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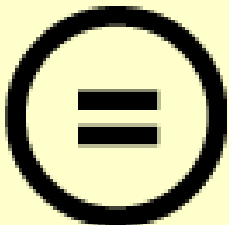
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**INFLUENCE OF HEAT STRESS AND CARBOHYDRATE
AVAILABILITY ON SUBSTRATE METABOLISM AND
EXERCISE TOLERANCE TIME IN HUMANS**

By

James Lee John Bilzon

A Doctoral Thesis

Submitted in partial fulfilment of the requirements for the award of
Doctor of Philosophy of Loughborough University

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This thesis is dedicated to my wife, Emma, and to my family.

ABSTRACT

Whilst the effects of environmental heat stress on the physiological responses of humans during exercise have been investigated for over half a century, the mechanisms responsible for fatigue during exercise in the heat are not well understood. There is increasing evidence that heat stress increases the reliance on carbohydrate (CHO), particularly muscle glycogen, as a fuel for prolonged exercise. The provision of CHO during exercise and during short-term recovery from exercise in the heat may theoretically offer some benefit. However, the literature available on the efficacy of CHO feedings during prolonged running in the heat is scarce. The aim of the experiments that are reported in this thesis were to investigate the effects of heat stress and CHO feeding regimens on substrate metabolism and exercise tolerance during prolonged running. An initial investigation revealed that the heat stress imposed by wearing a military protective clothing ensemble during prolonged running impaired exercise tolerance time and increased the reliance on CHO as a fuel. This response was associated with increases in circulating adrenaline and lactate concentrations, which may be indicative of an enhanced β -adrenergic receptor stimulation of muscle glycogenolysis. Thus, further studies into the efficacy of CHO supplementation regimens during exercise and recovery from exercise in the heat were performed. Rehydration with a carbohydrate-electrolyte solution (CES) during a 4-h recovery period markedly increased total CHO utilisation and exercise tolerance during subsequent exercise in the heat (35°C) compared to a sweetened placebo. Whilst there was no difference in post-recovery exercise tolerance time after ingesting 55-g or 220-g of CHO within a CES, ingesting 220-g lead to a five-fold increase in estimated glycogen synthesis during recovery, which increased CHO availability and utilisation during subsequent exercise. Ingesting a 12.5% glucose solution attenuated the increased reliance on endogenous CHO stores during exercise in the heat, but the associated increases in thermal and cardiovascular strain and gastric discomfort may have been responsible for the impairment of exercise capacity. These findings suggest that increases in endogenous CHO metabolism occur in response to exogenous heat stress during prolonged running. Whilst CHO ingestion during short-term recovery periods are associated with favourable changes in glycogen synthesis and tolerance to subsequent exercise in the heat, ingestion of a hypertonic glucose solution during exercise in the heat, may impair exercise capacity.

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PUBLICATIONS

The findings in this thesis have been reported, in part, in the following publications.

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Bilzon, J.L.J., Allsopp, A.J. and Tipton, M.J. (2001). Assessment of physical fitness for occupations encompassing load-carriage tasks. *J. Occ. Med.*, **51**: 357-361.

Bilzon, J.L.J., Murphy, J.L., Allsopp, A.J., Wootton, S.A. and Williams, C. (2002). Influence of glucose ingestion by humans during recovery from exercise on substrate utilisation during subsequent exercise in a warm environment. *Eur. J. Appl. Physiol.*, **87**: 318-326.

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Bilzon, J.L.J., Gill, C.A., Dalzell, J., Allsopp, A.J. and Williams, C. (2001). Influence of a military clothing ensemble on the metabolic responses to prolonged running in a warm environment. *J. Sports Sci.*, **19**: 626-627.

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CHAPTER ONE

INTRODUCTION

Ecology is the branch of biology concerned with the study of the relationship between an organism and the environment in which it lives. For humans, a sub-tropical, low altitude, air-breathing, homeothermic animal, most of planet Earth represents a hostile place. This is particularly true when humans are required to live and perform physical work in unaccustomed hot and humid climates. For the human body to remain in thermal balance, heat loss must equal the heat gain from metabolism and the environment. Whilst considerable physiological adaptation (heat acclimatisation) occurs following repeated (chronic) exposure to such climatic conditions, permitting the human body to thermoregulate more efficiently at higher levels of thermal stress, acute exposure to such conditions presents a considerable challenge to the human thermoregulatory system.

Upon heat exposure compensatory responses are initiated, such as increased peripheral blood flow, which help to maintain body temperature within a narrow prescriptive zone. When metabolic work (exercise) is performed in such conditions, the system is challenged further, and the circulatory system must respond accordingly to sustain the additional requirements for skeletal muscle and peripheral blood flow. If however, in stimulating these compensatory responses body temperature is driven beyond the prescriptive zone, the consequences may be profound, as in cardiovascular insufficiency consequent to heat exhaustion. Rowell (1986) clearly highlighted the profound challenge placed on the human cardiovascular system when exercise is performed in a hot environment, and the fatal consequences that may ensue if human behaviour is not adapted accordingly:

“Perhaps the greatest stress ever imposed on the human cardiovascular system (except for severe haemorrhage) is the combination of exercise and hyperthermia. Together these stresses can present life-threatening challenges, especially in highly motivated athletes who drive themselves to extremes in hot environments. A long history of heat fatalities gives stark testimony to the gravity of the problem and the failure of various organisations to recognise and deal with it effectively.”

Historically, the study of man's ability to perform work in extreme hot and humid environments (environmental physiology) is a well-established field of exercise physiology, and has received the attention of the scientific community for over half a century (Adolph 1947). Since this early work, it has become widely accepted that physiological fatigue occurs earlier during prolonged exercise in high environmental temperatures (Saltin *et al.* 1972; Febbraio *et al.* 1996a; Galloway and Maughan 1997). Indeed, the effects of environmental temperature have been extensively studied with respect to changes in body fluid spaces, temperature regulation and cardiovascular responses (Rowell *et al.* 1968; Bell *et al.* 1983; Nielsen *et al.* 1990). As a consequence, it is commonly accepted that the impairment of physiological function observed during exercise in the heat is directly related to thermoregulatory incapacity and the hyperthermia which ensues (Fink *et al.* 1975; Brück and Olszewski 1987; Nielsen *et al.* 1990), processes which are exacerbated by dehydration (Sawka 1992). Despite this understanding of the thermoregulatory and cardiovascular responses to exercise in the heat, the observations of Rowell (1986) still hold true today, and heat related illnesses remain a significant problem during military training (Joint Services Publication 539) and athletic competition (ACSM 1984). This may stem from the fact that the physiological mechanism(s) responsible for fatigue during prolonged exercise in the heat are not fully appreciated, making them inherently difficult to guard against.

As such, scientists have recently begun to explore the compensatory metabolic responses to exercise in the heat. Support for such an approach stems from the observation that energy depletion, consequent to the effects of increasing temperature on skeletal muscle function, has been implicated in the pathophysiology of heat stroke (Hubbard *et al.* 1987). Indeed, a number of initial studies demonstrated that environmental temperature affects intramuscular substrate utilisation during submaximal (Fink *et al.* 1975) and isometric exercise (Edwards *et al.* 1972). More specifically, Fink *et al.* (1975) observed an increase in intramuscular glycogen utilisation during intermittent cycling exercise in the heat. Such responses are worthy of further investigation as it is well established that physiological fatigue during prolonged exercise in a thermoneutral environment is associated with muscle glycogen depletion and/or hypoglycaemia (Coyle *et al.* 1986; Sahlin *et al.* 1990; Spencer *et al.* 1991; Constantin-Teodosiu *et al.* 1992). Furthermore, in thermoneutral environments, ingestion of exogenous carbohydrate during exercise can prolong

exercise tolerance time (Coyle *et al.* 1983, 1986; Coggan and Coyle 1987). Indeed, the importance of carbohydrate (CHO) availability and the critical role of muscle glycogen as a substrate for contracting skeletal muscle, is well established (Bergstrom and Hultman 1966; Bergstrom *et al.* 1967; Hultman 1967).

Since these initial studies (Edwards *et al.* 1972; Fink *et al.* 1975), others have examined the effects of environmental heat stress on substrate utilisation during prolonged exercise in greater detail. Consistent with the findings of Fink *et al.* (1975), others (Febbraio *et al.* 1994a, 1994b; Hargreaves *et al.* 1996a) have observed an augmented intramuscular CHO utilisation during cycling exercise in the heat (40°C) compared to a cooler environment (20°C). In addition, when the rise in core temperature is attenuated by acclimation (King *et al.* 1985; Young *et al.* 1985; Febbraio *et al.* 1994a), by preventing dehydration (Hargreaves *et al.* 1996b; Gonzalez-Alonso *et al.* 1999), by reducing ambient temperature (Febbraio *et al.* 1996b; Parkin *et al.* 1999), or by providing external cooling (Kozlowski *et al.* 1985), muscle glycogenolysis and CHO utilisation are reduced.

Given the observed increases in CHO and muscle glycogen utilisation during cycling exercise in the heat it is somewhat surprising that the affects of heat stress on substrate utilisation during prolonged running have not been assessed or quantified. Evidence suggests that prolonged running performance is more likely to be limited by muscle glycogen availability (Tsintzas *et al.* 1995, 1996a) than cycling, where physiological fatigue is often associated with hypoglycaemia (Coyle *et al.* 1983, 1986). Furthermore, whilst several studies appear to have investigated the influence of CHO ingestion on exercise performance in the heat (Millard-Stafford *et al.* 1992; Febbraio *et al.* 1996a; Carter *et al.* 2002, 2003), the findings were equivocal. Clearly, the influence of heat stress and CHO availability on substrate metabolism and exercise tolerance time during prolonged running warrants systematic investigation. Hence, the investigations that are reported in this thesis aim to test the following central hypothesis:

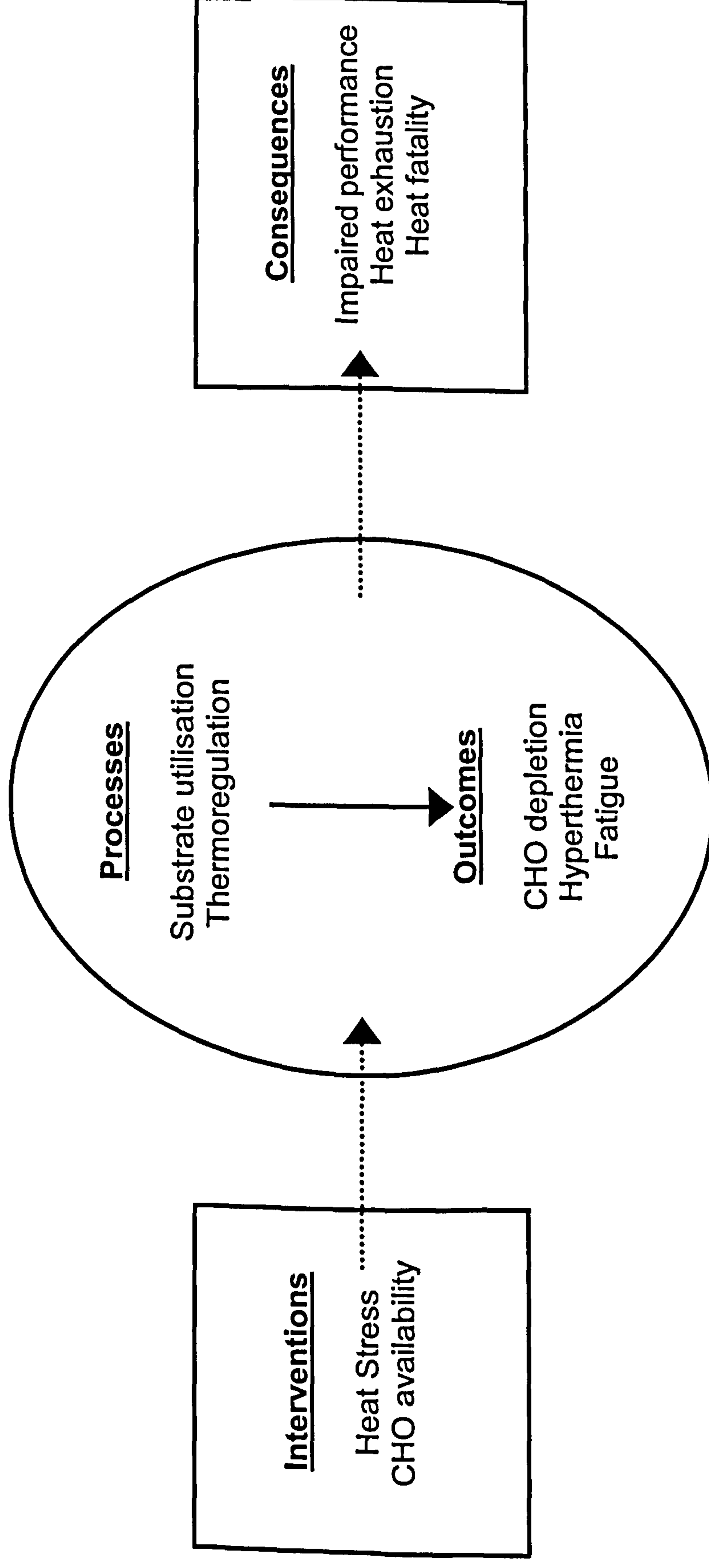
Carbohydrate intake can be manipulated sufficiently to stimulate glycogen synthesis during recovery and metabolism during prolonged running, increasing its availability to meet the increased requirements for exercise in the heat. As a consequence exercise tolerance time in the heat will be improved.

The central hypothesis is presented pictorially in Figure 1.1. This experimental model was used to manipulate heat stress and CHO availability, in order to assess their influence on the thermal and metabolic responses to prolonged running and exercise tolerance time. This research is not only pertinent to endurance sports performers, for whom heat stress poses a significant problem (ACSM 1987), but also to military personnel who are often required to perform repeated bouts of prolonged exercise whilst wearing protective clothing, even in hot and humid environments (Montain *et al.* 1994).

Following a review of the relevant literature (Chapter 2), there is an outline of the experimental procedures that were common between investigations (Chapter 3). The experimental Chapters of this thesis have been written as standalone papers, two of which have been published in the *Eur. J. Appl. Physiol.*, and a further one partly published in *J. Occ. Med.* The first experimental chapter that is reported in this thesis investigated the influence of a standard military protective clothing ensemble on the thermal and metabolic responses to exercise in the heat (Chapter 4). The following two chapters (Chapters 5 and 6) consider the influence of various CHO supplementation regimens during short-term recovery on aspects of substrate utilisation and exercise tolerance time during subsequent treadmill running in the heat. The final investigation assessed the influence of heat stress and glucose ingestion during exercise on substrate metabolism and glucose kinetics during prolonged submaximal treadmill running (Chapter 7). The use of stable isotope tracer techniques in chapters 6 and 7 permitted the contribution of CHO from various body pools to be quantified. The thesis is concluded in Chapter 8, with some reflection on the wider implications of the findings reported in each experimental chapter.

In view of the possible implications of this work for the performance of military personnel in the heat, this work has been sponsored by the Defence Catering Group (DCG) and the Corporate Research Programme (CRP) within the Ministry of Defence and conducted at the Institute of Naval Medicine. The military scenario in mind was the rapid deployment of specialised forces to a hot location, with limited logistical support and inadequate time to acclimatise.

Figure 1.1 Pictorial representation of the central hypothesis as an experimental model.



According to the model above, the studies described in this thesis will manipulate heat stress and carbohydrate (CHO) availability to study their effects on the processes of substrate utilisation and thermoregulation, in an attempt to alter the time-course for CHO depletion, the onset of hyperthermia and subsequent physiological fatigue. The results of these studies may have implications for delaying the onset of physiological fatigue and preventing heat-related illnesses and fatalities (consequences) during exercise in the heat.

CHAPTER TWO

REVIEW OF LITERATURE

2.1 Introduction

Whilst physiological fatigue is an inevitable accompaniment of prolonged strenuous exercise, there are a number of mechanisms which may cause fatigue. When prolonged exercise is performed in cool/temperate environments, the gradual depletion of endogenous glycogen reserves (Ahlborg *et al.* 1967a; 1967b) and dehydration (Armstrong *et al.* 1985) are the two primary causes of fatigue. Prolonged exercise is known to gradually deplete endogenous carbohydrate (CHO) reserves and contribute to the onset of fatigue (Bergstrom and Hultman 1966; Ahlborg *et al.* 1967a). Furthermore, exercise induced dehydration adversely affects cardiovascular function and temperature regulation (Montain and Coyle 1992; Gonzalez-Alonso *et al.* 1997). When similar bouts of exercise are performed in hot environments, the onset of fatigue may be related to thermoregulatory incapacity and the hyperthermia which ensues (Nielsen *et al.* 1993; Nielsen 1994). However, these thermal responses to exercise and heat stress are accompanied by compensatory increases in sweat loss (Sawka 1992) and endogenous glycogen utilisation (Febbraio *et al.* 1994a; 1994b). Fluid and CHO replacement regimens may therefore be as effective in enhancing exercise performance in the heat, as they are in temperate environments.

This thesis investigates the effects of heat stress and various CHO supplementation regimens on the metabolic responses and tolerance to prolonged running. As mentioned in Chapter 1, such scientific enquiry is necessary because of the limited knowledge of the effects of heat stress on substrate utilisation during prolonged running, and the potential benefits of CHO supplementation during short-term recovery periods and subsequent exercise in the heat. To begin with this review will briefly examine the circulatory and thermoregulatory responses to exercise and environmental heat stress. Dehydration and whole-body energy metabolism will then be considered, with particular emphasis on CHO (glucose and glycogen) utilisation during prolonged exercise, and the potential effects of heat stress. Finally, the effects of CHO and fluid replacement strategies will be considered.

2.2 Thermoregulation during exercise

2.2.1 *Metabolic Heat Production and Thermal balance*

The maintenance of internal temperature (homeothermic) is a unique feature of birds and mammals, a characteristic which has been acquired over the last 70 million years of evolution. As such, the body core temperature of the human is largely independent of the external environment. The price paid for such independence is a high metabolic rate which enables humans to maintain body temperature within a fairly narrow range. However, with a resting core temperature of approximately 37°C the human being lives its life only a few degrees removed from our upper limit of thermal viability compared with the lower end of the temperature scale. It is therefore not surprising that more elaborate thermoregulatory mechanisms exist to prevent overheating than to prevent overcooling (Sutton and Thompson 1998).

As exercise intensity increases, total body metabolic rate is increased to provide energy for skeletal muscle contraction. Metabolic rate can therefore increase more than 10 fold, from rest ($\dot{V}O_2 = 0.25$ to 0.35 l.min⁻¹) to sustained exercise intensities ($\dot{V}O_2 = 3$ to 4 l.min⁻¹). Since the mechanical efficiency of the body is essentially below 25%, approximately 75% of the total energy yield is converted to heat. The primary response to this increase in metabolic heat production is an increase in deep body temperature. Indeed, when steady-state exercise is performed in an environment with a temperature of 5 to 30°C (thermoneutral zone), there is a linear relationship between metabolic heat production and body temperature, which is largely independent of environmental temperature (Nielsen 1938; Robinson *et al.* 1941; Lind 1963). This relationship between exercise intensity and body temperature was found to be stronger when individual differences are accounted for by expressing exercise intensity in relative ($\% \dot{V}O_2$ max) terms (Astrand 1960; Saltin and Hermansen 1966).

Despite these large increases in metabolic heat production, the higher deep body temperature generally reaches a plateau within 60-min of steady-state exercise in a temperate environment (Robinson *et al.* 1941; Lind 1963). This response serves to establish a gradient for heat flux from core to surface and to stimulate peripheral

vasodilation and sweating. As a consequence, cardiac output must increase to maintain skeletal muscle blood flow and meet the additional requirements for peripheral blood flow and heat dissipation. Whilst this may be achieved by a reduction in renal, splanchnic and hepatic blood flow during heavy exercise (Rowell *et al.* 1965; Rowell 1986), thermal equilibrium can generally be achieved in a temperate environment. These responses are neurally controlled by the hypothalamus as the central point of integration.

If the heat content of the body is to remain in equilibrium, heat production and heat gain from the environment must therefore equal heat loss. There are essentially four mechanisms responsible for heat dissipation, three of which are bidirectional avenues for thermal exchange (conduction, convection and radiation), and one of which is unidirectional (evaporation). The relationship between body core temperature and the various avenues of heat dissipation were summarised in a heat storage equation (Winslow *et al.* 1936). Whilst these mechanisms allow humans to thermoregulate throughout a wide range of exercise intensities in temperate environments, there are a number of factors which may drive human thermoregulatory function beyond the state of equilibrium, resulting in significant hyperthermia. Such variables include dehydration, environmental factors (temperature, humidity, air velocity) and importantly for this thesis, clothing. When exercise is sustained under such conditions, core temperature continues to rise, hyperthermia ensues and exercise performance/capacity is impaired. In extreme circumstances, exercise induced hyperthermia may have profound clinical consequences, as in cardiovascular insufficiency consequent to heat exhaustion.

2.2.2 *Effects of dehydration*

Even at low ambient temperatures evaporation of sweat secreted onto the skin surface is one of the primary mechanisms for heat dissipation during exercise (Maughan 1985). The environmental temperature will determine the relative contributions of evaporative and dry heat exchange to total heat loss, and the hotter the environment the greater the dependence on eccrine sweat gland secretion and evaporative heat loss (Nielsen 1938). Evaporation of 1 litre of water from the skin surface will dissipate approximately 2.4 MJ (580 kcal) of heat. There are a number of variables which will

determine sweat rate during exercise, including the exercise intensity, the environmental conditions, the clothing worn, the degree of hyperthermia and the body mass and degree of acclimatisation of the individual (Molnar *et al.* 1946; Adolph 1947; Strydom *et al.* 1966; Shapiro *et al.* 1982). Whilst sweat rates may therefore vary considerably, the highest sweat rate reported in the literature, which was measured from Alberto Salazar during the 1984 Olympic marathon, is $3.7 \text{ l}\cdot\text{h}^{-1}$ (Armstrong *et al.* 1986). If fluid is lost at such a rate without adequate replacement, dehydration will rapidly ensue. However, even with high rates of fluid replacement, maximal gastric emptying rates are approximately 1 to $1.5 \text{ l}\cdot\text{h}^{-1}$ in an average male (Murray 1987; Mitchell and Voss 1991). As these rates may be significantly impaired during high intensity ($>75\% \dot{V}O_2 \text{ max}$) exercise (Costill and Saltin 1974), hypohydration (Neufer *et al.* 1989) and heat strain (Owen *et al.* 1986; Neufer *et al.* 1989), dehydration is difficult to prevent in many exercise scenarios.

The physiological effects of exercise-induced dehydration have been studied by comparing the physiological responses of subjects to exercise under various climatic conditions, with and without fluid ingestion, and more systematically, using various methods to induce a graded pre-exercise fluid deficit (hypohydration). There is little doubt that prolonged aerobic exercise and possibly maximal aerobic power are adversely affected by hypohydration via impairments in thermoregulatory and cardiovascular function (Sawka and Pandolf 1990). The effects of hypohydration on physical work capacity and maximal aerobic power are summarised in Table 2.1.

In comparison to euhydration, hypohydration results in an increased core temperature during exercise in temperate (Cadarette *et al.* 1984; Grande *et al.* 1959; Neufer *et al.* 1989; Sawka *et al.* 1980) as well as in hot (Claremont *et al.* 1976; Percy *et al.* 1956; Pitts *et al.* 1956; Sawka *et al.* 1988) environments. There is a graded increase in core temperature with increasing fluid deficit, and decrements in exercise capacity may be observed with a fluid deficit as little as 1% body mass (Ekblom *et al.* 1970). Whilst these responses could result from either a disproportionate increase in metabolic heat production or a disproportionate decrease in heat loss, a large body of evidence suggests that hypohydration probably does not influence metabolic rate during submaximal exercise (Saltin 1964; Greenleaf and Castle 1971; Sawka *et al.* 1979,

1983, 1985b). Therefore, it appears that a reduction in heat dissipation is responsible for the hypohydration-mediated increase in heat storage during exercise. This statement is supported by evidence demonstrating reductions in sweat rates during exercise at a given metabolic rate (Moroff and Bass 1965; Senay 1968; Strydom and Holdsworth 1968). Whilst a number of studies have also demonstrated no change in sweat rate (Strydom *et al.* 1966; Claremont *et al.* 1976), they tended to report increases in core temperature. Therefore, it appears that sweat rates are reduced for a given core temperature, and the potential for heat dissipation via sweat evaporation is reduced when hypohydrated.

Study	Year	Method of dehydration	Δ BM (%)	Test environment	Exercise mode	Max aerobic power	Work capacity
Armstrong <i>et al.</i>	1985	Diuretics,	-1	Neutral	TM	ND	↓6%
Caldwell <i>et al.</i>	1984	Exercise,	-2	Neutral	CY	ND	↓7W
		diuretics,	-3	Neutral	CY	↓8%	↓21W
		sauna	-4	Neutral	CY	↓4%	↓23W
Saltin	1964	Exercise,	-4	Neutral	CY	ND	↓?
		diuretics,					
		sauna					
Pinchan <i>et al.</i>	1988	Fluid	-1	Hot	CY	-	↓6%
		restriction	-2	Hot	CY	-	↓8%
			-3	Hot	CY	-	↓20%
Craig and Cummings	1966	Heat	-2	Hot	TM	↓10%	↓22%
			-4	Hot	TM	↓27%	↓48%
Buskirk <i>et al.</i>	1958	Exercise, heat	-5	Neutral	TM	↓0.2 l.min ⁻¹	-
Webster <i>et al.</i>	1988	Exercise, heat,	-5	Neutral	TM	↓7%	↓12%
		sauna					
Herbert and Ribisl	1971	?	-5	Neutral	CY	-	↓17%
Houston <i>et al.</i>	1981	Fluid	-8	Neutral	TM	ND	-
		restriction					

Table 2.1 Summary of the effects of hypohydration on maximal aerobic power and physical work capacity (from Sawka 1992).

The difficulty in identifying a mechanism responsible for these reductions in sweat rate and impairments in thermoregulatory function has been that, generally speaking, fluid deficits result not only in hypovolaemia, but also in hypertonicity

(hyperosmotic-hypovolaemia). The observed increases in plasma tonicity (and therefore osmotic pressure) occur because eccrine sweat is ordinarily hypotonic relative to plasma (Kirby and Convertino 1986). As a result, plasma becomes hypertonic when dehydration is induced by sweat output (Senay 1968; Kubica *et al.* 1983). Both the independent and combined effects of plasma hyperosmolality (Harrison *et al.* 1978; Candas *et al.* 1986; Liebert *et al.* 1988) and hypovolaemia (Fortney *et al.* 1981b, 1988; Morimoto 1990) have been suggested as mediating the reduced sweating response during exercise-heat stress.

The studies that have considered the combined and independent effects of these two variables have demonstrated that both core temperature and sweat rate responses are more strongly associated with tonicity and/or electrolyte concentrations (Senay 1968; Nielsen *et al.* 1971; Greenleaf *et al.* 1974, 1977; Sawka *et al.* 1985b) than with blood volume. Furthermore, most studies demonstrate that ingestion of sodium-containing solutions will elevate core temperature and reduce sweating responses in the heat, despite the maintenance of euhydration (Nielsen *et al.* 1971; Harrison *et al.* 1978). It is worthy of note that these impairments of thermoregulatory function are more likely to be observed during exercise in hot than in temperate environments (Nielsen 1974). Nevertheless, these increases in plasma tonicity appear to exert a powerful influence on sweat production and body temperature during exercise and heat stress, probably because they are indicative of changes in the extracellular fluid bathing the hypothalamic neurons (Senay 1979; Kozlowski *et al.* 1980). At least in rats, there appear to be pre-optic anterior hypothalamic neurons that are both thermo-sensitive and osmo-sensitive (Nakashima *et al.* 1984; Silva and Boulant 1984). These data suggest a central interaction between thermoregulation and body water regulation.

Whilst these studies demonstrate that hypertonicity exerts a powerful effect on thermoregulation, a number of studies have elicited an iso-osmotic hypovolaemia and demonstrated increases in core temperature and a reduction in the examined thermoregulatory effector response (Fortney *et al.* 1981a, 1981b; Nadel 1981). Whilst an evidence-based explanation for these responses remains elusive, Fortney *et al.* (1981b) hypothesised that hypovolaemia may reduce cardiac pre-load and alter the activity of baroreceptors, which have afferent input to the hypothalamus. Therefore, a reduced atrial filling pressure could modify neural information to the hypothalamic

thermoregulatory centre that control sweat rate. Indeed, hypohydration decreases blood volume, reduces central venous pressure (Kirsch *et al.* 1981; Morimoto 1990) and cardiac filling, which reduces stroke volume and requires a compensatory increase of heart rate to maintain cardiac output.

When exercise is performed in the heat, the maintenance of cardiac output may not be possible as hypovolaemia ensues. The combination of exercise and heat strain results in competition between the central and peripheral circulation for a limited blood volume (Nadel *et al.* 1980; Rowell 1986). As a result of the decreased blood volume and blood displacement to cutaneous vascular beds, central venous pressure, venous return and thus cardiac output will decrease below euhydrated levels (Nadel 1981; Sawka *et al.* 1979). Several investigations have demonstrated that these conditions reduce skin blood flow for a given core temperature and therefore decrease the potential for dry heat exchange (Nadel *et al.* 1980; Fortney *et al.* 1981a, 1984). Similar hypovolaemia-induced reductions in skin blood flow have been demonstrated in animal studies (Proppe 1990; Ryan and Proppe 1990). The restoration of blood volume can partially reverse the hypohydration mediated reduction in skin blood flow during exercise in the heat (Stephenson *et al.* 1983; Nose *et al.* 1990).

Whilst this review has partially discussed the combined effects of hypohydration and environmental heat stress during exercise, the independent effects of environmental conditions on human circulatory and thermoregulatory function and endurance exercise capacity will now be considered.

2.2.3 *Effects of the environment*

A number of studies have now demonstrated that fatigue occurs earlier during prolonged steady-state endurance exercise in the heat compared to a temperate environment (Saltin *et al.* 1972; Galloway and Maughan 1997). Whilst there are many theories as to precisely how environmental heat stress impairs endurance exercise capacity, evidence suggests that environmental heat stress impairs both circulatory and thermoregulatory function during exercise. In order to remain in a state of thermal equilibrium, the human must evoke physiological heat loss mechanisms, including increased skin blood flow and volume as well as increased sweat rate, to dissipate

heat whilst faced with the dual challenge of endogenous (metabolic) heat production and the external (environmental) heat load.

This increased circulatory demand presents a significant challenge, as the system must meet these demands for heat dissipation whilst maintaining circulation to active skeletal muscle to support metabolic processes. This increase in circulatory demand may lead to a reduction in central venous pressure, but in most cases this will be compensated for by either an increase in exercise heart rate (Williams *et al.* 1962; Klausen *et al.* 1967; Nadel *et al.* 1979) or vasoconstriction and redistribution of blood flow from the splanchnic, renal and hepatic regions or from non-exercising muscles (Radigan and Robinson 1949; Rowell *et al.* 1965, 1968; Rowell 1986). During moderate intensity exercise in the heat it is unlikely that active muscle blood flow will be compromised (Savard *et al.* 1988), although greater heat stress coupled with more intense exercise could lead to reduced muscle blood flow (Harrison 1985; Gonzalez-Alonso *et al.* 1999). Whilst it has been hypothesised that such reductions in active muscle blood flow could contribute to the impairment of performance during exercise, studies have demonstrated, throughout a wide range of exercise intensities, that heat stress does not impair active leg blood flow during exercise in a water perfused suit (Savard *et al.* 1988) or during uphill walking (Nielsen *et al.* 1990).

