimen compared to the PI supplementation regimen.

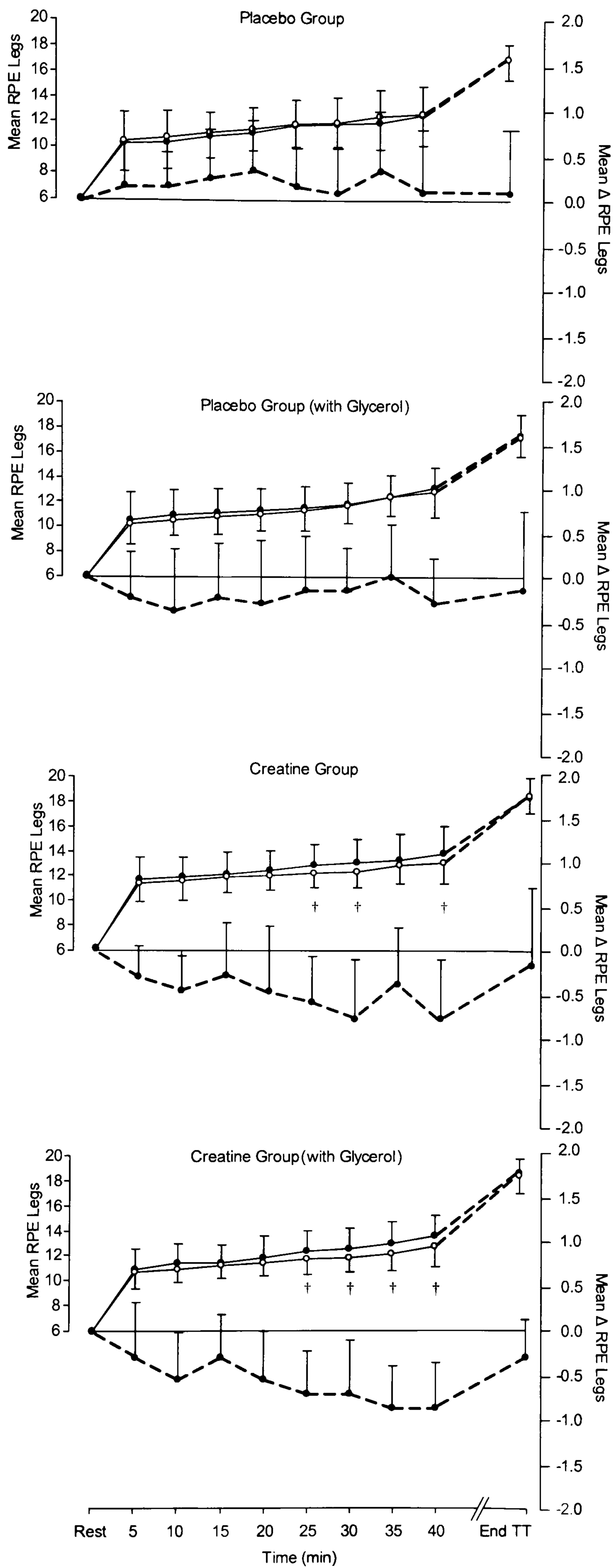


Figure 5.4 Mean RPE for perceived leg fatigue (solid lines) during exercise before (black circles) and after (white circles) supplementation in the two groups and mean within-individual changes (treatment-control) (dashed line). Data presented as the mean \pm s.d. †: indicates a significant difference pre- vs. post-supplementation.

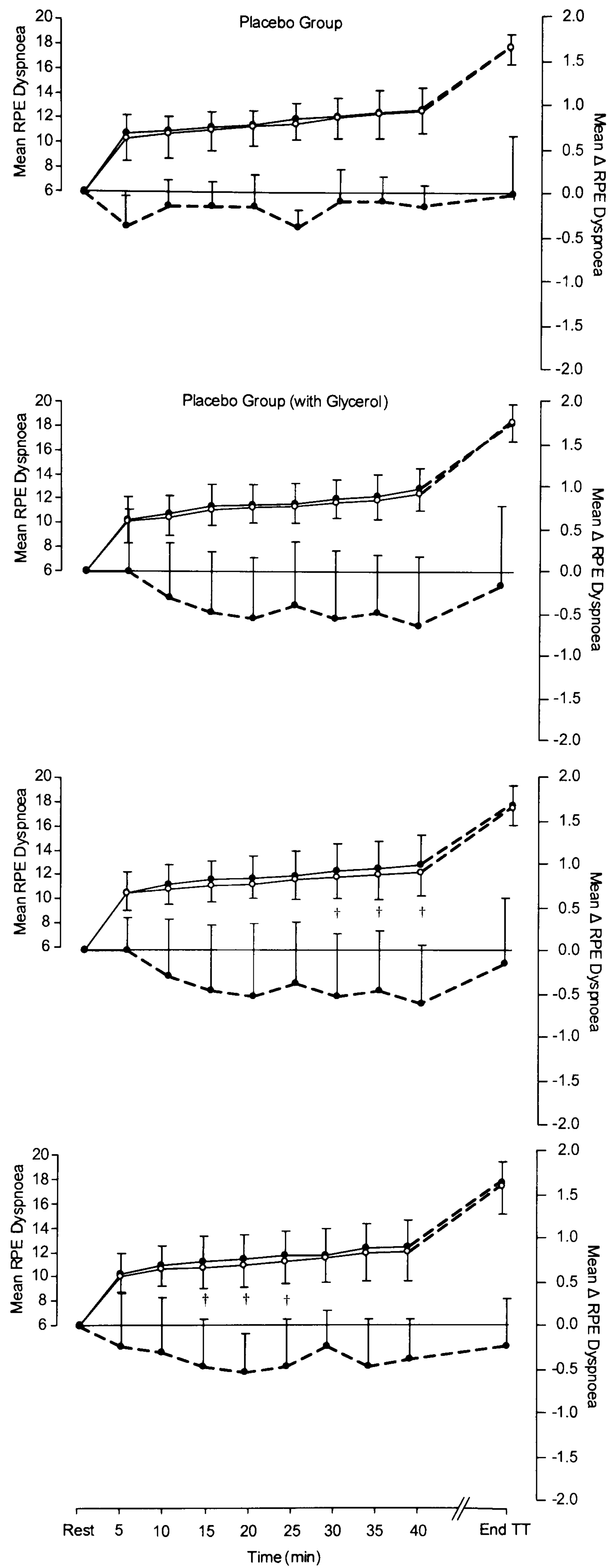


Figure 5.5 Mean RPE for perceived dyspnoea (solid lines) during exercise before (black circles) and after (white circles) supplementation in the two groups and mean within-individual changes (treatment-control) (dashed line). Data presented as the mean \pm s.d. †: indicates a significant difference pre- vs. post-supplementation

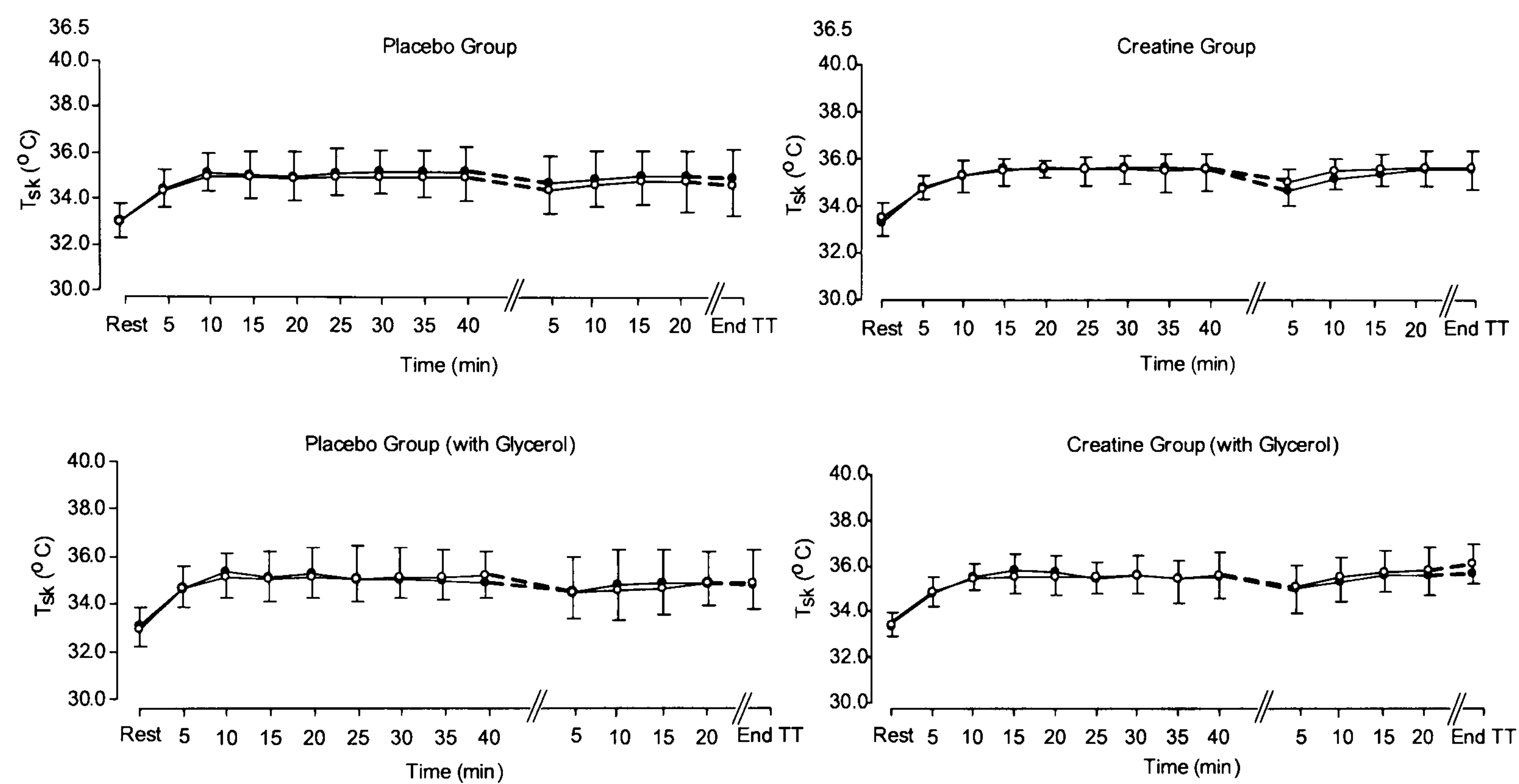


Figure 5.6 Mean skin temperature during exercise before (black circles) and after (white circles) supplementation in the two groups. Data presented as the mean \pm s.d.

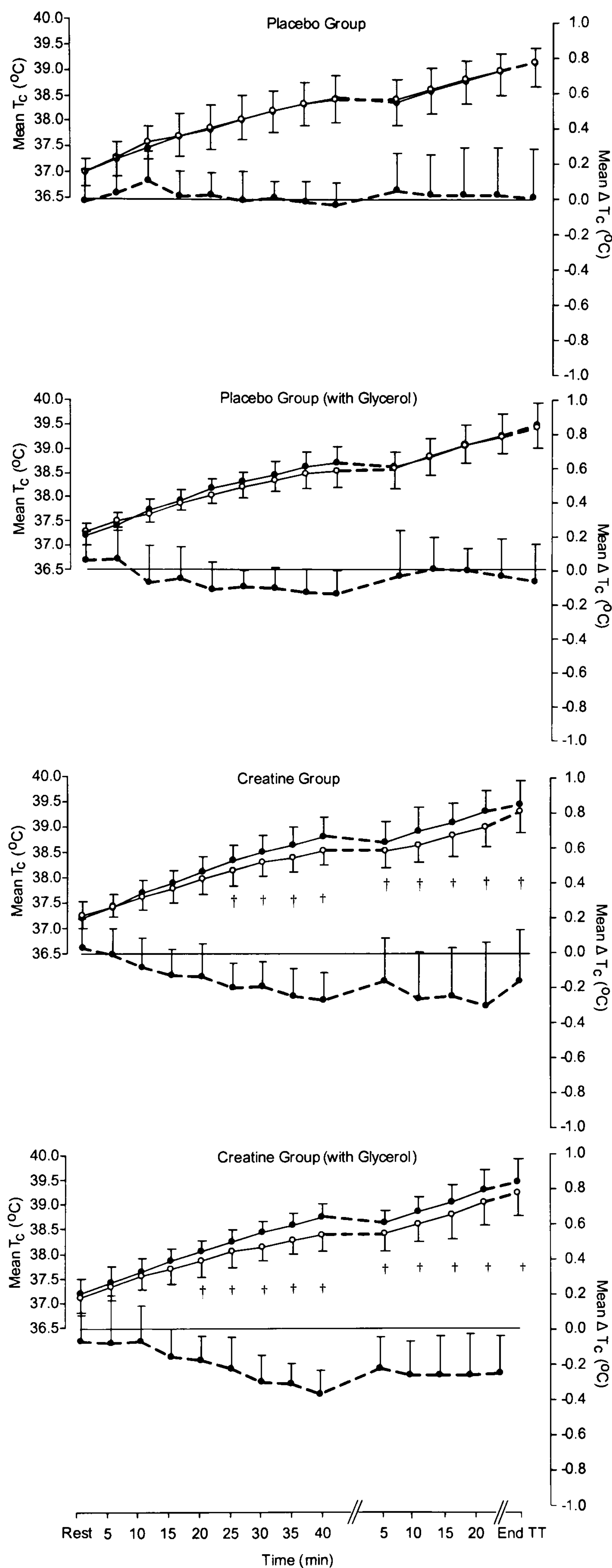


Figure 5.7 Mean rectal temperature (solid lines) during exercise before (black circles) and after (white circles) supplementation in the two groups and mean within-individual changes (treatment-control) (dashed line). Data presented as the mean \pm s.d. †: indicates a significant difference pre- vs. post-supplementation.

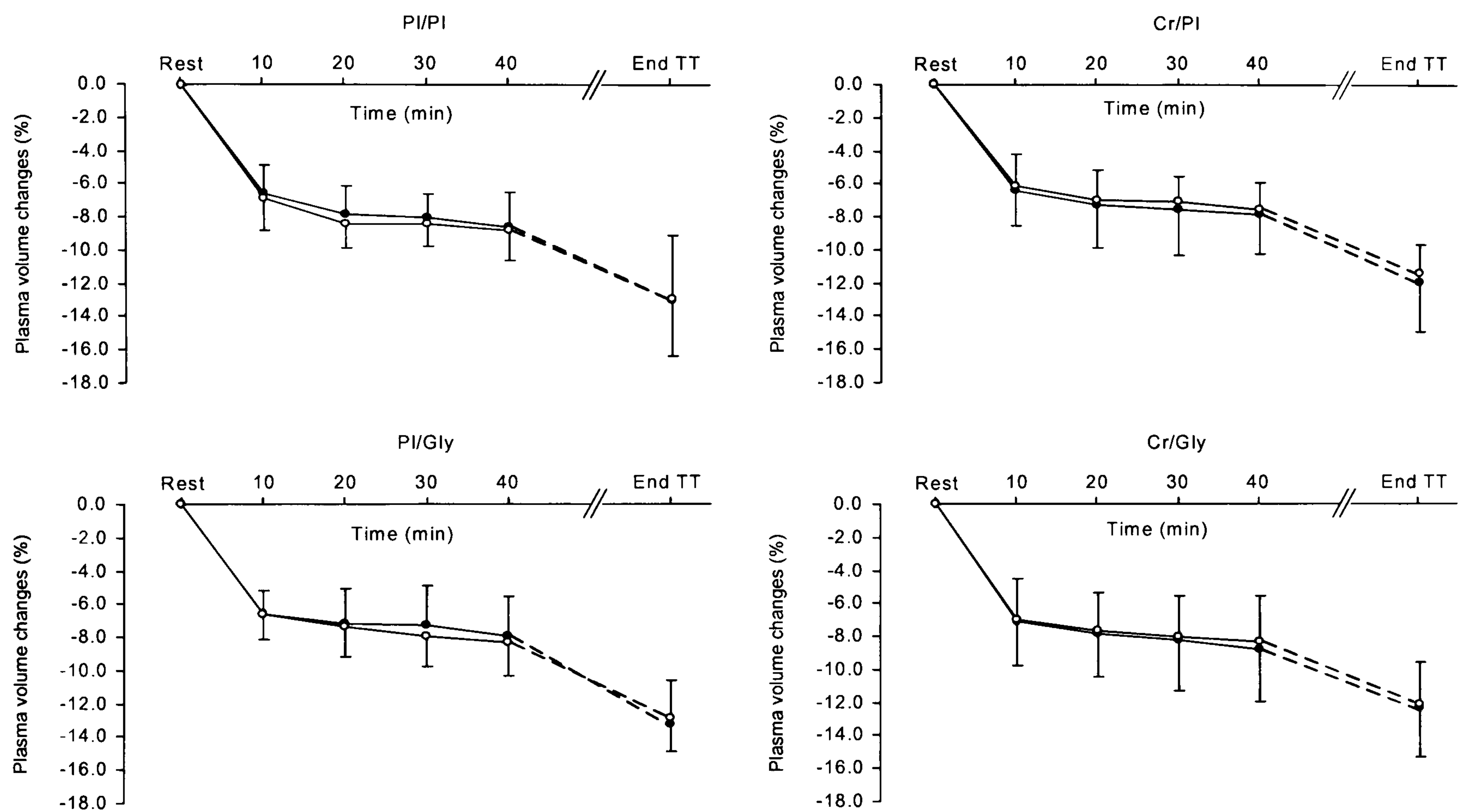


Figure 5.8 Percentage changes in plasma volume during exercise before (black circles) and after (white circles) supplementation in the two groups. Data presented as the mean \pm s.d.