As environmental temperature increases, the temperature gradient between the skin and the environment is reduced, and consequently dry heat dissipation is compromised. When ambient temperature exceeds skin temperature, heat is gained from the environment by physical transfer, leaving evaporative loss as the only mechanism available for heat loss. Whilst the potential problems of progressive dehydration, resulting from high sweat rates without adequate fluid replacement have already been discussed, sweat evaporation is a very effective mechanism for heat dissipation. However, if the ambient water vapour pressure, and therefore relative humidity, is also high, sweat evaporation will also be compromised. When prolonged exercise is performed in conditions of high ambient temperature and humidity, all routes of heat dissipation will become compromised, and metabolic heat production may exceed heat dissipation. If work rates are not reduced, then core temperature will continue to rise, a condition termed 'uncompensable heat stress'. Under such conditions, the ensuing hyperthermia is thought to limit endurance exercise capacity

and result in fatigue at a core temperature of $\sim 40^{\circ}\text{C}$ (Nielsen *et al.* 1990, 1993; Nielsen 1994). Whilst the physiological mechanisms by which hyperthermia may cause fatigue during prolonged exercise in the heat are not clear, it has been hypothesised that hyperthermia affects the central nervous system, reducing the mental drive for motor performance (Nielsen *et al.* 1990).

In certain physically demanding occupational activities, including military activities, there is the added complication that personnel are required to wear protective clothing which may serve to exacerbate the effects of high ambient temperature and humidity on thermoregulation during exercise. These effects will be briefly discussed here.

2.2.4 *Influence of protective clothing*

Donning clothing is, under normal circumstances, considered a behavioural response to prevent over-cooling when exposed to cold environments. However, many occupational groups are required to wear clothing ensembles to protect them from occupational hazards within their work environment, often whilst maintaining relatively high work rates for sustained periods. This is particularly true in the military environment, where personnel are required to perform repeated bouts of prolonged (1-2 hours), moderate intensity ($60\text{-}80\% \dot{V}O_2 \text{ max}$) exercise whilst wearing protective clothing and carrying auxiliary equipment, even in hot and humid environments. Whilst the available literature on the effects of different protective clothing ensembles is extensive, given the potential application of the findings of this thesis for the military, the generic effects of exercising in the heat whilst wearing protective clothing will be briefly discussed.

Whilst military personnel are occasionally required to work whilst wearing impermeable Nuclear Biological and Chemical (NBC) defence suits, work rates are usually low, and the duration relatively short. However, military personnel are also required to perform prolonged daily activities whilst wearing permeable cotton Combat Fighting Order (CFO). The exact thermal properties of such clothing are dependent on the number of layers worn, the permeability of the individual garments and the body surface area covered. Givoni and Goldman (1972, 1973) were probably

the first to investigate the rectal temperature and heart rate responses of military personnel to a wide range of metabolic work rates, environmental conditions and clothing ensembles. These data were used to produce models of maximal work times for the US military. Whilst not entirely preventing sweat evaporation, such permeable clothing ensembles reduce both evaporative heat loss and dry heat exchange with the environment, essentially producing a micro-climate within the clothing ensemble. Military personnel are therefore frequently exposed to uncompensable heat stress (Robinson *et al.* 1945; Belding and Kamon 1973).

2.2.5 Summary

When prolonged steady-state exercise is performed in a temperate environment, metabolic heat production gradually increases body core temperature to a higher plateau within 60-min, which is largely determined by the metabolic work rate, and is essentially independent of the ambient conditions. This occurs in order to establish a thermal gradient between core and skin temperature and to stimulate peripheral vasodilation and sweating, the primary mechanism for heat dissipation during exercise. Sweating can result in substantial losses of hypo-osmotic fluid (hypohydration) and result in a relative hyperosmotic-hypovolaemia, which may further impair cardiovascular function, thermoregulation and exercise capacity. When exercise is performed in a hot environment, the mechanisms responsible for dry heat exchange with the environment are gradually impaired, to the extent that when ambient temperature exceeds skin temperature, heat will be gained from the surrounding environment. As relative humidity increases, sweat evaporation will also be gradually reduced and core temperature will continue to rise. Both dry heat exchange and sweat evaporation may be further impaired by the micro-climate which develops within protective clothing, exposing personnel within such high-risk occupations to uncompensable heat stress, and in some circumstances, exertional heat illness. Having discussed thermoregulation and particularly metabolic heat production during exercise, the way in which substrates are utilised as fuel to support such metabolic work during prolonged exercise will be discussed, with particular emphasis on endogenous carbohydrate utilisation.

2.3 Carbohydrate metabolism during exercise

2.3.1 Historical overview

Since the early part of the 20th Century human scientists have become increasingly intrigued by the way in which nutrients are utilised during exercise and how nutritional supplements may influence exercise performance. Early studies were instrumental in developing indirect respiratory calorimetry techniques for the study of substrate metabolism, and demonstrated the importance of both fat and CHO as fuels for endurance exercise. It later became apparent that CHO was essential for the maintenance of high exercise intensities and to optimise exercise performance. This work was expanded in the 1960's with the re-introduction of the percutaneous muscle biopsy technique to examine skeletal muscle glycogen metabolism during exercise in humans (Bergstrom and Hultman 1966; Ahlborg *et al.* 1967a, 1967b;; Bergstrom *et al.* 1967; Hermansen *et al.* 1967; Hultman 1967). These studies demonstrated the critical role of muscle glycogen as a determinant of endurance exercise performance and the importance of increasing dietary CHO intake prior to endurance exercise. Indeed, a high (~70%) CHO diet elevates muscle glycogen content and enhances endurance exercise performance (Bergstrom *et al.* 1967; Pernow and Saltin 1971).

2.3.2 Plasma glucose metabolism during exercise

Even in lean people the fat stores of the body are large relative to the limited CHO stores, which amount to some 2000-2500 mmol glucosyl units, depending on body mass. Of this total, in the post-absorptive state, liver glycogen contributes 400-550 mmol (Hultman 1977), circulating plasma glucose approximately 90 mmol (DeFronzo *et al.* 1979), with the remainder stored as skeletal muscle glycogen. Thus, it is apparent that glucose transported through the plasma represents about a quarter of the CHO available to provide fuel for skeletal muscle during exercise. Despite this, under certain conditions, plasma glucose can supply >70% of the CHO oxidised during exercise (Wahren *et al.* 1971; Ahlborg *et al.* 1974; Broberg and Sahlin 1989), even at relatively high (75% $\dot{V}O_2$ max) exercise intensities (Coggan and Coyle 1987). The rate of plasma glucose utilisation during exercise is dependent upon many variables

including, the mode, intensity and duration of exercise, the availability of other substrates (pre-exercise diet), and the training status of the individual. However, before the regulation of plasma glucose metabolism and the role of the liver can be fully discussed, the relative merits of methods of measurement of plasma glucose kinetics/metabolism will be briefly discussed.

2.3.2.1 Methods of measuring glucose metabolism during exercise

Glucose metabolism during exercise has traditionally been studied by catheterising both the arterial blood supply and the venous drainage of a given tissue bed, thereby allowing measurement of the arteriovenous glucose difference and the rate of blood flow. While the product of these two parameters (i.e. net arteriovenous glucose balance) does not indicate the ultimate fate (or source) of the glucose taken up (or released), it does indicate the maximum possible contribution of glucose to (or from) the metabolic processes in the tissue bed under study. Whilst a useful technique for addressing certain experimental hypotheses, the experiments reported in this thesis were concerned with whole-body, as opposed to specific tissue, glucose utilisation during prolonged dynamic exercise (i.e. treadmill running). This method will therefore not be discussed further, but for a more thorough review see Coggan (1991).

Both stable and radioactive isotope tracer techniques are being used more frequently to measure whole-body glucose (and other substrate) kinetics and glucose metabolism during dynamic exercise. The use of stable isotope tracers has become particularly attractive for human metabolic studies, as stable isotopes do not carry with them the potential health risks associated with radioactive isotopes. Creation of an isotopic tracer involves the substitution of one or more naturally occurring atoms in specific position(s) in the tracee molecule with an isotope of that atom with a less common abundance. As such, the tracer is chemically identical to the compound of interest (the tracee), but distinct in some characteristic that enables its precise detection. In studies of human glucose metabolism the stable isotopes of hydrogen (^2H) and carbon (^{13}C) are commonly used as substitutes for the more commonly occurring isotopes (^1H and ^{12}C), thus acting as glucose tracers. These stable isotopes of ^2H and ^{13}C have a low natural abundance relative to ^1H and ^{12}C , and thus allow precise measurements of

glucose kinetics and metabolism from relatively small quantities of tracer. The natural abundance of the stable hydrogen and carbon isotopes are given in Table 2.2.

Element	Stable Isotope	Percent Natural Abundance
Hydrogen (H)	1	99.985
	2	0.015
Carbon (C)	12	98.89
	13	1.11

Table 2.2 List of stable isotopes of hydrogen (H) and carbon (C).

Calculation of glucose kinetics usually refers to the determination of the rate of appearance (Ra) of glucose and, at least in the steady state, the rate of disappearance (Rd) of glucose. The rate of whole-body glucose appearance (Ra), for example can therefore be detected from the dilution of constantly infused glucose molecules labelled with ^{13}C or ^2H . However, the ^2H isotope is often used to label glucose for kinetic analysis, as the isotope does not recycle in the process of gluconeogenesis. The rate of whole-body glucose disappearance (Rd) is then inferred from changes (or lack of) in plasma glucose concentration. When the concentrations of unlabelled and labelled glucose are not changing, the system is considered to be in steady-state, and Rd must equal Ra . However, when the concentrations of labelled and unlabelled glucose are changing, as is usually the case during exercise, the equations used to calculate Ra and Rd may be modified to reflect the non-steady-state conditions (Steele 1959; Wolfe 1992).

The use of stable-isotopes to study glucose metabolism has the advantage of being much less invasive than arteriovenous balance measurements. Furthermore, when using a glucose tracer labelled with ^{13}C , it is possible to follow the flux of carbon isotopes from glucose to CO_2 , making it possible to quantify the rate of glucose oxidation (Rox). This calculation, however, requires steady-state labelling of glucose and expired CO_2 , conditions which are difficult to achieve during exercise unless the exercise bout is relatively long (i.e. >90 mins) (Young *et al.* 1967; Coggan *et al.*

1990). However, the time-course for achieving this state of isotopic equilibrium can be substantially reduced by using a priming dose prior to the start of a constant infusion, a technique which was first described by Searle *et al.* (1954). Numerous studies have demonstrated that the use of an isotope prime does not affect the final equilibrium value, but significantly reduces the time required for equilibrium to be achieved (Kreisberg *et al.* 1970; Bortz *et al.* 1972; Wolfe *et al.* 1982). For a more detailed insight into the theoretical assumptions behind isotope tracer methodologies and calculations, the reader is referred to Wolfe (1992).

Although arterio-venous balance and isotope tracer methods have not been directly compared during exercise in humans, examination of the literature suggests that Ra may underestimate endogenous glucose production during exercise. Because Rd is derived from the measurement of Ra , the rate of whole-body glucose disposal may also be underestimated. A similar underestimation is often observed during euglycaemic glucose clamps, where Ra is often less than the rate of exogenous glucose infusion (Argoud *et al.* 1987; Cobelli *et al.* 1987; Finegood *et al.* 1987, 1988). However, a more recent study has used a dual isotope tracer technique (U-¹³C and 6,6-²H₂ glucose) to simultaneously quantify the Ra , Rd and Rox of plasma glucose during exercise at 50% $\dot{V}O_2$ max (Jeukendrup *et al.* 1999a). There were no statistically significant differences between these values from 60-120 min of exercise. The potential for underestimation of glucose production and utilisation from isotope tracer methodologies does not therefore invalidate its use, especially for within subject comparisons, where absolute rates are less important than the relative change.

2.3.2.2 Effect of exercise intensity and duration

At rest, skeletal muscle demonstrates a small net glucose uptake (Katz *et al.* 1986), which appears to be directed to non-oxidative pathways, as the respiratory quotient of muscle is close to 0.7, commensurate with predominantly fat oxidation (Klassen *et al.* 1970). Whilst there appears to be a net glucose release from skeletal muscle at the onset of exercise (Wahren 1970), within a few minutes this reverts to a net glucose uptake (Jorfeldt and Wahren 1970), the rate of which is positively related to the exercise intensity (Katz *et al.* 1986). During cycle ergometry, glucose uptake by the

leg increases exponentially from $<0.3 \text{ mmol}\cdot\text{min}^{-1}$ at rest to almost $8 \text{ mmol}\cdot\text{min}^{-1}$ during maximal exercise (Wahren *et al.* 1971; Katz *et al.* 1986). Whilst there is some debate over whether this exponential increase results from an increase in demand from each active muscle fibre (Nesher *et al.* 1985), or an increase in the number of active muscle fibres (Vollestad and Blom 1985), it is most likely a combination of the two. Because glucose uptake by muscle is concentration dependent, the increase in arterial glucose concentration, observed during intense exercise (Kjaer *et al.* 1986), probably contributes to this exponential rise in active muscle glucose uptake. As glucose uptake by the brain (Ahlborg and Wahren 1972), splanchnic bed (Wasserman *et al.* 1987) and resting muscle (Ahlborg *et al.* 1975) remain relatively constant during exercise, Rd glucose (Calles *et al.* 1983; Kjaer *et al.* 1986; Wolfe *et al.* 1986) shows a similar relationship to a-v difference measurements.

Plasma glucose uptake also increases with the duration of prolonged steady-state exercise (Jorfeldt and Wahren 1970; Ahlborg *et al.* 1974;). During submaximal cycle ergometer exercise, leg glucose uptake may double between 10 and 40-min (Wahren *et al.* 1971), but does not peak for several hours of exercise (Ahlborg *et al.* 1974). This gradual increase in plasma glucose utilisation partly compensates for, and may be related to, the progressive depletion of muscle glycogen stores during exercise (see section 2.3.3). Late in exercise, however, the rate of glucose utilisation may gradually decrease towards pre-exercise levels, due to a decline in hepatic glucose output and ensuing hypoglycaemia (Ahlborg *et al.* 1974; Coggan and Coyle 1987).

As plasma glucose utilisation increases with time and total CHO oxidation remains constant or decreases, plasma glucose utilisation represents a growing percentage of both total energy expenditure and total CHO oxidation during prolonged exercise. After 10-min of exercise at $25\text{-}30\% \dot{V}O_2 \text{ max}$, plasma glucose uptake by the legs accounts for approximately 20% of total energy expenditure and 50% of total CHO oxidation (Wahren *et al.* 1971). After 90-min of exercise, these percentages have risen to 45% and nearly 100%, respectively (Ahlborg *et al.* 1974). A similar pattern is observed during more intense exercise (Ahlborg and Felig 1982; Coyle *et al.* 1986). These calculations of the contribution of plasma glucose to total energy provision (from a-v difference and Rd) assume that most of the glucose taken up by the muscle

during exercise is immediately oxidised. This assumption appears to be reasonable, at least during mild to moderate intensity exercise (Young *et al.* 1967; Coggan *et al.* 1990; Jeukendrup *et al.* 1999a). During more intense exercise this assumption may not be true, as glucose appears to accumulate, presumably due to inhibition of hexokinase as a result of rapid glycogenolysis and accumulation of glucose-6-phosphate (Katz *et al.* 1986). Indeed, more recent evidence has demonstrated that during intense cycling exercise at $78\% \dot{V}O_2 \text{ max}$ muscle glucose uptake is higher following ingestion of a combined α - and β -adrenoceptor antagonist, suggesting that raised circulating adrenaline concentrations may reduce plasma glucose uptake and utilisation during intense exercise (Howlett *et al.* 2003). Plasma glucose uptake is similarly decreased when adrenaline is infused into adrenalectomised humans during exercise (Howlett *et al.* 1999).

2.3.2.3 Effects of exercise mode

In addition, to the effects of exercise intensity and duration on glucose uptake and oxidation, glucose utilisation also appears to be influenced by the mode of exercise, and care must therefore be taken in drawing generic conclusions from different studies. Specifically, arm exercise has been reported to result in a greater reliance on plasma glucose as an energy source than does leg exercise at the same absolute $\dot{V}O_2$ (Ahlborg *et al.* 1986). This difference does not appear to be related to the availability of other CHO sources (i.e. muscle glycogen), because muscle glycogen concentration is similar in the arm and leg muscle of untrained subjects (Gollnick *et al.* 1972). However, the arms tend to be used less than the legs during everyday activities, as a result the respiratory capacity of skeletal muscle tends to be lower in the arms. This lower skeletal muscle respiratory capacity may result in a greater utilisation of plasma glucose. Alternatively, the greater reliance on glucose during arm exercise may simply be a function of a relatively small active muscle mass, because glucose uptake by a given muscle group has been reported to be inversely related to the total amount of active muscle (Richter *et al.* 1988).

Similarly this may explain why, when all else is equal, subjects performing prolonged cycle ergometer exercise appear to be more susceptible to a decline in plasma glucose

concentration (i.e. hypoglycaemia) than subjects performing prolonged running or walking (Young *et al.* 1967; Ahlborg *et al.* 1974; Coyle *et al.* 1986; Dohm *et al.* 1986). Whilst this has never been proven experimentally, Achten *et al.* (2003) have recently demonstrated that rates of fat oxidation are approximately 28% higher when running compared to walking at exercise intensities ranging from 55-80 % $\dot{V}O_2$ max. It is still undetermined whether these responses are associated with higher rates of plasma glucose oxidation during cycling compared to running at the same relative exercise intensity.

2.3.2.4 Effect of other substrates

Whilst the regulation of skeletal muscle glycogen metabolism is discussed later (see section 2.3.3), it is important that the potential effects on plasma glucose metabolism are discussed here. Glucose-6-phosphate may accumulate within active skeletal muscle as a result of rapid glycogenolysis, the rate of which is largely dependent on muscle glycogen concentration (Gollnick *et al.* 1981; Richter and Galbo 1986). As such, the rate of plasma glucose oxidation in humans is inversely related to muscle glucose-6-phosphate concentrations and positively related to the percentage of muscle fibres that are glycogen depleted (Gollnick *et al.* 1981). This may explain why plasma glucose oxidation rates increase during exercise as skeletal muscle glycogen stores become progressively depleted. However, similar increases in plasma glucose oxidation are observed throughout exercise when muscle glycogen concentrations are substantially greater than (Richter and Galbo 1986) and less than (Gollnick *et al.* 1981) normal concentrations. Further research is therefore required to quantify the precise role of skeletal muscle glycogen in regulating plasma glucose oxidation.

In addition to the effects of muscle glycogen concentration, elevated plasma FFA concentrations have also been found to increase fat oxidation and suppress plasma glucose utilisation in resting humans (Thiebaud *et al.* 1982; Ferrannini *et al.* 1983). Randle *et al.* (1963) proposed that this occurs in response to inhibition of phosphofructokinase (PFK) by an increase in muscle citrate levels, resulting in accumulation of glucose-6-phosphate and therefore free intracellular glucose. However, this remains a controversial topic, as there is not a linear relationship

between FFA concentration and plasma glucose utilisation. Whilst one study reported that muscle glycogen and total carbohydrate oxidation rates were reduced when FFA levels were elevated by a high fat meal and heparin administration at 68% $\dot{V}O_2$ max (Costill *et al.* 1977), Ravussin *et al.* (1986) only observed such an effect during the first 30 min of a 150 min exercise bout at 44% $\dot{V}O_2$ max.

More recently, Hargreaves *et al.* (1988) decreased leg glucose uptake by approximately 40% during a bout of one legged knee extensor exercise, after elevating plasma FFA concentration from 0.5 to 1 mmol.l⁻¹ by infusion of a lipid emulsion and heparin. They hypothesised that elevated FFA exerts its inhibitory effect on plasma glucose utilisation due to a direct effect on membrane plasma glucose transport. Again, in human studies, whilst such responses are replicated during the first 30-60 min of exercise (Costill *et al.* 1977; Ravussin *et al.* 1986), such responses are not observed later in exercise when FFA concentrations are normally higher (Ravussin *et al.* 1986). The hypothesis of Hargreaves *et al.* (1988) therefore requires further experimentation.

2.3.2.5 Glucose production during exercise

With the liver as, apparently, the sole source of glucose into the circulation during exercise in the fasted state, the increase in skeletal muscle glucose utilisation during exercise poses a serious challenge to glucose homeostasis. Despite this challenge, plasma glucose concentration remains relatively constant during exercise that is moderate in intensity and duration, at least in a temperate environment. This implies that the previously described factors influencing glucose utilisation during exercise have similar effects on hepatic glucose production. In the early phases of prolonged exercise, glucose production in the liver stems predominantly from liver glycogenolysis (Wahren *et al.* 1971; Ahlborg *et al.* 1974), depleting liver glycogen stores to approximately 50% of concentrations found in subjects consuming a mixed diet (Hultman 1977) within 1 hour. Indeed, isotopic tracer estimates of gluconeogenesis indicate a relatively small contribution to Ra during exercise at 40% $\dot{V}O_2$ max (Stanley *et al.* 1988) and 50% $\dot{V}O_2$ max (Jeukendrup *et al.* 1999a). Probably due to the overall increase in demand for hepatic glucose output,

gluconeogenesis contributes relatively less to the total demand at higher exercise intensities (Wahren *et al.* 1971), despite large increases in circulating concentrations of the major gluconeogenic precursor, lactate. Whilst uptake of lactate, pyruvate and glycerol can account for almost 20% of total glucose release after 40-min of exercise at 30% $\dot{V}O_2$ max (Ahlborg *et al.* 1974), it contributes less than 10% after 40-min of exercise at 60% $\dot{V}O_2$ max (Ahlborg and Felig 1982).

After approximately 40-min of exercise there is a constant rise in uptake of gluconeogenic precursors across the splanchnic bed, primarily due to an increase in fractional extraction (Ahlborg and Felig 1982). Whilst the fractional extraction of glycerol remains relatively constant, as a result of the steady rise in arterial glycerol concentrations, the contribution of glycerol to gluconeogenesis also increases. Despite an observed increase in gluconeogenesis, maximal rates of glucose production from this source are between 0.7 and 1.7 mmol.min⁻¹ (Hultman 1977; Ahlborg and Felig 1982), and glucose production by the liver will therefore decrease markedly as liver glycogen stores become progressively reduced, and as a consequence plasma glucose concentrations may decline (Coyle *et al.* 1986; Coggan and Coyle 1987).

2.3.2.6 Hormonal regulation of hepatic glucose production

Hepatic glucose production during exercise is stimulated by glucagon and the catecholamines, and suppressed by insulin (Issekutz and Vranic 1980; Wasserman *et al.* 1989). There is also evidence to suggest that some auto-regulatory mechanism exists, such that glucose production by the liver is dependent on the prevailing plasma glucose concentration (Jenkins *et al.* 1985, 1986). Whilst these potential regulatory mechanisms are difficult to study in humans, animal studies have emphasised the importance of changes in the insulin/glucagon ratio for increasing hepatic glucose production during exercise. In dogs, when insulin concentrations are allowed to decrease normally and glucagon release is blocked, R_a is markedly decreased (Wasserman *et al.* 1989). A similar phenomenon is observed when glucagon is allowed to rise normally and insulin concentration is held constant (Wasserman *et al.* 1989), with both glucagon and insulin therefore appearing to play an important role in hepatic glucose production. In contrast, R_a increases normally in adrenalectomised

dogs for at least the first 90-min of exercise, suggesting that the catecholamines (particularly adrenaline) are not important for the initial increase in hepatic glucose production during exercise (Moates *et al.* 1988).

Care must be taken in interpreting these results because as in human studies changes in glucagon and insulin do not appear to be as critical in stimulating hepatic glucose production during moderate intensity (50 to 60% $\dot{V}O_2$ max) exercise (Bjorkman *et al.* 1981; Chrisholm *et al.* 1982). Hyperinsulinaemia appears to only partially suppress hepatic glucose production, and when glucagon secretion is blocked hepatic glucose production increases as normal (Bjorkman *et al.* 1981; Hoelzer *et al.* 1986). However, when catecholamine action is blocked and insulin and glucagon held constant, plasma glucose levels fall continuously (Hoelzer *et al.* 1986). The results of these studies suggest that in exercising humans, the catecholamines are of primary importance for initiating increased glucose production, and that changes in glucagon and insulin only play an important role when catecholamine action is deficient (Tuttle *et al.* 1988). The problem with interpreting the results of these studies is that the hormonal manipulations occurred in the presence of a decrease in plasma glucose concentration, which in itself has been shown to stimulate hepatic glucose production both at rest (Wolfe *et al.* 1986) and during exercise (Jenkins *et al.* 1985, 1986). This does not however, eliminate the possibility that catecholamines play an important central role in regulating plasma glucose production, particularly as the human liver is richly innervated with sympathetic nerves (Moghimzadeh *et al.* 1983). Many studies have focussed on gluco-regulation during relatively short-term exercise (40 to 60 min), and the catecholamines may therefore play an important role during longer and/or more intense exercise. Indeed, the effects of heat stress on catecholamine secretion during exercise suggest that these hormones play an important role in stimulating hepatic glucose production, even in the presence of exogenous glucose ingestion (Angus *et al.* 2001). These hormonal-induced effects of hyperthermia on hepatic glucose production and/or muscle glycogen metabolism will be fully discussed in section 2.4.

2.3.3 *Skeletal muscle glycogen metabolism during exercise*

Fatigue during prolonged intense exercise in a temperate environment appears to be associated with either skeletal muscle glycogen depletion (Hermansen *et al.* 1967) or hypoglycaemia (Coyle *et al.* 1986). As previously discussed, muscle glycogen and plasma glucose metabolism are tightly regulated during exercise. Muscle glycogen availability appears to be critical to the maintenance of a desired work rate, particularly during treadmill running (Tsintzas *et al.* 1996b). Whilst whole-body muscle glycogen utilisation can be estimated from stable isotope tracer measurements of plasma glucose and total CHO oxidation (Jeukendrup *et al.* 1999a), direct measurements of change in muscle glycogen concentration are determined from muscle biopsy tissue. However, caution must be exercised in comparing various studies, particularly as evidence now suggests that the reporting of mixed muscle fibre glycogen concentrations may mask substantial differences observed in the type I and type II fibres during exercise (Tsintzas *et al.* 1995). Thus analysis of mixed muscle fibre glycogen concentrations may lead to misinterpretation of metabolic events within active skeletal muscle.

2.3.3.1 *Type and intensity of exercise*

There appear to be a number of physiological and metabolic differences in the responses of humans to prolonged cycling and running (Hermansen *et al.* 1970; Derman *et al.* 1996). During prolonged cycling exercise, most studies demonstrate reductions in both blood glucose concentration and total CHO oxidation, both of which are maintained at higher levels when CHO is ingested during exercise (Coyle *et al.* 1983, 1986; Bjorkman *et al.* 1984). Ingestion of large quantities of CHO (120 to 300 g) during cycling at 70% $\dot{V}O_2$ max results in only modest increases in blood glucose concentration in the absence of substantial increases in circulating insulin concentrations. Interestingly, similar rates of muscle glycogen utilisation were observed in these studies (Coyle *et al.* 1986; Hargreaves and Briggs 1988; Mitchell *et al.* 1989). In the study by Coyle *et al.* (1986), subjects cycled to exhaustion at 71% $\dot{V}O_2$ max with and without CHO ingestion, and despite subjects reaching fatigue after 3 hours in the control trial, muscle glycogen utilisation (~ 440 mmol.kg⁻¹ dry

mass) was similar in both conditions. Subjects did not reach fatigue until 1 hour later in the CHO feeding trial, and despite the fact that a considerable amount of glycogen remained in the muscle ($\sim 160 \text{ mmol.kg}^{-1}$ dry mass), no further muscle glycogen was utilised. These data could lead to the conclusion that exogenous glucose feedings do not spare muscle glycogen during prolonged cycling exercise.

Indeed, such a conclusion is supported with evidence from a further study, which demonstrated no difference in muscle glycogen utilisation between control and experimental conditions where hyperglycaemia was maintained at 11 mmol.l^{-1} by infusion of glucose at a rate of 1.6 g.min^{-1} (Coyle *et al.* 1991). It therefore appears that, whilst exogenous glucose feedings may reduce hepatic glucose production (i.e. spare liver glycogen) during prolonged cycling at moderate and high intensities (Bosch *et al.* 1994; McConnell *et al.* 1994; Jeukendrup *et al.* 1999a), there are no effects on muscle glycogen utilisation, at least during the first hour of exercise. This may be in stark contrast to findings from running studies, which have mainly been conducted in the last decade.

During prolonged running without CHO ingestion, blood glucose concentrations do not appear to fall to the same extent as during cycling (Williams *et al.* 1990; Tsintzas *et al.* 1995). In fact, in most running studies, blood glucose concentrations do not even decline (Millard-Stafford *et al.* 1992; Wilber and Moffat 1992; Tsintzas *et al.* 1993) and CHO oxidation rates are maintained throughout exercise (Madsen *et al.* 1990; Williams *et al.* 1990; Millard-Stafford *et al.* 1992;). When CHO is ingested during prolonged running, CHO oxidation rates are either similar (Riley *et al.* 1988; Williams *et al.* 1990; Tsintzas *et al.* 1995) or higher (Sasaki *et al.* 1987; Wilber and Moffat 1992) than ingestion of a placebo solution. Furthermore, many studies have shown such CHO feedings to enhance endurance running capacity and performance (Macaraeg 1983; Sasaki *et al.* 1987; Millard-Stafford *et al.* 1992; Wilber and Moffat 1992; Tsintzas *et al.* 1993, 1995) in the absence of declining blood glucose and CHO oxidation rates. This may suggest that it is not the availability of plasma glucose which limits endurance running performance or capacity.

During prolonged running at $70\% \dot{V}O_2$ max for 60-min, ingestion of 50g of CHO in the form of a 5.5% solution resulted in a 28% sparing of glycogen in the vastus lateralis muscle (Tsintzas *et al.* 1995). This response was accompanied by an increase in blood glucose and serum insulin concentrations during the first 20-min of exercise. Importantly, the CHO feedings resulted in a 42% decrease in muscle glycogen utilisation from type I muscle fibres, with the concentrations in type II fibres unaffected. It seems, therefore, that skeletal muscle is capable of increasing blood glucose uptake, not only during the latter stages, but also during the initial stages of running, thereby reducing (sparing) muscle glycogen utilisation. This sparing appears to occur predominantly in type I muscle fibres, most of which appear to be recruited during exercise at $70\% \dot{V}O_2$ max (Vollestad *et al.* 1984). In a separate study the same experimental model was associated with a 14% improvement in run time to exhaustion following ingestion of the ~50g of CHO (Tsintzas *et al.* 1996a). The theory that muscle glycogen sparing by CHO ingestion can delay the onset of fatigue during prolonged running was assessed during a further study. After ~104 min of running, fatigue occurred in the water ingestion trial, whereas this did not occur until ~132 min in the CHO trial. Whilst muscle glycogen concentrations were similarly depleted at the point of fatigue, rates of utilisation were 24% lower in the CHO trial at the same time of fatigue as the water trial (Tsintzas *et al.* 1996b). As previously observed, the sparing was essentially limited to the type I muscle fibres. Muscle glycogen availability, particularly in type I muscle fibres, therefore appears to be critical to the maintenance of prolonged running at $70\% \dot{V}O_2$ max.