5.4 Discussion

This study has demonstrated that supplementation with either Cr, or a combination of Cr and Gly, significantly increased TBW by up to 1.4 L prior to exercise (Fig. 5.2) and reduced perception of effort (Fig. 5.4, 5.5), cardiovascular (Fig. 5.3) and thermoregulatory (Fig. 5.7) responses during exercise in the heat. Furthermore, combining Gly with a standard Cr supplementation regimen (Harris *et al.*, 1992) resulted in a significantly greater increase in TBW (0.87 ± 0.21 L) than either supplementation alone (Fig. 5.2). Gly supplementation alone resulted in a significant increase in TBW of 0.57 ± 0.28 L and attenuated heart rate during exercise without significantly influencing T_c or RPE. Despite the significant increase in TBW and consequently improved thermoregulatory responses during exercise, no hydration intervention had any effect on exercise performance.

In the present study, subjects experienced, on average, a TBW increase of 500 ± 240 ml (range 200-1000 ml) over the 7 day supplementation period when Gly alone was ingested (Pl/Gly); an average increase that falls within the range (400-800 ml) previously reported using a similar Gly dose (Montner *et al.*, 1996). However, previous studies utilised a single Gly dose combined with a bolus of water consumed between 2 and 3 hours prior to measurement, whereas in the present study Gly was administered for a period of 7 days with the final Gly dose administered 5 hours prior to exercise. The average peak [Gly] in the present study after Gly supplementation was 7.6 ± 1.36 mmol·L⁻¹ (range 5.6-9.9) mmol·L⁻¹ and 7.1 ± 1.41 (range 5.7-9.8) mmol·L⁻¹ in the Pl and Cr groups, respectively (Table 5.7), which is lower than the peak concentration reported by Montner *et al.* (1996) (11.4 mmol·L⁻¹) and Freund *et al.* (1995) (13.0 mmol·L⁻¹) but higher than the concentration reported by Murray *et al.* (1991) (2.8 mmol·L⁻¹). Differences in the size of the Gly dose and time between ingestion and measurement are likely to account for the noted differences in [Gly]. Gly supplementation resulted in a similar distribution of the retained fluid between intra- and extra-cellular water compartments (Fig. 5.2) owing to the free distribution of Gly in all body water compartments with the exception of cerebral spinal fluid and aqueous humor (Freund *et al.*, 1995; Seifert *et al.*, 1995; Tourtellotte *et al.*, 1972). In previous Gly hyperhydration studies, water retention was quantified by measuring the volume of urine produced (Anderson *et al.*, 2001; Freund *et al.*, 1995) which gives no indication as to where the retained water was distributed. This Gly-induced water retention has been attributed to an increased concentration of ADH (Freund *et al.*, 1995). Although [ADH] was not measured in the present study, previously reported differences in [ADH] between Gly and water interventions were small and only approached statistical

significance (Freund *et al.*, 1995). While an ADH mechanism cannot be ruled out, it is more likely that this Gly-induced water retention is mediated by the action of Gly on the kidneys. When blood [Gly] is at normal physiological levels, almost all filtered Gly is passively reabsorbed by the proximal and distal renal tubules of the kidneys (Sommer *et al.*, 1993). When blood [Gly] is increased with exogenous Gly ingestion, there is an increase in Gly and associated water reabsorption (Kruhoffer & Nissen, 1963). In the present study, Gly supplementation also induced a significant elevation in serum osmolality (Table 5.8), which is directly attributable to the increased plasma [Gly] as previously described (Freund *et al.*, 1995; Murray *et al.*, 1991). Cr supplementation alone on the other hand, significantly increased ICW with only a minor, non-significant increase in ECW, resulting in a TBW increase of approximately 630 ± 330 ml on average (range 100-1200 ml) (Fig. 5.2). The increases in body mass and TBW following Cr supplementation in the present study were of similar magnitude when compared to a previous study from this laboratory (Kilduff *et al.*, 2004) and the study by Kern *et al.* (2001). Several studies have now confirmed that the increase in TBW associated with Cr supplementation is confined predominantly to the intra-cellular compartments of skeletal muscle (Kilduff *et al.*, 2004; Ziegenfuss *et al.*, 1998). It has been suggested that an increase in body mass of greater than 0.2 kg identifies a 'responder' to Cr supplementation (Kilduff *et al.*, 2003). The individual increases in body mass after Cr supplementation in the present study (Table 5.2) would suggest that only 2 subjects were non-responders to Cr.

The present study is the first to show that the volume of water retained by ingesting either Cr or Gly can be significantly enhanced by combining these two hyperhydrating agents. This novel 'water-loading' strategy that combines Cr and Gly resulted in a mean TBW increase of approximately 870 ± 210 ml (range 600-1400 ml), a significantly larger volume than either Cr or Gly alone (Fig. 5.2). Additionally, the retained water was dispersed equally between the intra- and extra-cellular water compartments. It seems plausible that the water retained by combining the ingestion of Cr and Gly was mediated via a Cr-induced increase in ICW and an increase in ECW as a consequence of the added Gly. This hyperhydration induced by combined Cr and Gly supplementation is the highest directly measured increase in hydration reported in the literature to date (Kilduff *et al.*, 2004; Montner *et al.*, 1996). Therefore, these data would suggest that combined Cr and Gly supplementation is potentially the most effective method of hyperhydrating prior to exercise. Furthermore, this innovative 'water-loading' strategy is comprised of two agents that specifically target both intra- and extra-cellular body water compartments and in doing so overcomes the limitations of previous hyperhydration strategies. For example, Gly has been investigated as a potential hyperhydrating agent for a number of decades and

continues to be of great interest as evidenced by a number of recently published studies (Anderson *et al.*, 2001; Marino *et al.*, 2003). However, the benefits of Gly hyperhydration are equivocal with at least 23 original papers published on Gly hyperhydration to date providing conflicting results. In instances where hyperhydration was induced, the hydrating effects of Gly were transient due to the metabolism of Gly by the liver and kidneys. Of greater significance however, and major problem associated with Gly ingestion is the fact that it permeates the blood-brain barrier extremely slowly and thus causes cerebral dehydration and associated headaches (Tourtellotte *et al.*, 1972). Combining Gly with Cr overcomes this major problem as in contrast to Gly, Cr is taken up by the brain (Matthews *et al.*, 1999) and in doing so counteracts the negative effects associated with Gly ingestion, increasing the level of initial hydration but will also potentially prolong the period that hyperhydration will last. However, it is currently unknown whether Gly ingestion or infusion for prolonged periods of time may cause cerebral oedema. A single bolus of oral Gly supplementation is unlikely to be harmful due to delayed absorption by the brain and rapid metabolism by the liver and urinary excretion prior to Gly reaching cerebral circulation (Sommer *et al.*, 1993; Tourtellotte *et al.*, 1972). Although Gly was ingested for 7 days in the present study without incident, further research is required to examine the effects of Gly ingestion for prolonged periods of time on intracranial pressure. The mean TBW increase of 870 ± 210 ml produced by the combined ingestion is approximately 20% lower than the sum of the mean increase in TBW produced by Cr and Gly (i.e. 1130 ml), suggesting that the level achieved with the combined ingestion may represent the upper limit of hyperhydration. Under normal physiological conditions, water balance is controlled by sensitive osmoreceptors located in the hypothalamus possibly via ADH-mediated changes in water excretion in the urine and thirst-mediated changes in water ingestion (Burrell *et al.*, 1991). For example, Freund *et al.* (1995) reported that ingestion of a bolus of water resulted in a significant reduction in [ADH] and a subsequent increase in free water clearance. Ingestion of a bolus of water combined with Gly also resulted in a significant reduction in plasma [ADH], although the decrease tended to be attenuated. The decrease in [ADH] occurred despite a Gly induced increase in plasma osmolality, that the authors attributed to the dilutional effect of hyperhydration on plasma $[\text{Na}^+]$. Therefore, attempting to increase water retention further by hyperhydrating with combined Cr and Gly is likely to have a similar diluting effect on plasma $[\text{Na}^+]$ resulting in a further reduction of [ADH] and increased urine production, thus limiting the volume of water that can be retained. Furthermore, 5 days supplementation with 20 g of Cr has been reported to elevate skeletal muscle [Cr] stores by a margin dependent on the initial muscle [Cr] (Greenhaff *et al.*, 1994). Continued

supplementation with Cr after this period will not result in any appreciable further increases in the skeletal muscle Cr pool (Hultman *et al.*, 1996) and subsequently there will be no further increase in ICW retention.

Hyperhydration achieved through Gly and Cr supplementation in the present study was successful in attenuating the increase in heart rate by up to 5 and 7 beats·min⁻¹ respectively during constant-load exercise in the heat (Fig. 5.3). Yet despite a further increase in TBW when Cr and Gly were combined (compared to Cr alone) there was no further significant attenuation in heart rate (heart rate was reduced by up to 9 beats·min⁻¹) (Fig. 5.3). However, Cr/Gly supplementation increased TBW by an average of 240 ml more than Cr/Pl, which may not be large enough to significantly alter the physiological responses to exercise in the conditions of the present study. Previous studies examining the effects of either Gly or Cr supplementation on cardiovascular responses during exercise in the heat have been equivocal, with some showing a reduction in heart rate (Anderson *et al.*, 2001; Kilduff *et al.*, 2004; Montner *et al.*, 1996) and others finding no such effect (Kern *et al.*, 2001; Latzka *et al.*, 1998; Murray *et al.*, 1991). However, it is well established that dehydration results in an increased heart rate and reduced stroke volume and cardiac output during exercise (Gonzalez-Alonso *et al.*, 1995). Therefore, it would be expected that as the magnitude of body water loss increases through sweating, there would be an increase in T_{c} during exercise in the heat (Sawka *et al.*, 2001). The fluid loss from sweat is obtained in varying proportions from both the intra- and extra-cellular water compartments of the body in order to maintain blood volume (Sawka *et al.*, 2001). Nose *et al.* (1988) reported a strong association between the loss of water in sweat and urine and the decrease in intracellular fluid following prolonged exercise in the heat. In the present study, when Cr induced an increase in ICW, there was a significant attenuation in the rise in T_{re} by up to 0.35°C during exercise in the heat (Fig. 5.7). It is possible that this Cr-induced increase in ICW may have resulted in an increase in the specific heat capacity of the body, resulting in a greater capacity to store heat (Kilduff *et al.*, 2004). The potential physiological advantage from the hyperhydration-induced reductions in heart rate and T_{c} are unclear from the results of the present study. However, given the association between attainment of a 'critical T_{c} ' and the development of fatigue (Nielsen *et al.*, 1993) it is tempting to assume that hyperhydration would have resulted in an increased time to exhaustion had this experimental protocol been utilised. Furthermore, subjects who had supplemented with Cr had significantly lower ratings of perceived leg fatigue during the constant-load exercise (Fig. 5.4), suggesting that subjects were able to discern the benefits of the reduction in T_{c} mediated by Cr supplementation.

There are two published reports to date that appear to confirm the reduction in T_c during exercise in the heat following Cr supplementation (Kern *et al.*, 2001; Kilduff *et al.*, 2004). Conversely, Gly supplementation, which increased ICW to a lesser extent, did not significantly reduce the rise in T_{re} during the exercise period (Fig. 5.7). It is therefore unsurprising that there is considerable debate in the literature surrounding whether Gly ingestion can reduce T_c during exercise in the heat, with several studies reporting a reduced T_c during exercise (Anderson *et al.*, 2001; Lyons *et al.*, 1990; Seifert *et al.*, 1995) and numerous other studies finding no such effect (Hitchins *et al.*, 1999; Latzka *et al.*, 1998; Marino *et al.*, 2003; Murray *et al.*, 1991). This may also explain why several studies reported plasma volume expansion via saline or dextran infusion has no effect on heart rate, T_c , skin blood flow or performance during exercise in the heat (Grant *et al.*, 1997; Watt *et al.*, 2000). In the current study, Gly supplementation resulted in a significant increase in both sweat rate and total sweat loss in both the Pl and Cr groups. It may, therefore, be somewhat surprising that there was no reduction in T_{re} due to the expected increase in evaporative cooling in the Pl group after Gly supplementation. To date, the vast majority of studies conclude that Gly has no influence on sweat loss (Hitchins *et al.*, 1999; Murray *et al.*, 1991) with Lyons and colleagues the only authors to report both an increased sweat loss and decreased T_c during exercise in the heat as a direct result of Gly supplementation (Lyons *et al.*, 1990). However, the mean increase in total sweat loss in the investigation by Lyons *et al.* (1990) was significantly larger than in the present study (450 ml vs. 210 ml respectively).

Despite the reduction in T_c , heart rate and perceived leg fatigue during constant-load exercise in the present study after supplementation with Cr/Pl and Cr/Gly, time trial performance was not affected. Several studies have indicated that the increased heart rate and T_c resulting from dehydration can have a negative impact on exercise performance (Cheuvront *et al.*, 2005). For example, Cheuvront *et al.* (2005) determined that hypohydration was associated with an increased T_c and heart rate and a significant reduction in work performed during a 30 min cycling time trial, even in a temperate (20°C) environment. Therefore, if dehydration could be minimised then there would potentially be less of an associated reduction in exercise performance. As such, several studies have concluded that hyperhydration is associated with a significant improvement in exercise performance in the heat (Anderson *et al.*, 2001; Hitchins *et al.*, 1999; Kilduff *et al.*, 2004; Montner *et al.*, 1996). Subjects in the studies by Kilduff *et al.* (2004) and Montner *et al.* (1996) were required to cycle submaximally until exhaustion, whereas the studies by Hitchins *et al.* (1999) and Anderson *et al.* (2001) utilised a self paced time trial for 30 and 15 min respectively, to quantify performance. The findings of Hitchins *et al.* (1999) seem

particularly surprising given that cardiovascular and thermoregulatory responses during exercise were not different between Gly and water ingestion trials, meaning the authors could provide no explanation for the observed ergogenic effect of Gly. Subjects in the study by Anderson *et al.* (2001) were required to complete 90 min of steady state exercise prior to commencement of the time trial, more than twice as long as in the present study. The greater degree of dehydration that would occur during this prolonged submaximal exercise period may explain why pre-exercise hyperhydration resulted in a significant improvement in time trial performance in the study by Anderson *et al.* (2001). Therefore, it seems likely that the exercise trial in the present study was not of sufficient duration and therefore too high an exercise intensity for hyperhydration to have a significant effect on performance. Similarly, other studies find no effect of hyperhydration on exercise performance when compared to euhydration (Latzka *et al.*, 1998; Marino *et al.*, 2003). For example, Marino *et al.* (2003) found Gly hyperhydration had no effect on a 60 min cycling time trial in hot and humid conditions compared to pre-exercise water ingestion. Latzka *et al.* (1998) produced similar findings when subjects were asked to complete treadmill exercise at 55% $\dot{V}O_{2\text{ max}}$ until exhaustion. However, these authors also reported that after either Gly or water ingestion, exercise time to exhaustion was significantly greater than if no water had been consumed prior to exercise. Therefore, it would appear that commencing exercise in a hyperhydrated state may not confer any significant advantage in terms of exercise performance compared to euhydration or indeed modest dehydration (i.e. loss of 2-3% body mass). The results from the present study are compatible with such an idea, although further research is needed to determine the effects of hyperhydration on physiological responses and performance during a more prolonged exercise trial where a more marked degree of dehydration would be expected.

5.4.1 Conclusion

In the present study, supplementation with both Cr and combined Cr and Gly for 7 days was effective in increasing TBW and reducing heart rate and T_{c} during prolonged exercise in the heat. The key finding of this study was that the increase in TBW after combined Cr and Gly supplementation was significantly greater than Cr supplementation alone. Despite the increased hydration associated with combined Cr and Gly, there was no further attenuation in heart rate or T_{re} compared to Cr alone. Hyperhydrating prior to exercise through Cr, Gly or a combination of the two did not result in any significant improvement in 16.1 km time trial performance compared to euhydration. This may be because the time trial was too short to induce a degree of dehydration high enough to confer a significant

improvement in exercise performance as a result of the altered hydration status. Furthermore, hyperhydration may not offer any significant advantage in terms of exercise performance compared to euhydration or indeed modest dehydration (i.e. loss of 2-3% body mass).

Chapter 6

Rectal, telemetry pill and tympanic membrane thermometry during exercise heat stress.

6.1 Introduction

Hyperhydration with combined Cr and Gly supplementation has been shown to result in a significant reduction in T_c and heart rate during exercise in a hot climate chamber (Chapter 5). However, Noakes (2005) points out that physiological phenomena studied under environmental conditions have little practical application to competitive sport, and as such it is impossible to extrapolate research findings from the laboratory to the field. For example, in the study performed in Chapter 5, the facing air velocity was $6.5 \text{ km} \cdot \text{hr}^{-1}$ significantly lower than that experienced when actually racing out of doors (Di Prampero *et al.*, 1979). Recently, Saunders *et al.* (2005), compared the T_c of subjects cycling at $33.0 \pm 0.4 \text{ }^\circ\text{C}$ in four different wind velocities: $0.2 \text{ km} \cdot \text{hr}^{-1}$ - wind still conditions, $10 \text{ km} \cdot \text{hr}^{-1}$ - to replicate many laboratory studies, and 100% and 150% of calculated road speed facing wind velocities based on the equation of DiPrampero *et al.* (1979). The authors reported that in wind still or low facing wind velocities, excessive heat storage occurs whilst exercising at moderate and high intensities, due to a failure of the environment to absorb and dissipate heat (evidenced by higher sweat rates during wind still conditions) rather than thermoregulatory failure. Therefore, the extent to which supplementation with Cr or combined Cr and Gly reduced T_c and heart rate during cycling exercise in the heat may be significantly less when the exercise is performed outdoors in similar environmental conditions. Evidently, further field-based research is required to determine the ‘true’ effects of combined Cr and Gly supplementation on thermoregulation and exercise performance in the heat.

The development of heart rate telemetry systems and portable metabolic analyzers in the last two decades has enabled accurate assessment of physiological responses during unrestrained exercise in the field. However, until recently accurate measurement of T_c during exercise in the field has not been possible. Esophageal and T_{re} temperatures are typically considered to reliably estimate T_c during exercise (Sawka & Wenger, 1998) with T_{re} the most commonly published method in scientific research (Moran & Mendal, 2002). Yet, despite its widespread use, the relative invasiveness and social stigma attached to T_{re} coupled with necessary wire connections between the thermistor and the measuring device make T_{re} monitoring of subjects while exercising in the field problematic. The aforementioned problems of T_{re} measurement in the field and the need to continuously and accurately measure T_c during extreme conditions such as during space travel, led to rapid advances in this technology during the early 1990s and the subsequent development of an ingestible temperature sensor or ‘telemetry pill’ by the National Aeronautics and Space

Agency (Rav-Acha *et al.*, 2003). The telemetry system monitors T_c via a radio wave signal, transmitted from the ingested pill and sent to a small external receiver (Rav-Acha *et al.*, 2003). The telemetry pill has been shown to provide valid measurements of T_c compared to both esophageal temperature and T_{re} within the range of 36°C and 38°C during rest and prolonged cycling exercise lasting approximately 3 h in both warm and cold-water immersion trials (O'Brien *et al.*, 1998). However, the low exercise intensities (40 – 50% $\dot{V}O_{2\max}$) utilized in this (O'Brien *et al.*, 1998) and other studies (Kolka *et al.*, 1993; Kolka *et al.*, 1997; Lee *et al.*, 2000) induced only a modest degree of thermal strain, with a peak telemetry pill temperature (T_p) of 38.7°C reported in the literature (Kolka *et al.*, 1997). However, as intense exercise can regularly induce a rise in T_c greater than 40°C (Roberts, 2000), further validation of the telemetry pill during more severe exercise heat stress is required.

The telemetry pill method of temperature measurement is expensive relative to other methods, especially when large numbers of subjects need to be tested. On the other hand, T_c measurement by tympanic membrane thermometer has also been shown to accurately reflect T_{re} during hyperthermia in the clinical situation albeit in young children (van Staaij *et al.*, 2003) and is relatively inexpensive. The evidence supporting the reliable use of tympanic temperature (T_{ty}) during exercise is equivocal. For example, Deschamps *et al.* (1992) established that T_{ty} was significantly lower than esophageal temperature by approximately 0.5°C during cycle exercise and thus concluded that T_{ty} could not be used to assess exercise-induced hyperthermia. Conversely, Newsham *et al.* (2002) reported no differences between peak T_{re} and T_{ty} measurements on cessation of approximately 30 min stair-climbing exercise in the heat (32°C, 70% relative humidity), although T_{ty} increased to a greater extent than T_{re} during the exercise period. The studies by Deschamps *et al.* (1992) and Newsham *et al.* (2002) employed small numbers of subjects (i.e. 6 and 10) who completed the experimental protocol on only one occasion. The obvious disparity in the limited number of studies that have attempted to validate the use of a tympanic membrane thermometer during exercise in the heat clearly highlights the need for further research. Therefore, the aim of the present study was to compare T_c measurements obtained from the ingestible telemetry pill and the tympanic membrane thermometer with those from a rectal thermistor during rest and high intensity exercise conducted in a hot and humid environment (30°C and 70% relative humidity) intended to raise T_c above 39°C.

6.2 Methods

6.2.1 Subjects.

10 well-trained cyclists gave their written informed consent to take part in the present study which was approved by the local Ethics Committee. The subjects had the following characteristics (mean \pm s.d.): age: 31 ± 6 years, height: 1.74 ± 0.4 m, weight: 74.7 ± 6.6 kg, $\dot{V}O_{2\max}$: 4.1 ± 0.4 L \cdot min $^{-1}$. Subjects were recruited from local cycling clubs, and none were acclimatized to exercise in the heat at the time of study. The subjects were fully informed of any risks and discomforts associated with the experiments before giving their written informed consent to participate.

6.2.2 Experimental design and protocols.

Subjects completed four exercise trials consisting of 40 min constant-load exercise at 63% WR_{\max} followed by a 16.1 km (10 mile) time trial at ambient temperature $30 \pm 1^{\circ}\text{C}$ with a relative humidity of $70 \pm 3\%$ and air velocity of approximately 1.8 m \cdot s $^{-1}$. Tests were performed one week apart over four consecutive weeks and at the same time of day. On each of the experimental test days subjects ingested 500 ml of water 3 h prior to exercise and a further 500 ml of water 1 h prior to exercise in an attempt to ensure subjects were adequately hydrated prior to all exercise trials (Convertino *et al.*, 1996). A flexible rectal thermistor (IBLS bioelectronics unit, University of Glasgow, U. K.) was inserted 100 mm beyond the anal sphincter prior to exercise and attached to a recording device (C8600 10 channel microprocessor, Comark, Hertfordshire, U. K.). The rectal thermistor was calibrated by immersion in a water bath at three temperatures (30°C , 35°C and 40°C). The subject remained seated on the cycle ergometer for 5 min while resting T_{re} , T_{p} and T_{ty} were recorded. Subjects were then instructed to begin 5 min of unloaded cycling before the WR was increased in a 'single step' to the predetermined 63% WR_{\max} . Subjects were required to maintain a pedal cadence of 70 - 100 revs \cdot min $^{-1}$ for 40 min. On completion of the 40 min period, WR was decreased to 20 W and the subject asked to maintain cadence for 1 min. After a further 4 min rest period the subject was instructed to complete a 16.1 km (10 mile) self-paced time trial on a road mounted cycle (King Cycle Indoor Trainer, Buckinghamshire, U. K.). T_{re} and T_{p} were recorded at 5 min intervals throughout the exercise period. T_{ty} was measured every 10 min throughout the steady state exercise and immediately on completion of the time trial. Subjects were required to consume 2.14 ml cold-water \cdot kg $^{-1}$ body mass (5°C) every 10 min throughout the 40 min steady state period.

6.2.3 Telemetry pill system.

T_p was monitored using a CorTemp™ ingestible core body temperature pill, 20 mm in length and 12 mm diameter (CorTemp, HQ inc., Palmetto, Florida, U.S.A.) that subjects ingested 8 h before commencement of exercise (O'Brien *et al.*, 1998). Each pill transmits a low frequency radio wave that varies in wavelength depending on the temperature. This radio wave is received and converted to a digital format by a CorTemp™ data recorder. The manufacturer calibrated each individual pill, firstly by placing each in a water bath maintained at 35°C and allowing stabilization of temperature and frequency rate. The water bath was controlled digitally and had a temperature stability of $\pm 0.01^\circ\text{C}$ at 20°C. The digital temperature readout was calibrated at $\pm 0.05^\circ\text{C}$ over the range of 35 - 45°C. Each pill was then placed in a water bath maintained at a constant 45°C and allowed to stabilize to bath temperature. The frequencies and temperatures for the low and high temperature baths were figured into a proprietary formula to determine sensor offset and slope, which together constitute the 8-digit calibration number for each pill. Pill calibration numbers were tested to verify 0.1°C accuracy using a stabilized water bath at 40°C and a CorTemp™ recorder to report T_p readings. Pills falling outside $\pm 0.1^\circ\text{C}$ accuracy were returned to the calibration process for one additional calibration and test run. These pills were discarded if they fell outside accuracy parameters after the second calibration and test run.

6.2.4 Tympanic membrane thermometer.

T_{ty} was recorded using a Genius tympanic membrane thermometer (First Temp Genius Thermometer, Sherwood-Davis and Geck, St Louis, MO, U.S.A.). The tympanic membrane thermometer measures the infrared heat generated from tissues within view of the probe and aims to measure the temperature from the tissues surrounding the eardrum. The same investigator performed each T_{ty} measurement by pulling the ear upwards and backwards while inserting the probe as far as possible into the ear until a tight fit was achieved (van Staaij *et al.*, 2003). T_{ty} was recorded in rectal equivalent mode that utilizes an algorithm to predict T_{re} . However, this prediction equation is confidential because of its commercially sensitive nature; therefore, the equation used cannot be presented. The manufacturers calibrated the Genius tympanic thermometer and the calibration was validated by immersion in a water bath at three temperatures (30°C, 35°C and 40°C).

6.3 Results

The mean temperature at 5 min time points for T_{re} and T_p and 10 min intervals for T_{ty} is shown in Figure 6.1. There were no differences in temperature readings between methods at rest (T_{re} : $37.2 \pm 0.3^\circ\text{C}$; T_p : $37.2 \pm 0.2^\circ\text{C}$; T_{ty} : $37.1 \pm 0.3^\circ\text{C}$; $P=0.40$) (Fig. 6.1) and there was a high correlation between methods (T_{re} vs. T_p : $r=0.93$, $P<0.01$; T_{re} vs. T_{ty} : $r=0.80$, $P<0.01$). During exercise, temperature rose progressively peaking at $39.4 \pm 0.4^\circ\text{C}$, $39.4 \pm 0.4^\circ\text{C}$ and $38.9 \pm 0.5^\circ\text{C}$ for T_{re} , T_p and T_{ty} , respectively (Fig. 6.1). There were no differences between T_{re} and T_p measurements during the exercise period ($P=0.32$) (Fig. 6.1) and these temperature readings overall were highly correlated ($r=0.98$, $P<0.01$) (Fig. 6.2). There were no differences between T_{re} and T_{ty} at the 10 min ($P=0.11$) and 20 min ($P=0.06$) time points (Fig. 6.1) and these individual time points were significantly correlated ($r=0.67$, $P<0.01$ and $r=0.53$, $P<0.01$ respectively). T_{ty} was significantly lower than T_{re} at the 30 min (T_{ty} : $37.9 \pm 0.3^\circ\text{C}$; T_{re} : $38.2 \pm 0.3^\circ\text{C}$, $P<0.01$), 40 min (T_{ty} : $38.1 \pm 0.4^\circ\text{C}$; T_{re} : $38.5 \pm 0.3^\circ\text{C}$, $P<0.01$) and at the end of the time trial (T_{ty} : $38.9 \pm 0.5^\circ\text{C}$; T_{re} : $39.4 \pm 0.4^\circ\text{C}$, $P<0.01$) (Fig. 6.1) and were significantly correlated ($r=0.69$, $P<0.01$; $r=0.73$, $P<0.01$; $r=0.59$, $P<0.01$ respectively). T_{re} and T_{ty} temperature readings overall were also correlated ($r=0.92$, $P<0.01$) (Fig. 6.2).

The Bland-Altman analysis of the intermethod differences in T_c measurement is depicted in Figure 6.3 (for individual time points) and Figure 6.4 (overall). The overall mean difference between T_{re} and T_p was 0.02°C with 95% confidence interval -0.04 to 0.01°C and LOA -0.37 to 0.33°C indicating a high level of agreement between these two methods (Fig. 6.4). The overall mean difference between T_{re} and T_{ty} was 0.26°C with 95% confidence interval 0.22 to 0.30°C and the LOA -0.35 to 0.87°C (Fig. 6.4). The mean difference and the LOA between methods increased as temperature increased (Figs. 6.3 and 6.4) with the largest difference between methods occurring between T_{re} and T_{ty} at the end of the time trial (mean difference: 0.43°C ; 95% confidence interval: 0.29 to 0.56°C ; LOA: -0.38 to 1.23°C) (Figs. 6.3 and 6.4).

T_p was not detected from a subject on one occasion prior to commencement of exercise. On three occasions, T_p was significantly reduced below 30°C immediately after ingestion of water, suggesting that the telemetry pill was still located in the upper part of the gastrointestinal tract and thus was being transiently cooled by the water. On all four occasions, these data were not included in the statistical analyses and figures.