2.3.3.2 Pre-exercise nutritional status

In most studies that have assessed the influence of exogenous CHO supplementation during exercise on muscle CHO metabolism, subjects were fasted for 12 to 16 hours prior to the experimental trials (Coyle *et al.* 1986; Tsintzas *et al.* 1995; Hargreaves and Briggs 1998). The effects of CHO ingestion on muscle glycogen utilisation were assessed 3 hours after a high CHO meal (2.5 g CHO per kg body mass). Whilst the pre-exercise meal increased muscle glycogen concentration by 11%, muscle glycogen utilisation during the subsequent 60-min run at $70\% \dot{V}O_2$ max was not different between the CHO and placebo trials (Chryssanthopoulos *et al.* 1995). However, it

may be that the ingestion of a pre-exercise meal *per se*, which elevates plasma insulin concentration, spares existing skeletal muscle glycogen stores. Indeed, the rates of muscle glycogen utilisation during this study (Chryssanthopoulos *et al.* 1995) were approximately half of those reported following an overnight fast (Tsintzas *et al.* 1995). If one therefore assumes that muscle glycogen availability is the limiting factor during prolonged running, these results may suggest that CHO feedings during exercise performed 3 hours after a high CHO meal would have no effect on endurance exercise capacity. However, endurance exercise capacity does appear to be enhanced by CHO ingestion compared to a placebo, when exercise is performed 3 hours after a high CHO meal (Wright *et al.* 1991; Chryssanthopoulos *et al.* 1994). Unfortunately, post exercise muscle biopsy samples were not collected during these studies and a mechanistic explanation for this improvement in exercise capacity remains elusive.

2.3.3.3 Pre-exercise muscle glycogen concentrations

One of the differences between cycling studies (Coyle *et al.* 1986; Flynn *et al.* 1987; Mitchell *et al.* 1989) and treadmill running studies (Tsintzas *et al.* 1995, 1996b), that have examined the effects of CHO ingestion on muscle metabolism, is that subjects had significantly higher pre-exercise glycogen concentrations in the cycling studies. In rat (Hespel and Richter 1990) and human (Hargreaves *et al.* 1995) studies, there is a direct positive relationship between resting muscle glycogen concentration and the rate of utilisation during subsequent exercise. These increases in pre-exercise muscle glycogen concentration do not appear to influence muscle glucose uptake (Bosch *et al.* 1993; Hargreaves *et al.* 1995), which is in direct contrast to the results of previous investigations (Richter and Galbo 1986; Hespel and Richter 1990). Further studies are required to systematically address whether different pre-exercise muscle glycogen concentrations (300 to 800 mmol.kg⁻¹ dry mass) influence plasma glucose and exogenous glucose oxidation during cycling and running.

2.3.4 Summary

From the application of a-v difference, stable isotope and muscle biopsy techniques combined with indirect respiratory calorimetry, the importance of hepatic and circulating endogenous glucose and muscle glycogen for muscle energy metabolism

during endurance exercise has been well established. In addition to the numerous variables which may influence the rate at which these substrates are utilised, the hormonal regulation has also been discussed. The available evidence suggests that prolonged constant pace running capacity is limited more by muscle glycogen availability, particularly in type I muscle fibres, whereas cycling tends to be limited by hepatic glucose availability and consequent hypoglycaemia. This may be related to the relative active muscle mass involved in these two activities. Furthermore, it appears that subjects are more able to mobilise and oxidise free fatty acids during constant pace running than in cycling.

Having discussed thermoregulation and skeletal muscle energy metabolism during prolonged constant-pace exercise, the evidence suggesting that exercise in the heat alters skeletal muscle energy metabolism will now be discussed.

2.4 Effects of heat stress on energy metabolism during exercise

As previously discussed, exercise in the heat increases both cardiovascular and thermoregulatory strain, because of the additional requirement to increase peripheral blood flow for evaporative heat dissipation. Because exercise capacity during exercise in the heat is thought to be related to cardiovascular and thermoregulatory insufficiency, researchers have traditionally focussed their attention on these physiological systems. In contrast, research into the effects of environmental heat stress on human muscle metabolism and associated mechanism(s) has only received significant attention over the last 15-years.

2.4.1 Effect on carbohydrate metabolism

Probably the earliest study to demonstrate an affect of environmental temperature on intramuscular substrate utilisation, showed that muscle glycogen utilisation was higher and triglyceride utilisation lower during 60-min of intermittent exercise in a 41°C compared to a 9°C environment (Fink *et al.* 1975). Consistent with these findings, more recent studies have demonstrated an augmented intramuscular CHO utilisation during 40-min of continuous cycle exercise at 70% $\dot{V}O_2$ max in the heat

(40°C) compared to a cooler (20°C) environment (Febbraio *et al.* 1994a; Hargreaves *et al.* 1996a). When the degree of hyperthermia is reduced by heat acclimation (Febbraio *et al.* 1994a; King *et al.* 1985; Young *et al.* 1985), by preventing dehydration (Hargreaves *et al.* 1996b; Gonzalez-Alonso *et al.* 1999), by reducing the ambient temperature (Febbraio *et al.* 1996b; Parkin *et al.* 1999) or by providing external cooling (Kozlowski *et al.* 1985), muscle glycogenolytic rate and/or total CHO oxidation is/are reduced. It should be noted, however, that several studies have observed no augmentation of muscle glycogen utilisation during exercise and heat stress (Nielsen *et al.* 1990; Yaspelkis *et al.* 1993; Young *et al.* 1995; Maxwell *et al.* 1999). There are a number of methodological differences between these studies which may go some way to explaining the observed differences.

As previously demonstrated, exercise in itself results in a relative hyperthermia (Febbraio *et al.* 1996a), and the difference in body core temperature between experimental conditions must therefore be of sufficient magnitude to induce altered metabolic responses to exercise. Whilst Yaspelkis *et al.* (1993) found no difference in the rate of muscle glycogenolysis, the difference in ambient temperature was only 10°C and resulting in a maximum difference in core temperature of 0.4°C between experimental conditions. Furthermore, muscle glycogenolytic rate is influenced by pre-exercise muscle glycogen concentration (Hespel and Richter 1992; Chesley *et al.* 1995; Hargreaves *et al.* 1995). It is therefore not surprising that in studies where pre-exercise glycogen concentrations were higher before exercise in the cool environment, relative to the warmer, no differences were observed in muscle glycogen utilisation (Nielsen *et al.* 1990; Young *et al.* 1995). Whilst muscle glycogen utilisation was not different in the hot and cool environments in the study by Young *et al.* (1995), intramuscular lactate accumulation was elevated during exercise in the hot environment, suggesting that anaerobic glycolysis was accelerated in these conditions. Furthermore no differences in intramuscular substrate metabolism are observed during supramaximal exercise in the heat compared to a cooler environment (Maxwell *et al.* 1999), probably because metabolic heat production is at such a high rate that environmental heat stress has little impact on the degree of hyperthermia. It therefore appears that during submaximal exercise, a marked increase (>0.5°C) in core temperature is required to augment intramuscular CHO metabolism.

The increase in skeletal muscle glycogen utilisation during exercise in the heat appears to involve both oxidative and non-oxidative pathways. Muscle lactate accumulation is greater (Febbraio *et al.* 1994a, 1994b; Gonzalez-Alonso *et al.* 1999; Parkin *et al.* 1999) during exercise in the heat. Whilst these studies suggest that anaerobic glycolysis is augmented, few studies have assessed lactate efflux from contracting skeletal muscle and uptake by other organs and tissues during exercise and heat stress. Nielsen *et al.* (1990) reported no differences in a-v lactate production or lactate release from contracting skeletal muscle in the heat compared to a cooler environment, but these data are difficult to interpret because 60-min of exercise in the heat (which would have increased core temperature) was followed by 30-min exercise in a cooler environment. However, research from the same laboratory have recently demonstrated a higher post-exercise muscle lactate content in the presence of an augmented lactate release, which was induced by dehydration-induced hyperthermia (Gonzalez-Alonso *et al.* 1999).

The consistent observation of a higher respiratory exchange ratio (RER) suggests that CHO, particularly glycogen, is also oxidised to a greater extent during exercise in the heat, possibly at the expense of lipid oxidation (Young *et al.* 1985; Dolny and Lemon 1988; Febbraio *et al.* 1994a, 1994b; Hargreaves *et al.* 1996a;). In support of this, studies using isotopic tracer and a-v difference techniques have recently demonstrated that *Rd* glucose is not increased during exercise in the heat, despite higher rates of total CHO oxidation, thus concluding that intramuscular glycogen oxidation is increased (Hargreaves *et al.* 1996a; Gonzalez-Alonso *et al.* 1999). The combined results from these studies suggest that flux through the pyruvate dehydrogenase (PDH) pathway is up-regulated during exercise in the heat because some of the pyruvate formed during exercise is being oxidised. Whether PDH activity is up-regulated during exercise in the heat has not, to date, been investigated.

2.4.2 Lipid and protein metabolism

Whilst relatively few studies have assessed the effects of environmental heat stress on lipid metabolism during exercise, free fatty acid (FFA) concentrations appear unaltered during exercise with thermal stress (Fink *et al.* 1975; Nielsen *et al.* 1990; Yaspekis *et al.* 1993). However, these concentrations only reflect a balance between

whole-body lipolysis and FFA uptake. Indeed, Fink *et al.* (1975) demonstrated similar circulating FFA concentrations during exercise in the heat and cool environment, but intramuscular triglyceride utilisation was reduced during exercise in the heat. There do not appear to have been any subsequent attempts to quantify the effects of heat stress on intramuscular triglyceride utilisation during exercise. However, Gonzalez-Alonso *et al.* (1999) have recently reported a lower FFA uptake by skeletal muscle during the latter stages of dehydration-induced hyperthermia, compared to exercise in the euhydrated state. It therefore appears that exercise and acute heat stress increases intramuscular glycogen utilisation through aerobic and anaerobic pathways, resulting in a reduction in lipid metabolism, with little change in plasma glucose oxidation.

Little attention has been given to the effects of heat stress on protein metabolism during exercise, possibly due to the minimal contribution of protein to total energy turnover during exercise. However, indirect evidence of increased intramuscular ammonia accumulation (Febbraio *et al.* 1994b) in the absence of any difference in IMP accumulation, suggests that protein degradation may be increased during exercise in the heat. This interpretation should be treated with caution until more direct evidence on protein oxidation during exercise in the heat is available. Indeed, other studies suggest that whilst protein oxidation may contribute ~10% (Lemon and Mullin 1980) to total energy turnover during cycling at 61 % $\dot{V}O_2$ max, this is reduced to less than 5% during prolonged cycling in the heat (Dolny and Lemon 1988).

2.4.3 Glucose availability and utilisation

A number of studies have now demonstrated a relative hyperglycaemic response to exercise in the heat (Fink *et al.* 1975; Febbraio *et al.* 1994a; Hargreaves *et al.* 1996a; Yaspelkis *et al.* 1993; Febbraio *et al.* 1996a). Indeed, using a dye infusion technique, Rowell *et al.* (1968) demonstrated an augmented hepatic glucose production during exercise in the heat. These results were confirmed using isotope tracer (Hargreaves *et al.* 1996a) and $a - \bar{v}$ difference (Gonzalez-Alonso *et al.* 1999) techniques. Hepatic glucose production (HGP) during exercise is regulated by a complex interplay between neural and hormonal factors (see review by Kjaer 1995), such that euglycaemia is usually maintained by HGP to meet the metabolic need for glucose

during prolonged exercise. Consequently, increasing circulating glucose concentrations by exogenous feedings (McConnell *et al.* 1994; Jeukendrup *et al.* 1999a, 1999b) or infusion (Howlett *et al.* 1998), HGP is reduced, and may be completely suppressed. Interestingly, when isotope labelled glucose is ingested during exercise in the heat, glucose appearance from the gastrointestinal tract results in a relative hyperglycaemia, but HGP is unaffected (Angus *et al.* 2001). This study highlights the powerful feed-forward control of thermal stress (Febbraio 2001).

2.4.4 Mechanisms responsible for alterations in energy metabolism

A number of mechanisms have been proposed to account for the shift towards increased CHO metabolism during exercise in the heat, including reductions in skeletal muscle blood flow. It is clear that the increased demand for peripheral blood flow during exercise in the heat reduces splanchnic (Rowell *et al.* 1968), hepatic (Rowell *et al.* 1965), renal (Radigan and Robinson 1949) and inactive skeletal muscle (Rowell 1986) blood flow. Researchers have also hypothesised that active muscle blood flow may be compromised in favour of peripheral blood flow (Fink *et al.* 1975; Nielsen *et al.* 1990; Kozlowski *et al.* 1985), which would reduce oxygen delivery and cellular respiration, but this remains an issue of some controversy (Nielsen *et al.* 1990; Bell *et al.* 1983). To date however, human studies have not demonstrated a reduction in active muscle blood flow during exercise in the heat, measured by thermodilution techniques (Nielsen *et al.* 1990, 1993, 1997; Savard *et al.* 1988), plethysmography and doppler flowmetry (Smolander and Louhevaara 1992). One study has recently demonstrated a reduced leg blood flow during exercise with dehydration and hyperthermia compared to euhydrated subjects (Gonzalez-Alonso *et al.* 1999). However, since dehydration results in hypovolaemia, these alterations may therefore have occurred as a result of reduced blood volume as opposed to a hyperthermia-induced reduction in leg blood flow *per se*.

Animal studies have demonstrated that during dehydration induced hyperthermia, oxygen extraction is increased to maintain muscle oxygen uptake despite a reduction in limb blood flow (Schumacker *et al.* 1987). It therefore appears that reduced oxygen uptake by active skeletal muscle is not the main factor mediating the augmented glycogenolysis and lactate accumulation during moderate intensity exercise in the

heat. This however does not rule out the possibility that reduced leg blood flow may influence metabolic processes during exercise in the heat. Clark *et al.* (1995) have demonstrated that functional vascular shunts exist in skeletal muscle such that metabolism is altered via alterations in the rate of supply of nutrients and removal of metabolic by-products.

Higher muscle lactate concentrations have been observed in the absence of alterations in muscle glycogen utilisation, and a correlation between the percentage of type II fibres and the difference in post-exercise lactate accumulation during exercise in the heat (Young *et al.* 1985). This has led to the hypothesis that either, exercise in the heat increases type II muscle fibre recruitment or, these fibres are more sensitive to changes in temperature (Sawka *et al.* 1985a; Young *et al.* 1985). However, a subsequent study has demonstrated that muscle fibre recruitment patterns are similar during exercise in hot and temperate environments, and have failed to replicate the correlation between lactate accumulation and fibre type (Febbraio *et al.* 1994a).

During exercise intramuscular temperature rises in relation to metabolic rate (Saltin and Hermansen 1966) and is augmented during exercise in the heat (Febbraio *et al.* 1994a, 1994b; Hargreaves *et al.* 1996b; Gonzalez-Alonso *et al.* 1999; Parkin *et al.* 1999). It has been suggested that such rises in muscle temperature could directly up-regulate the activity of key enzymes and alter muscle metabolism (Kozlowski *et al.* 1985; Young 1990). Commonly, Q_{10} values for enzyme mediated reactions are 2 to 3. Therefore, for a typical increase of muscle temperature of 2°C during exercise in the heat, a 30 to 40% increase in enzyme reaction is possible. It is particularly difficult to study the effects of muscle temperature on muscle metabolism *in vivo*, because limb heating tends to increase core temperature and adrenaline secretion, which may increase glycogenolysis during exercise (Jansson *et al.* 1986; Greenhaff *et al.* 1991). However, a more recent study has used heated pads and water perfused cuffs to manipulate muscle temperature independently of changes in core temperature and circulating catecholamine concentrations, demonstrating increased glycogenolysis and lactate accumulation during 2-min of exercise at 115% $\dot{V}O_2$ max (Febbraio *et al.* 1996c). Similar responses were demonstrated during more moderate intensity exercise (70% $\dot{V}O_2$ max), when one leg was cooled and the other heated for 40-min prior to and

20-min during exercise (Starkie *et al.* 1999). Changes in muscle temperature and associated enzyme activity may therefore play a role in augmenting intramuscular glycogen utilisation during high and moderate intensity exercise.

2.4.5 *Effects of catecholamines*

It is widely acknowledged that adrenaline secretion increases during exercise (see Galbo 1983), and that this increase is augmented with heat stress (Febbraio *et al.* 1994a; Hargreaves *et al.* 1996a; Gonzalez-Alonso *et al.* 1999). As glycogen phosphorylase activity is increased by β -adrenergic receptor stimulation (Richter *et al.* 1982), any increase in circulating adrenaline concentration may result in a concomitant increase in muscle glycogenolysis. Indeed, most (Febbraio *et al.* 1994a, 1996c; Hargreaves *et al.* 1996b; Gonzalez-Alonso *et al.* 1997), but not all (Nielsen *et al.* 1990) studies have shown that muscle glycogen utilisation closely matches plasma adrenaline concentration during exercise. Animal studies have demonstrated that adrenaline infusion increases glycogen utilisation during voluntary submaximal exercise or electrical stimulation (Richter *et al.* 1981, 1982; Issekutz 1985). Furthermore, removal of the adrenal medulla (Hashimoto *et al.* 1982) or β -adrenergic receptor blockade (Issekutz 1984), reduces muscle glycogen utilisation.

Human studies that have manipulated adrenaline concentration by infusion have produced conflicting results. Those that have demonstrated an effect on muscle glycogen utilisation have infused supra-physiological doses of adrenaline (Jansson *et al.* 1986; Spriet *et al.* 1988). However, two studies have shown that glycogen utilisation is not increased during intense dynamic (Chesley *et al.* 1995) or prolonged (Wendling *et al.* 1996) exercise when adrenaline is infused to mimic normal physiological concentrations. The study by Chesley *et al.* (1995) was conducted at $85\% \dot{V}O_2 \text{ max}$, which was likely to have activated glycogen phosphorylase irrespective of adrenaline concentration. Furthermore, an increase in glycogen utilisation during exercise and heat stress has been observed in circumstances where there has been little, if any, disruption of the intracellular milieu (Febbraio *et al.* 1994b). It should also be highlighted that adrenaline increases glycogenolytic rate of type I but not type II fibres (Greenhaff *et al.* 1991), and heat stress augments the use

of glycogen in type I fibres only. In addition, when adrenaline is infused to mimic concentrations observed during cycle exercise at $70\% \dot{V}O_2 \text{ max}$ in a 40°C environment, intramuscular glycogen utilisation is increased (Febbraio *et al.* 1998).

2.4.6 Summary

During prolonged moderate intensity exercise in a hot environment, the ensuing hyperthermia appears to be associated with increases circulating adrenaline and glucose concentrations, total CHO oxidation, muscle glycogen utilisation, muscle lactate accumulation. Indeed, HGP appears to be maintained even when exogenous glucose is ingested. There is therefore strong evidence to suggest that both the increase in HGP and muscle glycogen utilisation observed during exercise in the heat may be mediated by an augmented sympatho-adrenal response, secondary to an increase in circulating adrenaline concentration. Whether such responses are responsible, even in part, for the impairment of exercise performance/capacity during exercise in the heat remains debatable. It should be highlighted, however, that whilst differences in the pattern of substrate utilisation between prolonged running and cycling may exist, almost all of the studies that have considered the effects of heat stress on muscle metabolism during prolonged exercise have used cycling as the exercise modality. Whether we can make generic conclusions from the results of these studies to all modes of exercise is debatable.

Having discussed temperature regulation and substrate utilisation during exercise, and the potential differences in substrate utilisation which may result from dehydration and/or exercising in a warm environment, the efficacy of CHO and fluid intake regimens during exercise and short-term recovery will be discussed.

2.5 Carbohydrate and fluid intake regimens

Due to the high rates of endogenous liver and muscle glycogen utilisation, and high sweat rates which occur during prolonged exercise, particularly when exercise is performed in a hot environment, a vast amount of research has been undertaken on the efficacy of CHO and fluid replacement strategies both during and immediately after

exercise. The main focus of this research has been on rapid rehydration and glycogen synthesis post-exercise, and on the maintenance of hydration status and exogenous glucose availability during exercise. The effectiveness of these regimens on muscle metabolism, thermoregulation and exercise performance/capacity will be discussed.

2.5.1 Fluid replacement during recovery

As previously discussed, dehydration during exercise is difficult to prevent, as sweat rates may be greater than gastric emptying rate during exercise, even if adequate quantities of fluid are ingested. The resultant hypovolaemic-hyperosmotic state may compromise heat dissipation mechanisms and impair thermoregulatory function. Thus rapid restoration of body fluid is essential, particularly if further exercise is to be performed on the same or successive days. Whilst the effectiveness of the rehydration process is determined by many factors, the nature and composition of the fluid, the physiological responses to ingestion and the behavioural response to fluid intake appear to be the most important. The terms water and carbohydrate-electrolyte solution (CES) will be used to highlight, where necessary, generic differences in the nature of fluid ingested during experiments.

2.5.1.1 Gastric emptying

The availability of ingested fluid (and exogenous CHO) depends mainly on the rate at which fluids are emptied from the stomach into the small intestine together with the rate of intestinal absorption. Thus, gastric emptying presents a rate limiting step for the absorption of ingested fluids and their content (Costill and Saltin 1974). Whilst the precise effect of each factor determining the rate of gastric emptying is still unclear, the volume (Mitchell and Voss 1991; Noakes *et al.* 1991; Mitchell *et al.* 1994), temperature (Fone *et al.* 1990), energy content and osmolality (Cosill and Saltin 1974; Foster *et al.* 1980; Brener *et al.* 1983; Hunt *et al.* 1992; Vist and Maughan 1995) of the solution are known to influence the rate. In addition, the level of dehydration of the subject may play a role (Neufer *et al.* 1989; Rehrer *et al.* 1990a).

Most studies have demonstrated that the rate of gastric emptying of most solutions follows an exponential time course (Hunt and Spurrell 1951; Leiper and Maughan

1988; Rehrer *et al.* 1989; Vist and Maughan 1995). The volume of fluid emptied per unit time is therefore directly proportional to the volume present in the stomach. As such, the interpretation of results from different studies is strongly influenced by the sampling frequency and time of sampling.

The two main responses that stimulate gastric emptying are the nerve impulses that occur in response to stomach distension (Brener *et al.* 1983), and the action of gut hormones (Minami and McCallum 1984). The composition of the fluid ingested does, however, strongly influence the gastric emptying rate. Receptors embedded in the gastric musculature, and in the walls of the duodenum and jejunum, are sensitive to changes in volume, osmolality, pH, fat and amino acid concentrations (Murray 1987). As such, when fluid is ingested intra-gastric pressure increases and the receptors in the distended gastric musculature respond by increasing the rate of emptying (Hunt and MacDonald 1954; Minami and McCallum 1984). The inverse-exponential rate of gastric emptying indicates the important role of gastric volume in regulating the rate of gastric emptying. As such, when large volumes of fluid are maintained in the stomach by repetitive drinking, the gastric emptying is maintained at a higher rate (Rehrer *et al.* 1990b; Noakes *et al.* 1991).

Interestingly, prescribed fluid intake during relatively short post-exercise periods (2 to 4 hours), does not result in complete rehydration even when fluid ingestion is equal to the post-exercise fluid deficit (Costill and Sparks 1973; Nielsen *et al.* 1986; Gonzalez-Alonso *et al.* 1992). However, one study compared the effectiveness of ingested fluid volumes equivalent to 100 and 150% of fluid deficit during a 3-hour rehydration period, resulting in 48 and 68% whole-body rehydration, respectively. The gastric emptying rates indicate that the cumulative volume of fluid emptied from the stomach was significantly greater at each hour during the 3-hour rehydration period (Mitchell *et al.* 1994). Furthermore, positive fluid balance appears to be achievable when substantially greater volumes of fluid are ingested compared to those lost (150 to 200%). It remains to be established whether such levels of rehydration are effective in enhancing subsequent endurance exercise capacity/performance.

Another important determinant of gastric emptying rate is the CHO content of the solution, and generally speaking, as the concentration increases, gastric emptying rate

declines (Hunt and Knox 1968; Barker *et al.* 1978; Foster *et al.* 1980). Whilst some studies suggest that gastric emptying rate is impaired when the CHO content of a CES exceeds 2.5% (Costill and Saltin 1974; Foster *et al.* 1980), others have demonstrated that CES solutions up to 10% CHO concentration do not impair the rate of gastric emptying compared to water (Owen *et al.* 1986; Rehrer *et al.* 1989). Whilst there therefore appears to be conflicting results from various studies, more recent investigations (Maughan and Leiper 1990; Vist and Maughan 1995) have shown quite clearly that gastric emptying rate is slowed, both in relation to the CHO content of the CES and the volume remaining in the stomach.

2.5.1.2 Intestinal absorption

Two of the major factors regulating net water transport in the small intestine are osmolality (Hunt *et al.* 1991; Wapnir and Lifshitz 1985) and solute flux (Malawer 1965). Solutions hypertonic to human plasma (normally $>280 \text{ mOsm.kg}^{-1}$) reduce fluid absorption and increase secretion while hypotonic solutions promote fluid absorption (Farthing 1990; Hunt *et al.* 1992), with the optimal osmolality being in the range of $200\text{-}250 \text{ mOsm.kg}^{-1}$ (Wapnir and Lifshitz 1985; Leiper and Maughan 1986). Indeed, a negative correlation between fluid absorption and osmolality has been repeatedly observed in studies involving only a single transportable substrate (Wapnir and Lifshitz 1985; Farthing 1988; Hunt *et al.* 1991).

Fluid absorption and secretion are also related to solute transport, which in turn, is related to the type of CHO in the CES. Per mole, glucose stimulates more net water and Na^+ absorption than fructose (Fordtran 1975). However, water absorption appears to be independent of CHO type in CES solutions containing up to 6% CHO, which were iso-osmotic and iso-caloric (Gisolfi *et al.* 1992). Increasing the CHO content to 8% reduces water absorption from iso-caloric solutions of glucose and corn syrup solids, but not from 8% solutions of sucrose or maltodextrin. It appears that the use of glucose and fructose in the same solution, which have the advantage of being transported by separate non-competitive pathways, might enhance total CHO absorption. Indeed, a recent study has demonstrated that exogenous CHO oxidation rates may reach 1.3 g.min^{-1} when fructose and glucose are ingested simultaneously at high rates during cycling exercise (Jentjens *et al.* 2004), compared to 0.8 to 1 g.min^{-1}

with glucose alone (Wagenmakers *et al.* 1993a; Jentjens *et al.* 2004). These data suggest that total exogenous CHO absorption is increased when ingesting multiple source CHO solutions during exercise, which may also be beneficial for glycogen resynthesis during recovery.

Sodium is the major ion of the extracellular fluid space and replacement of Na^+ may therefore be necessary to restore the extracellular fluid volume lost. Indeed, the Na^+ concentration appears to regulate the extracellular fluid volume (Nose *et al.* 1988a, 1990). As such, ingestion of water during post-exercise rehydration rapidly dilutes the plasma volume, diminishing both the volume-dependent and osmotic drive for drinking and increases urine excretion (Nose *et al.* 1988b). Costill and Sparks (1973) also demonstrated that ingestion of a CES resulted in a smaller urine production and a greater restoration of plasma volume compared to water, data which have more recently received wider support (Gonzalez-Alonso *et al.* 1992; Maughan and Leiper 1995). It appears however, that the optimal solution would contain 40-60 mmol.l^{-1} Na^+ (Maughan and Leiper 1995), which may reduce the palatability of the solution. More dilute Na^+ solutions are not as effective for rapid rehydration and may be detrimental to subsequent exercise performance (Mitchell *et al.* 1994).

2.5.2 *Glycogen synthesis during recovery*

In addition to the advantages of appropriate fluid intake for post-exercise rehydration, the use of CES has become popular practice to simultaneously promote rapid synthesis of depleted endogenous glycogen reserves. The time course for the restoration of muscle glycogen stores post-exercise is probably the most important factor determining the time needed to recover. Indeed, post-exercise muscle glycogen synthesis appears to have such high metabolic priority that intramuscular triglycerides are broken down at an increased rate to supply fuel for oxidative muscle metabolism (Kiens and Richter 1998).

2.5.2.1 *Regulation of muscle glycogen synthesis*

Resynthesis of muscle glycogen stores requires glucose, the majority of which must be orally ingested and transported across the muscle cell membrane. In skeletal

muscle, glucose is transported by facilitated diffusion, predominantly using the glucose carrier protein GLUT-4 (Goodyear and Kahn 1998), which is independently induced to the cell surface in the presence of insulin or muscular contraction (Lund *et al.* 1995; Thorrell *et al.* 1999). The maximal rate of muscle glucose transport appears to be determined by the total GLUT-4 concentration and the proportion that is translocated to the cell membrane (MacLean *et al.* 2000). For more detailed reviews of this topic the reader is referred to Ivy and Kuo (1998) and Richter *et al.* (2001).

Several studies have demonstrated that the pattern of muscle glycogen synthesis following exercise-induced glycogen depletion occurs in a biphasic manner (Piehl *et al.* 1974, 2000; Maehlum *et al.* 1977; Blom *et al.* 1987; Ivy *et al.* 1988b; Price *et al.* 1994). The rapid phase appears to persist for 30-60 min and can proceed without the presence of insulin (Piehl *et al.* 1974; Maehlum *et al.* 1977), and may only occur when post-exercise glycogen concentrations are low (Maehlum *et al.* 1977) and when CHO is provided immediately after exercise (Ivy *et al.* 1988a). After this rapid phase, muscle glycogen synthesis occurs at a much slower rate, is dependent on the availability of CHO and high insulin concentrations, and can last for several hours (Ivy 1991). Low post-exercise glycogen concentrations may stimulate an increase in the number of GLUT-4 transporters at the plasma membrane and in glycogen synthase activity, both of which are likely to be responsible for the rapid phase of glycogen synthesis. The slow phase of glycogen synthesis appears to be dependent on CHO availability and, particularly, high insulin concentrations. The sensitivity of the muscle to insulin also appears to be dependent on muscle glycogen concentration, with low concentrations increasing sensitivity (Nielsen *et al.* 2001).

2.5.2.2 Timing of carbohydrate intake

It has been demonstrated that delaying post-exercise CHO intake results in a 45% lower muscle glycogen synthesis compared to ingestion immediately after exercise (Ivy *et al.* 1988a). The authors suggested that this was a result of lower muscle glucose uptake in the delayed intake trial. Indeed, in animal studies, the exercise induced increase in post-exercise glucose transporters associated with the cell membrane may return to normal within 2-hours in the absence of CHO ingestion (Cartee *et al.* 1989; Goodyear *et al.* 1990). It is therefore not unlikely that in the study

by Ivy *et al.* (1988a), when CHO feeding was delayed, the number of glucose transporters at the plasma membrane would have been significantly reduced, thus contributing to the lower muscle glycogen synthesis. A more recent study concluded that there was no difference in post-exercise glycogen synthesis when a high glycemic index meal was ingested either immediately after or 2-hours after exercise (Parkin *et al.* 1997). However, glycogen synthesis was calculated as an average rate over an 8-hour period and it is therefore possible that muscle glycogen synthesis would have been higher in the immediate post-exercise feeding trial, had samples been taken between 2 and 4 hours post-exercise.