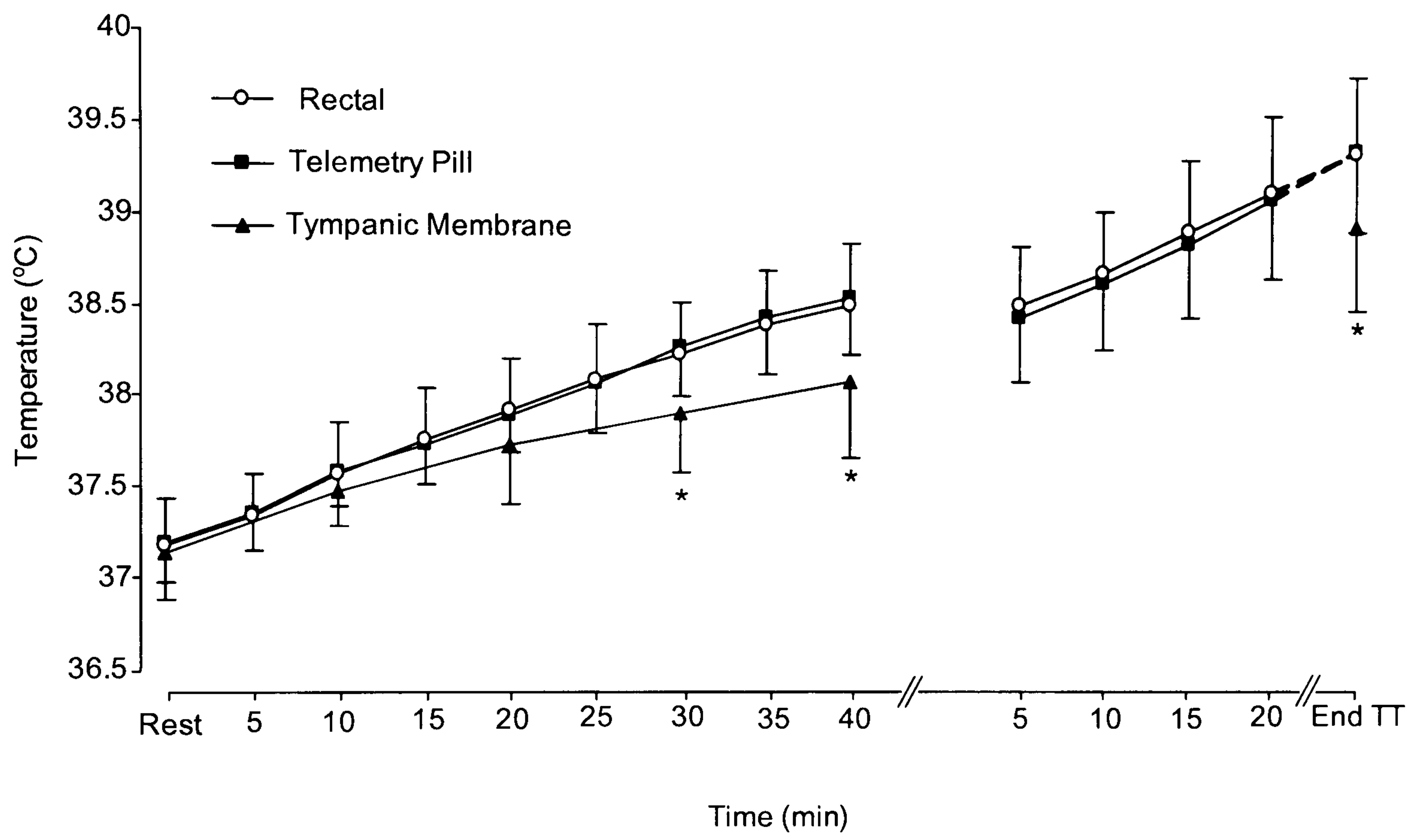


Figure 6.1 Rectal, telemetry pill and tympanic membrane temperature during exercise. Data presented as the mean \pm sd. *: indicates a significant difference between T_{re} and T_{ty} .

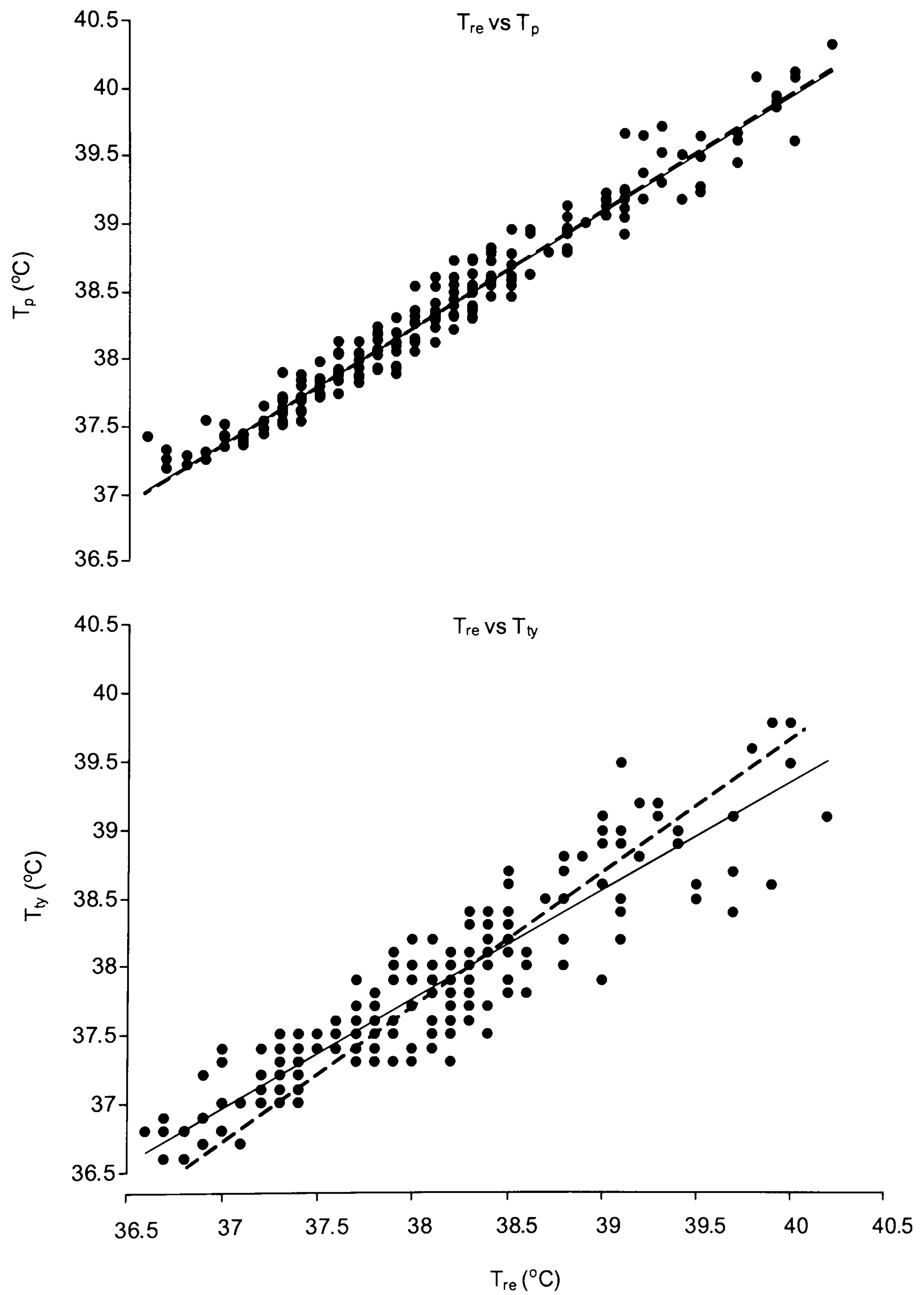


Figure 6.2 Correlation of rectal vs. telemetry pill temperatures and rectal vs. tympanic membrane temperatures with line of equality (dashed line) and line of best fit (solid line).

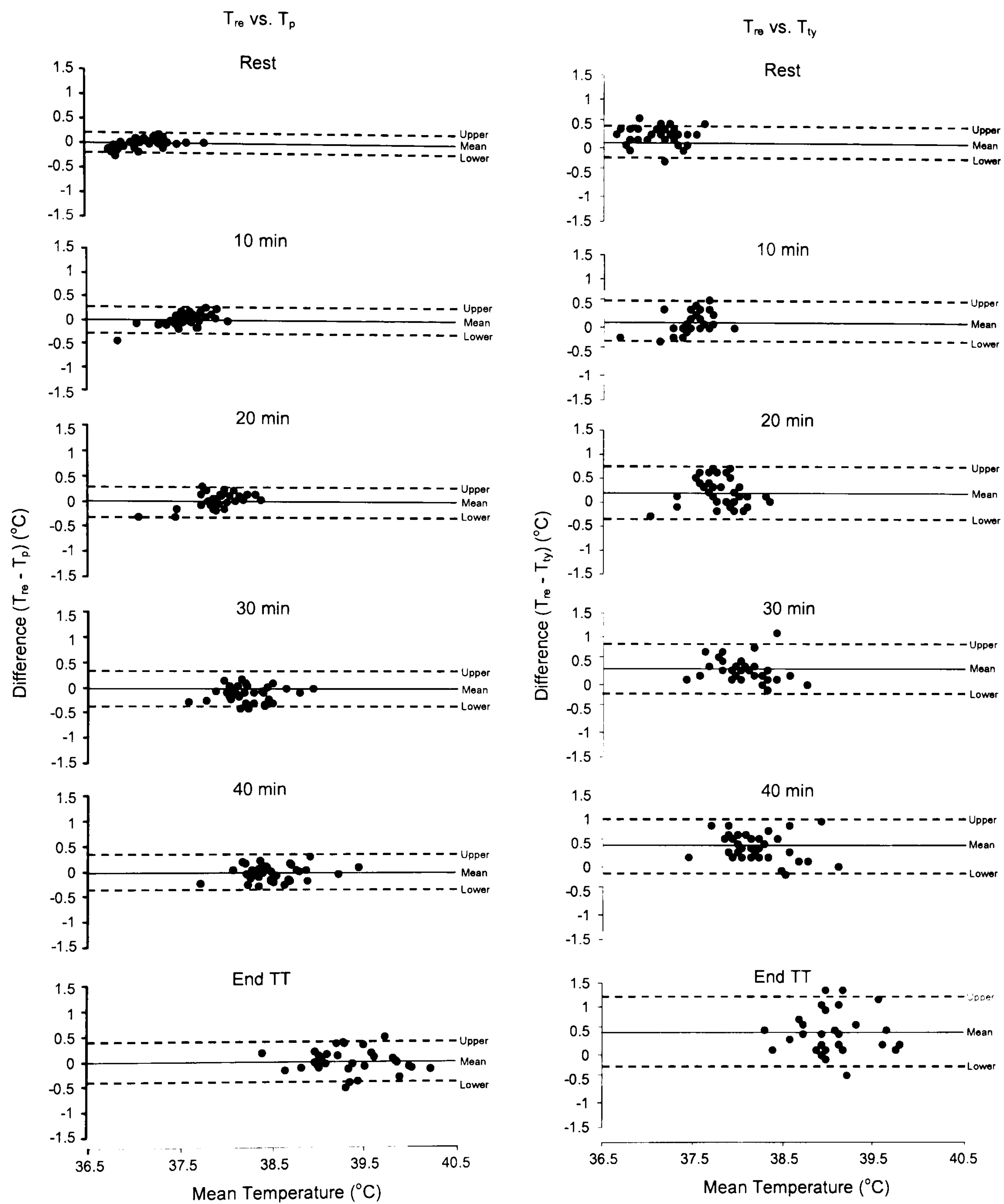


Figure 6.3 Bland-Altman plots of rectal vs. telemetry pill temperatures and rectal vs. tympanic membrane temperatures at each 10 min time point, with means (*solid lines*) and limits of agreement (*dashed lines*).

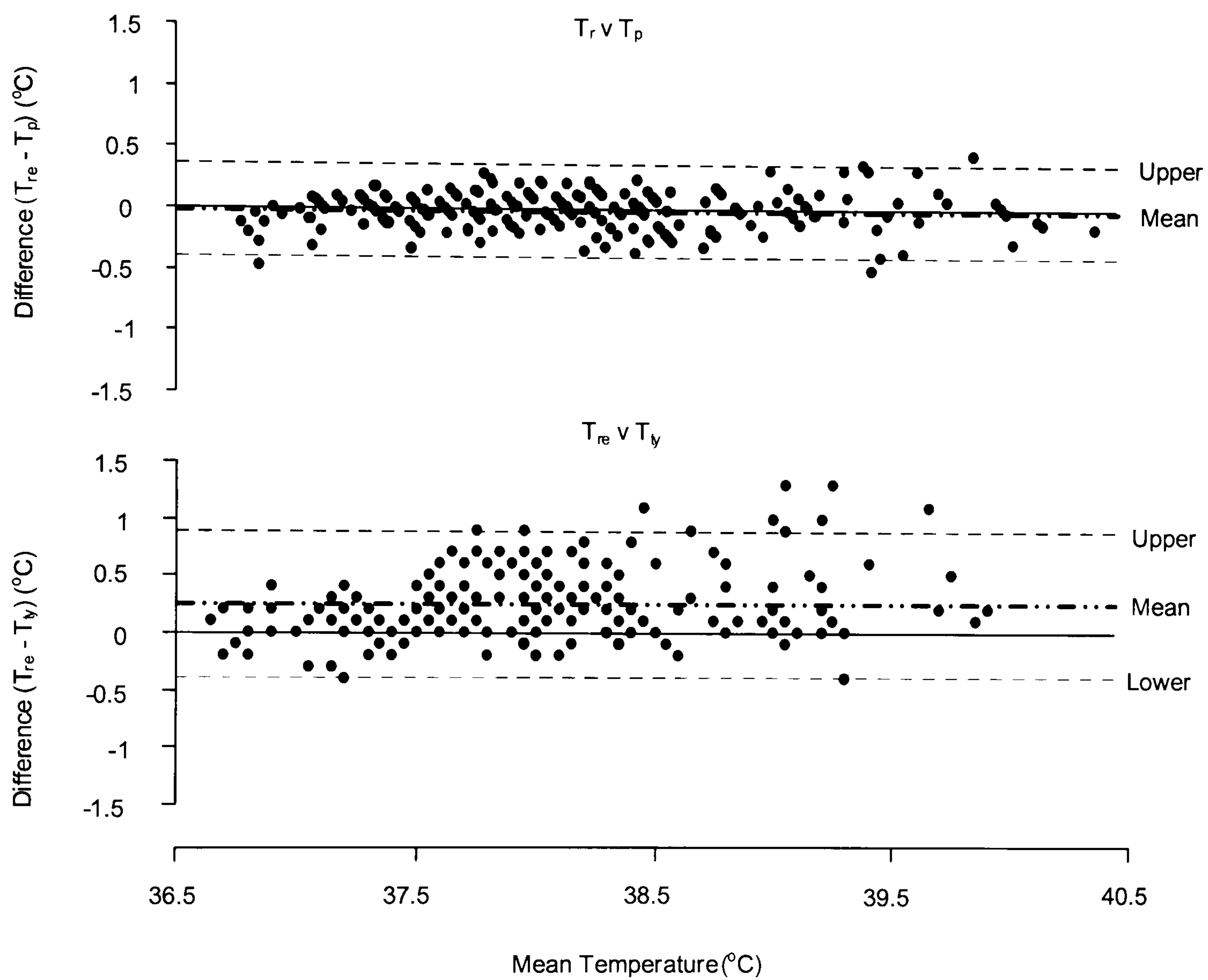


Figure 6.4 Bland-Altman plots of overall rectal vs. telemetry pill temperatures and rectal vs. tympanic membrane temperatures, with means (*solid lines*) and limits of agreement (*dashed lines*).

6.4 Discussion

This study examined the validity of both an infrared tympanic membrane thermometer and the telemetry pill system for T_{re} measurement during rest and exercise-induced hyperthermia. The exercise trials were successful in inducing significant thermal strain in subjects with a mean peak T_{re} recorded at the end of exercise of 39.4°C (range: $38.8 - 40.2^{\circ}\text{C}$) (Fig. 6.1). This study is the first to show that the telemetry pill system can provide a valid measurement of T_c during periods of severe heat stress induced by exercise. Furthermore, the present study confirmed early observations that T_{ty} is significantly lower than T_c during exercise in the heat (Deschamps *et al.*, 1992) suggesting that a mechanism of selective brain cooling may be present in humans (Cabanac, 1993).

The close agreement between T_{re} and T_p during exercise observed in the present study is consistent with most previous studies (Kolka *et al.*, 1997; Lee *et al.*, 2000; O'Brien *et al.*, 1998). However, this study is the first to offer evidence of a close agreement between T_p and T_{re} when temperature exceeds 39°C (Fig. 6.1). Furthermore, Bland-Altman plots (Figs. 6.3 and 6.4) confirm that the telemetry pill system provides similar predictions of T_c to T_{re} during a period of severe thermal strain. Conversely, studies by Kolka *et al.* (1993) and Sparling *et al.* (1993) concluded that the ingestible telemetry pill was not a valid method of T_c measurement, with the latter study demonstrating consistently lower T_p readings compared to T_{re} during both rest (T_p : $36.9 \pm 0.4^{\circ}\text{C}$; T_{re} : $37.5 \pm 0.2^{\circ}\text{C}$) and 30 to 90 min of steady state cycle or treadmill exercise to exhaustion. However, the short time delay (approximately 3-9 h) between pill ingestion and commencement of exercise in some subjects potentially contributed to these compromised results due to probable temperature fluctuations in the upper part of the gastrointestinal tract (Kolka *et al.*, 1993). Furthermore, telemetry pill technology was in its infancy when this study was published and all subsequent studies have found the telemetry pill system to accurately measure T_c during exercise in the heat (Kolka *et al.*, 1997; Lee *et al.*, 2000; O'Brien *et al.*, 1998).