2.5.2.3 Amount of carbohydrate

When no CHO is ingested after exercise the rate of glycogen synthesis is low (7-12 $\text{mmol.kg}^{-1(\text{dw})}.\text{h}^{-1}$) (van Hall *et al.* 2000; Ivy *et al.* 1988a; Maehlum *et al.* 1978; Tarnopolsky *et al.* 1997) compared to when CHO is ingested immediately after exercise (20-50 $\text{mmol.kg}^{-1(\text{dw})}.\text{h}^{-1}$) (Maehlum *et al.* 1977, 1978; Blom *et al.* 1987; Tarnopolsky *et al.* 1997; Piehl *et al.* 2000). Blom (1989) demonstrated that although increasing CHO consumption from 0.18 to 0.35 $\text{g.kg}^{-1}.\text{h}^{-1}$ increased the rate of glycogen synthesis by more than 150%, a further increase to 0.7 $\text{g.kg}^{-1}.\text{h}^{-1}$ CHO did not further increase glycogen synthesis rate. Whilst they suggested that the maximum rate of glycogen synthesis had been achieved by feeding 0.35 $\text{g.kg}^{-1}.\text{h}^{-1}$ CHO, this is unlikely given that numerous other studies have reported greater rates (Casey *et al.* 1995; McCoy *et al.* 1996; van Hall *et al.* 2000; Piehl *et al.* 2000). Blom (1989) did report incredibly high glycogen synthesis rates in the 0.35 $\text{g.kg}^{-1}.\text{h}^{-1}$ CHO trial, which may explain why no further increases were observed.

A further study has, however, found no difference in muscle glycogen synthesis rate when either 0.75 or 1.5 $\text{g.kg}^{-1}.\text{h}^{-1}$ of CHO was provided during a 4-h recovery period (Ivy *et al.* 1988b). Whilst the findings of this study provide some support for the findings of Blom (1989), van Loon *et al.* (2000) have recently demonstrated that when the rate of CHO ingestion is increased from 0.8 to 1.2 $\text{g.kg}^{-1}.\text{h}^{-1}$ the rate of muscle glycogen synthesis was approximately 2-fold greater. In the latter study, CHO feedings were provided at 30-min intervals during recovery, whilst the studies that had observed no difference in glycogen synthesis, feedings were given at 2-hour

intervals. It has been suggested that 2-hour intervals are not sufficient to adequately increase and maintain blood glucose and insulin concentrations for 2-hours (Ivy 1998). This may be related to the rate of gastric emptying, which whilst initially faster with a large fluid volume (Rehrer *et al.* 1994), repetitive feedings are more likely to prevent the inverse-exponential fall in the rate of gastric emptying with decreasing volume (Vist and Maughan 1995).

2.5.2.4 Type of carbohydrate ingested

Carbohydrates may be functional classified according to the extent to which they increase blood glucose. The blood glucose response is quantified after ingesting a given food product with a given amount of glucose (usually 50 g), compared to an isocaloric amount of free glucose, and is termed the glycemic index (GI) (Jenkins *et al.* 1981; Wolever *et al.* 1991). This measure reflects the rate of digestion and absorption of CHO rich foods, making it easier to compare the functional effect of different CHO feedings or meals. A number of studies have demonstrated that ingestion of fructose results in lower rates of glycogen synthesis compared to glucose, probably because fructose has a low GI (Blom *et al.* 1987; Conlee *et al.* 1987). Indeed, fructose is absorbed more slowly from the intestine (Henry *et al.* 1991) and requires conversion to glucose by the liver before it can be metabolised in skeletal muscle. As such, glucose is the preferred form of CHO when high rates of muscle glycogen synthesis are required, although fructose may be of more benefit for liver glycogen synthesis (Nilsson and Hultman 1974; Conlee *et al.* 1987).

A number of studies have observed similar rates of muscle glycogen synthesis when either glucose or sucrose was ingested (Blom *et al.* 1987; Casey *et al.* 2000). Whilst this is surprising given that sucrose contains equimolar amounts of glucose and fructose, it has been suggested that because fructose is preferentially metabolised in the liver, more of the free glucose is available for muscle glycogen synthesis (Blom *et al.* 1987). Whilst it is difficult to compare different studies because the quantity and feeding rate of CHO also vary, it appears that when moderate to large quantities of sucrose are ingested, this can result in muscle glycogen synthesis rates which are similar to those obtained with glucose ingestion (Blom *et al.* 1987; Casey *et al.* 2000; van Hall *et al.* 2000).

2.5.2.5 *Effects on subsequent exercise*

Relatively few studies have considered the effects of CHO feedings during short-term recovery periods on subsequent endurance exercise performance or capacity. Two studies have demonstrated that whilst muscle glycogen stores are almost completely restored after 22 to 24-hours of recovery on a high CHO intake regime, maximal physical work capacity is still lower compared to the initial exercise bout (Keizer *et al.* 1987; Fallowfield and Williams 1993). Other studies, using shorter recovery periods (4-h) have demonstrated that exercise capacity cannot be fully restored by rehydration with CES or water (Fallowfield *et al.* 1995; Wong *et al.* 1996a, 1996b). However, these short-term recovery studies have clearly demonstrated that post-recovery endurance exercise capacity is greater when rehydration is achieved with CES compared to water (Fallowfield *et al.* 1995), although ingestion of a 50-g bolus dose of CHO immediately after exercise may be sufficient to produce a maximum benefit in subsequent endurance exercise capacity after 4-h (Wong *et al.* 1996a). Whilst it is unfortunate that muscle glycogen synthesis rates were not measured during these studies, they report maintenance of high levels of CHO oxidation during subsequent exercise following CES ingestion (Fallowfield *et al.* 1995, Wong *et al.* 1996a).

2.5.3 *Fluid and CHO intake during exercise*

The detrimental effects of exercise induced dehydration (and hypohydration) on human cardiovascular and thermoregulatory function have already been discussed. From a thermoregulatory perspective, the primary aim of fluid intake during exercise is to prevent dehydration and the associated consequences, particularly during exercise in the heat. Whilst these factors will not therefore be discussed here, the use of fluids (CES) to provide an alternative source of exogenous CHO during prolonged exercise has become common practice among athletes. As such, the metabolic responses to and mechanisms for improvements in exercise performance/capacity associated with CHO intake during exercise will be reviewed. Indeed, the number of studies concluding that CHO intake during exercise improves exercise performance/capacity ensures that this view point is widely accepted, at least during exercise in temperate environments.

2.5.3.1 Feeding schedule

When CES are ingested from the onset of exercise, exogenous CHO oxidation is observed within the first 5-min and gradually increases over the first 75 to 90-min of exercise, as more CHO is emptied from the stomach and absorbed. The plateau which occurs after 75 to 90-min appears to be largely uninfluenced by the timing or frequency of CHO feedings. The administration of a large glucose load (100 g) at the beginning of exercise (Pirnay *et al.* 1977; Krzentowski *et al.* 1984; Guezennec *et al.* 1989; Jandrain *et al.* 1989) appears to result in a quantitatively similar pattern of exogenous CHO oxidation as the same load given as repetitive feedings (Massicotte *et al.* 1989, 1990, 1994; Moodley *et al.* 1992; Burelle *et al.* 1999). In these studies the maximum rate of exogenous CHO oxidation ranged from 0.48 to 0.65 g.min⁻¹. Whilst repetitive feeding strategies are often adopted, as they purportedly accelerate the rate of gastric emptying (Rehrer *et al.* 1990b; Noakes *et al.* 1991), gastric emptying does not usually limit exogenous CHO oxidation (Moodley *et al.* 1992; Rehrer *et al.* 1992). This may be more of a consideration during exercise in the heat, where fluid availability becomes increasingly important. However, the feeding schedule should be such that high exogenous CHO rates are achieved as soon as possible, as delaying this may prove ineffective (McConnell *et al.* 1996).

2.5.3.2 Type of carbohydrate

A number of studies have now compared the oxidation rates of various types of ingested CHO during exercise. There has become increasing interest in the use of fructose as it generally improves the palatability of CES, and results in a 20 to 30% smaller increase in plasma insulin concentrations compared with glucose (Samols and Dormandy 1963). This may be important for the maintenance of lipolysis. Used as a pre-exercise feeding, fructose may also prevent the rebound hypoglycaemia sometimes observed with the use of glucose (Guezennec *et al.* 1989; Okano *et al.* 1988). However, numerous studies have demonstrated that fructose results in a 25 to 45% lower rate of exogenous CHO oxidation during exercise, compared to glucose (Massicotte *et al.* 1989; Guezennec *et al.* 1989; Jandrain *et al.* 1993; Adopo *et al.* 1994; Burelle *et al.* 1997). As previously discussed, this probably relates to the fact that fructose is absorbed more slowly and subsequently has to be converted to glucose in

the liver. Similarly, ingestion of galactose within CES results in a lower rate of exogenous CHO oxidation ($0.41 \text{ g}\cdot\text{min}^{-1}$) compared to glucose ($0.85 \text{ g}\cdot\text{min}^{-1}$), probably for the same reasons as fructose (Leijssen *et al.* 1995).

There is evidence to suggest that both maltose and sucrose are oxidised at similar rates to glucose. Indeed, Hawley *et al.* (1992) demonstrated that ingesting 180 g of maltose or glucose during 90-min of exercise at $70\% \dot{V}O_2 \text{ max}$ resulted in similar exogenous CHO oxidation rate of approximately $1 \text{ g}\cdot\text{min}^{-1}$. Similarly, sucrose ingestion results in similar exogenous CHO oxidation rates to glucose during moderate intensity exercise (65 to $70\% \dot{V}O_2 \text{ max}$), both when the intake is moderate (Moodley *et al.* 1992) and relatively high (Wagenmakers *et al.* 1993a). The oxidation rates of maltose and sucrose appear to be similar to that of glucose, and the efficacy of using these sources of CHO may therefore be similar.

Maltodextrins have seen increasing popularity by sports drink manufacturers because of their relatively low osmotic value for equivalent energy density. In theory, a maltodextrin based CES would therefore empty from the stomach and be absorbed more rapidly than an equivalent glucose solution. However, the maximum exogenous CHO oxidation rate of a 17% glucose or maltodextrin solution was similar (0.75 to $0.80 \text{ g}\cdot\text{min}^{-1}$) during 80-min of exercise at $70\% \dot{V}O_2 \text{ max}$ (Rehrer *et al.* 1992). Interestingly, the rate of gastric emptying and thus the rate of delivery of CHO to the intestine were similar with both solutions. Similarly, no differences in exogenous CHO oxidation appear to be observed between glucose and maltodextrin solutions throughout a range of concentrations from 4 to 16%, both resulting in maximum oxidation rates of approximately 1.0 to $1.1 \text{ g}\cdot\text{min}^{-1}$ (Wagenmakers *et al.* 1993a). There appears to be no additional increase in oxidation rate by feeding $1.2 \text{ g}\cdot\text{min}^{-1}$ of either glucose or maltodextrin.

It has been suggested that the inclusion of two or three different CHO (glucose, fructose and sucrose) within a CES may increase fluid and CHO absorption, because they have separate transport mechanisms across the intestinal wall (Shi *et al.* 1995). Indeed, it was demonstrated that the addition of fructose (50-g) to a glucose (50-g) solution ingested at the onset of 2-hours of exercise at $61\% \dot{V}O_2 \text{ max}$ increased,

exogenous CHO oxidation rate by 21% compared to an iso-caloric glucose solution (Adopo *et al.* 1994). Further support for these data were recently published to demonstrate that the simultaneous ingestion of high rates ($1.8 \text{ g}\cdot\text{min}^{-1}$) of CHO from multiple sources (glucose and fructose or glucose and sucrose) resulted in exogenous CHO oxidation rates (1.25 to $1.3 \text{ g}\cdot\text{min}^{-1}$) which were significantly higher than when an iso-caloric glucose solution (0.83 to $1.06 \text{ g}\cdot\text{min}^{-1}$) was ingested (Jentjens *et al.* 2004a, 2004b).”

2.5.3.3 Amount of carbohydrate

It has been assumed that the maximum amount of CHO that needs to be ingested to maximise exercise performance is the amount which will elicit maximal rates of exogenous CHO oxidation. The relationship between CHO intake and oxidation appears to be relatively complex, expressing a non-linear relationship. Feeding a 4.5% glucose solution (58-g glucose) compared to a 17% glucose solution (220-g glucose) during 80-min of exercise at $70\% \dot{V}O_2 \text{ max}$ results in a relatively small difference in exogenous CHO oxidation (42 vs. 32-g, respectively) (Rehrer *et al.* 1992). Hence, despite a 4-fold increase in CHO feeding, exogenous CHO oxidation was only increased by $\sim 31\%$. Similarly, when subjects ingest 0.6 , 1.2 , 1.8 and $2.4 \text{ g}\cdot\text{min}^{-1}$ of CHO in a maltodextrin based CES during 2-hours of exercise at $65\% \dot{V}O_2 \text{ max}$, oxidation rates appear to plateau after an intake of $1.2 \text{ g}\cdot\text{min}^{-1}$ (Wagenmakers *et al.* 1993a). Interestingly, when using single CHO sources, none of the more recent studies have observed CHO oxidation rates to exceed $1.1 \text{ g}\cdot\text{min}^{-1}$, despite feeding up to $3.0 \text{ g}\cdot\text{min}^{-1}$ (Wagenmakers *et al.* 1993a; Jeukendrup *et al.* 1999b).

2.5.3.4 Factors affecting exogenous carbohydrate oxidation

With the increasing reliance on CHO metabolism with increasing exercise intensity, it would seem logical that exogenous CHO oxidation rates would be higher during more intense exercise. It appears that whilst this essentially true, the rate of exogenous CHO oxidation appears to plateau between 51 and $64\% \dot{V}O_2 \text{ max}$ (Pirnay *et al.* 1982). There appears to be no further increase in the rate of exogenous CHO oxidation when the exercise intensity is increased from $60\% \dot{V}O_2 \text{ max}$ ($0.51 \text{ g}\cdot\text{min}^{-1}$) to $75\% \dot{V}O_2 \text{ max}$

($0.42 \text{ g}\cdot\text{min}^{-1}$) (Pirnay *et al.* 1995). More recently, van Loon *et al.* (1999) observed no difference in exogenous CHO oxidation rates when comparing exercise at 38 and 55% $\dot{V}O_2$ max. It is therefore possible that a reduction in exogenous CHO oxidation rate is only observed at very low exercise intensities, when the reliance on CHO metabolism is lower, and glucose may be stored rather than oxidised.

There seems to be a consistent finding that maximum exogenous CHO oxidation rates are limited between 1.0 and $1.1 \text{ g}\cdot\text{min}^{-1}$, when using radioactive (Hawley *et al.* 1992; Bosch *et al.* 1994) or stable (Pirnay *et al.* 1982; Wagenmakers *et al.* 1993a; Jeukendrup *et al.* 1996, 1997, 1999a) isotope tracer techniques. Whilst one of the limitations could be gastric emptying, this is unlikely given that Rehrer *et al.* (1992) showed that 80-min after ingesting 220-g of CHO, 120-g had been delivered to the duodenum and only 38-g had been oxidised. Using slightly different protocols, other studies have confirmed these results (Moodley *et al.* 1992; Saris *et al.* 1993).

Another limiting factor could be the rate of intestinal absorption of CHO. Using the triple lumen technique to measure duodenojejunal glucose absorption a 6% CES, it was estimated that the maximal rate of intestinal absorption was between 1.3 and $1.7 \text{ g}\cdot\text{min}^{-1}$ (Duchman *et al.* 1997). Using stable isotope techniques to measure gut Ra , it appears that when a low dose of glucose is ingested, gut Ra ($0.43 \text{ g}\cdot\text{min}^{-1}$) is equal to that ingested (Jeukendrup *et al.* 1999b). However, when CHO was ingested at a rate of $3 \text{ g}\cdot\text{min}^{-1}$, the rate of gut Ra was approximately one third of the rate of ingestion (0.96 to $1.04 \text{ g}\cdot\text{min}^{-1}$). Thus, whilst only part of the ingested CHO appeared in the systemic circulation, 90 to 95% of it was oxidised. These data are supported by Hawley *et al.* (1994), who infused glucose directly into the systemic circulation and was able to increase exogenous CHO oxidation above $1.0 \text{ g}\cdot\text{min}^{-1}$. These data therefore suggest that exogenous CHO oxidation is limited by the rate of digestion, absorption and subsequent transport of glucose into systemic circulation.

2.5.4 Carbohydrate intake during exercise in the heat

Having already discussed the shift towards CHO metabolism, particularly muscle glycogen metabolism, during exercise in the heat, the efficacy of fluid and CHO

feedings during exercise in the heat will be discussed. A recent study by Hargreaves *et al.* (1996b) has demonstrated that fluid intake alone, reduces the core temperature response and muscle glycogen utilisation during exercise in a temperate environment. However, such a study has not been replicated during exercise in the heat. Whilst it is traditionally thought that exercise performance in the heat is limited by thermoregulatory incapacity and ensuing hyperthermia, some studies have shown that consuming a high CHO diet (Pitsiladis and Maughan 1999) and feeding CHO during exercise (Davis *et al.* 1988; Millard-Stafford *et al.* 1990), enhances exercise performance in the heat. However, another study has found no influence of CHO ingestion on exercise performance at 70% $\dot{V}O_2$ max (Febbraio *et al.* 1996a).

There is clearly some conflicting evidence, and the mechanism responsible for such a potential ergogenic effect remains unclear. A recent study demonstrated that whilst glucose ingestion increases exogenous glucose oxidation during exercise in the heat, such feedings do not spare endogenous liver glycogen reserves or reverse the hyperthermia-induced increase in muscle glycogen utilisation (Jentjens *et al.* 2002). Despite this lack of an obvious metabolic mechanism, Carter *et al.* (2003) have recently demonstrated that carbohydrate supplementation improves both moderate (60% $\dot{V}O_2$ max) and high (73% $\dot{V}O_2$ max) intensity exercise capacity by 14.5 and 13.5%, respectively. The authors suggest that, in the absence of a clear metabolic explanation, a central effect involving an increased tolerance of rising deep body temperature merits further investigation.

2.5.5 Summary

It is clear that CES offer advantages, both for rehydration and the replenishment of endogenous glycogen stores during recovery, and for preventing dehydration and providing an alternative source of CHO during exercise. Indeed, CHO intake during the early stages of recovery is essential if the rapid phase of glycogen synthesis is to be capitalised upon, and CHO intake during exercise can contribute up to $\sim 1.3 \text{ g}\cdot\text{min}^{-1}$ towards the metabolic requirements for exercise. There is little doubt that such nutritional strategies can enhance exercise performance and capacity.

Whilst heat stress increases the reliance on CHO, particularly muscle glycogen metabolism during exercise, the efficacy of CHO feedings during exercise and short-term recovery remains debatable. Whilst early indications look positive, the mechanisms are not clear and there have been very few studies looking at the effectiveness of CHO supplementation regimens for prolonged running exercise in the heat. As such, the studies reported in this thesis have been undertaken to assess the effects of heat stress and CHO supplementation regimens during exercise and recovery, on substrate utilisation and exercise tolerance during prolonged running.

CHAPTER THREE

GENERAL METHODS

3.1 Introduction

All experiments described in this thesis were conducted in the Environmental Medicine Unit's (EMU) environmental chamber at the Institute of Naval Medicine (INM), with each study being approved by the Ministry of Defence (Navy) Personnel Research Ethics Committee (MOD(N) PREC). The specific design of each experiment is outlined in the subsequent experimental chapters, whereas this chapter will describe many of the methods which were common to some or all of the studies.

3.2 Subjects

Forty-four male military personnel (aged 20 to 39 years) volunteered to participate in the experiments described in this thesis. Each was involved in various physical training programmes of which submaximal running was a central feature. They were recruited by advertisement within local military establishments. Subjects were informed about the nature and demands of each experiment and gave their written informed consent (Appendix 1 and 2) once they had fully understood the details of each study.

Subsequently, subjects were required to complete a medical history questionnaire, specifically designed for screening subjects for exposure to exercise and/or heat stress (Appendix 3), and to provide information about current weekly training schedules. Subjects with a history of heat illness were excluded from further participation in the trials. Finally, subjects underwent a full medical examination including a 12 lead electrocardiograph (ECG), conducted by a qualified paramedic and reviewed by an Independent Medical Officer (IMO). The mean \pm SEM physical characteristics of the subjects ($n = 44$) were: age, 31.1 ± 0.7 years; height, 178.2 ± 1.0 cm; body mass, 78.6 ± 1.5 kg; body fat, 15.5 ± 0.6 %; and maximal oxygen uptake ($\dot{V}O_2 \text{ max}$), 59.9 ± 1.2 ml.kg⁻¹.min⁻¹.

3.3 Anthropometry

Height was measured using a fixed stadiometer and recorded to the nearest 0.1 cm (Holtain Ltd., UK). Subjects removed their shoes and socks and stood with their heels together and resting against the stadiometer. Subjects were asked to inhale deeply and the headboard was lowered until it made contact with the superior point of the head. Gentle traction was applied to the mastoid processes and occipital bone in order to compensate for any shrinkage of the inter-vertebral discs.

Body mass was measured on a beam balance to the nearest 1 g (Sartorius, Göttingen, Germany). During the preliminary measurements subjects were required to wear only light clothing during this measurement, and to remain as stationary as possible. Before and after each of the exercise trials, subjects were weighed nude in order to calculate weight loss attributable to sweat loss. Upon completion of exercise, subjects were required to towel dry themselves prior to this measurement. During the 4-h recovery (rehydration) periods (Chapters 5 and 6), subjects were also weighed nude, in order to calculate weight gain attributable to fluid intake and retention. During the experiment reported in Chapter 4, clothed body mass was also recorded in order to estimate evaporative sweat loss.

Skinfold thicknesses were measured to the nearest 0.1 mm using skinfold callipers (Holtain Ltd., UK). All measurements were made in triplicate on the dominant side of the body. Skinfold thickness was measured at four different sites (bicep, tricep, subscapular, and suprailiac), and the sum of the four was used to determine body density using previously described gender and age related equations (Durnin and Womersley 1974). In order to determine the appropriate site for biceps measurement, subjects were requested to flex their arm to an angle of 90°, and the midpoint between the acromion and the olecranon processes was marked. The measurement of skinfold thickness was determined with the arm relaxed by the side of the body (anatomical position), with palms facing forwards. The measurement of triceps skinfold was taken at the same level as the bicep, again with the arm relaxed at the side of the body. The subscapular skinfold was taken at the inferior angle of the scapular, also with the arms in a relaxed position. The suprailiac skinfold was measured superior to the iliac crest, with the arm slightly away from the body to facilitate easier access. Percentage body

fat (%BF) was subsequently estimated from calculations of body density (BD), using previously described methods (Siri 1956):

$$\%BF = \left(\frac{4.95}{BD} - 4.50 \right) \times 100 \quad (3.1)$$

3.4 Treadmills

Two motor-driven treadmills (Woodway ELG70ERGO and ELG55ERGO, Weil am Rhein, Germany) were used in all of the studies reported in this thesis. The treadmills have a range of speed from 0 to 30 kph, and a range of gradient from 0 to 30%, which fulfilled the requirements for all of the investigations reported.

Prior to the start of each study, the treadmills were calibrated by measuring both the treadmill belt length (in metres) and the time taken (in seconds) to complete 50 revolutions at various speeds spanning the experimental range. Using the relationship between distance, time and speed, the actual speed of the treadmill was validated and the reliability of the integral digital speedometer confirmed.

3.5 Preliminary tests

3.5.1 *Familiarisation*

During the first visit to the laboratory, subjects were introduced to running on a motorised treadmill, the laboratory environment and the experimental procedures. Subjects were also invited to observe the experiment, and in some circumstances this was invaluable in helping to alleviate feelings of anxiety as to what was required and whether any trial could be completed efficaciously. Since treadmill running was the exercise mode used in all of the reported investigations, subjects were thoroughly familiarised with running on the specified treadmill. They were also introduced to the methods of collecting expired gas samples.

3.5.2 *Metabolic demand of submaximal running*

Following the full medical examination, each subject completed two preliminary tests before the main experimental trials. The purpose of the first preliminary test was to determine the oxygen cost ($\dot{V}O_2$) of treadmill running over a range of submaximal speeds. The actual speeds were 7, 9, 11 and 13 kph. Each subject exercised for 4 min at each speed during a continuous 16-min treadmill test. Expired gases were collected during the last 90 sec of each 4 min period, and indirect respiratory calorimetry data reported at 20 sec intervals (Sensormedics Vmax29, CA, USA). Heart rate and ECG were also monitored throughout the exercise period. Using linear regression analysis, the relationship between running speed and $\dot{V}O_2$ was established for each subject.

3.5.3 *Maximal oxygen uptake ($\dot{V}O_{2,max}$)*

The second preliminary test was performed to determine maximal oxygen uptake ($\dot{V}O_{2,max}$) using a continuous, incremental graded uphill treadmill running test to volitional exhaustion. The protocol was modified from that described by Taylor *et al.* (1955). The submaximal running speed was kept constant (13 kph) throughout the test. After the first 2 min of running on the level treadmill, the inclination was increased by 1% every minute. Maximal exertion was expected in this test and subjects were required to run for as long as possible. Expired gases were collected continuously and data reported at 20 sec intervals (Sensormedics Vmax29, CA, USA) throughout the test. The highest 20 sec average value for $\dot{V}O_2$ obtained during the last 2 min of exercise was considered to be the $\dot{V}O_{2,max}$ value. Heart rate and ECG were also monitored throughout the exercise period. Strong verbal encouragement was given from the experimental team throughout the test, particularly in the final 2 min. The criteria used to establish that a 'true' $\dot{V}O_{2,max}$ had been achieved were a plateau in $\dot{V}O_2$ with increasing exercise intensity, a final respiratory exchange ratio (RER) of 1.15 or above, a final heart rate within 10 $b \cdot \text{min}^{-1}$ of age-predicted maximum ($220 - \text{age}$), and volitional exhaustion (Bird and Davison 1997).

From the results of the submaximal $\dot{V}O_2$ test and the $\dot{V}O_{2,max}$ test, a treadmill speed that would elicit a relative metabolic demand equivalent to 60% $\dot{V}O_{2,max}$ was individually derived by means of linear regression (Chapters 5, 6 and 7). For the experiment reported in Chapter 4, these data were used to determine a treadmill speed that would elicit a metabolic demand equivalent to that observed during running at 9.5 kph whilst wearing the full military clothing ensemble and carrying the external backpack load.

3.6 Control of training and diet

Subjects were specifically instructed to avoid consuming caffeine and alcohol for 48 h prior to an experimental session. With the exception of the 24 h period prior to each experimental session, subjects were asked to consume their normal diet, keeping it as constant as possible during the experimental period. For the experiments reported in Chapter 6 and 7, subjects were also asked to avoid consuming foodstuffs derived from plants with the C_4 photosynthetic cycle, which are naturally enriched with ^{13}C (Péronnet *et al.* 1990), for 5 days before and throughout the experimental period. This was done so as to minimise possible shifts in background enrichment as a result of change in endogenous substrate utilisation and differences in background ^{13}C enrichment of endogenous fuel stores. Each subject was provided with a pre-packaged meal (3680 kcal: 55% carbohydrate; 35% fat; 10% protein; 170 mmol.d⁻¹ Na⁺) and instructions for its consumption during the 24 h period prior to each experimental session. Additional fluid requirements were met from the ingestion of water.

Subjects were instructed to consume ~ 10 ml.kg⁻¹ body weight of tap water on waking, and report to the laboratory after an overnight fast of at least 10h. With the exception of Chapter 7, subjects consumed a standardised breakfast meal of cereals and toast (685 kcal: 70% carbohydrate; 20% fat; 10% protein) on arrival at the laboratory. At least 120 min were allowed to elapse between consuming the breakfast and starting an exercise period.

Heavy physical exercise is known to reduce muscle (Tsintzas *et al.* 1995, 1996a) and liver glycogen (Bosch *et al.* 1994; McConnell *et al.* 1994) content, and cause

hypovolaemia (Montain and Coyle 1992) and haemoconcentration (Van-Beaumont *et al.* 1973) due to sweat loss without adequate fluid replacement. These factors will influence thermoregulation and exercise capacity, and may affect substrate metabolism. Therefore, in order to reduce any residual effects of prior physical activity on experimental measurements, subjects were asked to maintain a constant training programme throughout the experimental period and refrain from arduous training for 48-h prior to an experimental trial. Such practices have been shown to result in consistent pre-test endogenous glycogen concentrations (Miller *et al.* 1983).

3.7 Environmental conditions

All experiments were conducted within the Environmental Medicine Unit main environmental chamber at the Institute of Naval Medicine. The chamber has full temperature (5 to 50°C) and relative humidity (30 to 85%) control. It comprises a main laboratory area of approximately 50 m² where the treadmills were located for the experiments. Environmental conditions were maintained constant throughout the experiments, and data were recorded via wet bulb globe temperature (WBGT) monitors, positioned directly adjacent to the subjects torso on the frame of the treadmill. The following mean \pm standard error of the mean (SEM) environmental conditions were recorded during each of the experiments:

Chapter 4: a constant dry bulb temperature of 30.6 ± 0.1 °C, wet bulb temperature of 20.9 ± 0.1 °C, a relative humidity of 40.2 ± 0.2 % and air velocity of 3.1 ± 0.1 m.s⁻¹.

Chapter 5: a constant dry bulb temperature of 35.1 ± 0.1 °C, wet bulb temperature of 24.1 ± 0.1 °C, a relative humidity of 40.3 ± 0.2 % and air velocity of 3.1 ± 0.1 m.s⁻¹.

Chapter 6: a constant dry bulb temperature of 34.8 ± 0.1 °C, wet bulb temperature of 23.7 ± 0.1 °C, a relative humidity of 39.8 ± 0.1 % and air velocity of 3.1 ± 0.1 m.s⁻¹.

Chapter 7: *Temperate environment.* A constant dry bulb temperature of 19.3 ± 0.2 °C, wet bulb temperature of 12.2 ± 0.1 °C, a relative humidity of 40.2 ± 0.1 % and air velocity of 3.1 ± 0.1 m.s⁻¹.

Warm environment. A constant dry bulb temperature of 33.9 ± 0.1 °C, wet bulb temperature of 23.3 ± 0.1 °C, a relative humidity of 40.0 ± 0.1 % and air velocity of 3.1 ± 0.1 m.s⁻¹.

3.8 Standardised recovery protocol

A standardised recovery protocol was used in the experiments reported in Chapters 5 and 6, to investigate the influence of carbohydrate and fluid replacement regimens on recovery and the thermal and metabolic responses to subsequent exercise. This procedure is a modified version of that described by Fallowfield *et al.* (1995), and involved an initial constant pace treadmill run at 60% $\dot{V}O_2, max$ (T1), for 90-min or until aural temperature (T_{aur}) reached 39°C, whichever came first. This was followed by a 4-h rehydration-recovery period (REC), and a subsequent bout of exercise (T2) at the same intensity, to volitional fatigue or, until T_{aur} reached 39°C (Figure 3.1).

After the preliminary tests, subjects were required to complete two experimental trials in which two treadmill runs at 60% $\dot{V}O_2, max$ were performed in each experimental condition. Trials were separated by at least 7 days. The order of the experimental trials was randomised and administered in a double-blind crossover design.

The purposes of the T1 exercise periods were threefold: firstly, to collect initial response baseline data; secondly, to reduce endogenous carbohydrate (CHO) stores, particularly muscle glycogen (Ivy *et al.* 1988a; Tsintzas 1993; Tsintzas *et al.* 1996a); and finally, to induce body fluid losses, which if not replaced, impair subsequent thermoregulatory function and exercise capacity (Sawka 1992). The duration of REC was selected for comparison with similar recovery periods in previous investigations that assessed the effects of various fluid and CHO replacement regimens on recovery and subsequent exercise performance (Nielsen *et al.* 1986; Keizer *et al.* 1987; Ivy *et al.* 1988b; Lambert *et al.* 1992; Fallowfield *et al.* 1995; Wong *et al.* 1995, 1996a,b).

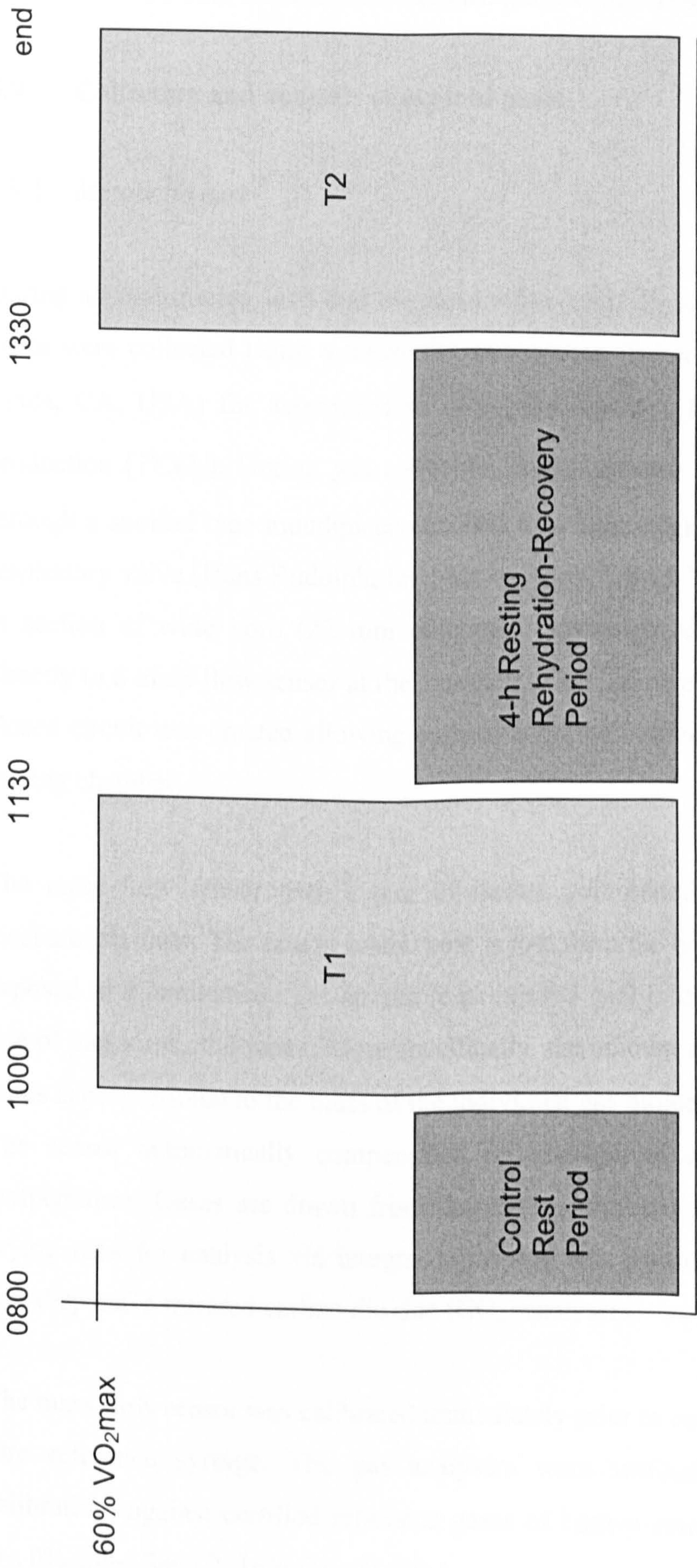


Figure 3.1 Schematic representation of the standardised recovery protocol.

It has been demonstrated that, at least in thermoneutral conditions, the standardised protocol adopted in these studies, provides a reliable measure for assessing short-term recovery from a bout of endurance exercise (Fallowfield 1994).

3.9 Collection and analysis of expired gases

3.9.1 *Metabolic cart*

During all preliminary tests and the main experiment reported in Chapter 7, expired gases were collected using a metabolic cart system (Sensormedics VMax29, Yorba Linda, CA, USA) for determination of oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$). During gas collection, subjects wore a nose clip and breathed through a snorkel type mouthpiece attached to a lightweight two-way low resistance respiratory valve (Hans Rudolph, Inc., Kansas City, USA). This was attached to a 1.5 m section of wide bore (22 mm diameter) lightweight dilution tubing connected directly to a mass flow sensor at the entrance to the 2.6 litre mixing chamber. Thus, a closed circuit was created allowing expired air to be continuously analysed from the mixing chamber.

The mass flow sensor uses a pair of heated gold-plated stainless steel wires to measure gas flow. The rate at which heat is lost from the heated wires when they are exposed to a laminarised gas stream (e.g. expired gas) is directly related to the flow rate of gas across the wires. More specifically, the amount of heat extracted from the wires is proportional to the mass of the individual gas molecules flowing across them. The sensor automatically compensates for changes in ambient and expired gas temperatures. Gases are drawn from the mixing chamber via a Nafion Perma Pure drying tube for analysis via integral rapid-response paramagnetic oxygen (O_2) and non-dispersive infrared carbon dioxide (CO_2) analysers.

The mass flow sensor was calibrated immediately prior to each exercise test using a 3-litre reference syringe. The gas analysers were similarly calibrated (two point calibration) against certified reference gases of known concentrations (Span 1, 26% O_2 , 0% CO_2 ; Span 2, 16% O_2 , 4% CO_2).

Using the Haldane transformation formula (Consolazio *et al.* 1963), the measured gas volumes were automatically corrected to standard temperature and atmospheric pressure for a dry gas, by the integral software. This allowed the calculation of oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), minute ventilation ($\dot{V}E$) and the respiratory exchange ratio (RER).

3.9.2 Douglas bag

During the main experimental trials reported in Chapters 4, 5 and 6, expired gas samples were collected in 150 litre capacity Douglas bags (Harvard Equipment Ltd.) for determination of $\dot{V}O_2$ and $\dot{V}CO_2$. During gas collection, subjects similarly wore a nose clip and breathed through a snorkel type mouthpiece attached to a lightweight two-way low resistance respiratory valve (Hans Rudolph, Inc., Kansas City, USA), attached to a 1.5 m section of wide bore (22 mm diameter) tubing. Again, a closed circuit was established, allowing expired gases to be collected over a measured time period (5 min for resting samples and 1 min for exercise samples).

The fraction of expired O_2 and CO_2 were measured using a paramagnetic oxygen analyser and an infrared CO_2 analyser (Taylor Servomex, Series 1400). Both analysers were calibrated against certified reference gases of known concentrations (BOC gases Ltd., Guildford, Surrey, UK) and room air immediately prior to each series of gas analyses. The O_2 and CO_2 analysers were connected in series to a Harvard digital dry gas meter and gas was drawn through the system by an evacuation pump (Moulinex vacuum pump 237) fitted to a flow regulator. The dry gas meter had been calibrated against a Tissot Model 6001 spirometer (Collins Ltd., USA). Expired gas temperature was determined during evacuation by a thermistor placed in the air outlet pipe of the dry gas meter and connected to a digital squirrel data logger (Grants Instruments Ltd., Cambridge, UK). The digital readout of the thermistor was calibrated prior to each experiment in a water bath at 25 and 40 °C.

Again, using the Haldane transformation formula (Consolazio *et al.* 1963), the measured gas volumes were corrected to standard temperature and atmospheric pressure for a dry gas, and $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}E$ and RER calculated.

3.9.3 Isotope Ratio Mass Spectrometry

In the experiments reported in Chapters 6 and 7, subjects ingested glucose containing [U-¹³C]-glucose in order that exogenous glucose oxidation rates could be calculated from the change in ¹²C/¹³C ratio of breath CO₂. As such, expired gas samples were collected into 10-ml Vacutainers, in triplicate, and were subsequently analysed for ¹³C/¹²C ratio of expired CO₂ by Isotope Ratio Mass Spectrometry (IRMS). In the study reported in Chapter 6, these analyses were performed in the Institute of Human Nutrition of Southampton General Hospital on an ANCA system (Europa Scientific, Crewe, England). In the study reported in Chapter 7, these analyses were performed in the Stable Isotope Research Centre (SIRC) of Maastricht University on a Finnigan MAT 252 (Bremen, Germany).

The isotope enrichment of expired CO₂ was then expressed as the delta per mil (mil = 1000) difference between the ¹³C/¹²C ratio of the sample and a known laboratory reference standard, according to the formula:

$$\delta^{13}C = \left(\left(\frac{{}^{13}C/{}^{12}C \text{ sample}}{{}^{13}C/{}^{12}C \text{ standard}} \right) - 1 \right) \cdot 10^3 \quad (3.2)$$

Twenty samples were analysed in duplicate in order that the intra-assay coefficient of variation (CV) could be determined. The mean ¹³C enrichment of the 20 samples was -20.6 ‰ vs. PDB and the intra-assay CV was 0.6%.

3.10 Substrate oxidation rates

In each of the experiments reported in this thesis the principle of indirect respiratory calorimetry was used to estimate substrate oxidation rates and energy expenditure. Although the technique has been used mainly for the determination of metabolic rate, it has been recognised for almost a century that measurements of $\dot{V}O_2$ and $\dot{V}CO_2$ can also give information on the type and rate of fuel oxidation within the body (Lusk 1923). The calculation of rates of glucose, fat and protein oxidation, based on measurements of gaseous exchange together with urinary nitrogen excretion, has seen

increasing use recently in clinical (Owen *et al.* 1980) and exercise physiology (Tsintzas *et al.* 1995, 1996a; Jeukendrup *et al.* 1999a). However, the use of such calculations is based on a number of principles and assumptions which must be considered.

Firstly, during exercise studies, protein oxidation is often considered to be negligible, despite the fact that it can be estimated from nitrogen excretion in urine and sweat (Lemon and Nagle 1981). Evidence suggests that in fed subjects, protein oxidation may contribute as little as ~5% to the total energy requirements of moderate intensity exercise (Lemon and Mullin 1980), and that this contribution is less when exercise is performed in a warm environment (Dolny and Lemon 1988). It therefore seems reasonable to assume that protein oxidation was small and negligible in the trials reported in this thesis.

Although computations provide an estimation of the amount of carbohydrate oxidised, expressed as an amount of glucose, no conclusion can be drawn concerning the source of the glucose which is oxidised (e.g. muscle or liver glycogen, exogenous glucose). Similarly, the amount of fat oxidised is expressed as an amount of free-fatty acid(s), representative of the composition of triacylglycerol stores, but no conclusion can be drawn concerning the type of fats actually oxidised or the source of this substrate (e.g. adipose stores, muscle triacylglycerol).

It is also assumed that other metabolic processes, which involve the production and/or utilisation of O₂ and/or CO₂ (e.g. gluconeogenesis and lipogenesis) are quantitatively negligible compared to glucose and fat oxidation (Ferrannini 1988; Frayn 1983). Furthermore, it is assumed that the RER adequately reflects the respiratory quotient. That is, there is no change in carbon dioxide production measured at the mouth due to hypo or hyperventilation or changes in body CO₂ content from the bicarbonate pool (Frayn 1983). As the O₂ reserves of the body are relatively small in comparison to the rate of O₂ consumption at moderate exercise intensities, and arterial blood O₂ concentration remains fairly constant at rest and during exercise, it is also assumed that whole body $\dot{V}O_2$ is a direct reflection of O₂ consumption in the tissues. As a result, previously described non-protein respiratory quotient calculations, revised

from those proposed by Lusk (1923), were used to calculate substrate oxidation rates in this thesis (Péronnet and Massicotte 1991):

$$\text{glucose oxidation (g.min}^{-1}\text{)} = 4.585 \dot{V}CO_2 - 3.226 \dot{V}O_2 \quad (3.3)$$

$$\text{fat oxidation (g.min}^{-1}\text{)} = 1.695 \dot{V}O_2 - 1.701 \dot{V}CO_2 \quad (3.4)$$

3.11 Skin and deep body temperature

3.11.1 Mean skin temperature (T_{skin})

Four interchangeable skin temperature thermistors (Grants Instruments Ltd., Cambridge, UK) were used to monitor skin temperature, following calibration in a water bath at 20, 30 and 40°C. They were placed on the posterior of the calf, quadriceps, upper arm and chest as described by Mitchell and Wyndham (1969). Thermistors were connected to a digital squirrel data logger (Grants Instruments Ltd., Cambridge, UK) and data recorded to the nearest 0.05°C at 1 min intervals throughout all exercise periods. Weighted mean skin temperature (T_{skin}) was subsequently calculated according to the method described by Ramanathan (1964):

$$T_{skin} = (0.3 \times (T_{chest} + T_{arm})) + (0.2 \times (T_{calf} + T_{thigh})) \quad (3.5)$$

3.11.2 Deep body temperature

Deep body temperature, commonly referred to as core temperature can be measured at several anatomical locations, including the rectum, tympanic membrane, aural canal, oral cavity and axilla. Whilst oral and axillary measurements are easily accessible, they do not always accurately reflect core temperature. Recently, tympanic membrane temperature (T_{ty}) and aural canal temperature (T_{aur}) have been widely used as an index of deep body temperature because of their ease of access and alleged correlation with brain or hypothalamic temperature (Benzinger 1969; Baker *et al.* 1972; Brengelmann *et al.* 1977; Germain *et al.* 1987; Burges *et al.* 1988). However, T_{ty} and T_{aur} have been observed to fluctuate with changes in facial skin temperature and may therefore underestimate the degree of hyperthermia (Roberts 1994). On the other hand, rectal temperature (T_{rec}) is generally regarded as a good indicator of core temperature

because of its independence from changes in skin and environmental temperature (Livingstone *et al.* 1983; Deschamps *et al.* 1992).

In order to compare T_{aur} and T_{rec} measurements, both were simultaneously recorded throughout exercise periods in the study reported in Chapter 4. Rectal temperature was measured with a rectal thermistor (Grants Instruments Ltd., Cambridge, UK), inserted to a depth of 10 cm, which had previously been calibrated in a water bath at 30, 40 and 50°C. Aural canal temperature was measured by placing similarly calibrated thermistors into the aural canal on the left and right sides (Shiraki *et al.* 1986), and the aural canal insulated with cotton wool. Thermistors were connected to a data logger (Grants Instruments Ltd., Cambridge, UK) and data recorded to the nearest 0.05°C at 1 min intervals throughout exercise.

The relationship between T_{aur} and T_{rec} is given in Figure 3.2. Aural canal temperature was consistently lower than rectal temperature and the magnitude of this difference was dependent on skin temperature. When subjects exercised whilst wearing the protective clothing ensemble (CFO), skin temperature was higher (35.5 ± 0.3 °C) than in the control (CON) trial (33.8 ± 0.2 °C). In the CFO trial, the magnitude of the difference between T_{rec} and T_{aur} was less than in the CON trial, particularly at higher deep body temperatures, providing further evidence that T_{aur} is influenced by changes in skin temperature.

In the experiments reported in Chapters 5 and 6, the environmental conditions and skin temperatures were similar in all experimental conditions and T_{aur} was therefore deemed to be a suitable method of assessing deep body temperature. In the experiment reported in Chapter 7, subjects were exposed to two different environments (19 and 34 °C). In such circumstances, differences in skin temperature were expected and T_{rec} was therefore used to assess deep body temperature. This permitted the comparison of deep body temperature between experimental conditions which were independent of skin temperature.

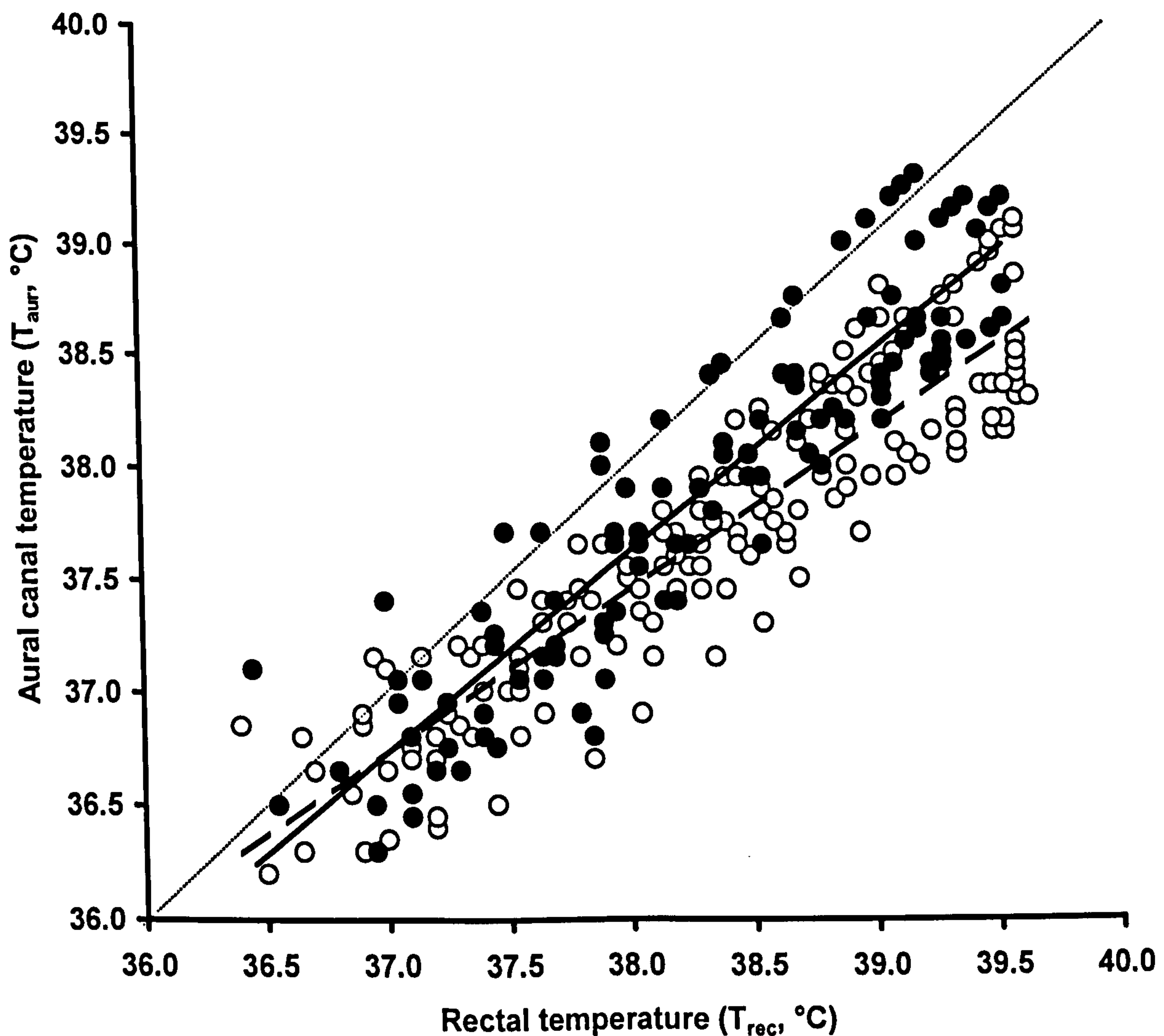


Figure 3.2 Relationship between rectal temperature (T_{rec}) and aural canal temperature (T_{aur}) during the CON (○) and CFO (●) trials reported in Chapter 4. The linear relationship between T_{rec} and T_{aur} are indicated as follows: 1-1 relationship (.....); CFO trial (—); CON trial (— —).

3.12 Fluid balance

3.12.1 Sweat rate during exercise

In all of the trials reported in this thesis, total sweat rate during each exercise period was calculated. This value was derived from the change in nude body mass from pre to post exercise, corrected for fluid intake and urinary excretion. This value was then expressed as a function of exercise time (rate), as follows:

$$\text{Sweat rate (g.min}^{-1}\text{)} = ((\Delta BM + FI) - UE) / t \quad (3.6)$$

where ΔBM is the change in body mass from pre to post exercise (g), FI is the total volume of fluid ingested during exercise (g), UE is the volume of urine excreted during exercise (g) and t is the exercise time (min).

Values were further corrected for respiratory water vapour loss during exercise, using a previously described method (Mitchell *et al.* 1972):

$$\dot{m}e \text{ (g}\cdot\text{min}^{-1}\text{)} = 0.019 \dot{V}O_2 (44 - Pa) \quad (3.7)$$

where $\dot{m}e$ is the rate of evaporative water loss in expired air, $\dot{V}O_2$ is the oxygen uptake ($\text{l}\cdot\text{min}^{-1}$ STPD) and Pa is the ambient water vapour pressure (mm Hg).

3.12.2 Rehydration during recovery

In Chapters 5 and 6, body fluid balance after the 4-h recovery period was estimated according to the method described by Gonzalez-Alonso *et al.* (1992). The percent gain in body mass during the recovery period relative to the loss of mass during the preceding exercise period provided an index of rehydration. Net fluid balance was also calculated from body mass loss, and volumes of ingested fluid and urine excreted. Subjects passed urine into a sterilised 24-h urine collection bottle throughout the recovery period.

3.13 Perceptual variables

During all exercise trials reported in this thesis, subjects were asked to provide their rating of perceived exertion (RPE) at 15-min intervals. This was conducted using the twenty point linear rating scale described by Borg (1973). In order to monitor the subjects' perception of thermal discomfort, a ten point linear rating scale was also used, where 1 denoted "comfortable" and 10 denoted the perception of being "unbearably hot".

3.14 Blood sampling

3.14.1 *Capillary blood sampling*

The haemodynamic responses to changes in posture are well documented (Harrison 1985; Shirrefs and Maughan 1994). On moving from an upright to a supine position, a marked increase in plasma volume occurs as a result of the altered capillary filtration pressure, and conversely, this effect is reversed on standing. These haemodynamic alterations are essentially complete within 15 to 20 min. Prior to each resting capillary blood sample (Chapters 5 and 6 only) subjects were therefore required to adopt a standing position for at least 20 minutes. After this period pre-exercise duplicate 50 μ l capillary blood samples were collected from the finger of a pre-warmed hand using an auto-clix automatic lancet (Boehringer Mannheim UK Ltd.). Further capillary blood samples were similarly obtained at 30-min intervals during and at the end of each exercise period. Samples were mixed thoroughly for 3 min and immediately analysed for blood lactate and glucose concentrations (Analox P-GM7, Analox Instruments, UK).

3.14.2 *Venous blood samples*

In the studies reported in Chapters 5 and 6 venous blood samples (10 ml) were drawn from an antecubital vein (by venepuncture) before and after each bout of exercise. During the experiment reported in Chapter 6, blood samples were similarly drawn after 60 and 180 min of the 4-h recovery period. As all of these samples were resting blood samples, they were drawn after subjects' had been lying supine for a period of at least 20-min.

In the studies reported in Chapters 4 and 7, venous blood samples were obtained before and at 15-min intervals during exercise, from an indwelling cannula (18 gauge Venflon). Cannulae were kept patent by infusion of heparinised saline solution (0.9% w/v sodium chloride, Steripak Ltd., UK) following each sample and remained *in situ* throughout the experimental trial. As noted previously, subjects were required to remain standing for a period of 20-min prior to the collection of each resting blood sample in order to minimise the effect of hydrostatically induced changes in

haemoconcentration, and allow comparisons to be made between resting and exercising data.

All venous blood samples were drawn into sterile syringes and dispensed into blood collection tubes (Becton Dickinson Vacutainers, Meylan, Cedex-France). Serum was collected by allowing whole blood to clot for 1-h (Chapters 4, 6 and 7), followed by chilled centrifugation (4°C) at 3000 rpm for 15 min (Labofuge 400R, Heraeus Instruments, Osterode, Germany). The remaining whole-blood was added to tubes containing either lithium-heparin or ethylenediaminetetra-acetic acid (EDTA) as anticoagulants. A 2 ml aliquot of blood was immediately analysed for haemoglobin concentration and packed cell volume (see section 3.15). The remaining tubes were subsequently centrifuged at 3000 rpm for 15-min (4°C) to obtain plasma. Aliquots of plasma were dispensed into 2-ml cryogenic vials and immediately stored at -70°C and later analysed for various metabolites and hormones (see section 3.15).

3.15 Analysis of blood samples

Some of the biochemical assays were performed in the Environmental Medicine Unit at the Institute of Naval Medicine. However, measurements of plasma free fatty acids (FFA) and glycerol and serum insulin, cortisol and aldosterone were performed in the Department of Physical Education, Sports Science and Recreation Management of Loughborough University. In addition, measurements of plasma [¹³C]-glucose and [²H₂]-glucose enrichment were performed in the Stable Isotope Research Centre (SIRC) of Maastricht University (Chapter 7).

Haemoglobin concentrations were measured in triplicate by the cyanmethaemoglobin method (Boehringer Mannheim GmbH Diagnostica, Germany). Haematocrit (packed cell volume) was determined in triplicate using a micro-haematocrit reader (Hawksley Ltd., UK), following centrifugation at 3000 rpm for 15-min. Haemoglobin and haematocrit values were used to estimate percentage change in plasma volume (%ΔPV), using the method of Dill and Costill (1974):

$$\% \Delta PV = \left[\frac{hbg_1 \times (1 - hct_2)}{hbg_2 \times (1 - hct_1)} - 1 \right] \times 100 \quad (3.8)$$

where $\% \Delta PV$ is the percentage change in plasma volume, hbg is the haemoglobin concentration, hct is the haematocrit ratio or packed cell volume and subscripts 1 and 2 indicate baseline and individual time point values, respectively.

In the experiments reported in Chapters 5 and 6, glucose and lactate concentrations were determined from whole capillary blood samples. Samples were mixed thoroughly for 3-min and subsequently analysed using a commercially available method (Analox P-GM7, Analox Instruments, UK). In the experiments reported in Chapters 4 and 7 plasma samples were thawed at room temperature, mixed thoroughly and centrifuged at 3000 rpm for 3-min prior to analysis. Glucose and lactate concentrations were subsequently determined using a commercially available enzymatic method (Abx Diagnostics, Montpellier, France) and performed on an automated system (COBAS Mira Plus, Roche Diagnostic Systems, Switzerland).

Plasma free fatty acid (FFA) concentrations were similarly determined on an automated system using a commercially available enzymatic method (NEFA-C test kit, Wako, Japan). Plasma glycerol concentrations were determined using a fluorimetric method described by Laurrell and Tibbling (1966) on a standard fluorimeter. In the experiment reported in Chapter 4, plasma ammonia concentrations were also determined using a commercially available spectrophotometric method (Boehringer Mannheim GmbH Diagnostica, Germany). These analyses and the haemoglobin method described earlier, were carried out on a standard spectrophotometer (HACH DR/4000U, Camlab Ltd., Cambridge, UK).

Serum insulin, aldosterone and cortisol concentrations were determined using commercially available I^{125} radio-immunoassays (Coat-A-Count, Diagnostic Products Corporation, USA). Radioactivity was measured using an automated gamma counter (Cobra II, Packard Instruments Co., USA).

3.15.1 High-performance liquid chromatography

Plasma adrenaline and noradrenaline concentrations were determined by high-performance liquid chromatography (HPLC), using a commercially available method (Bio-Rad Laboratories, CA, USA), by electrochemical detection. The HPLC system consisted of a pump and integrator (Hewlett Packard Series 1100, Germany) coupled to an electrochemical detector (Hewlett Packard 1049, Germany).

3.15.2 Mass spectrometry

For determination of $^{13}\text{C}/^{12}\text{C}$ ratios of plasma glucose, glucose was first extracted with chloroform-methanol-water and derivatisation was performed with butyl-boronic acid and acetic anhydride (Pickert *et al.* 1991). Thereafter, the derivative was measured by gas chromatography isotope ratio mass spectrometry (GC-IRMS) on a Finnigan MAT 252 (Bremen, Germany). By establishing the relationship between the enrichment of a series of glucose standards of variable enrichment (by combustion-IRMS; Carlo Erba-Finnigan MAT 252, Bremen, Germany) and the enrichment of the glucose trimethylsilyl derivative of these standards, the enrichment of plasma glucose samples was determined. Plasma $^2\text{H}_2$ -glucose enrichment was determined by gas chromatography mass spectrometry (GC-MS) analysis of the derivatives on a Finnigan INCOS-XL (Bremen, Germany). For ^2H glucose enrichment, ion masses of 200 and 202 were selectively monitored.

3.15.3 Coefficient of variation

The intra-assay coefficient of variation ($(SD / mean) \times 100$) for each assay is given in Table 3.1. Each coefficient of variation was determined using at least 15 samples measured in duplicate. The mean concentration of each series of analysis is also included in Table 3.1.

Assay	Units	Mean concentration	Number (<i>n</i>)	CV (%)
Haematocrit	%	40	50	1.8
Haemoglobin	g.dl ⁻¹	145.1	50	0.9
Lactate (Analox method)	mmol.l ⁻¹	1.3	25	8.0
Lactate (Abx method)	mmol.l ⁻¹	1.3	25	1.7
Glucose (Analox method)	mmol.l ⁻¹	5.2	25	3.4
Glucose (Abx method)	mmol.l ⁻¹	5.5	25	1.8
FFA	mmol.l ⁻¹	0.50	25	2.6
Glycerol	mmol.l ⁻¹	0.25	25	5.9
Ammonia	nmol.l ⁻¹	70.2	15	9.1
Insulin	μIU.ml ⁻¹	25.1	15	8.5
Aldosterone	pmol.l ⁻¹	619	15	6.5
Cortisol	nmol.l ⁻¹	409	15	7.4
Adrenaline	nmol.l ⁻¹	0.90	15	9.5
Noradrenaline	nmol.l ⁻¹	11.2	15	7.6
¹³ C-glucose enrichment	δ‰ vs. PDB	56.2	15	0.4
² H ₂ -glucose enrichment	APE	5.8	15	0.4

Table 3.1 Intra-assay coefficient of variation (CV%) for all biochemical assays.

3.16 Statistical analysis

All analyses were conducted using a software programme (Statistical Package for the Social Sciences, SPSS Version 10.0).

Statistical comparisons were made after establishing the normality or otherwise of the data distribution using the Anderson-Darling Goodness of Fit test (Anderson and Darling 1954). Where normality of the data distribution could not be assumed, a Wilcoxon signed ranks test was used to compare group means. Where normality of the data distribution could be assumed, parametric statistics were preferred, since this permitted the use of more powerful statistical techniques. In these circumstances, statistical significance was assessed by Analysis of Variance (ANOVA) for repeated measures techniques. The assumptions underlying this technique are that the variances are equal and that any covariances are zero. Descriptive data are presented as mean \pm standard error of the mean (SEM).

F-ratio values were adjusted by the Greenhouse-Geisser Epsilon correction in repeated measures ANOVA where the assumption of the sphericity appeared to be violated. Use of the Greenhouse-Geisser gives a more conservative estimate of significance. In the case of a significant *F*-ratio, a Tukey post-hoc test was applied to identify differences. Differences between and within trials were considered significant when $P < 0.05$.

CHAPTER FOUR

INFLUENCE OF A PROTECTIVE CLOTHING ENSEMBLE ON SUBSTRATE METABOLISM
AND EXERCISE TOLERANCE TIME DURING PROLONGED RUNNING
IN A WARM ENVIRONMENT**4.1 Introduction**

Military personnel are often required to perform repeated bouts of prolonged (1-2 hours), moderate intensity (60-70 % $\dot{V}O_{2max}$) exercise on the same or successive days. During such activities personnel have the additional burden of exercising whilst wearing protective clothing and carrying auxiliary equipment, even in hot and humid environments. As such, personnel are often exposed to uncompensable heat stress (Montain *et al.* 1994) and are unable to achieve thermal equilibrium. The ensuing hyperthermia may lead to an earlier onset of physiological fatigue (Nielsen *et al.* 1993; Nielsen 1994) and in extreme circumstances, heat illness. Exertional heat illness (EHI) therefore remains a substantial problem during military operations and training (Joint Services Publication 539), and generally occurs during sustained physical activity in hot, humid environments (Gardner *et al.* 1996). In an attempt to prevent such illnesses occurring, military guidelines for the avoidance of heat illness, based on maximum Wet Bulb Global Temperature (WBGT), were introduced (Defence Council Instructions JS 122/01).