In the present study, ingestion of cold water immediately reduced T_p below normal physiological limits (below 30°C) on three separate occasions with no such effect observed on either T_{re} or T_{ty} , suggesting the pill was still located in the upper part of the gastrointestinal tract. This possible confounding factor emphasizes the need for a long transition period (8-12 h) between pill ingestion and T_p measurement as suggested by O'Brien *et al.* (1998). The results from the present study confirm the accuracy of the telemetric pill system to monitor T_c during exercise-induced conditions of extreme heat

stress, however the requirement of a long delay between ingestion and accurate T_c measurement means its use to measure T_c at the point of collapse in athletes suffering from suspected heat exhaustion is limited as a rapid measurement of T_c is required for diagnosis. Additionally, once inside the gastrointestinal tract, the crystal sensor within the telemetry pill vibrates producing a magnetic flux making it impossible to carry out a magnetic resonance imaging scans on a collapsed athlete. However, these potential limitations can be overcome by using the telemetry pill as a suppository. Furthermore, data from the present study indicates that the telemetry pill system could be employed as a preventative measure in order to reduce incidences of heat injury in military personnel and amateur athletics competitors subjected to severe environmental heat stress as suggested by Byrne *et al.* (2006). The ingestible telemetry pill system has been used in a number of recent studies to measure T_c of athletes exercising in the field (Edwards & Clark, 2006; Fowkes Godek *et al.*, 2004; Laursen *et al.*, 2006). Indeed, Fudge *et al.* (2007) recently used the telemetry pill system to assess thermal strain in elite Kenyan endurance athletes during a week of intense altitude training.

Although the tympanic membrane thermometer provided a valid estimation of T_{re} during rest and the initial stages of exercise in the current study, differences between measurements began to occur as temperature exceeded 37.5°C (Fig. 6.1). The tympanic membrane thermometer began to significantly underestimate T_{re} after 30 min of exercise and the level of disagreement increased with further increases in temperature (Figs. 6.3 and 6.4). Furthermore, Bland-Altman analysis reveals that T_{ty} may be 1.23°C below or 0.38°C above T_{re} (Fig. 6.3), which has led previous authors to conclude that measurement of T_c by tympanic membrane thermometer is not accurate for research or clinical purposes (Deschamps *et al.*, 1992). One possible explanation for the observed differences in T_c and T_{ty} during hyperthermia is the presence of a selective brain cooling mechanism in humans. Selective brain cooling is known to occur in many species of mammals in the form of thermal panting, which causes water to evaporate from the upper airways and subsequent heat loss from the head such that brain temperature is reduced (Baker, 1979). The human head sweats more than the rest of the body (Cabanac & Brinnet, 1988) which when combined with the heat lost from the upper airways may be sufficient to cause selective brain cooling (Cabanac, 1993). Therefore, as the tympanic membrane shares the blood supply with the vasculature of the hypothalamus through the internal carotid artery (Benzinger, 1959; 1969), T_{ty} may actually provide a better estimation of brain temperature. Indeed, Mariak *et al.* (1994) reported a direct relationship between T_{ty} and brain temperature during a surgical procedure in an anaesthetised subject and thus concluded that in most clinical situations T_{ty} offers the best approximation of brain temperature among the

externally accessible body temperatures (Mariak, 2002). As both T_{re} and T_p are measured some distance from the ear it is likely that these methods of T_c measurement provide a measurement of trunk temperature and the disparity with T_{ty} would seem to support the existence of selective brain cooling in humans. Despite these findings, some researchers refute the existence of selective brain cooling in humans and propose that the gap between T_{ty} and T_{re} observed during hyperthermia is caused by contamination of T_{ty} by T_{sk} (Brenngelmann, 1993). However, during short term exposure to cold air, T_{ty} does not change yet T_{sk} drops significantly (Brinnet & Cabanac, 1989). Furthermore, when T_{ty} and T_{re} were recorded simultaneously in comatose patients from 33°C to 42°C, they evolved similarly up until 38°C, from which point T_{ty} became lower than T_{re} (Brinnet & Cabanac, 1987). This would suggest that the disparity between T_{ty} and T_{re} during hyperthermia in humans is caused not by contamination of T_{ty} by a lower T_{sk} but instead, by the existence of selective brain cooling.

These results from the present study are in stark contrast to the findings of the only published study to date where T_{ty} was compared to T_{re} during exercise in the heat when T_{ty} was recorded in rectal equivalent mode that utilizes an algorithm to predict T_{re} (Newsham *et al.*, 2002). Newsham and colleagues established that peak T_{re} (38.9°C) was not significantly different from T_{ty} (39.2°C) after a period of self-paced stair-climbing exercise in a hot environment (32°C, 70% relative humidity) (Newsham *et al.*, 2002). However, the many methodological differences between the present study and that of Newsham *et al.* (2002) are likely to account for some of these differences. For example, Newsham *et al.* (2002) utilized a Thermoscan Pro-1 tympanic thermometer (San Diego, U.S.A.) whereas a First Temp Genius Thermometer was used to measure T_{ty} in the present study. Despite these two brands of tympanic membrane thermometer estimating T_c in patients suffering from a fever by a similar degree of accuracy (Hoffman *et al.*, 1999), there is no existing data comparing different brands of tympanic membrane thermometer during exercise. However, this is the first study to measure the accuracy of the Genius tympanic membrane thermometer during exercise whereas different brands of tympanic thermometer may use different equations to predict T_{re} . The difference in exercise modalities between the study of Newsham *et al.* (2002) and the present study may also have contributed to the observed differences in the accuracy of T_{ty} as a predictor of T_c . As T_{re} is affected by blood flow from exercising leg muscle and thus heat transfer (Saltin *et al.*, 1968), intense cycle exercise would potentially affect T_{re} to a greater extent than stationary stair-climb exercise due to the recruitment of larger muscle groups. Alternatively, the constant facing air velocity of 1.8 m·s⁻¹ in the present study may have artificially reduced T_{ty} due to an increase in convective cooling, whereas experimental trials in the study by Newsham *et al.* (2002)

were conducted in a still environment. Several investigators have confirmed that changes in T_{sk} brought about by fanning or selective cooling of the skin rapidly decreases T_{ty} with little or no effect on T_{re} (Deschamps *et al.*, 1992; Greenleaf & Castle, 1972; Livingstone *et al.*, 1983). However, there is some evidence to suggest that the effect of the external environment on T_{ty} can be minimized by insulating the external ear canal with cotton wool (Rasch & Cabanac, 1993). Although this procedure was not performed in the present study, Hansen *et al.* (1996) argue that cotton wool ear pads do not prevent decreased T_{ty} when T_c is increasing, providing further evidence for the existence of selective brain cooling in humans. The possibility that experimental error, such as inaccurate placement of the tympanic membrane thermometer in either study could explain the observed differences in T_{ty} accuracy does not seem likely as both groups of experimenters utilized a similar technique of T_{ty} measurement.

The extent to which the tympanic membrane thermometer underestimates T_c increased with exercise duration peaking in a difference of $0.43 \pm 0.81^\circ\text{C}$ at the end of the time trial. Although this difference may not be clinically significant, medical personnel assessing heat strain should be aware of the existence of a temperature gradient between T_{ty} and core temperature measured externally elsewhere in the body. Despite the documented differences between T_{ty} and core temperature in this and other studies (Deschamps *et al.*, 1992), several other experimental trials involving exercise have been published with T_{ty} as the sole measurement of core temperature (Hsu *et al.*, 2005; Voltaire *et al.*, 2003). However, experimenters should continue to use caution when employing this technique to measure core temperature during exercise as selective cooling of the brain may cause T_{ty} to underestimate trunk temperature and thus mask the occurrence of a dangerously high body temperature. The differences between T_{ty} and T_c highlighted in the present study apply solely to the exercise situation, whereas the accuracy of the tympanic membrane thermometer in the clinical situation to measure viral induced increases in T_c was outside the scope of this study.

6.4.1 Conclusions

These results demonstrate that the ingestible telemetry pill system provides valid measurements of T_c during both rest and exercise-induced hyperthermia up to the limits of T_c measurement and therefore can be used in the field where T_{re} and esophageal temperatures cannot be taken. While the infrared tympanic membrane thermometer closely matched T_{re} measurements at rest and in the early stages of exercise, T_{ty} appeared to significantly underestimate thermal strain once T_c exceeded 37.5°C . Further experimental

evidence is required to determine whether the disparity between T_{ty} and T_{re} is merely be due to imperfections in the tympanic membrane thermometer methodology or due to the existence of selective brain cooling in humans.

Chapter 7

General Discussion

The primary objectives of the experiments described in the previous chapters were:

- i. To investigate the effects of ingesting two different fluid retaining agents simultaneously on body fluid balance and in doing so determine whether combining Cr and Gly can induce a greater degree of hyperhydration than either Cr or Gly alone. This was achieved by designing a series of studies that measured the effects of all combinations of supplements on TBW, ECW, ICW and plasma volume.
- ii. To develop the optimal hyperhydration strategy for use during conditions of restricted water access or exercise induced heat stress. This was achieved by comparing a Cr/Gly supplementation strategy based on previously established protocols from the literature with novel methodologies.
- iii. To assess the effects of these novel ‘water-loading’ strategies on metabolism, cardiovascular and thermoregulatory responses and performance during exercise in the heat. This was achieved by examining the effects of combined Cr and Gly hyperhydration on physiological responses during steady state exercise in a hot and humid environment (30°C and 70% relative humidity) and performance in a 16.1 km time trial and in doing so providing further insight into the relationship between dehydration and performance.
- iv. To validate a new method of T_{c} measurement for use outwith the laboratory in training and competitive situations. This was achieved by comparing T_{c} measurements obtained from an ingestible telemetry pill and an infrared tympanic membrane thermometer with those from a rectal thermistor during rest and high intensity exercise conducted in a hot and humid environment (30°C and 70% relative humidity) intended to raise T_{c} above 39°C. This will allow future research examining the effects of Cr, Gly and combined Cr and Gly hyperhydration on T_{c} and heart rate and exercise performance to be completed in the field.

Despite no significant increases in TBW (Figs. 3.2, 4.2) or change in plasma volume after Cr/Gly ingestion following loading protocols 1 and 2 (LP1 and LP2) described in Chapter 3 (a) and 4 (a) respectively, there was an increase in body mass (Figs. 3.2, 4.2), suggesting a significant retention of fluid. This may be explained by water requiring longer than 3 hours from ingestion of the final Cr/Gly supplement to disperse throughout the body water compartments (Fig. 4.7). However, because the final supplement was consumed 2 and 3

hours prior to exercise in LP1 and LP2 respectively this explains why subjects displayed a significant increase in body mass without a concomitant increase in TBW. Supplementation with combined Cr and Gly using the third loading protocol (LP3) where the final supplement was ingested 5 hours prior to exercise as described in Chapter 5, significantly increased body mass, TBW and resulted in a percentage increase in plasma volume (Fig. 5.2). Additionally, this study is the first to show that the increase in TBW resulting from ingestion of this novel ‘water-loading’ strategy that combines Cr and Gly using LP3 is significantly larger than the volume retained with either Cr or Gly alone (Fig. 5.2). The increase in TBW by up to 1.4 L induced by combined Cr and Gly supplementation using LP3 also resulted in a reduction in perceived effort (Fig. 5.4, 5.5), cardiovascular (Fig. 5.3) and thermoregulatory (Fig. 5.7) responses during exercise in the heat. Yet despite the reduction in physiological stress, hyperhydration via Cr and Gly did not improve exercise performance, assessed by a 16.1 km cycling time trial. The series of experiments presented in this thesis have resulted in an extremely effective ‘water-loading tool’ that can be used to reduce T_{c} and heart rate during exercise in the heat, and potentially reduce the risk of heat injury. Others who work in hot conditions with restricted access to fluids, such as rescue workers, fireman, soldiers and astronauts, may also benefit from prolonged periods of hyperhydration induced by this novel water loading strategy. In this chapter, the findings and general conclusions of this series of studies are discussed along with suggestions for future research.

9.1 Hyperhydration

Subjects experienced a body mass increase of 1.59 ± 0.21 kg after 7 days of supplementation with Cr/Gly in LP1 and 1.20 ± 0.37 kg in LP2 (Figs. 3.2 and 4.2 respectively). This exceeds the 0.4-1.0 kg increase in body mass observed in other hyperhydration studies (Kilduff *et al.*, 2004; Lyons *et al.*, 1990; Montner *et al.*, 1996). However, these increases in body mass were not accompanied by any increases in TBW indicating an increase in water retention that was not measured by bioimpedance analysis. These results are not in agreement with other hyperhydration studies that demonstrated an increase in fluid retention as reflected by a significant increase in TBW (Kern *et al.*, 2001; Kilduff *et al.*, 2004; Seifert *et al.*, 1995). For example, subjects in the study by Kilduff *et al.* (2004) experienced a 0.8 kg increase in body mass accompanied by a 0.6 L increase in TBW after 7 days of Cr supplementation. Conversely, combined Cr/Gly supplementation following LP3 resulted in a significant 0.97 ± 0.28 kg increase in body

mass accompanied by a 0.87 ± 0.21 L (range 0.6-1.4 L) increase in TBW (Fig. 5.2, Table 5.2). However, it remains a possibility that the number of subjects ($n=6$) who completed LP1 and LP2 was not high enough to identify a significant increase in TBW. Nevertheless, the small increases in TBW resulting from Cr/Gly supplementation in LP1 and LP2 (0.32 L and 0.15 L respectively) were considerably smaller than those reported in other hyperhydration studies (e.g. Freund *et al.*, 1995; Kern *et al.*, 2001; Kilduff *et al.*, 2004; Lyons *et al.*, 1990). On the other hand, a statistical power calculation utilising the mean TBW increase following Cr/Gly supplementation in LP3 revealed that $n=6$ subjects (80% power) would be required in order to observe a significant TBW increase ($P<0.05$), indicating that the lack of TBW increase in LP1 and LP2 may be due to a difference in the loading protocol methodology. The results from LP3 also demonstrate that both the increase in body mass and the volume of water retained by ingesting either Cr or Gly can be significantly enhanced by combining these two hyperhydrating agents (Fig. 5.2). Additionally, the retained water was dispersed equally between the intra- and extra-cellular water compartments (Fig. 5.2). It seems plausible that the water retained by combining Cr and Gly was mediated via a Cr-induced increase in ICW and a Gly-induced increase in ECW. This innovative ‘water-loading’ strategy comprised of two agents that specifically target both ICW and ECW compartments and as such, may overcome the limitations of previous hyperhydration strategies. For example, the hyperhydrating effects of previous strategies were transient due to the metabolism of Gly by the liver and kidneys. Of greater concern is the potential side-effect of cerebral dehydration, due to the relatively impermeability of the blood-brain barrier to Gly, with resulting headaches (Tourtellotte *et al.*, 1972). Combining Gly with Cr may overcome this, as in contrast to Gly, Cr is taken up by the brain (Matthews *et al.*, 1999) and in doing so counteracts the negative effects associated with Gly ingestion, increasing the level of initial hydration but also potentially prolonging the period that hyperhydration will last. Furthermore, it has been suggested that the response to Cr supplementation is highly individual (Myburgh, 2000). Studies suggest that the populations that have been studied fall more or less equally (that is 25%) into one of four groups: nonresponders, low responders, average responders, and high responders (Myburgh, 2000). For example, an increase in body mass of greater than 0.2 kg identifies a ‘responder’ to Cr supplementation (Kilduff *et al.*, 2003). Therefore, subjects supplementing with combined Cr and Gly who are nonresponders to Cr, would still actively retain water due to the inclusion of Gly, and hence would still benefit during heat stress from Cr/Gly ingestion. There also appears to be an individualised response to water retention due to Gly supplementation (Koenigsberg *et al.*, 1995), as Pl/Gly supplementation in LP3 resulted in a mean increase in TBW of 0.57 ± 0.28 L (Fig. 5.2)

with a range of 0.03-1.00 L (Table 5.2). However, it is currently unknown whether the vastly diverse effects of Gly supplementation on fluid retention are due to anatomical, metabolic or genetic differences between subjects.