It is apparent that WBGT guidelines alone are inadequate to prevent heat related illnesses occurring during military exercises. Indeed, environmental heat stress is but one factor in the risk of developing heat illnesses. The effects of clothing and load-carriage during exercise are equally, if not more important causes of heat strain. It is widely accepted that unless work rates are reduced, load carriage increases the metabolic demand of exercise (Goldman and Iampietro 1962). In addition, both clothing and load carriage impair heat dissipation, particularly sweat evaporation, which is the primary mechanism for heat dissipation during exercise in a hot environment (Fox *et al.* 1966). Although the influence of clothing and load carriage on the thermoregulatory responses to exercise are well documented (Givoni and

Goldman 1972, 1973), relatively little is known regarding their influence on other physiological mechanisms relating to the onset of fatigue during prolonged exercise.

Previous investigations have shown that during exercise and environmental heat stress, the drive to exercise is diminished by increased core temperature (Nielsen *et al.* 1990; Sawka 1992). More recent studies have demonstrated that metabolic alterations may account, in part, for physiological fatigue during exercise induced hyperthermia. Data suggest that at higher core temperatures, the rate of carbohydrate (CHO) oxidation (Febbraio *et al.* 1994a), muscle glycogen utilisation (Febbraio *et al.* 1994b) and blood and muscle lactate accumulation (Young *et al.* 1985) increase during prolonged cycling exercise. This is important because carbohydrate, particularly muscle glycogen availability, is a primary determinant of endurance exercise capacity (Bergstrom *et al.* 1967).

Whilst various mechanisms have been proposed for the influence of heat stress on substrate metabolism, increases in circulating catecholamine concentrations have been consistently observed (Febbraio *et al.* 1994a, 1996; Hargreaves *et al.* 1996a, 1996b; Gonzalez-Alonso *et al.* 1999; Parkin *et al.* 1999). Indeed, the response of the adrenal medulla, which secretes these hormones, to exercise is greater with increasing heat strain (Galbo *et al.* 1979). Furthermore, adrenaline infusion has been shown to stimulate glycogenolysis in the type I fibres of electrically stimulated rat (Richter *et al.* 1982) and human skeletal muscle (Greenhaff *et al.* 1991; Mora-Rodriguez *et al.* 2001). Catecholamine induced changes in glycogenolysis and muscle fibre recruitment have therefore been proposed as mechanisms responsible for increased CHO oxidation during exercise in a hot environment (Febbraio *et al.* 1994a, 1994b).

Despite these findings, we are unaware of any studies that have examined the effects of heat strain imposed by a protective clothing ensemble on whole-body metabolism during sustained exercise. Furthermore, no study to date has examined the influence of heat strain on substrate metabolism during prolonged running. Therefore, the purpose of this experiment was to test the hypothesis that the heat strain induced by a military clothing ensemble would increase CHO oxidation rates and circulating catecholamine concentrations, and reduce exercise tolerance time during prolonged running in a warm environment.

4.2 Methods

4.2.1 Subjects

Eleven healthy male volunteers acted as subjects for this study, which was carried out with the approval of the Ministry of Defence (Navy) Personnel Research Ethics Committee (MOD(N) PREC). Written consent to participate was provided by all subjects after the nature of the study had been explained to them. All were involved in various training programmes, including prolonged submaximal running and marching in military protective clothing ensembles. Mean \pm SEM ($n = 11$) physical characteristics of the subjects were: age, 29.7 ± 1.4 years; height, 180.8 ± 2.3 cm; body mass, 81.1 ± 2.9 kg; body fat, 15.8 ± 1.0 %.

4.2.2 General design

All Subjects were randomly assigned, within a crossover design, to complete two trials, at least 7-d apart. During the Combat Fighting Order (CFO) trial subjects dressed in denim trousers, shirt and combat jacket (~ 2 -kg) and carried a 15-kg load, whilst running at 9.5-kph on a level treadmill. During the control trial (CON) subjects dressed in shorts and running shoes, and ran at a treadmill velocity individually calculated to elicit an equivalent metabolic demand (10.8 ± 0.2 kph). Subjects were required to run until volitional exhaustion, or until T_{rec} reached 39.5°C , whichever came first. All exercise periods took place in an environmental chamber which was maintained at a mean \pm SEM: dry bulb temperature, 30.6 ± 0.1 °C; wet bulb temperature, 20.9 ± 0.1 °C; relative humidity, 40.2 ± 0.2 %; and air velocity, 3.1 ± 0.1 m.s⁻¹. The environment was selected to approximate the highest permissible wet bulb globe temperature (WBGT) in which military personnel are permitted to exercise for up to 60 min.

4.2.3 Preliminary measurements

Following a full medical examination and the estimation of percentage body fat from four skinfold measurements (Durnin and Womersley 1974), subjects were familiarised with treadmill running and with the experimental procedures. Each subject then completed three preliminary tests at least 7 d before the two main trials.

The first two were to assess the oxygen cost of submaximal running and maximal oxygen uptake (mean \pm SEM $\dot{V}O_{2max}$ 58.4 ± 1.5 ml.kg⁻¹.min⁻¹), as described in Chapter 3. Following a 20-min recovery period, a third test was performed to determine 'steady-state' $\dot{V}O_2$ during treadmill running at 9.5-kph whilst wearing a military (Combat Fighting Order; CFO) clothing ensemble (see procedures for description). Using the linear relationship between $\dot{V}O_2$ and treadmill velocity, a running speed was calculated to elicit the same $\dot{V}O_2$ during the CON trial. This ensured that metabolic heat production was approximately the same during both trials. These conditions were used to simulate the relative metabolic demand of 6-mile military speed march.

4.2.4 *Procedures*

On reporting to the laboratory subjects were seated in a room maintained at 22°C, where they consumed a standard breakfast (685 kcal: 117 g CHO) and 1 l of water. After lying recumbent for approximately 10 min an 18 gauge teflon catheter was inserted into an antecubital vein and flushed with 0.9% saline. Ninety minutes later subjects emptied their bladder and nude body mass was recorded (Salter SD100). Following instrumentation with a rectal thermistor (inserted to a depth of 10 cm; T_{rec}), two aural thermistors (T_{aur}), four skin thermistors (Ramanathan 1964; T_{skin}) and three ECG chest electrodes, subjects dressed in the appropriate clothing ensemble and clothed body mass was recorded.

Subjects entered the environmental chamber and stood in position on the treadmill for 15 min before, baseline T_{rec} , T_{aur} , T_{skin} and heart rate were recorded, a 5 min expired gas sample was collected (Douglas bag) and a 20 ml blood sample was drawn. The treadmill speed was then increased accordingly and further venous blood samples (20 ml) and expired gas samples (2 min) were collected at 15-min intervals throughout and at the end of exercise. Upon cessation of exercise clothed and nude body masses were recorded, and subjects lay recumbent in the recovery room (22°C) whilst being actively cooled with electronic fans.

4.2.5 *Experimental measurements and calculations*

The fraction of oxygen (FeO_2) in expired gas samples was measured by a paramagnetic O_2 analyser, percentage carbon dioxide (FeCO_2) was measured by an infrared CO_2 analyser (Taylor Servomex, Series 1400) and minute ventilation was measured by a dry gas meter (Harvard, Kent UK). From gas analyses, $\dot{V}E$, $\dot{V}O_2$ and $\dot{V}CO_2$ were determined and the respiratory exchange ratio (RER) calculated. Rates of total carbohydrate and fat oxidation (CHO-ox and FAT-ox) were calculated from $\dot{V}O_2$ and $\dot{V}CO_2$, as previously described (Péronnet and Massicotte 1991):

$$\text{CHO-ox (g.min}^{-1}\text{)} = 4.585 \dot{V}CO_2 - 3.226 \dot{V}O_2 \quad (4.1)$$

$$\text{FAT-ox (g.min}^{-1}\text{)} = 1.695 \dot{V}O_2 - 1.701 \dot{V}CO_2 \quad (4.2)$$

Total sweat loss (g) over the exercise period was calculated from the difference between pre- and post-exercise nude body mass, corrected for fluid intake, urine production and respiratory water vapour. The amount of sweat which was evaporated (g) during exercise was calculated from the difference between pre- and post-exercise clothed body mass. By expressing these data relative to exercise duration, total sweat rates and sweat evaporation rates (g.min^{-1}) were subsequently derived.

4.2.6 *Analytical methods*

Part (6 ml) of each venous blood sample was decanted into EDTA-containing tubes and used for the determination of haemoglobin (cyanmethaemoglobin method: Boehringer Mannheim, GmbH Diagnostica) and spun microhaematocrit, in triplicate. These data were used for the determination of changes in plasma volume ($\%\Delta\text{PV}$) relative to pre-exercise (Dill and Costill 1974). The remaining EDTA sample was centrifuged for 15-min at 4°C . Aliquots of plasma were decanted into separate tubes and either immediately analysed for ammonia concentration (Boehringer Mannheim, GmbH Diagnostica) or frozen at -70°C and later analysed for free-fatty acid (FFA; Wako Chemical GmbH) lactate and glucose (Boehringer Mannheim, GmbH Diagnostica) concentrations, using commercially available kits. A further 4-ml of each sample was left to clot for 1-h at 10°C and the serum separated by centrifugation for 15-min at 4°C . Serum was stored at -70°C and later analysed for insulin, cortisol

and aldosterone concentration (^{125}I radioimmunoassay; Coat -A-Count, DPC, CA). The remainder of each sample (10-ml) was decanted into lithium heparin tubes and plasma obtained by centrifugation for 15 min at 4°C. After storage at -70°C each sample was analysed for catecholamine concentrations (adrenaline and noradrenaline), by high performance liquid chromatography (HPLC), using a commercially available kit (Bio-Rad Laboratories, UK).

4.2.7 *Statistical analysis*

Statistical comparisons were made after establishing the normality or otherwise of the data distribution using the Anderson-Darling Goodness of Fit test (Anderson and Darling 1954). A two-way analysis of variance (ANOVA) with repeated measures was used to compare results between treatments and across time. Where significant *F*-ratios were found, a Tukey post-hoc test was used to determine the cause of the variance. When there were only single comparisons, a Student's *t*-test for correlated data was used to determine whether differences between treatments existed. Descriptive data are presented as mean \pm standard error of the mean (SEM). Differences between and within trials were considered significant when $P < 0.05$.

4.3 Results

4.3.1 *Exercise tolerance time*

The mean \pm SEM duration of the exercise periods was 25.8 ± 4.5 min longer during the CON (65.9 ± 5.2 min, range 47.5 to 90.0 min) compared to the CFO (40.1 ± 3.2 min, range 30.0 to 63.3 min) trial ($P < 0.01$). Eight of the eleven CFO trials were terminated with subjects showing signs and symptoms indicative of heat exhaustion (e.g. dizziness, nausea, hyperventilation and disorientation). In contrast, only one subject showed similar symptoms during the CON trial.

4.3.2 *Sweat production*

Total sweat production was greater ($P < 0.01$) in the CON (2067 ± 205 g) compared to the CFO (1567 ± 137 g) trial. When expressed relative to exercise time, sweat rates were higher ($P < 0.01$) in the CFO (39.3 ± 1.8 g.min $^{-1}$) compared to the CON (31.2 ± 1.4 g.min $^{-1}$) trial. Furthermore, the total amount of sweat which was evaporated was lower ($P < 0.01$) in the CFO (884 ± 79 g) compared to the CON trial (1722 ± 170 g).

Thus, the percentage of sweat produced which was evaporated was lower ($P < 0.01$) in the CFO ($56 \pm 2 \%$) compared to the CON trial ($83 \pm 2 \%$).

4.3.3 *Metabolic responses*

There was no difference in mean exercise $\dot{V}O_2$ between the CON and CFO trials at any time point during exercise (Table 4.1). After 15-min of exercise subjects were working at the same approximate $\dot{V}O_2$ in the CON ($68.6 \pm 2.1 \%$ $\dot{V}O_{2, \max}$) and CFO trials ($69.3 \pm 2.3 \%$ $\dot{V}O_{2, \max}$), respectively. Values were observed to increase over time in both trials ($P < 0.01$). Mean exercise $\dot{V}CO_2$ was greater ($P < 0.05$) after 45-min of the CFO compared to the CON trial. The RER was greater after 30-min and 45-min ($P < 0.05$) of exercise during the CFO compared to the CON trial.

Total carbohydrate oxidation rates (*CHO-ox*) increased approximately tenfold from rest ($\sim 0.35 \text{ g}\cdot\text{min}^{-1}$) to exercise ($\sim 3.5 \text{ g}\cdot\text{min}^{-1}$) and were higher ($P < 0.05$) at rest and after 30-min and 45-min of exercise during the CFO compared to the CON trial (Table 4.1). Total fat oxidation rates (*Fat-ox*) were also observed to increase approximately fivefold from rest ($\sim 0.08 \text{ g}\cdot\text{min}^{-1}$) to exercise ($\sim 0.4 \text{ g}\cdot\text{min}^{-1}$) in both trials. There were no differences between the CFO and CON trials. Therefore, carbohydrate oxidation contributed approximately $84 \pm 10 \%$ and $77 \pm 10 \%$ ($P < 0.01$) of the total energy requirement during the CFO and CON trials, respectively.

4.3.4 *Biochemical responses*

Expressed as percentage change from rest, plasma volume ($\% \Delta \text{PV}$) was reduced by $6.2 \pm 1.2 \%$ and $5.8 \pm 1.1 \%$ at the end of the CON and CFO trials, respectively. Plasma volume was reduced by $3.7 \pm 1.0 \%$ after 45 min in the CON trial, compared to $5.8 \pm 1.1 \%$ at the end of the CFO trial (comparable time points), although values were not statistically different ($P = 0.11$).

		Exercise time (min)									
		rest		15		30		45		end	
		mean	±SEM	mean	±SEM	mean	±SEM	mean	±SEM	mean	±SEM
$\dot{V}O_2$ (l.min ⁻¹)	CON	0.45	±0.04	3.21	±0.27	3.37	±0.33	3.47	±0.39	3.43	±0.35
	CFO	0.43	±0.03	3.25	±0.29	3.44	±0.34			3.67	±0.48
$\dot{V}CO_2$ (l.min ⁻¹)	CON	0.39	±0.03	2.98	±0.28	3.10	±0.30	3.21	±0.38	3.15	±0.35
	CFO	0.39	±0.04	3.08	±0.31	3.25	±0.35			†3.46	±0.44
RER	CON	0.87	±0.02	0.93	±0.03	0.92	±0.03	0.92	±0.03	0.92	±0.03
	CFO	†0.92	±0.02	0.95	±0.04	†0.95	±0.04			†0.94	±0.03
CHO-ox (g.min ⁻¹)	CON	0.33	±0.02	3.29	±0.18	3.34	±0.16	3.52	±0.20	3.44	±0.18
	CFO	0.43	±0.02	3.65	±0.22	†3.82	±0.24			†4.03	±0.20
FAT-ox (g.min ⁻¹)	CON	0.10	±0.01	0.38	±0.06	0.44	±0.06	0.43	±0.06	0.48	±0.05
	CFO	0.06	±0.01	0.29	±0.05	0.33	±0.07			0.33	±0.06

Table 4.1 Oxygen uptake ($\dot{V}O_2$, l.min⁻¹), carbon dioxide production ($\dot{V}CO_2$, l.min⁻¹), respiratory exchange ratio (RER), carbohydrate and fat oxidation rates (g.min⁻¹) at each time point during the CON and CFO trials. Values are mean ± SEM ($n = 11$). † Denotes that values are significantly different from CON ($P < 0.05$).

Plasma glucose concentrations were similar at rest (~4.2 mmol.l⁻¹), but appeared to be elevated in the CFO compared to the CON trial (Figure 4.1). Values were statistically different after 15-min of exercise ($P < 0.05$), but not thereafter. Serum insulin concentrations were similar at rest (~20 μ U.ml⁻¹) and fell to ~10 μ U.ml⁻¹ during the first 15-min of exercise in both trials (Figure 4.2). Concentrations remained relatively

constant for the remainder of each exercise period and there were no differences between trials.

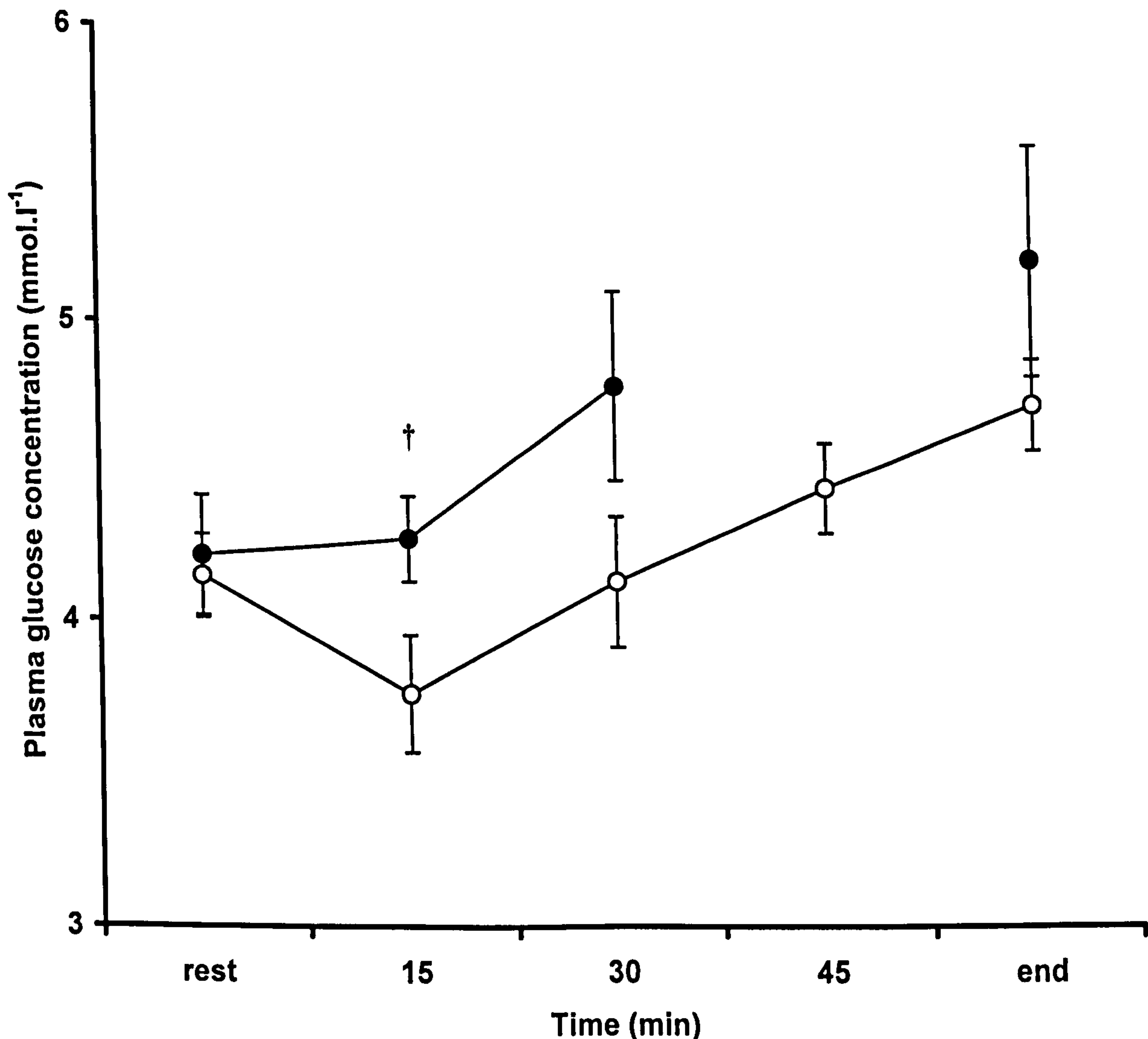


Figure 4.1 Plasma glucose concentrations (mmol.l^{-1}) during the CFO (●) and CON (○) trials. Values are mean \pm SEM ($n = 11$). † Denotes a significant difference between the CFO and CON trials ($P < 0.05$).

Plasma lactate concentrations increased over time from rest ($\sim 2.5 \text{ mmol.l}^{-1}$) to exercise and were greater after 15 ($P < 0.05$) and 30 min ($P < 0.01$) and at the end ($P < 0.01$) during the CFO compared to the CON trial (Figure 4.3). Whilst plasma FFA concentrations were similar at rest ($\sim 120 \text{ } \mu\text{mol.l}^{-1}$) and after 15-min of exercise ($\sim 100 \text{ } \mu\text{mol.l}^{-1}$), there was a linear increase thereafter (Table 4.2). Concentrations peaked at the end (CON, $271 \pm 30 \text{ } \mu\text{mol.l}^{-1}$; CFO $182 \pm 21 \text{ } \mu\text{mol.l}^{-1}$) in both trials, with values being greater ($P < 0.01$) at exhaustion during the CON trials.

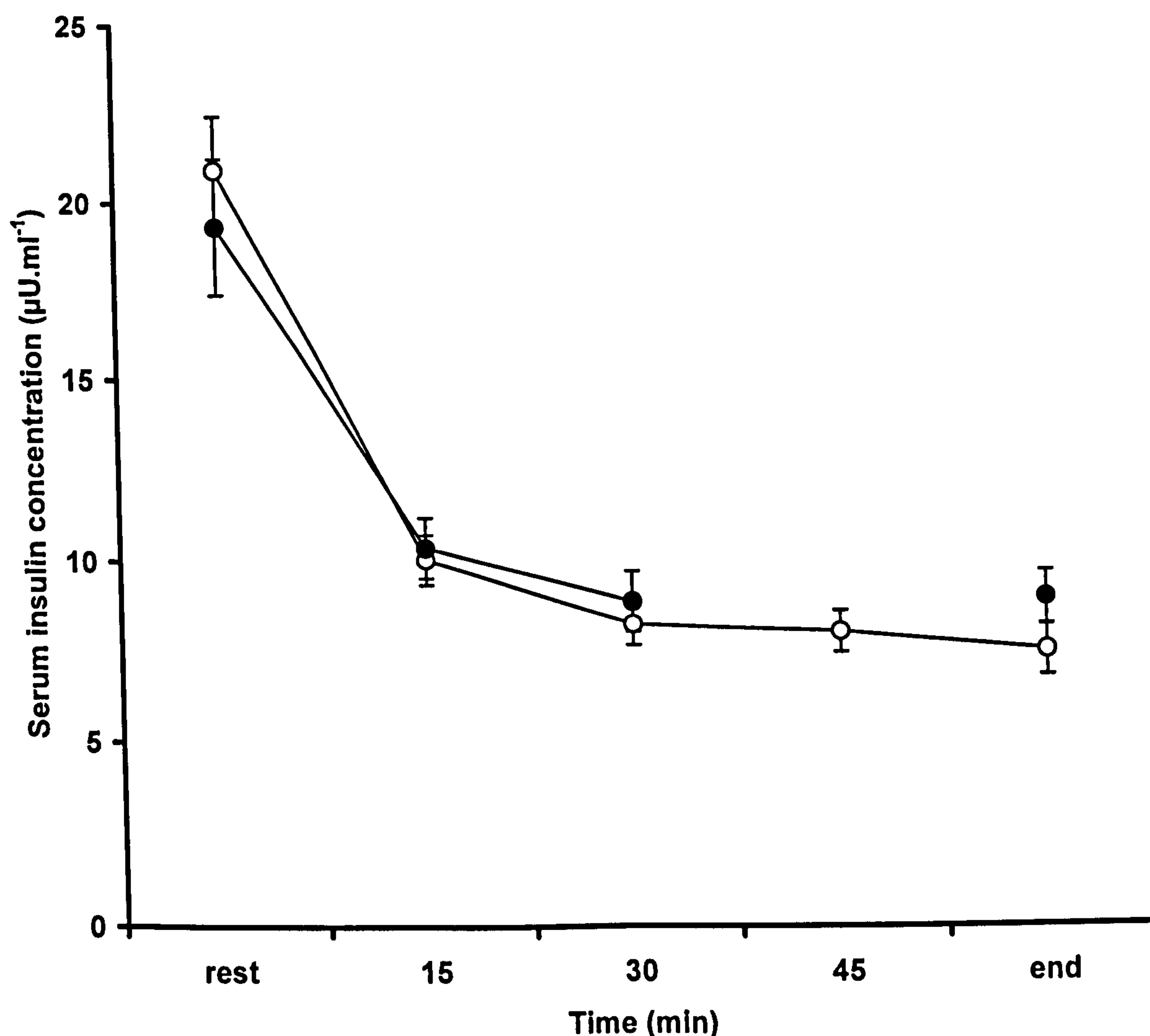


Figure 4.2 Serum insulin concentrations ($\mu\text{IU}\cdot\text{ml}^{-1}$) during the CFO (●) and CON (○) trials. Values are mean \pm SEM ($n = 11$).

Serum cortisol concentrations were similar at rest and after 15-min of exercise, but whilst they remained fairly linear in the CON trial, values continued to increase in the CFO trial and were greater after 30-min and at the end of exercise (Table 4.2). Plasma adrenaline concentrations increased from rest throughout each of the exercise periods (Figure 4.4). Whilst there were no differences between trials after 15-min of exercise, values were elevated in the CFO compared to the CON trial after 30-min ($P < 0.01$) of exercise. However, values were similar in the CON ($1.77 \pm 0.12 \text{ nmol}\cdot\text{l}^{-1}$) and CFO ($1.93 \pm 0.09 \text{ nmol}\cdot\text{l}^{-1}$) trials at exhaustion. Plasma noradrenaline concentrations showed a similar pattern of change to adrenaline, increasing throughout exercise in both trials (Figure 4.4). However, concentrations were elevated in the CFO compared to the CON trial after 15 ($P < 0.05$) and 30-min ($P < 0.01$) of exercise. Again, there was no difference between final concentrations measured at the end of exercise in the CON ($19.0 \pm 1.7 \text{ nmol}\cdot\text{l}^{-1}$) and CFO ($22.6 \pm 1.2 \text{ nmol}\cdot\text{l}^{-1}$) trials.

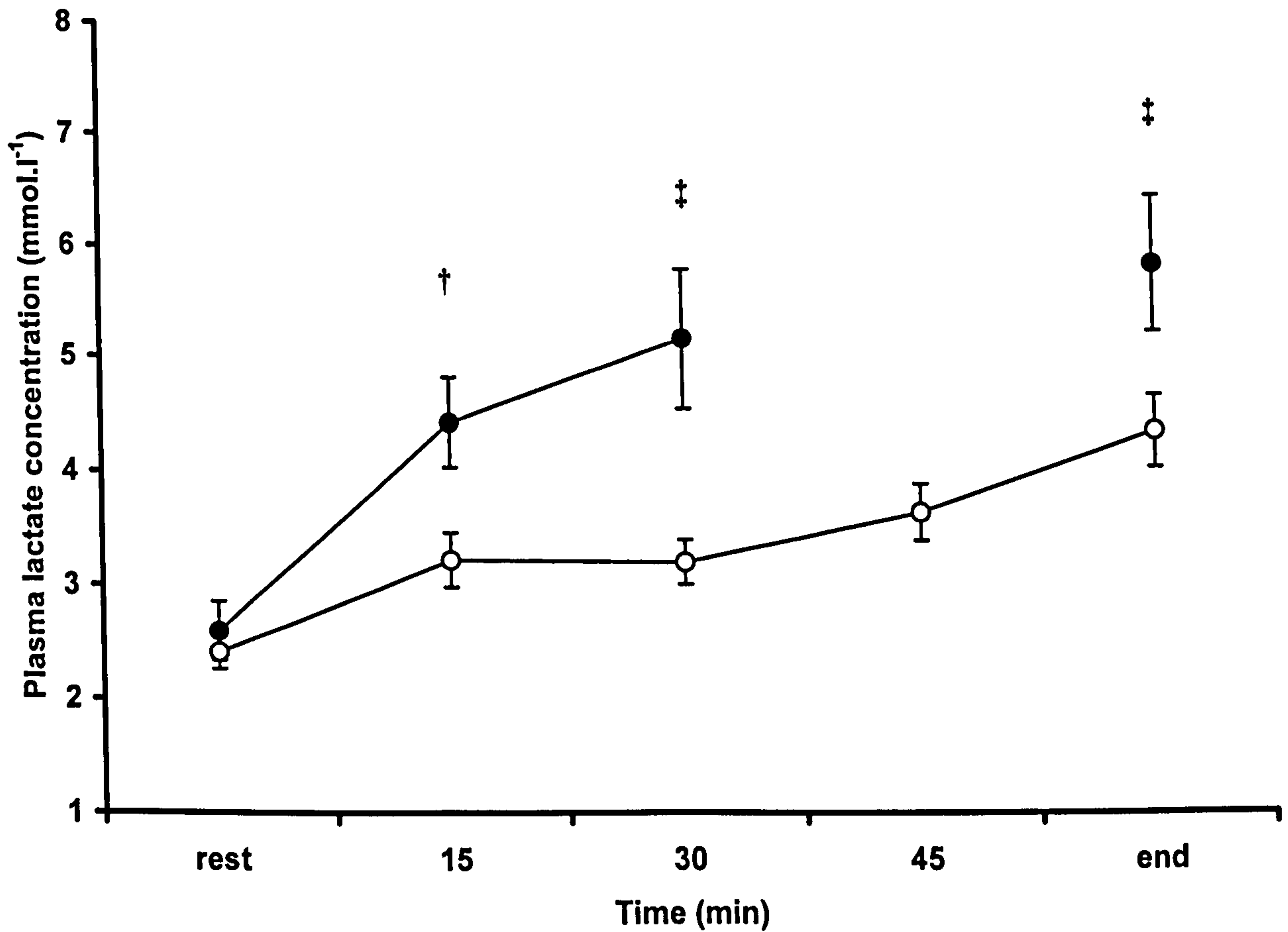


Figure 4.3 Plasma lactate concentrations (mmol.l⁻¹) during the CFO (●) and CON (○) trials. Values are mean ± SEM (*n* = 11). Symbols denote a significant difference between the CFO and CON trials: † (*P*<0.05); ‡ (*P*<0.01).

Exercise time (min)

		Exercise time (min)									
		rest		15		30		45		end	
		mean	±SEM	mean	±SEM	mean	±SEM	mean	±SEM	Mean	±SEM
FFA (μmol.l ⁻¹)	CON	114	± 9	92	± 8	139	± 12	205	± 18	271	± 30
	CFO	124	± 10	110	± 11	153	± 18			182	± 21
Cortisol (nmol.l ⁻¹)	CON	374	± 37	378	± 38	317	± 30	374	± 55	476	± 64
	CFO	441	± 44	416	± 37	†495	± 57			†581	± 54

Table 4.2 Plasma FFA (μmol.l⁻¹) and cortisol (nmol.l⁻¹) at each time point during the CON and CFO trials. Values are mean ± SEM (*n* = 11). † Denotes that values are significantly different from CON (*P*<0.01).