A key finding of the present series of studies is the importance of the methodology that led to an increase in TBW. The only difference between LP2 and LP3 was the length of time between the ingestion of the final supplement and testing. Thus, it would seem apparent that a period of time in excess of 3 hours is required for the retained water to be distributed within body water compartments (Fig. 4.7). Gly has been investigated as a potential hyperhydrating agent for a number of decades (Anderson *et al.*, 2001; Lyons *et al.*, 1990). However, the benefits of Gly hyperhydration are equivocal with at least 23 original papers published on Gly hyperhydration providing conflicting results. Previous studies examining the effectiveness of Gly supplementation as a hyperhydration method have consistently utilised a single Gly bolus mixed with water and ingested between 2-3 hours prior to analysis (Lyons *et al.*, 1990; Montner *et al.*, 1996). However, when Gly was delivered in a similar fashion alongside a Cr hyperhydration protocol previously shown to be successful in our lab (Kilduff *et al.*, 2004), no increase in hydration was measured (Fig. 3.2). Previous Gly hyperhydration studies have quantified water retention by the volume of urine produced (Anderson *et al.*, 2001; Freund *et al.*, 1995), which provides no information as to where the retained water was distributed. Body compartment analysis by multifrequency bioimpedance combined with changes in body mass used in the present study, provides data indicating fluid changes in both the intra- and extra-cellular water compartments. Furthermore, the mechanism by which bioimpedance estimates body water provides insight into the confounding data from LP1 and LP2. Since hypertonic solutions such as the Cr/Gly combination ($965 \pm 61 \text{ mosmol} \cdot \text{kg}^{-1}$) cause an initial net movement of fluid into the intestinal lumen (Gisolfi *et al.*, 1990), there is a loss of ECW and thus TBW, which ultimately leads to some degree of dehydration, albeit temporarily. This is confirmed by the small percentage reductions in plasma volume that occurred after supplementation with both Pl/Gly and Cr/Gly in LP1 and LP2 (Chapters 3(a) and 4(a)). Interestingly, fluid changes in the trunk have little effect on bioimpedance measurements as the trunk only accounts for 5-12% of total body impedance (Kushner, 1992). This is confirmed by the relatively small impact on bioimpedance measurements of up to 2 L of fluid within the abdominal cavity (Kushner *et al.*, 1996). Additionally, the profoundly high osmolality of the Cr/Gly solution may have inhibited gastric emptying (Costill & Saltin, 1974), further contributing to the lack of increase in TBW 2-3 hours after Cr/Gly ingestion as demonstrated in LP1 and LP2. Although Cr/Gly supplementation results in significant water retention, a period of time greater than 3 hours is required after ingestion of the final

Cr/Gly supplement before significant hydrating effects are discerned throughout the body water compartments. Furthermore, it is possible that a similar effect would have been observed after LP1 and LP2 had a longer period of time been left between ingestion of the final supplement and testing. The results from the present series of experiments provides an explanation as to why the results of Gly hyperhydration studies to date have been equivocal in nature (e.g. Latzka *et al.*, 1997; Lyons *et al.*, 1990; Marino *et al.*, 2003; Montner *et al.*, 1996). That is, inconsistencies with the period of time between ingestion of the Gly bolus and commencement of exercise (Robergs & Griffin, 1998) coupled with the highly individualised response to Gly ingestion (Table 5.2) has resulted in some but not all subjects receiving the physiological benefits of increased TBW prior to the exercise induced heat stress. Therefore, if the period of time between ingestion of the Gly bolus is short (i.e. less than 3 hours) then there will not be adequate time for the retained water to be dispersed within body compartments in many of the subjects. For example, in a set of studies by Latzka *et al.* (1997;1998), 8 subjects were required to ingest $1.2 \text{ g Gly} \cdot \text{kg}^{-1}$ body mass only 1 hour prior to exercise in a hot environment. The small subject number combined with the short period of time between ingestion of Gly and exercise commencement may explain why these researchers found no physiological benefits of Gly ingestion compared to water. The findings of the present series of investigations not only provide invaluable information to researchers but to coaches who wish to pursue Gly as a means to hyperhydrate athletes prior to exercise.

Although an expansion of TBW induced by combined Cr/Gly supplementation may provide athletes with a physiological advantage (Figs 5.3, 5.4, 5.5, 5.7), there has been some anecdotal evidence published in the media suggesting a link between Cr use and muscle strains, muscle cramps, heat intolerance, and other side effects (e.g. Tocci, 2005). Although these findings are not well supported by the scientific literature, there was one isolated incident of muscle cramping (gastrocnemius) during supplementation with Cr/Gly in the present series of studies (Chapter 3(a)). However, it is unclear whether Cr supplementation was causative of muscle cramping in this case. Furthermore, data from previous studies (Kern *et al.*, 2001; Kilduff *et al.*, 2004) and the present investigations (Chapter 5) suggest that Cr actually provides protection from heat stress as opposed to heat intolerance as suggested by (Tocci, 2005). The majority of studies conducted in athletes and soldiers indicate a substantial level safety of both short- and long-term Cr use in healthy adults (Bennett *et al.*, 2001; Greenwood *et al.*, 2003a; Greenwood *et al.*, 2003b; Kreider *et al.*, 2003; Poortmans & Francaux, 1999; Robinson *et al.*, 2000). There have also been some concerns that high dose Cr usage may cause kidney damage, although these are based solely on two case reports in which one of the affected individuals was

suffering from existing underlying renal disease (Koshy *et al.*, 1999; Pritchard & Kalra, 1998). Both comprehensive literature reviews and expert panels have maintained that there is no conclusive evidence to support the notion that Cr may adversely affect kidney function in healthy individuals (Farquhar & Zambraski, 2002; Pline & Smith, 2005; Poortmans & Francaux, 2000; Terjung *et al.*, 2000; Yoshizumi & Tsourounis, 2004). On the other hand it is currently unknown whether Gly ingestion or infusion for prolonged periods of time may cause cerebral oedema. A single bolus of oral Gly supplementation is unlikely to be harmful due to delayed absorption by the brain and rapid metabolism by the liver and urinary excretion prior to Gly reaching cerebral circulation (Sommer *et al.*, 1993; Tourtellotte *et al.*, 1972). Although Gly was ingested for 7 days in the present series of experiments without incident, further research is required to examine the effects of Gly ingestion for prolonged periods of time on intracranial pressure. Furthermore, there is some concern that prolonged elevation of blood [Gly] for periods of weeks or months may pose a risk to kidney function and fluid regulation. However, Frank *et al.* (1981) reported that adverse reactions to Gly were associated with intravenous, subcutaneous or intraperitoneal injection, with no occurrences with oral ingestion. Nevertheless, individuals with diabetes, kidney failure, migraine, cardiovascular or liver disorders or who are pregnant should avoid Gly ingestion due to acute symptoms that may pose a risk for associated complications (Robergs & Griffin, 1998). Additionally, athletes who intend to exercise at high altitude should also avoid combined Cr and Gly ingestion due to reported associations between plasma volume expansion and the development of high altitude pulmonary oedema (Luks *et al.*, 2007).

9.2 Cardiovascular, thermoregulatory and metabolic responses during exercise in the heat

There is mounting evidence to suggest that a direct relationship exists between the level of dehydration and the elevations in heart rate, T_c (Montain & Coyle, 1992b), muscle glycogen use (Hargreaves *et al.*, 1996), blood concentrations of fluid regulating hormones (McConell *et al.*, 1997) and discomfort during exercise in the heat (Noakes, 1993). However, it is currently unclear from the existing data whether this relationship is spurious or indeed if dehydration is causative of the above noted increases in physiological responses. The results from the present series of studies would seem to support the hypothesis of Montain & Coyle (1992a) that dehydration does cause an increase in T_c during exercise. For example, in Chapters 3 (a) and 4 (a), supplementation with combined Cr and Gly did not significantly alter body water levels, and subsequently physiological

responses during exercise in the heat were not different compared to pre-supplementation values. However, when Cr and Gly were ingested using LP3, TBW increased significantly which resulted in a significant attenuation in heart rate (Fig. 5.3), T_c (Fig. 5.7) and perception of effort (Figs. 5.4, 5.5) during exercise in the heat compared to the pre-supplementation exercise trial. Metabolic responses were not different following Cr/Gly supplementation (Table 5.4, 5.5, 5.6), and therefore alterations in hydration status provide the only reasonable explanation for the reductions in T_c and heart rate reported in Chapter 5. However, the hypothesis of Noakes *et al.* (1991) that metabolic rate not percentage dehydration is the most important determinant of T_c during exercise in the heat cannot and indeed should not be discounted. Indeed the high correlation between relative exercise intensity (i.e. % $\dot{V}O_{2\max}$) and T_c during exercise has been known for 40 years (Saltin & Hermansen, 1966). Furthermore, Pugh *et al.* (1967) measured T_{re} , sweat rates and body mass loss during a marathon run in warm conditions. The authors reported that the winner's compared to average race finishers mean speed was higher (16 vs. 13 km·hr⁻¹), estimated $\dot{V}O_2$ was higher (54 vs. 44ml kg⁻¹·min⁻¹), body mass loss was double (5.23 vs. 2.85 kg) and post-race T_{re} was higher (41.1 vs. 39.0 °C). Therefore, the implication of these findings are that successful runners who employ a higher work rate, have higher energy expenditure and consequently higher T_c . However, in the present series of investigations metabolic rate was not different between exercise trials (Tables 3.1, 4.2, 5.4) and therefore no valid conclusions regarding alteration of this variable can be drawn.

Supplementation with combined Cr and Gly using LP3 also resulted in a small but significant increase in plasma volume of 2.4%, from pre- to post-supplementation. This is in contrast to the majority of previous studies that utilised Cr or Gly alone to hyperhydrate, which concluded that there were no changes in plasma volume compared to water or a Pl (Freund *et al.*, 1995; Kilduff *et al.*, 2004; Lyons *et al.*, 1990). In agreement with these previous studies, there were no appreciable changes in plasma volume in the present study in LP3 from pre- to post-supplementation when either Cr or Gly alone were ingested. However, the fact that combined Cr and Gly resulted in a significantly greater retention of fluid than Cr or Gly alone and the unique way in which this novel hyperhydration strategy retains fluid, may account for these notable differences. Nevertheless, the proposed benefits of an expansion in plasma volume prior to exercise in the heat remain unclear at present. Montain and Coyle (1992b) have indicated that plasma volume drops significantly during prolonged cycling at 63-67% $\dot{V}O_{2\max}$ with a corresponding rise in T_c . Therefore it is reasonable to assume that any expansion of plasma volume prior to exercise would be of cardiovascular and thermoregulatory benefit to subjects cycling in the heat as maintenance

of plasma volume would sustain stroke volume and provide an adequate avenue for heat loss. The results of the present series of experiments appear to confirm such a hypothesis as plasma volume dropped by approximately 12% following 40 min cycling exercise at 63% WR_{max} and a 16.1 km time trial (Figs. 3.6, 4.6, 5.8) resulting in a significant increase in T_c . However, when plasma volume was expanded by 2.4% prior to exercise via ingestion of Cr and Gly (Chapter 5) there was a significant attenuation in both T_c (Fig. 5.7) and heart rate (Fig. 5.3). Nevertheless, this plasma volume hypothesis fails to explain how Cr supplementation alone can result in a significant attenuation in T_c and heart rate during exercise in the heat despite no percentage increase in initial plasma volume in both the present experiments (Fig. 5.7) and previous studies (Kern *et al.*, 2001; Kilduff *et al.*, 2004). Furthermore, given that plasma volume does not continue to drop beyond the initial fall due to postural changes during running either in the field or on a treadmill despite large reductions in TBW (4-7%) it is unclear what benefit, if any may be gained from plasma volume expansion prior to running in the heat. Therefore, it remains a distinct possibility that the reductions in T_c (Fig. 5.7) observed after Cr and combined Cr and Gly supplementation may have been due to an increased specific heat capacity of the body, resulting in a greater capacity to store heat (Kilduff *et al.*, 2004). Given that it takes 0.83 kcal of heat production per kg of body mass to increase T_c by 1°C; a Cr/Gly induced expansion of TBW (and hence, increase in body mass), could lead to a more efficient distribution of heat within the body (Kilduff *et al.*, 2004). Several published studies lend support for this hypothesis, and indeed further propose that fluid ingestion enhances performance in the heat by increasing the heat storage capacity of the body (Kay & Marino, 2000; Kilduff *et al.*, 2004; Sawka, 1992).

The recent reports of heat related injuries and deaths in athletics competition and during military service have highlighted the need for an adequate intervention. Methods such as heat acclimatisation, pre-cooling or plasma volume expansion via saline infusion are not readily accessible or indeed practical for the amateur runner. Furthermore, fluid replacement during the race itself can be problematic; too little could potentially result in an elevated T_c and heat stroke, too much could result in frequent visits to the lavatory (as demonstrated by Paula Radcliffe stopping to urinate during the race in the London Marathon, 2005) and a progressive fluid overload, leading to dilution of blood [sodium] below 130 mmol·L⁻¹ and ultimately hyponatraemic encephalopathy and even death (Noakes, 2005). Therefore, increasing body water stores prior to exercise with combined Cr and Gly is a simple way in which to provide a fluid reservoir for each athlete, protecting against dehydration and limiting the volume of fluid that would have to be replaced during the race. However, the dissimilarity in wind conditions between laboratory conditions and

the external environment mean the extent by which supplementation with Cr or combined Cr and Gly reduced T_c and heart rate during cycling exercise in the heat may be significantly less when the exercise is performed outdoors. Evidently, further field-based research using portable recording devices such as the ingestible telemetry pill, is required to determine the ‘true’ effects of combined Cr and Gly supplementation on thermoregulation and exercise performance in the heat.

9.3 Exercise performance

Several studies have indicated that the increased heart rate and T_c resulting from dehydration can have a negative impact on exercise performance (Cheuvront *et al.*, 2005). Yet, despite the reductions in T_c (Fig. 5.7), heart rate (Fig. 5.3) and perceived leg fatigue (Fig. 5.4) during constant-load exercise induced by supplementation with Cr/Pl and Cr/Gly, time trial performance was not affected (Chapter 5). It seems likely that the exercise trial in the present study was not of sufficient duration and therefore too high an exercise intensity for hyperhydration to have a significant effect on performance. For example, the mean sweat loss recorded in the present series of studies only equated to 2.5%, 2.0% and 2.0% of body mass in Chapters 3(a), 4(a) and 5 respectively, all within the so called ‘tolerable range’ of dehydration (Cheuvront & Haymes, 2001). Therefore, it would appear that commencing exercise in a hyperhydrated state may not confer any significant advantage in terms of exercise performance compared to euhydration or indeed modest dehydration (i.e. loss of 2-3% body mass). The results from the present series of studies are compatible with such an idea, although further research is needed to determine the effects of hyperhydration on physiological responses and performance during a more prolonged exercise trial where a more marked degree of dehydration would be expected.

There is also theoretical opinion that dehydration within a tolerable range will not have a negative impact on exercise performance, but may even confer an advantage by preventing inevitable increases in body mass due to consumption of large volumes of fluid (Armstrong *et al.*, 1985; Noakes, 2001). Therefore if body mass can be reduced while power output remains constant there will be a concomitant reduction in the energy cost of exercise, especially in weight bearing activities. However, Armstrong *et al.* (2006) recently examined the effects of pronounced dehydration (~5.6% body mass) on physiological responses and running economy during a 10 min treadmill run at 70% and 80% $\dot{V}O_{2\max}$ in an ambient temperature of 23°C. Compared with euhydration, subjects in the hypohydrated state experienced a significantly higher heart rate and T_{re} concurrent with a reduction in

cardiac output and stroke volume. Furthermore, these authors found no differences in running economy between hydration conditions suggesting that dehydration in the region of 5.6% body mass and below will not confer any significant advantage in terms of reducing energy cost (Armstrong *et al.*, 2006). However, these tests were conducted in thermoneutral conditions (23°C) for a relatively short period of time (10 min), resulting in only a small 0.6°C increase in T_{re} . Given that T_c can rise in excess of 39°C during exercise in the heat (Fig. 5.5) and even beyond 43°C following prolonged exercise (Armstrong *et al.*, 1996) the possible effects of hyperthermia on $\dot{V}O_2$ cannot be ignored. Further, Noakes (2005) argues that findings from laboratory research completed on a treadmill with no facing wind such as those presented by Armstrong *et al.* (2006) cannot be extrapolated to the field. It is also presently unknown whether the reductions in heart rate and T_c caused by combined Cr and Gly supplementation would outweigh the potential negative impact of an increase in body mass during weight bearing sports such as running. However, there is some anecdotal evidence to suggest that hyperhydration may be of benefit during elite endurance performance. One of the noticeable results of the recent Olympic Games in Athens, was American runner Deena Kastor's bronze in the women's marathon. Afterwards it was revealed that she had ingested a Gly solution as part of her pre-race preparation in a bid to enhance and maintain hydration in the scorching heat of Athens. Could this have been a factor in her success? Further experimental research is required to determine the effects of an increased body mass induced by fluid retention on running economy and endurance performance performed in hot and humid conditions.