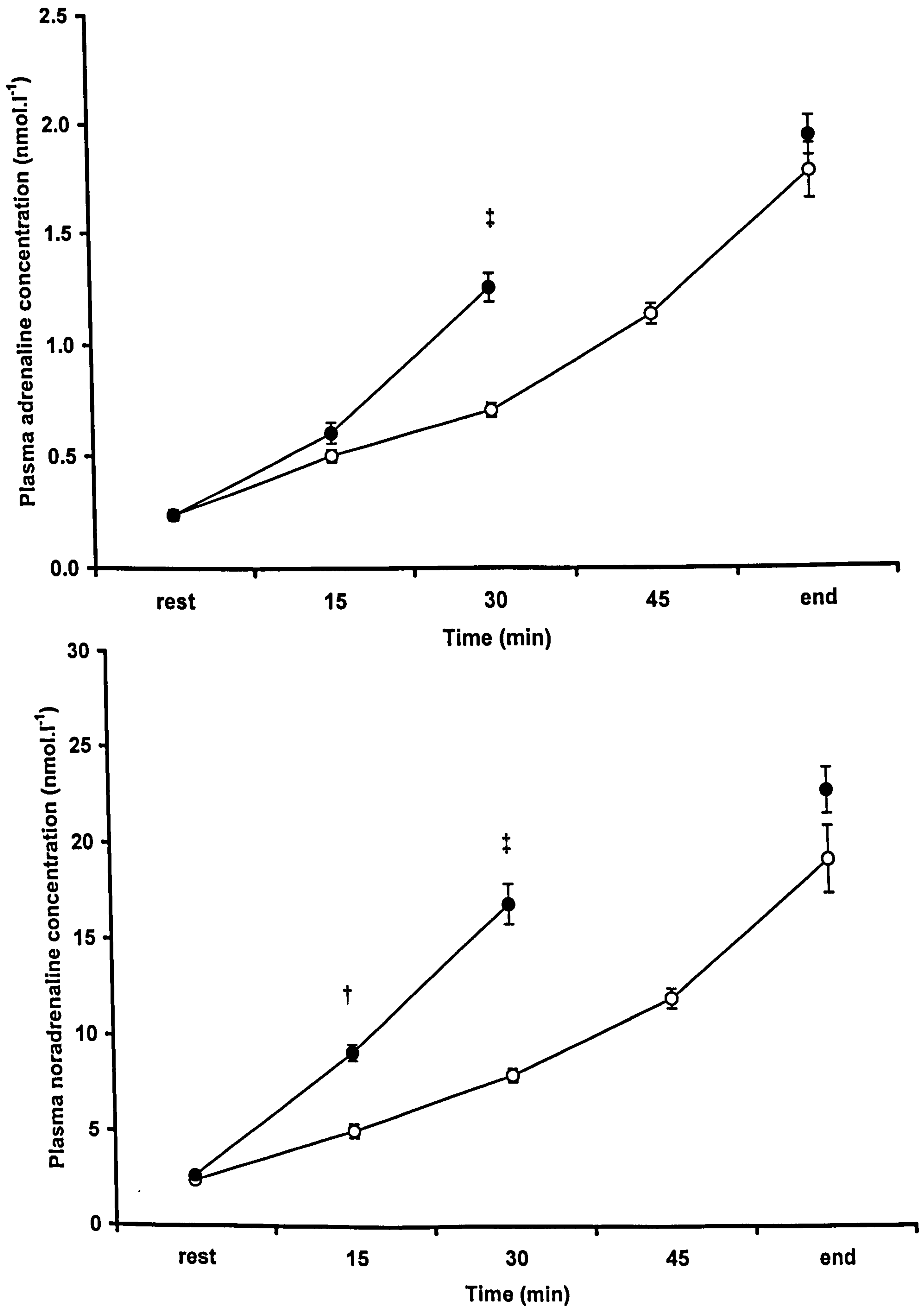


Figure 4.4 Plasma adrenaline and noradrenaline concentrations (nmol.l⁻¹) during the CFO (●) and CON (○) trials. Values are mean ± SEM (*n* = 11). Symbols denote a significant difference between the CFO and CON trials: † (*P* < 0.05); ‡ (*P* < 0.01).

4.3.5 *Thermal, cardiovascular and subjective responses*

Mean skin temperature (T_{skin}) was higher ($P < 0.01$) throughout the CFO ($\sim 35.5 \pm 0.3$ °C) compared to the CON trial (33.8 ± 0.2 °C), with little change over time in either trial (Figure 4.5). Rectal temperature (T_{rec}) increased over time in both trials ($P < 0.01$), being greater from 20 min onwards during the CFO compared to the CON trial. The rate of rise of T_{rec} was therefore greater in the CFO (3.0 ± 0.4 °C.h⁻¹) compared to the CON (2.2 ± 0.3 °C.h⁻¹) trial ($P < 0.01$). Aural temperature (T_{aur}) showed a similar pattern of change to T_{rec} , but was greater from 15 min onwards during the CFO compared to the CON trial (Figure 4.5). Aural temperature (T_{aur}) was consistently lower than T_{rec} at all time points and in both trials.

Heart rate responses showed a similar relationship to T_{rec} and T_{aur} , increasing over time in both trials ($P < 0.01$) and being greater at rest ($P < 0.01$) and at each subsequent time point ($P < 0.01$) during the CFO compared to the CON trials (Table 4.3). Values at exhaustion were similar in the CON (181 ± 2 beats.min⁻¹) and CFO (185 ± 3 beats.min⁻¹) trials. Similarly, ratings of perceived exertion (RPE) increased over time in each trial ($P < 0.01$) and were greater at rest ($P < 0.05$) and at each subsequent time point during exercise in the CFO compared to the CON trials (Table 4.3). Values reported at the point of exhaustion were similar in the CON (15 ± 1) and CFO (16 ± 1) trials. Perceived thermal discomfort was similar at rest and increased over time in each trial ($P < 0.01$). Values were greater after 15-min of exercise ($P < 0.05$), and at each subsequent time point during the CFO compared to the CON trial (Table 4.3). Again, values reported at the point of exhaustion were similar in both trials.

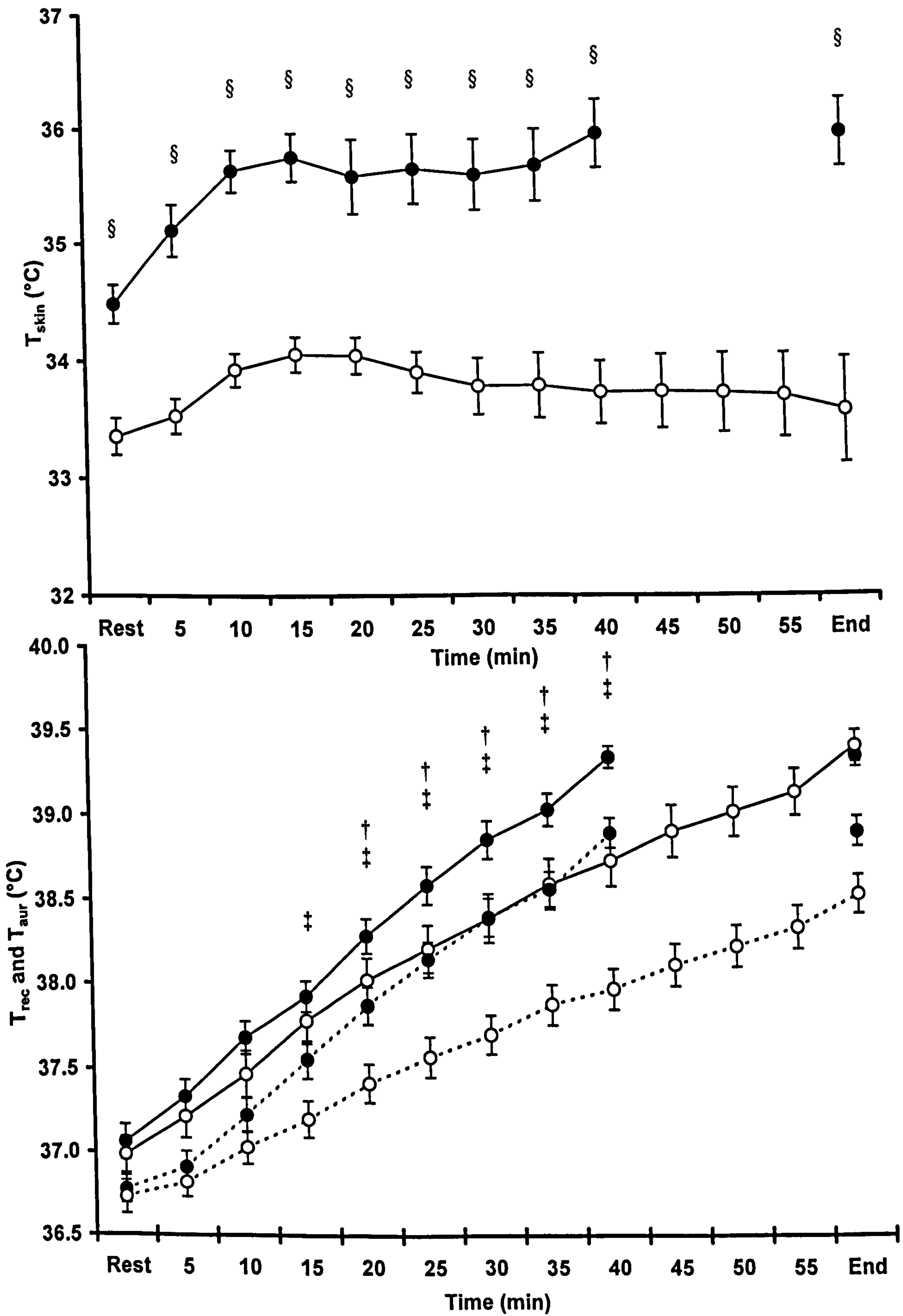


Figure 4.5 Mean skin (T_{skin}), rectal (T_{rec} —) and aural temperatures (T_{aur} ) during the CFO (●) and CON (○) trials. Values are mean \pm SEM ($n = 11$). Symbols denote a significant difference between the CFO and CON trials: § in T_{skin} ($P < 0.01$); † in T_{rec} ($P < 0.01$); ‡ in T_{aur} ($P < 0.01$).

		Exercise time (min)									
		rest		15		30		45		end	
		mean	±SEM	mean	±SEM	mean	±SEM	mean	±SEM	Mean	±SEM
HR (beats.min ⁻¹)	CON	68	±3	153	±4	160	±4	172	±3	181	±2
	CFO	‡75	±3	‡166	±3	‡178	±4			185	±3
RPE	CON	6.0	±0.0	9.1	±0.5	10.8	±0.6	12.6	±0.7	15.0	±0.6
	CFO	†6.5	±0.2	†10.1	±0.5	‡13.9	±0.8			15.5	±0.7
TD	CON	0.9	±0.3	3.4	±0.4	4.9	±0.5	6.3	±0.7	8.3	±0.5
	CFO	1.9	±0.4	†4.5	±0.5	‡7.2	±0.5			9.0	±0.4

Table 4.3 Heart rate (beats.min⁻¹), ratings of perceived exertion (RPE) and thermal discomfort (TD) at each time point during the CON and CFO trials. Values are mean ± SEM ($n = 11$). Symbols denote that values are significantly different from CON: † ($P < 0.05$); ‡ ($P < 0.01$).

4.4 Discussion

The results of the present study demonstrate that running at approximately 69% $\dot{V}O_2$ max whilst wearing a military protective clothing ensemble (CFO), imposes a significant heat strain on the wearer, increasing both thermal (T_{rec} and T_{aur}) and cardiovascular strain. In addition perceived ratings of thermal discomfort and exertion were increased whilst exercising in such clothing. As such, exercise tolerance time was reduced by approximately 26-min (40%) in the CFO compared to the CON trial. The associated increases in the rate of CHO oxidation and plasma lactate accumulation in the CFO trial are indicative of an increased rate of skeletal muscle glycogenolysis. Previous investigations have reported similar thermal and metabolic responses during cycling exercise in a hot compared to a thermoneutral environment (Febbraio *et al.* 1994a, 1994b; Young *et al.* 1985; Fink *et al.* 1975; King *et al.* 1985;

Kirwan *et al.* 1987). The data obtained in the present study support the experimental hypothesis and are consistent with the suggestion that the increase in CHO metabolism during exercise and endogenous heat strain are largely mediated by increases in circulating catecholamine concentrations.

The influence of increased thermal strain on reduced exercise tolerance time has been observed previously (Saltin *et al.* 1972). It has been suggested that when exercise is performed in conditions of increased endogenous heat strain, the drive to exercise is directly impaired by the hyperthermia which ensues (Nielsen *et al.* 1990; Fink *et al.* 1975). The results of the present study support this notion, as T_{rec} at the point of exhaustion was similar in the CON (39.4 ± 0.1 °C) and CFO (39.3 ± 0.1 °C) trials, despite the difference in exercise tolerance time. However, the physiological mechanism(s) by which hyperthermia leads to fatigue during exercise remains a matter of some debate. It was postulated that hyperthermia directly causes fatigue, affecting the central nervous system and reducing the mental drive for motor performance (Nielsen *et al.* 1990). Data to support this hypothesis are somewhat limited, whilst there is increasing evidence that metabolic alterations may account, at least in part, for the impairment of physiological function observed as hyperthermia ensues more rapidly (Young *et al.* 1985; Febbraio *et al.* 1994a, 1994b).

The CHO oxidation rates observed during exercise in the present study ($3-4$ g.min⁻¹) are higher than those previously reported ($2.5-3$ g.min⁻¹) for running at $70\% \dot{V}O_{2max}$ (Tsintzas *et al.* 1995), and are probably as a result of the high CHO breakfast meal consumed 2-h prior to exercise. Indeed, serum insulin concentrations at the start of exercise were elevated (~ 20 μ U.ml⁻¹) compared to those usually observed when subjects are fasted (~ 8 μ U.ml⁻¹). In addition, resting plasma FFA concentrations were lower in the present study (~ 100 μ mol.l⁻¹) compared to those observed at rest in fasted subjects (~ 300 μ mol.l⁻¹). Carbohydrate feedings have previously been observed to induce such responses, reducing fat oxidation and increasing the rate of CHO oxidation during exercise (Costill *et al.* 1977; Mikines *et al.* 1988).

The alterations in energy metabolism observed in the present study are similar to those previously observed during exercise and environmental heat stress, and include

an increased contribution of CHO to the metabolic demand of exercise, from 77% (CON) to approximately 84% in the CFO trial. A number of possible mechanisms have been proposed to account for the shift towards increased CHO metabolism during exercise and endogenous heat stress. These include a reduction in O₂ and substrate delivery secondary to a reduction in muscle blood flow (Rowell 1974), an alteration in neuromuscular recruitment pattern (Sawka *et al.* 1984; Young *et al.* 1985), a direct effect of temperature on enzyme-mediated reaction rates (Kozlowski *et al.* 1985; Young *et al.* 1985) and the effect of increased circulating catecholamine concentrations (King *et al.* 1985; Yaspelkis *et al.* 1993). Whilst it is difficult to accept or refute any of the above mechanisms on the basis of the results obtained in the present study, the current data support the notion that increased circulating catecholamine concentrations play a role in altering energy metabolism during exercise and heat stress.

It is well known that adrenaline secretion increases during exercise (Galbo 1983) and that this increase is augmented with heat stress (Febbraio *et al.* 1994a, 1994b; Hargreaves *et al.* 1996a). The data obtained in the present study also demonstrate increases in circulating adrenaline concentrations during exercise, which were greater with increased heat strain (CFO trial). Indeed, the adrenaline concentrations corresponding to the time of fatigue in the present CON and CFO trials are not dissimilar from those previously reported during exhaustive exercise in thermoneutral (Williams *et al.* 1992) and hot environments (Febbraio *et al.* 1994a), respectively. Since glycogen phosphorylase activity is enhanced by β -adrenergic receptor stimulation (Richter *et al.* 1982) any increase in circulating adrenaline concentration may, theoretically, result in a concomitant increase in intramuscular glycogen utilisation. Whilst muscle glycogen degradation was not measured in the present trial, the observed increases in total CHO oxidation and plasma lactate accumulation are indicative of an augmented rate of glycogenolysis, a response which is supported by previous findings.

Fink *et al.* (1975) were the first to demonstrate that an increased rate of intramuscular glycogen utilisation during submaximal exercise in a hot (41°C) compared to a thermoneutral environment (9°C). Since then, intramuscular glycogen utilisation has consistently been reported to closely match the plasma adrenaline response to exercise

and heat stress in most (Febbraio *et al.* 1994a, 1996b; Hargreaves *et al.* 1996a; Gonzalez-Alonso *et al.* 1997), but not all studies (Nielsen *et al.* 1990). Furthermore, adrenaline increases glycogenolytic rate in type I but not type II muscle fibres (Greenhaff *et al.* 1991), and heat stress only augments the use of glycogen in type I fibres (Febbraio *et al.* 1994a). Interestingly, the onset of physiological fatigue during prolonged running is associated with muscle glycogen depletion in type I muscle fibres (Tsintzas *et al.* 1996a). It is therefore feasible that an increased rate of muscle glycogen degradation in type I muscle fibres contributed to the earlier onset of physiological fatigue in the CFO compared to the CON trial.

Whilst an increased rate of muscle glycogenolysis consequent to an augmented sympatho-adrenal response seems the most likely mechanism by which CHO oxidation was increased in the CFO trial, other factors may play a role. These include an increase in endogenous glucose production (EGP) and plasma glucose oxidation or a reduction in free-fatty acid (FFA) release and fat oxidation. An increase in EGP and plasma glucose oxidation is a plausible explanation given that plasma glucose concentrations were greater in the CFO compared to the CON trial, albeit only significantly after 15-min of exercise. Indeed, increases in EGP have previously been observed during exercise and heat stress (Hargreaves *et al.* 1996a; Rowell *et al.* 1968), a process which may also be mediated by an augmented sympatho-adrenal response. Furthermore, studies in exercising rats have previously demonstrated an effect of adrenaline on EGP (Richter *et al.* 1981; Sonne *et al.* 1985). However, recent studies have used isotopic tracer methods (Hargreaves *et al.* 1996a) and arteriovenous difference techniques (Gonzalez-Alonso *et al.* 1999) and shown that, despite increases in EGP, muscle glucose uptake and oxidation are not influenced by heat stress in exercising humans.

Whilst increased heat stress does not appear to alter plasma FFA concentrations (Fink *et al.* 1975; Nielsen *et al.* 1990; Yaspelkis *et al.* 1993), it has been shown to reduce contracting leg muscle FFA uptake (Gonzalez-Alonso *et al.* 1999). Taken together, these findings may suggest that FFA release by adipocytes is reduced during exercise and heat stress. This is potentially an interesting phenomenon, given the marked augmented sympatho-adrenal response in the CFO trial, as adrenaline is a powerful lipolytic agent (Martin 1996). This may be a consequence of reduced blood flow to

adipocytes, which would limit albumin availability and promote fatty acid re-esterification within adipocytes. However, Yaspelkis *et al.* (1993) observed similar plasma glycerol concentrations during exercise with and without endogenous heat stress. Given the hydrophilic nature of this metabolite, it is unlikely that fatty acid re-esterification accounts for the similar FFA concentrations in plasma and more likely that during exercise in the heat, lipolysis is reduced. However, it is unlikely that a reduction in lipolysis and fat oxidation lead to an increase in CHO oxidation in the current CFO trial, particularly given that rates of fat oxidation and plasma FFA concentrations were not different between the CON and CFO trials during exercise.

In summary, the results of this study support our hypothesis that the heat strain induced by a military clothing ensemble (CFO) reduces exercise tolerance time and increases the reliance on carbohydrate (CHO) as a fuel for prolonged running in a warm environment. Given the increases in circulating catecholamine and lactate concentrations observed in the CFO trial, the most likely explanation for this influence of heat strain on energy metabolism is an increased rate of intramuscular glycogen degradation consequent to an augmented sympatho-adrenal response. Such responses are not only pertinent to military personnel, but may also be important for sports performers who perform repeated bouts of prolonged exercise on the same or successive days, in hot environments, without prior acclimatisation. Given the critical role of muscle glycogen for sustained exercise performance, and the importance of CHO intake for glycogen resynthesis during recovery, the influence of CHO feedings during short-term recovery from exercise in the heat should be investigated.

CHAPTER FIVE

SHORT-TERM RECOVERY FROM PROLONGED CONSTANT PACE RUNNING
IN A WARM ENVIRONMENT: THE EFFECTIVENESS OF A
CARBOHYDRATE-ELECTROLYTE SOLUTION**5.1 Introduction**

Substantial losses of water and electrolytes occur through sweating during prolonged exercise, especially when the ambient temperature is high (Adolph *et al.* 1947; Lentner 1981). The ensuing dehydration decreases blood volume (Harrison 1985), impairs thermoregulatory function (Pandolf *et al.* 1994) and exercise capacity (Sawka 1992). Indeed, previous investigations suggest that volitional fatigue during prolonged exercise in a warm environment is directly related to dehydration, thermoregulatory incapacity and the hyperthermia which ensues (Nielsen *et al.* 1993; Sawka 1992). Recently, cycling in a warm environment (Febbraio *et al.* 1994a) and progressive dehydration (Hargreaves *et al.* 1996a) have been shown to increase the rate of total carbohydrate (CHO) and muscle glycogen degradation. In addition, the heat stress imposed by a military clothing ensemble increased CHO oxidation and blood lactate accumulation during running, responses which are indicative of an increased rate of skeletal muscle glycogenolysis (Chapter 4). These metabolic alterations may account, in part, for the earlier onset of physiological fatigue observed when exercising in a warm environment. Inclusion of carbohydrate within a rehydration beverage may therefore be essential when repeated bouts of exercise are performed in a warm environment, on the same or successive days.

Previous research has clearly demonstrated that ingesting carbohydrate-electrolyte solutions (CES) during short (4-h) and long-term (22.5-h) recovery periods, enhances subsequent endurance capacity when exercise is performed in a thermoneutral (20°C) environment (Fallowfield *et al.* 1995; Fallowfield and Williams 1993). The availability of such a solution is determined by gastric emptying, intestinal absorption, and subsequent fluid retention (Mitchell and Voss 1991). These processes are influenced by several factors, including the solute content of a fluid (Costill and Saltin 1974) and the volume ingested (Noakes *et al.* 1991). Although, the addition of CHO

increases the solute content and therefore the osmotic load of a solution, it does not, at least in low concentrations (5-10%), appear to significantly compromise gastric emptying (Maughan and Leiper 1990; Rehrer *et al.* 1989). As such, exogenous CHO can be absorbed quickly, without compromising the rehydration process (Maughan and Leiper 1990).

Adequate CHO intake following exercise is essential for recovery and rapid repletion of endogenous reserves (Bergstrom *et al.* 1967). This is particularly true in the 2-h after exercise, when the rate of muscle glycogen resynthesis is somewhat greater than normal (Ivy *et al.* 1988a). Ingestion of CES between bouts of physical activity in a warm environment may therefore alleviate the symptoms of fatigue (Carter and Gisolfi 1989) and assist in the maintenance of performance during subsequent exercise. The influence of ingesting CES during recovery from exercise, on subsequent exercise capacity in a warm environment has not been investigated. The aim of the present experiment was to test the hypothesis that ingestion of a CES during recovery from exercise in the heat (35°C, 40% RH) would prolong exercise tolerance time 4-h later, compared to a sweetened placebo.

5.2 Methods

5.2.1 Subjects

Thirteen healthy male volunteers acted as subjects for this study, which was carried out with the approval of the MOD (Navy) Personnel Research Ethics Committee. Written consent to participate was provided by all subjects after the nature of the study had been explained to them. All were involved in various training programmes of which submaximal running was a central feature and had taken part in previous laboratory trials involving treadmill running to exhaustion. Mean \pm SEM ($n = 13$) physical characteristics of the subjects were: age, 32.3 ± 1.4 years; height, 178.2 ± 1.4 cm; body mass, 79.4 ± 2.8 kg; body fat, 16.5 ± 1.0 %.

5.2.2 General design

All Subjects were randomly assigned, within a crossover design, to complete two trials, at least 7-d apart. On each occasion, subjects completed a morning and

afternoon run on a motorised treadmill, separated by a 4-h recovery/rehydration period, according to the standardised recovery protocol (Section 3.8). During the 4-h recovery period subjects consumed either a 6.9% carbohydrate-electrolyte solution (CES) or a sweetened placebo (P) solution equivalent to 140% body mass loss from the first exercise period. All exercise trials were conducted in an environmental chamber controlled at a mean \pm SEM temperature 35.1 ± 0.1 °C, relative humidity 40.3 ± 0.2 % and air velocity of 3.1 ± 0.1 m.s⁻¹.

5.2.3 Preliminary measurements

Following a full medical examination and the estimation of percentage body fat from four skinfold measurements (Durnin and Womersley 1974), subjects were familiarised with treadmill running and with the experimental procedures. Each subject then completed two preliminary tests before the two main trials, to assess the oxygen cost of submaximal running and maximal oxygen uptake (mean \pm SEM $\dot{V}O_{2,max}$ 60.3 ± 3.3 ml.kg⁻¹.min⁻¹), as described in Chapter 3. Using the linear relationship between $\dot{V}O_2$ and treadmill velocity, a running speed was calculated to elicit 60% $\dot{V}O_{2,max}$ during the CES and P trials. Subjects maintained a constant training level throughout the experimental period and refrained from strenuous activity for 48-h prior to an experimental day.

5.2.4 Procedures

On reporting to the laboratory subjects were seated in a room maintained at 22°C, where they consumed a standard breakfast (685 kcal: 70% CHO; 20% fat; 10% protein) and water (550 ± 22 ml). After 90-min subjects entered the environmental chamber and were asked to empty their bladder. Nude body mass was then measured to the nearest 5-g (Sauter SD100) before dressing in shorts, socks and running shoes. Two aural thermistors (insulated with cotton wool) and three ECG chest electrodes were attached to the subjects for the determination of aural canal temperature (T_{aur}) and heart rate (HR), respectively. Immediately after sitting in a relaxed position for 15 min an initial 10 ml venous blood sample was drawn from an antecubital vein, without venostasis. Subsequent venous blood samples were obtained in the same manner. Duplicate 20- μ l capillary blood samples were simultaneously drawn from the finger.

After recording baseline T_{aur} and HR, subjects stood on the treadmill and the velocity was then increased to elicit a metabolic demand of 60% $\dot{V}O_2 \text{ max}$ (T1). During T1 subjects were required to run for 90 min, or until volitional fatigue, whichever was reached first. Ingestion of water was permitted at a rate of 2 ml.kg⁻¹ body mass every 15 min. Expired gas samples were collected over a 1 min period at 15-min intervals during T1 using the Douglas bag technique. Percentage oxygen (FeO₂) content was measured by a paramagnetic O₂ analyser, percentage carbon dioxide (FeCO₂) was measured by an infrared CO₂ analyser (Taylor Servomex, Series 1400) and minute ventilation was measured by a dry gas meter (Harvard, Kent, UK). From gas analyses, $\dot{V}E$, $\dot{V}O_2$, and $\dot{V}CO_2$ were determined and the respiratory exchange ratio (RER) calculated. Rates of total CHO (CHO_{tot}) and fat (Fat_{tot}) oxidation were calculated from $\dot{V}O_2$ and $\dot{V}CO_2$, using previously described methods (Péronnet and Massicotte 1991). Ratings of perceived exertion (RPE, Borg 1973) and thermal discomfort (TD) were similarly recorded at 15-min intervals. Duplicate capillary blood samples were drawn at 30 and 60 min of exercise. Both T_{aur} and HR were recorded at 60-s intervals throughout exercise. Post exercise venous and capillary samples were obtained at the end of T1. Following the assessment of nude body mass subjects left the environmental chamber to begin their controlled 4-h recovery period (REC) in a room maintained at 22°C.

During REC subjects ingested 3 equal feedings of CES or P (equivalent to 100% weight loss) at 0, 1 and 2-h. After 3-h subjects were asked to void their bladder, nude body mass was recorded, and a bolus dose equivalent to the remaining fluid deficit was ingested. Ingested volumes were therefore equivalent to 140% of body mass loss, providing 138 ± 12 g or 0 g of CHO during the CES and P trials, respectively. No food and only the prescribed fluids were consumed during the 4-h period. Duplicate capillary blood samples were similarly drawn at 60-min intervals throughout the recovery period. Urine was collected throughout the recovery period and subjects were asked to finally void their bladder upon entering the environmental chamber for a second time. Following the assessment of nude body mass subjects were dressed and instrumented as before. After venous and capillary blood samples were drawn, subjects stood in position on the treadmill and the speed was gradually increased to elicit a metabolic demand equivalent to 60% $\dot{V}O_2 \text{ max}$. Subjects were required to run

until volitional exhaustion to assess exercise tolerance time (T2). The frequency of data collection during T2 was identical to T1. At the end of the trial venous and capillary blood samples were drawn and body mass recorded.

5.2.4 *Analytical methods*

Samples of venous blood were dispensed into lithium heparin tubes and used for the determination of haemoglobin and haematocrit (Technicon H1, USA). Changes in plasma volume were estimated using the method described by Dill and Costill (1974). Whole capillary blood samples were collected in capillary tubes containing heparin, fluoride and nitrate and mixed for approximately 3 min. Duplicate capillary blood glucose and lactate concentrations were immediately determined (Analox P-GM7, Analox Instruments UK).

5.2.5 *Statistical analysis*

Statistical comparisons were made after establishing the normality or otherwise of the data distribution using the Anderson-Darling Goodness of Fit test (Anderson and Darling 1954). A two-way analysis of variance (ANOVA) with repeated measures was used to compare results between treatments and across time. Where significant *F*-ratios were found, a Tukey post-hoc test was used to determine the cause of the variance. When there were only single comparisons, a Student's *t*-test for correlated data was used to determine whether differences between treatments existed. Descriptive data are presented as mean \pm standard error of the mean (SEM). Differences between and within trials were considered significant when $P < 0.05$.

5.3 Results

5.3.1 *Exercise tolerance time*

Mean \pm SEM run times during T1 did not differ between the CES (74.8 ± 4.6 min, range 43.6 to 90.0 min) and P trials (72.5 ± 5.2 min, range 31.0 to 90.0 min). The coefficients of variation for the T1 runs of the CES (22.1%) and P (25.8%) trials were similar and there was a strong correlation between these two sets of data ($r=0.93$, $P < 0.01$). This provides an indication that subjects ran the same approximate time during T1 of both trials. During T2 subjects exercised 16 ± 3.4 min longer during the CES compared to the P trial ($P < 0.01$). Exercise tolerance time during T2 was

therefore $35 \pm 7\%$ greater for the CES trial (60.9 ± 5.5 min, range 37.0 to 92.4 min) compared to the P trial (44.9 ± 3.0 min, range 31.0 to 65.4 min). Exercise tolerance time was also greater during the first compared to the second exercise period in both conditions ($P < 0.01$).

5.3.2 *Fluid balance*

Body mass prior to T1 did not differ between the two conditions (CES, 78.82 ± 2.88 kg; P, 78.42 ± 2.90 kg). Fluid losses equivalent to $2.9 \pm 0.3\%$ and $2.7 \pm 0.2\%$ of pre-exercise body mass were incurred during T1 of the CES and P trials, respectively (Table 5.1). Similar quantities of fluid were consumed during the T1 exercise periods and subjects were therefore dehydrated by $1.9 \pm 0.2\%$ and $1.7 \pm 0.2\%$ of pre-exercise body mass during the CES and P trials, respectively. During the subsequent recovery period subjects consumed similar volumes (CES, 2006 ± 176 ml; P, 1830 ± 165 ml) of the appropriate rehydration solution, providing 138 ± 12 g (CES) or 0 g (P) of CHO. Urine production (CES, 515 ± 69 ml; P, 527 ± 89 ml) and post-recovery net fluid balance (CES, $+58 \pm 26$ g; P, -4 ± 68 g) did not differ between the two conditions. The two solutions were therefore equally effective in restoring body mass during REC (CES, 78.88 ± 2.87 kg; P, 78.42 ± 2.91 kg).