The vociferous debate surrounding the proposed benefits of rehydration on exercise performance in the heat (e.g. Cheuvront *et al.*, 2003; Coyle, 2004; Maughan & Shirreffs, 2004; Noakes, 2005; Noakes & Martin, 2002; Shirreffs *et al.*, 2004) looks set to continue for several years to come. One of the main reasons for the equivocal nature of the data in this area is the vastly different methodological approaches employed to answer the same question; namely does dehydration have a negative impact on exercise performance? For example, in the present set of experiments reducing the extent of dehydration via Cr and Gly hyperhydration did not improve performance in a simulated 16.1 km cycling time trial in 30°C, 70% relative humidity (Chapter 5). Conversely, Cheuvront *et al.* (2005) concluded that dehydration in the region of 3% body mass significantly reduced the total amount of work (kJ) completed in 30 min by $7.6 \pm 5.9\%$ compared to euhydration in a 20°C environment. However, the opposite findings of these different experiments are clearly only relevant to the individual populations and conditions (environmental and physiological) examined in that particular study. Therefore, extrapolation to competitive

endurance exercise contested in different environments is not possible. Only by comparing the effects of different levels of dehydration in the same athletes running at the their same speeds in the same environmental conditions on two or more different occasions, can researchers determine the independent effects of dehydration when all other variables that could potentially influence that relationship are controlled and hence identical (Noakes, 2005).

9.4 Applications

Many other vocations out with the area of sport and exercise also experience regular problems with fluid balance. For example, the potential danger of dehydration and heatstroke in the military situation have been recognised since the first half of the twentieth century. The first set of studies examining the effects of heat stress and dehydration on T_{re} , heart rate, exercise performance and physiological well being were published in 1938 and 1947 using soldiers as subjects (Adolph, 1938; Adolph, 1947; Adolph & Dill, 1938). These studies concluded that soldiers marching in desert heat developed dehydration despite free access to fluids, which subsequently resulted in premature fatigue. Furthermore, heart rate and T_{re} rose as a linear function of the level of dehydration. Adolph (1947) suggested that there were no immediate health risks associated with the dehydration to the extent of 7-10% of body mass but there was a risk of serious organ failure should dehydration exceed 15%. Yet despite these early findings, half a dozen American soldiers died in 1 week during the recent Iraq conflict because of the mind-baking 130° heat (Wyatt, 2004). Inside the tents, soldiers live in a heat index of 150° plus and soldiers tell stories of dizziness and of passing out facedown into their food in the dining hall (Wyatt, 2004). Medics at one unit report treating a dozen cases of kidney stones a day caused by dehydration. The official statistics show that more soldiers were treated for heat stroke and dehydration than gun shot wounds in the recent Gulf War conflict (Wyatt, 2004). Soldiers are currently required to consume over 15 L of water per day to avoid dehydration (Wyatt, 2004), a strategy that is both time consuming and hugely impractical. Ingestion of combined Cr and Gly may provide the military with a more practical solution, as soldiers will actively retain a larger volume of the ingested fluid (Fig. 5.2). Furthermore, it is possible that a Cr-induced increase in ICW (Fig. 5.2) may result in an increased specific heat capacity of the body, which will allow a greater capacity to store heat (Kilduff *et al.*, 2004). Therefore, soldiers may be provided with a greater degree of protection from heat stress than they would from simply replacing the fluid lost through sweat. However, further research investigating the effects of combined Cr and Gly on thermal and cardiovascular responses during extreme

heat stress while performing military exercises in both the laboratory and field situations, including in the confined space of a tank or armoured vehicle, is required before any definitive conclusions can be drawn.

Space travel leads also to severe physiological stresses that have profound effects on the wellbeing of astronauts (Lane & Feedback, 2002). Many studies have attempted to quantify the exact nature of the problems encountered by astronauts during and on return from prolonged space travel, though few practical solutions have been offered. Astronauts routinely experience a reduction in body mass of 1-3 kg on return from space flight (Leach *et al.*, 1996; Smith *et al.*, 1997) with losses up to 10 kg reported after long-duration missions (Grigoriev *et al.*, 1996). The cause of this loss in body mass is believed to be multi-factorial, with insufficient fluid and energy intake and losses in musculo-skeletal mass considered the main contributors (Lane & Feedback, 2002). Several studies have reported a significant reduction in TBW on return from space travel (Grigoriev *et al.*, 1996; Leach *et al.*, 1975; Leach & Rambaut, 1977). This water loss may largely be accounted for by loss of body mass (Grigoriev *et al.*, 1996) but also reflects water loss unrelated to muscle atrophy. Microgravity has been shown to remove all hydrostatic gradients within the body (Thornton *et al.*, 1977), resulting in a cephalad fluid shift of approximately 1-2 L and a further shift of fluid from extra-cellular to intra-cellular water compartments (Leach *et al.*, 1996). In addition, there is indirect evidence to suggest that the thirst response is reduced in space to such an extent that fluid lost through respiration and sweating is not adequately replaced, resulting in a reduction in TBW and plasma volume by up to 17% (Lane *et al.*, 2000; Leach *et al.*, 1996; Smith *et al.*, 1997). The extent of plasma volume reduction incurred during space travel may be further exacerbated by the practice of 'voluntary dehydration' undertaken by some astronauts prior to both take-off and re-entry to avoid the need to urinate (Seddon *et al.*, 1994). The effects of a significant reduction in plasma volume may be profound, for example decreasing the thermoregulatory capacity of the body, resulting in an increased heart rate and T_c during exercise and a reduced exercise tolerance (Fortney *et al.*, 1998). Additionally, up to two thirds of astronauts suffer from orthostatic intolerance (inability to maintain a standing posture) on return to earth (Buckey, Jr. *et al.*, 1996b) due to the combined effects of reduced central venous pressure (Buckey, Jr. *et al.*, 1996a) and skeletal muscle atrophy that ensues from a lack of static exercise associated with prolonged exposure to microgravity (Convertino *et al.*, 1989).

One experimental approach to maintaining plasma volume during space travel has been to infuse isotonic saline. In a terrestrial environment this maintained central blood volume,

resulting in an increased skin blood flow and associated convective heat loss (Fortney *et al.*, 1988). The medical expertise required to insert and maintain a venous infusion and the restricted mobility that would result, makes this method of rehydration impractical during space travel. Currently, dehydration prior to re-entry into the earth's atmosphere is treated by oral intake of sodium chloride and water (Lane & Smith, 1999). However, this treatment has limited efficacy and may be detrimental in astronauts who have dehydration-induced hypernatremia. While these current fluid strategies may be partially successful in replacing some of the fluid loss after dehydration has occurred, it may be more desirable to 'fluid-load' with combined Cr and Gly supplementation prior to take-off to minimise subsequent dehydration during flight. Furthermore, the Cr and Gly oral hyperhydration regimen described in Chapter 5 would provide astronauts a very simple way in which to increase fluid stores during the relative confinement of space travel improving the likelihood of astronaut compliance.

However, given that plasma volume can decrease during space travel by as much as 17%, the 2.4% expansion induced by Cr and Gly in the present study (Chapter 5) may not be large enough to offset this deficit. Further research is required to examine whether plasma volume expansion via Cr and Gly hyperhydration can minimise the adverse effects of plasma volume reduction during space travel and orthostatic intolerance on return to earth. Nevertheless, combined Cr and Gly supplementation does result in a significant increase in body mass of up to 1.6 kg (Figs. 3.2, 3.9, 4.2, 4.7, 5.2) which would counteract the majority of the losses reported after a space flight (Leach *et al.*, 1996; Smith *et al.*, 1997). Furthermore, when Cr and Gly are ingested according to LP3, there is a significant increase in TBW (Fig. 5.2) which would potentially offset the reduction in TBW that occurs during space flight (Grigoriev *et al.*, 1996; Leach *et al.*, 1975; Leach & Rambaut, 1977). Despite the relatively minor percentage increases in plasma volume associated with combined Cr and Gly ingestion (Chapter 5), astronauts encounter several other physiological problems during space travel that this novel hyperhydration strategy may potentially counteract (Lane & Feedback, 2002). For example, the effect of plasma volume loss in space may result in excessively high T_c for astronauts wearing protective garments during launch and landing. Dehydrated ($> 3\%$ body mass) unacclimatised individuals will exhibit excessive heat strain (T_c exceeding 39°C) during the pre-launch and launch of the space shuttle (Pandolf *et al.*, 1995). T_c has been measured in crewmembers during landing and found to be significantly elevated to approximately 38°C despite the use of a liquid cooling garment (Rimmer *et al.*, 1999). The finding that ingestion of Cr and Gly can significantly reduce heart rate (Fig. 5.3) and T_c (Fig. 5.7) during exercise induced heat stress insinuate that this simple hyperhydration strategy could improve physical and mental

performance during space travel and offer astronauts some protection from heat injury. Additionally, Cr supplementation prior to and during prolonged periods of immobilisation (hypokinesia) significantly attenuated skeletal muscle atrophy (Aoki *et al.*, 2004). Hypokinesia encountered during microgravity has been shown to induce muscle atrophy, slow-to-fast twitch muscle fibre shift and a decrease in force generation capacity (Aoki *et al.*, 2004;Caiozzo *et al.*, 1994). One of the proposed mechanisms for the protective effect of Cr on skeletal muscle includes higher mitotic activity in satellite cells (Dangott *et al.*, 2000). In addition, it is also possible that Cr is able to activate signalling pathways, protecting skeletal muscle against proteolysis (Vierck *et al.*, 2003). Alternatively, the increase in cellular hydration that occurs after Cr supplementation may also have a role in controlling protein turnover as this can act as a signal, stimulating protein synthesis and attenuating protein breakdown (Haussinger *et al.*, 1993). However, further research is required to examine the exact effects of Cr on skeletal muscular atrophy alongside a structured weight-training program during prolonged space travel.

Additionally, clinical complications arising in patients due to insufficient or incorrect hydration and nutritional replacement regimens during acute and chronic disease states or during postoperative surgical care are commonplace in many hospitals (Lennard-Jones, 2000). Some regularly recorded effects of chronic under-nourishment and dehydration are reduced muscle power, diminished force of coughing, immobility, apathy, loss of morale and depression, cool pale peripheries with prolonged capillary return time, decreased skin turgor, deep breathing, increased thirst, irritability, sunken eyes, dry mucus membranes and sunken fontanelle (Keys *et al.*, 1950). However, many of these conditions are reversible simply by delivering adequate fluid and nutrition to the affected person (Lennard-Jones, 2000). The effects of nutritional and hydrational depletion delay recovery and increase liability to complications (Lennard-Jones, 2000). The physiological consequences of fluid depletion are well known and described elsewhere in this thesis, but a further consideration in a clinical situation is to maintain patient comfort by preventing them feeling thirsty. This symptom does not necessarily correlate with general hydration and volume of fluid intake and can often best be relieved by sips of fluid (Lennard-Jones, 2000). However, in many situations (i.e. recovery from major surgery) patients are unable to manually ingest any liquids and therefore must have their fluids replaced by intravenous infusion or a naso-jejunal enteral feeding tube (Page *et al.*, 2002). The recent development of a procedure known as haemodynamic optimisation may minimise the extent of dehydration and resultant hypovolemia by using an ultrasonic probe inserted down the throat of the patient to accurately measure fluid levels in real time (Moss, 2006). This practice allows surgeons to replace the desired volume of fluid during the surgical procedures to prevent

dehydration occurring during the post-operative state (Moss, 2006). However, conscious patients who are subjected to periods of rigorous bed rest will remain greatly at risk from hydration and nutritional related concerns as they are often left to consume food and fluid *ad libitum* (Zorbas *et al.*, 2003). Indeed several of the physiological problems associated with prolonged periods of bed rest are similar to those encountered during space travel, a fact that ground based space medicine researchers use to their advantage when examining the efficacy of any clinical intervention strategies. It is well established that prolonged exposure to bed rest results in the significant increase in excretion of fluid and electrolytes in urine, which as a direct result causes a reduction in plasma volume (Greenleaf *et al.*, 1977a; Greenleaf *et al.*, 1977b; Zorbas *et al.*, 2003). A chronic reduction of plasma volume with associated hypovolemia, and a reduction in red cell mass, can impair the function of the cardiovascular system and decrease human performance (Balke *et al.*, 1954).

Given that supplementation with combined Cr and Gly supplementation results in a significant increase in TBW (Fig. 5.2) and percentage increase in plasma volume (Chapter 5) it is tempting to assume that patients exposed to prolonged periods of bed rest would benefit from ingestion of this unique hyperhydration strategy. The added calorific intake from ingesting glucose along with the Cr and Gly would also lessen the chances of patients developing malnutrition. Of course it is currently unknown whether a sterile Cr/Gly solution could be developed enabling delivery to unconscious patients by venous infusion. However, Page *et al.* (2002) have concluded that enteral feeding via a naso-jejunal tube is safe and well tolerated and at least as effective as intravenous hydration, and therefore would provide a means to deliver the Cr and Gly solution to patients in a coma or in recovery following a surgical procedure. Additionally, the restricted movement during bed rest can also cause a significant decrease in skeletal muscle mass and associated reductions in force production (Aoki *et al.*, 2004; Caiozzo *et al.*, 1994) which may be minimised via the anabolic effects of Cr supplementation as previously discussed (Aoki *et al.*, 2004). Indeed the effects of Cr supplementation on the exercise performance and general health of patients suffering from chronic obstructive pulmonary disease have been recently examined (Fuld *et al.*, 2005). Cr supplementation led to increases in fat-free mass, peripheral muscle strength and endurance, health status, but not exercise capacity compared to a Pl (glucose). These findings led the authors to conclude that 'Cr may constitute a new ergogenic treatment in chronic obstructive pulmonary disease' (Fuld *et al.*, 2005). However, controlled clinical trials during prolonged bed rest studies are required before the proposed physiological benefits of combined Cr and Gly supplementation can be confirmed.

9.7 General Conclusions

A number of conclusions can be drawn from the studies presented in the previous chapters. Although these conclusions are based on physiological evidence from well-controlled cycling studies in the laboratory situation, they may not allow extrapolation of these results to other populations, sports and environments.

- i. Supplementation with a combination of a Cr and Gly using loading protocols previously established in the literature (20g of Cr for 6 days and 1 g Gly·kg⁻¹ body mass diluted in 500 ml of water 2 hours prior to the start of the experimental trial) resulted in a significant increase in body mass with no change in TBW, ICW, ECW or RPE, heart rate and T_c during exercise in the heat compared to pre-supplementation. Given that previous Cr supplementation studies performed in this laboratory have consistently resulted in significant increases in TBW it can be hypothesised that the Gly administered prior to exercise had in some way negated the Cr induced increase in TBW.
- ii. Supplementation with a combination of a Cr and Gly using a novel loading protocol (ingestion of both Cr and Gly for 7 days) resulted in a significant increase in body mass TBW, ICW and ECW compared to pre-supplementation. Therefore, ingesting both Cr and Gly over several days may be the most effective method of fluid loading as there will be sufficient time for the retained fluid to be dispersed within body compartments.
- iii. Supplementation with a combination of a Cr and Gly (6 days of Cr and Gly ingestion, with the final supplement consumed 3 hours prior to measurement) resulted in a significant increase in body mass. Yet despite ingesting both Cr and Gly over several days to allow sufficient time for the retained fluid to be dispersed within body compartments there was no change in TBW, ICW, ECW or RPE, heart rate and T_c during exercise in the heat compared to pre-supplementation. It is probable that ingestion of a hypertonic solution such as the Cr and Gly mixture resulted in slowing of gastric emptying and an initial efflux of water from the plasma into the intestinal lumen. Therefore, the timing of ingestion is evidently critical, with the final supplement requiring to be consumed longer than 3 hours prior to the need for hyperhydration.

- iv. Consumption of both Cr and Gly over several days and ingestion of the final supplement 5 hours prior to exercise is the most effective method of fluid loading. This will allow sufficient time for the retained fluid to leave the stomach, pass across the intestinal lumen wall and be dispersed within body compartments.
- v. Supplementation with both Cr and combined Cr and Gly for 7 days using the loading protocol described in the previous chapter was effective in increasing TBW and reducing heart rate, T_c and perception of effort during prolonged exercise in the heat. The key finding of this study was that the increase in TBW after combined Cr and Gly supplementation was significantly greater than either Cr or Gly supplementation alone. Despite the increased hydration associated with combined Cr and Gly, there was no further attenuation in heart rate or T_{re} compared to Cr alone. Hyperhydrating prior to exercise through Cr, Gly or a combination of the two did not result in any significant improvement in 16.1 km time trial performance compared to euhydration. This may be because the time trial was too short to induce a degree of dehydration high enough to confer a significant improvement in exercise performance as a result of the altered hydration status. Furthermore, hyperhydration may not offer any significant advantage in terms of exercise performance compared to euhydration or indeed modest dehydration (i.e. loss of 2-3% body mass).
- vi. An ingestible telemetry pill system provides valid measurements of T_c during both rest and exercise-induced hyperthermia up to the limits of T_c measurement and therefore can be used in the field where T_{re} and esophageal temperatures cannot be taken. This will allow the effect of combined Cr and Gly supplementation on thermoregulatory responses during field studies to be precisely quantified. While the infrared tympanic membrane thermometer closely matched T_{re} measurements at rest and in the early stages of exercise, T_{ty} appeared to significantly underestimate T_c once T_c exceeded 37.5°C . Further experimental evidence is required to determine whether the disparity between T_{ty} and T_{re} is merely be due to imperfections in the tympanic membrane thermometer methodology or due to the existence of selective brain cooling in humans.

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UNIVERSITY OF GLASGOW

ETHICS COMMITTEE FOR NON CLINICAL
RESEARCH INVOLVING HUMAN SUBJECTS

RESEARCH SUBMISSION

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

Position held **Lecturer in IBLS**

Department/Group/Institute/Centre - **Centre for Exercise Science and
Medicine (CESAME), IBLS**

Name of Principal Researcher (if different from above)

Position held _____

Date of submission: **September, 2003**

Project Title: **The effects of combined creatine and glycerol
supplementation on hydration, thermoregulation and exercise
performance in the heat in endurance-trained subjects**

1. Describe the basic purposes of the research proposed.

Methyl guanidine acetic acid, or creatine (Cr), is synthesised naturally in the body (1). At rest there is approximately 120 g of Cr in the body, of which 95% is located in skeletal muscle (2). Creatine is synthesised from the amino acids arginine and glycine in a two-stage process (1,3,4). First, an amidine group is transferred from arginine to glycine forming guanidinoacetic acid in a reversible reaction catalysed by the enzyme glycine transaminase. The second non-reversible stage involves the transfer of a methyl group from S-adenosylmethionine, catalysed by guanidinoacetate methyltransferase, resulting in the methylation of guanidinoacetate and the formation of Cr. In humans, this process occurs in the pancreas and liver (5). While the mechanism responsible for the uptake of Cr by skeletal muscle is presently unclear, tissue Cr uptake is enhanced by insulin (6,7) and muscle contraction (8). There are, however, problems associated with the use of a crossover design in experiments involving Cr supplementation. The long washout period for Cr from muscle makes it difficult to interpret results obtained from placebo trials administered as the second treatment. This methodological problem has resulted in many studies using matched groups of subjects. With these concerns in mind, the balance of available evidence from Cr supplementation studies would suggest that Cr loading has no effect on peak power output during a single 30 sec maximal bout of cycling exercise (9), but can improve high intensity exercise performance when repeated exercise bouts are carried out (10,11,12,13,14). A finding common to most studies (including a study previously approved by the ethics committee of Glasgow University (November 1999) is a Cr-induced increase in intra-cellular water.

Another method of acute hyperhydration under current study involves the consumption of a small amount of glycerol [1-1.2 g/kg body mass (BM)] along with a large fluid bolus (25-35 ml/kg BM) in the hours prior to exercise. Glycerol, a three-carbon alcohol synthesised naturally in the body, provides the backbone to triglyceride molecules and is released during lipolysis. Normal plasma levels of glycerol are 0.05 mM at rest and may rise to 0.5 mM during prolonged exercise. Within the body it is evenly distributed throughout fluid compartments and exerts an osmotic pressure. When consumed orally, it is rapidly absorbed and distributed among body fluid compartments before being slowly metabolised via the liver and kidneys. When consumed in combination with a substantial fluid intake, the osmotic pressure will enhance the retention of this fluid and expansion of the various body fluid spaces. Typically, this allows a fluid expansion or retention of ~600 ml above a fluid bolus alone, by reducing urinary volume. A review of glycerol as a hyperhydrating agent is provided by Robergs and Griffin (15).

As dehydration is one of the primary causes of fatigue during exercise in the heat, the aim of the proposed study is to investigate the effects of a Cr-induced increase in intra-cellular water, along with a glycerol-induced increase in extra-cellular water, on metabolism, thermoregulation and exercise performance in the heat. In a recent study combining Cr and glycerol (approved by the ethics committee of Glasgow University), acute glycerol administration reversed the Cr-induced increase in intra-cellular water in an attempt to maintain osmotic balance between intra-cellular and extra-cellular water compartments. In this revised proposal, we seek to build on the previous findings and repeatedly administer glycerol during a period of Cr supplementation in order to overcome the initial reversal by glycerol of the Cr-induced increase in intra-cellular water and potentially increase extra-cellular water. The effects of this revised Cr and glycerol regimen on metabolism and exercise performance in the heat will also be investigated.

2. Outline the design and methodology of the project.

Methods/Design of investigation

We propose to study 24 endurance-trained male subjects (17-35 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.

Testing will take place in the Environmental Chamber in the West Medical Building. A series of assessments will be carried out. These will include (see protocols): body composition using standard anthropometric methods (bioelectrical impedance); extra-cellular water and total body water using multifrequency bioelectrical impedance; maximal O_2 uptake (VO_2 max) and lactate threshold (LT); and six cycle ergometer performance tests at an ambient temperature of $30^\circ C$, with relative humidity maintained at 70%. The first two performance tests will be familiarisation trials aimed to familiarise subjects with the exercise protocol and experimental procedures. The four subsequent performance tests will be carried out on days 1, 8, 15, and 22. Subjects will be assigned in a double blind fashion to either a Cr group or placebo Cr group: subjects will be matched into pairs based on BM and randomly assigned so that one member of each pair is in the Cr group and the other in the placebo group. Each Cr supplement will consist of 11.4 g of Cr H_2O (equivalent to 10 g Cr) and 75 g of glucose polymer made up in 500 mls of warm to hot water (x 2 times daily). The placebo supplement will consist of 170 g/d of glucose polymer (85 g x 2 times daily). During the first and third week of the experimental regimen, subjects in both groups will receive either 1 g/kg BM glycerol or an equivalent amount of placebo diluted in each of the 500 mls of Cr or placebo supplements. On each of the experimental test days (i.e. 1, 8, 15, 22), subjects will ingest the glycerol or placebo 4 hrs before the start of exercise. Subjects will also ingest approximately 500 ml of water each subsequent hour prior to exercise. The placebo group will follow the same procedure as the Cr group with regard to the preparation of the supplements. Subjects will also be required to consume at least 2 L of additional water each day.

During the supplementation period 24 hr urinary collections will be made. The volume of urine collected each 24 hr period will be measured and a representative sample stored for subsequent analysis of Cr and creatinine concentrations. Subjects will be instructed to carry out a weighed intake of food and an activity diary during the study period.

Protocols

Maximal Incremental Exercise Test: A direct measurement of VO_2 max and the LT will be determined on a computer controlled cycle ergometer on the first visit to the laboratory. This test will involve a step-wise increase in work rate (15-20 watts/min) until volitional exhaustion. The results from this test will allow the LT to be estimated using gas exchange criteria. Subjects will be given a warm-up before the test and a warm-down after the test. Subjects will be given a familiarisation trial and a warm-up before the test and a warm-down after the test.

Cycle Ergometer Performance Trial: On all four testing days (and familiarisation trials), subjects will perform 40 minutes of constant-load exercise at a moderate exercise intensity (approximately 63% of Maximum Work Rate) at an ambient temperature of $30^\circ C$, with relative humidity maintained at 70%. Following this 40 minute exercise bout, the subject will then undertake a 16.1 km (10 mile) time trail on a specialised cycle ergometer. Heart rate, oxygen uptake (and related cardiorespiratory measurements) and skin and rectal temperature will be measured throughout exercise as previously approved by the ethics committee.

Bioelectrical impedance: Extra-cellular water and total body water will be measured prior to and following each exercise test using multifrequency bioelectrical 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 (16).

All procedures with exception of the repeated glycerol administration have been previously approved by the University Ethics Committee.

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 for at least 15 min 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 100 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 healthy individuals without a history of any significant medical problem(s). All subjects will be endurance-trained and therefore accustomed to strenuous exercise to exhaustion. The good health of each subject will be established prior to the study by subjects undergoing a medical examination (as previously approved by the University Ethics Committee), which is supported by a written assurance from the subject. 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.

Close supervision of the subject is ensured at all times by the supervising investigator. The well-being of the subject is established at frequent intervals throughout all tests by asking the subject "Is everything alright?" Subjects are instructed, prior to the test, to respond to this question with a thumbs-up sign if everything is fine, and a thumbs-down sign if there is problem. If a problem is indicated, the investigator will ask further questions to establish whether there is a technical problem that could lead to potential hazard or whether the subject is feeling unwell. In either case, the test is immediately halted. All subjects are routinely instructed to cease exercising if they experience any discomfort or have any concern for their well-being.

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.)

Exercise has negligible risk in healthy adults, although maximal exercise has a small risk of inducing myocardial ischaemia.

The subjects will complete a medical questionnaire and provide their written consent with the option to withdraw from training or testing 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 and urine 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 (15). No side effects were reported in our previously approved study.

The only known 'side effect' of oral Cr supplementation that has been reported is an increase in body weight (12, 17).

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

It is envisaged that this research will benefit the identification of the physiological mechanisms of exercise tolerance (*i.e.* the ability of individuals to perform exercise) in the heat. The minimal 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 are their qualifications and experience?

Dr Yannis Pitsiladis PhD MMedSci BA, Chris Easton BSc, Mr John Wilson, Mrs Heather Collins (Technicians), and 3 BSc Honours Project Students. The principal investigators have wide ranging experience of exercise testing over periods of up to 10 years without incident. The principal researchers have conducted Cr 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 are identified from information held by another party (for example, a doctor or hospital) describe the arrangements whereby you gain access to this information.

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.

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

No.

11. Will payment be made to any research subject? If so, please state the level of payment to be made, and the source of the funds to be used to make the payment.

No.

12. Describe the procedures to be used in obtaining a valid consent from the subject. Please supply a copy of the information sheet provided to the individual subject.

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 Cr and glycerol supplementation and the placebo 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. Give details of the measures which will be adopted to maintain the confidentiality of the research subject.

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 information gained be anonymized? If not, please justify.

Yes

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

No.

17. Date on which the project will begin **(September, 2003)** and end **(September, 2005)**

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

The Environmental Chamber and Laboratory of Human Physiology (Lab 245), West Medical Building.

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University of Glasgow

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INFORMATION SHEET

Study title: The effects of combined creatine and glycerol supplementation on hydration, thermoregulation and exercise performance in the heat in endurance-trained subjects

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 previously used (by athletes) substances may increase your ability to work hard in the heat, by reducing dehydration that exercise in the heat induces. We will measure your ability to perform strenuous exercise lasting approximately 1 hour. 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), and **glucose** (Placebo). 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. Twenty four 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. If you decide to take part you are still free to withdraw at any time and without giving a reason.

What will happen to me if I take part? You will be asked to visit the laboratory on seven occasions over a five week period (see Table). The first test will last approximately 1 hour. All subsequent tests will last approximately 2 hours. You will be familiarised to the cycle test during the first three visits to the laboratory. After this practice period you will be randomly assigned to either a creatine group or a placebo group for the three week experimental phase of the study. After the first experimental cycle test you will consume either 20 g of creatine or 20 g of glucose a day (to be consumed with a glucose powder dissolved in one pint of warm to hot water before and after each daily training session) during the first and third week of the experimental phase. During the first and third week of the

experimental regimen, you will receive either glycerol (0.75 g per kg of body mass) or an equivalent amount of placebo diluted in each of the 500 mls of Cr or placebo supplements. You will also be asked to ingest an additional 2 litres of water per day. You will start the first supplementation period on the day after your pre-supplementation cycle test (visit 4) and will finish 6 days later, on the day before the post-supplementation cycle test. Following this one week period you will not take any supplements for 6 days, and after which you will complete a cycle test. For the final week you will take the opposite supplement to what you were taking initially. So, if you were initially taking glycerol, you will then change to the placebo supplement. You will not know which group you are in, until all the tests have been completed. The protocol for the second supplement is the same as the first. You will take the supplement for 6 days, before completing the final post-supplementation cycle test. 4 hrs prior to the last two performance trials you will consume your appropriate solution. If you received the glycerol solution prior to your second trial you will get the placebo solution prior to the last performance trial and vice versa.

On your first visit to the lab you will be asked to complete two confidential questionnaires; the first will allow us to obtain information related to your general health; and the second will allow us to quantify your past exercise/activity involvement.

During each exercise test and at regular intervals, we would like to take a small amount of blood from an intravenous line in the back of your hand. Intravenous lines may cause some bruising and subsequent soreness over the site of puncture and, rarely, a small wound which takes a few days to heal.

Your skin and core (internal) body temperature will be measured throughout exercise. For the measurement of core temperature, a rectal temperature probe will be inserted (in private and before the experiment) 10 cm beyond the anal sphincter. Skin temperature will also be measured by taping a probe to the chest, triceps, thigh and calf muscles on the right hand side of the body. This will allow core and skin temperature to be monitored throughout each experiment with only minor discomfort.

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 1 week and to keep a diary of your physical activity.

You will be required to collect all urine passed each 24 hour period (in containers to be provided) throughout the supplementation period (i.e. **all urine passed over the 7 days**). The volume will be measured and a representative sample analysed for creatine and creatinine concentration. We plan to use this information to assess the amount of creatine taken up by your body.

Finally, you will not be able to 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? Slight nausea, gastrointestinal distress (i.e. diarrhea) and headaches have previously been reported among some subjects. The only known 'side effect' of oral creatine supplementation that has been reported is an increase in body weight. This increase in body weight is mostly due to an increase in water retention.

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 inducing myocardial ischaemia ("heart attack"). The primary symptom of myocardial ischaemia is chest pain on exertion. If you experience any unusual sensations in your chest during the experiment, you should cease exercising immediately.

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 snorkeling. You will also wear a noseclip. Your heart rate may be monitored via adhesive surface electrodes for the additional monitoring of the heart's electrical activity (the "electrocardiogram"). You may experience difficulty swallowing while breathing through a mouthpiece and wearing a noseclip, due to some pressure in the ears. Some subjects experience increased salivation when breathing through a mouthpiece. Some subjects experience mild discomfort from prolonged sitting on the seat of the cycle ergometer.

Intravenous lines through which blood is collected, may cause some bruising and subsequent soreness over the site of puncture and, rarely, a small wound which takes a few days to heal.

You may experience some mild discomfort when inserting the rectal probe.

What are the possible benefits of taking part? We hope that you will find out more about how your body responds to supplementation with combined creatine-glycerol supplementation and subsequent strenuous exercise. This information may help us better understand the mechanisms associated with fatigue and hydration during strenuous exercise.

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.

Table: Schedule of visits and proposed tests.

| Visit | Test | Duration (hrs) |
|-------|---|-------------------|
| 1 | Maximal Progressive Exercise Test | 1 |
| 2 | Performance Cycle Test (Familiarisation 1) | 2 |
| 3 | Performance Cycle Test (Familiarisation 2) | 2 |
| 4 | Performance Cycle Test (Pre Supplementation 1) | 2 |
| 5 | Performance Cycle Test (Post Supplementation 1) | 2 |
| 6 | Performance Cycle Test (No Supplementation) | 2 |
| 7 | Performance Cycle Test (Post Supplementation 2) | 2 |

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

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Consent Form

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