Despite the longer exercise duration during T2 of the CES compared to the P trial, there was no difference in total sweat loss. Average sweat rate was therefore greater during T2 of the P trial, compared to CES ($P < 0.05$). Subjects were dehydrated by $1.4 \pm 0.2\%$ of pre-exercise body mass following T2 in both conditions.

5.3.3 *Metabolic responses*

There was a progressive increase in $\dot{V}O_2$ ($l \cdot min^{-1}$) over time during each of the exercise periods ($P < 0.01$), although there were no differences between conditions. Consequently, after 15-min of exercise during T1 subjects were exercising at $59.6 \pm 1.4\%$ (CES) and $61.1 \pm 1.7\%$ (P) of $\dot{V}O_{2, max}$, which rose to $64.3 \pm 1.7\%$ (CES) and $64.5 \pm 1.9\%$ (P) by the end of exercise ($P < 0.01$). Similar changes took place between 15-min and the end of T2 in both conditions ($P < 0.01$).

The nature of energy metabolism did not differ between the two treatments during T1 (Table 5.2), but RER values were higher at all time points during T2 of the CES

compared to the P trial ($P<0.01$). The rate of CHO oxidation was therefore higher ($P<0.01$) and fat oxidation lower ($P<0.01$) at all time points during T2 of the CES compared to the P trial. Carbohydrate oxidation contributed 64% (P) and 65% (CES) of the total energy requirement during T1, while during T2 carbohydrate oxidation contributed less in the P trial (47%) compared to the CES (74%) trial ($P<0.01$).

	CES		P	
	mean	±SEM	mean	±SEM
T1				
Pre-exercise body mass (kg)	78.82	±2.88	78.42	±2.90
Post-exercise body mass (kg)	77.39	±2.91	77.11	±2.92
Fluid intake during exercise (g)	757	±50	728	±50
Sweat loss (g)	2167	±156	2035	±152
Sweat rate (g.min ⁻¹)	29.2	±2.1	28.6	±1.8
Net fluid balance (g)	-1433	±126	-1307	±118
----- REC				
Post-recovery body mass (kg)	78.88	±2.87	78.42	±2.91
Fluid intake (g)	2006	±176	1830	±165
Urine production (g)	515	±69	527	±89
Net fluid balance (g)	58	±26	-4	±68
----- T2				
Post-exercise body mass (kg)	77.79	±2.88	77.34	±2.95
Fluid intake during exercise (g)	615	±57	452	±35
Sweat loss (g)	1705	±187	1525	±127
Sweat rate (g.min ⁻¹)	29.1	±3.5	†33.8	±1.8
Net fluid balance (g)	-1032	±170	-1077	±148

Table 5.1 Data relating to changes in net fluid balance throughout the experimental period during the CES and P trials. Values are means ± SEM ($n = 13$). † Denotes that values are significantly different from CES ($P<0.05$).

	T1				T2			
	15	30	45	end	15	30	end	
	Mean	mean	mean	mean	mean	mean	mean	mean
	±SEM	±SEM	±SEM	±SEM	±SEM	±SEM	±SEM	±SEM
RER								
CES	0.91 ±0.02	0.89 ±0.02	0.88 ±0.01	0.86 ±0.01	0.95 ±0.02	0.93 ±0.02	0.89 ±0.02	
P	0.91 ±0.01	0.90 ±0.02	0.89 ±0.02	0.87 ±0.02	†0.84 ±0.01	†0.83 ±0.01	†0.83 ±0.01	
CHO oxidation rate (g.min⁻¹)								
CES	2.65 ±0.22	2.42 ±0.19	2.32 ±0.19	2.13 ±0.17	2.95 ±0.19	2.91 ±0.21	2.49 ±0.28	
P	2.65 ±0.19	2.49 ±0.17	2.37 ±0.18	2.19 ±0.21	†1.77 ±0.15	†1.60 ±0.14	†1.52 ±0.13	
Fat oxidation rate (g.min⁻¹)								
CES	0.42 ±0.06	0.51 ±0.07	0.59 ±0.07	0.70 ±0.07	0.32 ±0.05	0.38 ±0.07	0.61 ±0.10	
P	0.43 ±0.07	0.48 ±0.07	0.57 ±0.08	0.68 ±0.09	†0.75 ±0.08	†0.83 ±0.08	†0.88 ±0.09	

Table 5.2 Respiratory exchange ratio (RER), carbohydrate and fat oxidation rates (g.min⁻¹) at each time point during T1 and T2 of the CES and P trials. Values are means ± SEM (*n* = 13). † Denotes that values are significantly different from CES (*P* < 0.01).

5.3.4 *Biochemical responses*

Plasma volume was reduced after T1 (CES, $-6.0 \pm 0.9\%$; P, $-5.4 \pm 1.0\%$) compared to pre-exercise ($P < 0.01$), in both conditions. There were no differences between the CES and P trials in the magnitude of the calculated decrease in plasma volume (Figure 5.1). Over the subsequent 4-h fluid replacement period, plasma volume was restored to pre-exercise levels. Plasma volume was similarly decreased ($P < 0.01$) during T2 of the CES and P trials.

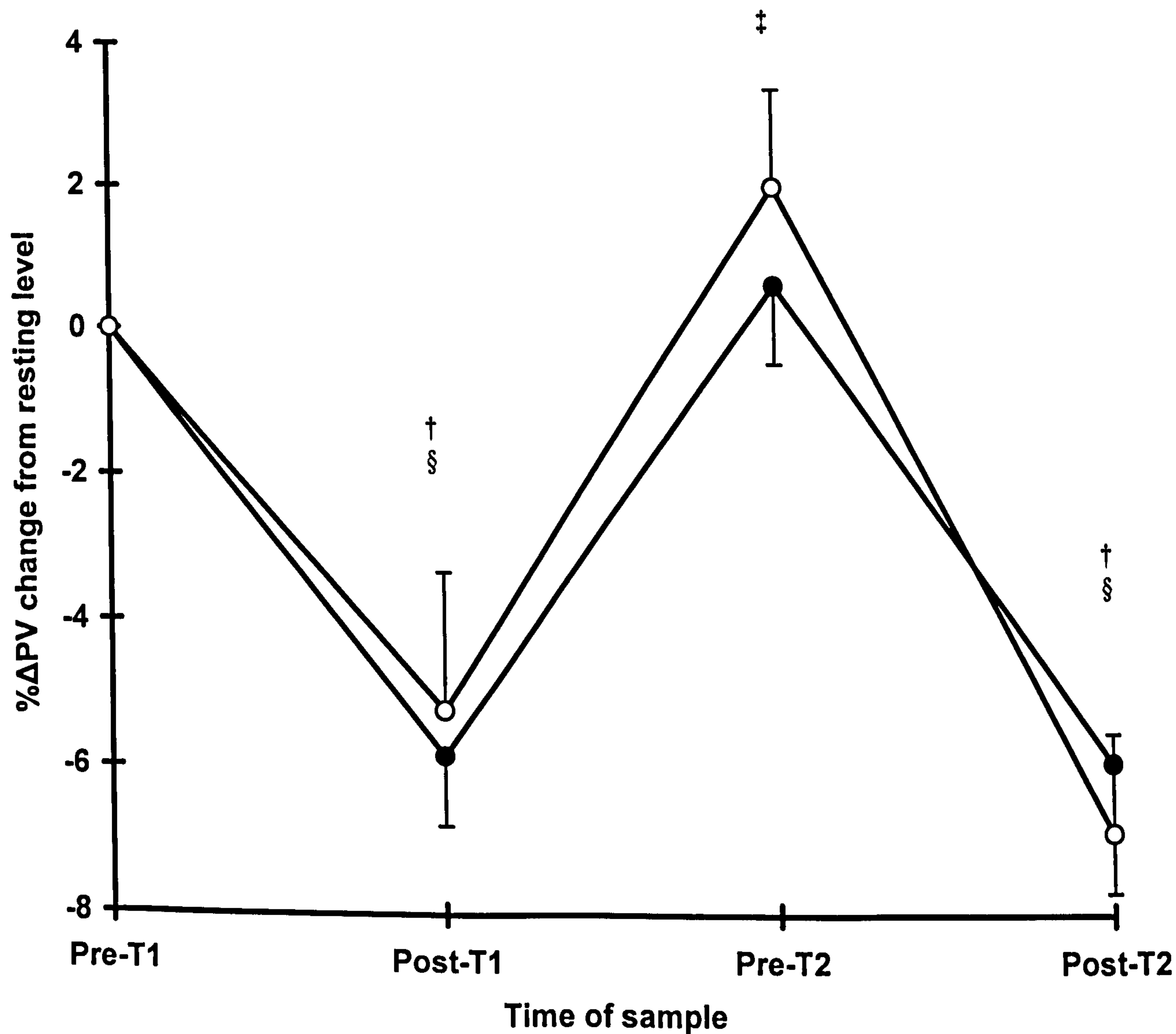


Figure 5.1 Estimated percentage changes in plasma volume ($\% \Delta PV$) during T1 and T2 of the CES (\bullet) and P (\circ) trials. Values are means \pm SEM ($n = 13$). Significant differences between time points are indicated as follows: \dagger from pre-T1; \ddagger from post-T1; \S from pre-T2.

Pre-exercise (T1) blood glucose concentrations did not differ between the CES and P trials, and remained similar throughout the T1 exercise period (Figure 5.2). During recovery blood glucose concentration was elevated after 60 min ($P<0.01$), 120 min ($P<0.01$) and 180 min ($P<0.05$) of the CES compared to the P trial. Although the blood glucose concentration had returned to resting values prior to the start of the second exercise period (T2), a moderately severe hypoglycaemic response was observed after 30-min and at the end of T2 during the CES but not during the P trial ($P<0.01$).

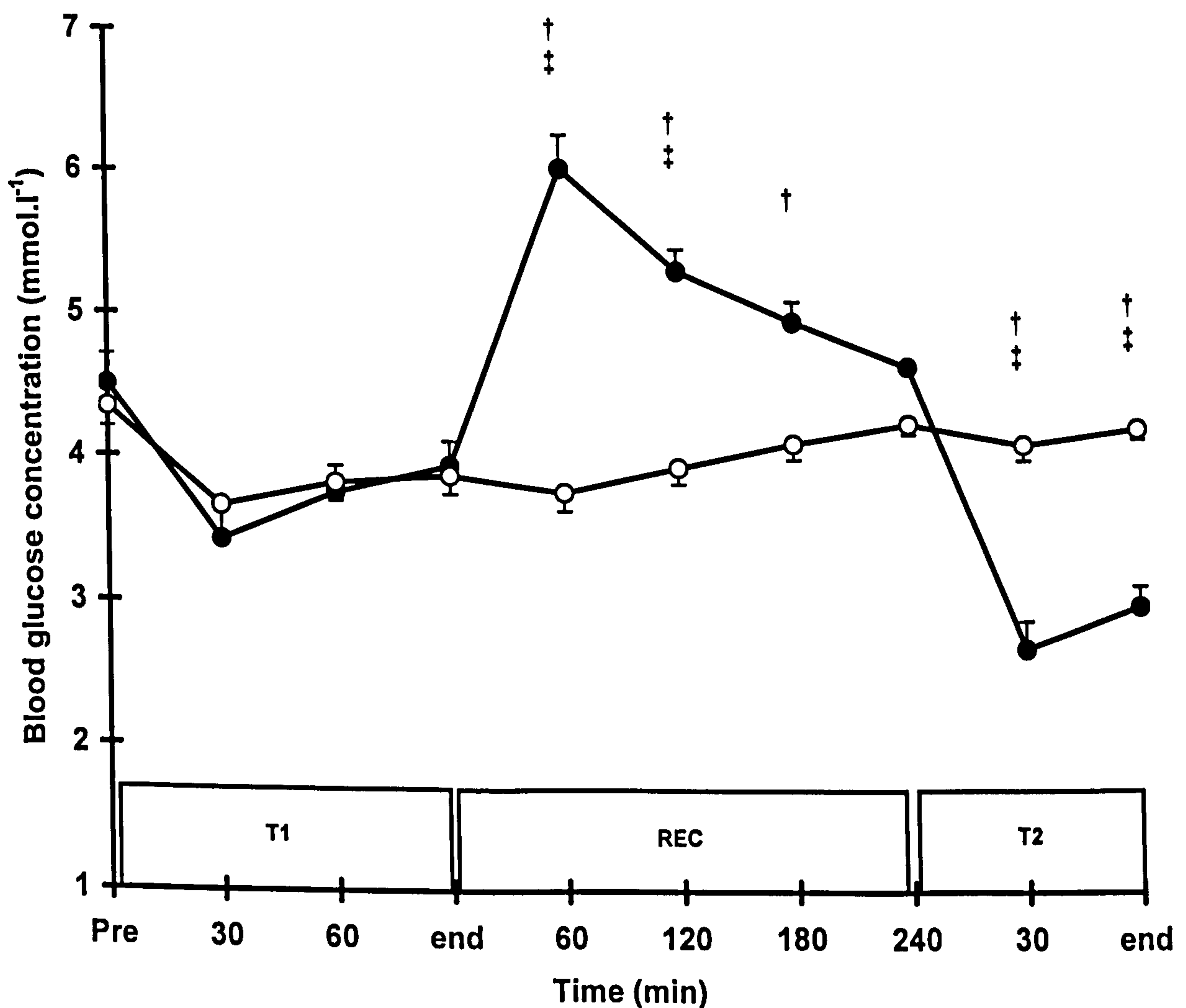


Figure 5.2 Plasma glucose concentrations (mmol.l^{-1}) during T1, REC and T2 of the CES (\bullet) and P (\circ) trials. Values are means \pm SEM ($n = 13$). \dagger Denotes a significant difference between the CES and P trials ($P<0.01$). \ddagger Denotes that values are significantly different from the pre-T1 value for that trial ($P<0.01$).

Blood lactate concentration increased from resting levels of 0.9 ± 0.1 and 1.0 ± 0.1 mmol.l^{-1} to 2.0 ± 0.1 and 2.2 ± 0.1 mmol.l^{-1} ($P < 0.01$) after 30 min of exercise during the CES and P trials, respectively. There were no differences in the magnitude of this increase between conditions. Normal resting blood lactate concentrations were restored during the recovery period. There were no differences in blood lactate concentrations between the CES and P trials, at any time point during T2.

5.3.5 *Thermal, cardiovascular and subjective responses*

There were no significant treatment effects in T_{aur} , HR, RPE or TD during the T1 exercise periods (Table 5.3). Heart rate increased by approximately 20 beats.min^{-1} between 15 min and the end of the T1 exercise periods ($P < 0.01$). Increases in T_{aur} ($P < 0.01$), RPE ($P < 0.01$) and TD ($P < 0.01$) were also observed during the T1 exercise periods. HR and T_{aur} were greater after 30-min of exercise during T2 of the P ($P < 0.05$) compared to the CES trial. Consequently, the rate of increase in heart rate (CES, 43 ± 5 beats.min^{-1} ; P, 59 ± 4 beats.min^{-1} ; $P < 0.01$) and T_{aur} [CES, 1.7 ± 0.2 $^{\circ}\text{C}$; P, 2.2 ± 0.2 $^{\circ}\text{C}$; $P < 0.01$] per hour of exercise was lower during T2 of the CES compared to the P trial. Subjects reported feeling more exerted (RPE) after 30 min ($P < 0.01$) and at the end of T2 ($P < 0.01$) during the P compared to the CES trial. Similarly, subjects reported feeling warmer (TD) at the end of T2 during the P compared to CES trial ($P < 0.05$).

	T1				T2			
	15	30	45	end	15	30	end	end
	Mean	±SEM	mean	±SEM	mean	±SEM	mean	±SEM
HR (beats.min⁻¹)								
CES	153 ±2	162 ±3	170 ±3	174 ±3	158 ±3	165 ±3	178 ±3	
P	154 ±3	162 ±4	170 ±4	174 ±4	163 ±3	†171 ±3	†185 ±3	
RPE								
CES	8 ±0.5	10 ±0.5	12 ±0.4	14 ±0.6	10 ±0.5	12 ±0.4	15 ±0.6	
P	9 ±0.5	11 ±0.3	13 ±0.4	15 ±0.7	11 ±0.3	†15 ±0.3	†18 ±0.3	
T_{aur} (°C)								
CES	37.6 ±0.1	37.9 ±0.1	38.2 ±0.1	38.4 ±0.1	37.5 ±0.1	37.8 ±0.1	38.4 ±0.1	
P	37.4 ±0.1	37.9 ±0.1	38.2 ±0.1	38.4 ±0.1	37.9 ±0.1	†38.3 ±0.1	38.6 ±0.1	
TD								
CES	2 ±0.2	4 ±0.2	6 ±0.3	7 ±0.6	3 ±0.5	5 ±0.4	8 ±0.6	
P	2 ±0.3	4 ±0.3	6 ±0.5	7 ±0.6	4 ±0.4	5 ±0.4	†10 ±0.6	

Table 5.3 Heart rate (HR, beats.min⁻¹), rating of perceived exertion (RPE, Borg 1973), aural temperature (T_{aur}) and thermal discomfort (TD) at each time point during T1 and T2 of the CES and P trials. Values are means ± SEM (*n* = 13). † Denotes that values are significantly different from CES (*P* < 0.01).

5.4 Discussion

The main finding of this study was that ingesting a carbohydrate-electrolyte solution during recovery from prolonged running in a warm environment improved exercise tolerance time 4-h later, compared to flavoured, sweetened water. As subjects were similarly rehydrated and euvolaemic prior to the second exercise period in both trials, the subsequent differences in exercise capacity appear to be related to the provision of CHO rather than electrolytes. The subjects' performance and physiological responses to T1 were similar during the two trials. Although the T1 run times were shorter than those previously reported (Fallowfield *et al.* 1995), this would appear to be in response to the external heat stress imposed during the present study. However, subjects exercised for 16.0 ± 3.4 min longer during T2 of the CES compared to the P trial. The magnitude of the difference in performance is similar (22.2 ± 3.5 min) to that found by Fallowfield *et al.* (1995), who assessed the influence of a CES on post-recovery running capacity in a laboratory environment maintained at approximately 20°C.

Despite similar results, there are a number of distinct methodological differences between this study and the earlier investigation of Fallowfield *et al.* (1995). Most notably, during the present trial subjects ingested a breakfast containing 114-g of CHO, 90-min prior to the start of T1, whereas subjects had fasted for at least 10-h prior to the start of the earlier investigation. Coyle *et al.* (1985) reported a 42% increase in the glycogen content of vastus lateralis muscle in subjects' fed 4-h prior to exercise compared to when fasted. Such pre-exercise feedings have been shown to negate the metabolic impact of subsequent CHO feedings (Widrick *et al.* 1993). Secondly, subjects exercised in a warmer environment during the present study (35°C) compared to the Fallowfield *et al.* (1995) study (20°C). Previous investigations suggest that exercise capacity in such warm environmental conditions is limited by the hyperthermia which ensues (Nielsen *et al.* 1993; Nielsen 1994) a problem which is exacerbated by progressive dehydration (Montain and Coyle 1992). During the present investigation subjects consumed fluid during exercise, which offset dehydration and probably enabled them to thermoregulate more effectively. Despite exercising in a warm environment and ingesting a high CHO pre-trial

breakfast, the provision of CHO during REC of CES still enhanced subsequent exercise tolerance time compared to P.

It was estimated from indirect calorimetry that 178 ± 17 g of CHO was oxidised in T1 of the CES and P trials. This estimation is similar to those previously reported from indirect calorimetric calculations (Wong *et al.* 1996b) and changes in muscle glycogen content during prolonged treadmill running, at a higher exercise intensity but in lower environmental temperatures (Tsintzas *et al.* 1995). The placebo solution ingested during the 4-h recovery of the P trial did not contain CHO. However, the CHO ingested during the CES trial, was sufficient to replace approximately 74% of that estimated to have been metabolised during T1. Previously published observations suggest that the volumes of fluid and substrate ingested were emptied from the stomach and available for absorption within the 4-h recovery period (Maughan and Leiper 1990; Gonzalez-Alonso *et al.* 1992). Assuming that 85% of the CHO emptied escaped the liver (Maehlum *et al.* 1978) and that the central nervous system used approximately $5 \text{ g}\cdot\text{h}^{-1}$ (Shreeve *et al.* 1956), approximately 96 g of CHO would have been available to the muscle during the CES trial.

Absorption of the exogenous CHO during REC of the CES trial is evident from the increases in blood glucose concentration, compared to the P trial. This apparent hyperglycaemia has been consistently reported to cause hyperinsulinaemia (Ivy *et al.* 1988a; Wong *et al.* 1996a). Together, hyperglycaemia and hyperinsulinaemia have been shown to increase muscle glucose uptake (Mikines *et al.* 1988) and muscle glycogen resynthesis to 300% of the basal level during the first 2-h of REC (Ivy *et al.* 1988a). It can therefore be assumed that muscle glycogen resynthesis would have been near the theoretical maximum of $6 \text{ mmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ during CES, compared to $1\text{-}2 \text{ mmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ during the P trial.

Consequently, during T2 of the P trial subjects used less CHO as a fuel for exercise, metabolising only half of that used during the CES trial. Although it is generally accepted that, when readily available, CHO is preferentially used as a fuel for exercise (Wagenmakers *et al.* 1993a), the insulinaemic response to CHO ingestion can incur further shifts in substrate metabolism (Wasserman *et al.* 1991). As subjects ingested the test beverages throughout REC, it is feasible that the CES group were

hyperinsulinaemic at the onset of T2. This response increases carbohydrate oxidation (Mikines *et al.* 1988), decreases plasma free fatty acid (FFA) concentration and total fat oxidation (Costill *et al.* 1977). More importantly, physiological hyperinsulinaemia increases glucose uptake by the contracting muscles at a time when hepatic glucose output is reduced and can therefore lead to hypoglycaemia during exercise (Ahlborg and Bjorkman 1987). A moderately severe hypoglycaemic response to exercise was apparent during T2 of the CES but not the P trial.

Two subjects reported symptoms of nausea and muscular weakness in response to the hypoglycaemia observed during the initial 20-min of T2 during the CES trial. Despite this hypoglycaemic response, symptoms were not reported after this initial 20-min period and exercise capacity was subsequently enhanced rather than impaired. Although the obvious mechanism for the enhanced exercise capacity would be related to the increased storage and subsequent availability of carbohydrate for metabolism *per se*, subjects also maintained a lower T_{aur} throughout T2 during CES. It appears that such a thermoregulatory response to exercise following carbohydrate ingestion has not been previously reported. A tentative explanation is that mild hypoglycaemia caused an earlier increase in peripheral blood flow and therefore evaporative heat loss (Gale *et al.* 1981; Macdonald *et al.* 1982) during CES. Evidence suggests that the magnitude of this response is dependent upon the severity of the hypoglycaemia (Gale *et al.* 1983). Such a mechanism has only been demonstrated in resting man under thermoneutral conditions, and therefore warrants further investigation.

In conclusion, ingesting a carbohydrate-electrolyte solution in sufficient quantities to replace body mass loss following prolonged, constant pace running in a warm environment improved exercise tolerance time 4-h later. The 6.9% carbohydrate-electrolyte solution facilitated rehydration as effectively as the flavoured, sweetened water solution. Provided that an adequate hydration status is maintained inclusion of CHO and electrolytes within a rehydration solution will delay the onset of fatigue during a subsequent bout of prolonged submaximal running in a warm environment. However, it remains unknown how much CHO is required to promote improvements in exercise tolerance time, and how this substrate is stored and subsequently utilised during further bouts of exercise.

CHAPTER SIX

INFLUENCE OF GLUCOSE INGESTION BY HUMANS DURING RECOVERY
FROM EXERCISE ON SUBSTRATE UTILISATION DURING SUBSEQUENT
EXERCISE IN A WARM ENVIRONMENT**6.1 Introduction**

It has been demonstrated that performing prolonged moderate intensity exercise in a warm environment increases total carbohydrate (CHO) oxidation and muscle glycogen degradation compared to exercising in a temperate environment (Febbraio *et al.* 1994a, 1994b). Similar responses are observed when thermoregulatory function is impaired by progressive dehydration (Hargreaves *et al.* 1996b) or by wearing protective clothing (Chapter 4) during exercise. These metabolic responses may therefore account, at least in part, for the early onset of physiological fatigue observed during exercise in a warm environment. As such, the inclusion of CHO within a rehydration beverage may increase endurance capacity when repeated bouts of exercise are performed in such environments on the same or successive days.

A number of studies have demonstrated that the ingestion of CHO within a rehydration solution during a short-term recovery period (4-h) enhances subsequent exercise capacity, both in a temperate (Fallowfield *et al.* 1995; Wong *et al.* 1995) and in a warm environment (Chapter 5). Such feedings may be effective in restoring exercise capacity because the rate of glycogen resynthesis is somewhat greater when CHO is ingested during the initial 4 to 6-h post-exercise (Blom *et al.* 1987; Ivy *et al.* 1988b). However, a dose-response relationship between CHO intake and the restoration of exercise capacity does not appear to exist. During a 4-h recovery period, as little as 50-g of CHO appears to be sufficient to produce a maximum improvement in endurance capacity during subsequent exercise (Wong *et al.* 1996b). This is despite the fact that a larger dose (175-g CHO) results in a 2.5-fold greater muscle glycogen resynthesis compared to a 50-g dose (Tsintzas *et al.* 1999). Whilst this imbalance between CHO intake, short-term glycogen resynthesis and exercise capacity is now well documented, the physiological mechanisms are less clear, and such responses have not been examined during exercise in a warm environment.

We are not aware of any studies to date, which have simultaneously examined the effects of CHO feedings on substrate utilisation and storage during recovery and utilisation during subsequent exercise in a warm environment. Therefore, the aim of the present experiment was to compare the effects of large (4 × 55-g; 220-g) and small (55-g) amounts of glucose on substrate storage and utilisation during recovery and subsequent exercise in a warm environment. By giving naturally enriched ¹³C-glucose, it was possible to estimate total glycogen synthesis (GS) and breakdown (GB) during recovery and quantify the oxidation rate of orally ingested CHO during subsequent exercise (Schneider *et al.* 1995). Such an approach provided further insight into the influence of CHO feeding regimens on short-term recovery and substrate utilisation during prolonged exercise.

6.2 Methods

6.2.1 Subjects

A group of 11 healthy male volunteers acted as subjects for this study, which was carried out with the approval of the Ministry of Defence (Navy) Personnel Research Ethics Committee. Written consent to participate was provided by all subjects after the nature of the study had been explained to them. All were involved in various training programmes of which submaximal running was a central feature. The mean ± SEM (*n* = 11) physical characteristics of the subjects were: age, 32.9 ± 1.6 years; height, 177.6 ± 2.3 cm; body mass, 74.0 ± 3.2 kg; body fat, 13.7 ± 0.9 %.

6.2.2 General design

All Subjects were randomly assigned, within a crossover design, to complete two trials, at least 7-d apart. On each occasion, subjects completed a morning and afternoon run on a motorised treadmill, separated by a 4-h recovery/rehydration period, according to the standardised recovery protocol (Section 3.8). During the 4-h recovery period subjects consumed 55-g of naturally enriched ¹³C-glucose in the form of a 7.5% carbohydrate-electrolyte solution (CES, mass 667-g, containing 24 mmol.l⁻¹ Na⁺, 2.6 mmol.l⁻¹ k⁺, 1 mmol.l⁻¹ Cl⁻, 300 mosmol.kg⁻¹) immediately after the first exercise period (T1). The subjects then consumed either:

- (i) The same quantity of CES at 60, 120 and 180 min of the 4-h recovery period, giving a total dose of 220 g CHO (C220), or
- (ii) An equivalent volume of a glucose-free electrolyte placebo (C55), at 60, 120 and 180-min of the 4-h recovery period.

The $^{13}\text{C}/^{12}\text{C}$ ratio of the ingested glucose was expressed relative to laboratory standards of known concentration and had a high natural [^{13}C]-abundance ($-10.48 \delta \text{ pm}$ vs. Pee Dee Belemnite (PDB)) relative to the mean \pm SEM abundance in breath gases at rest ($-26.14 \pm 0.19 \delta \text{ pm}$ vs. PDB). All exercise trials were conducted in an environmental chamber controlled at a mean \pm SEM temperature $34.8 \pm 0.1 \text{ }^\circ\text{C}$, humidity $39.8 \pm 0.1 \%$ and air velocity of $3.1 \text{ m}\cdot\text{s}^{-1}$.

6.2.3 Preliminary measurements

Following a full medical examination and the estimation of percentage body fat from four skinfold measurements (Durnin and Womersley 1974), subjects were familiarised with treadmill running and with the experimental procedures. Each subject then completed two preliminary tests before the two main trials, to assess the oxygen cost of submaximal running and maximal oxygen uptake (mean \pm SEM $\dot{V}O_{2 \text{ max}}$ $60.7 \pm 1.8 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), as described in Chapter 3. Using the linear relationship between $\dot{V}O_2$ and treadmill velocity, a running speed was calculated to elicit 60% $\dot{V}O_{2 \text{ max}}$ during the C55 and C220 trials. Subjects maintained a constant training level throughout the experimental period and refrained from strenuous activity for 48-h prior to an experimental day. Subjects were carefully instructed to refrain from consuming foodstuffs of C_4 metabolic origin with a high natural [^{13}C]-abundance (e.g. corn flakes, cane sugar and sports drinks) for 5-d before and during the experimental period.

6.2.4 Procedures

On reporting to the laboratory subjects were seated in a room maintained at 22°C , where they consumed a standard breakfast (685 kcal (2877 kJ): 70% carbohydrate, 20% fat, 10% protein) and water (approximately 780 g). After 90 min the subjects entered the environmental chamber and were asked to void their bladder. Nude body mass was then measured to the nearest 5 g (Sauter SD100, Germany) before dressing in shorts, socks and running shoes. The subjects were instrumented with two aural thermistors (insulated with cotton) and three ECG chest electrodes for the determination of tympanic membrane

temperature (T_{aur}) and heart rate (HR). After sitting in a relaxed position for 15 min an initial 15 ml venous blood sample was drawn from an antecubital vein. All blood samples were obtained without interruption of the circulation. Duplicate 50 μ l capillary blood samples were drawn from the thumb or finger and a 5 min expired gas sample collected.

After recording baseline T_{aur} (Grants squirrel data logger) and HR (S&W Diascope) subjects stood in position on the treadmill and the velocity was gradually increased to elicit a metabolic demand equivalent to 60% $\dot{V}O_2, max$ (T1). During T1 subjects were required to run for 90 min, until volitional fatigue, or until T_{aur} reached 39°C, whichever was reached first. Ingestion of water was permitted at a rate of 2 ml.kg⁻¹ body mass every 15 min. Expired gas samples were collected over a 1-min period at 15 min intervals during T1 using the Douglas bag technique. Percentage oxygen (O₂) content was measured by a paramagnetic O₂ analyser, percentage carbon dioxide (CO₂) was measured by an infrared CO₂ analyser (Taylor Servomex, Series 1400) and minute ventilation was measured by a Harvard dry gas meter. From gas analyses, $\dot{V}E$, $\dot{V}O_2$ and $\dot{V}CO_2$ were determined and the respiratory exchange ratio (RER) was calculated. Expired gas samples were also collected for the subsequent determination of ¹³C/¹²C ratio of expired CO₂. Duplicate 50 μ l capillary blood samples were drawn at 30 and 60 min of exercise. The T_{aur} and HR were recorded at 1-min intervals throughout the exercise period. Following T1, nude body mass was assessed, venous and capillary blood samples were drawn, and subjects left the environmental chamber to begin their controlled 4-h recovery period (REC) in a room maintained at 22°C.

During REC of both conditions, no food and only the prescribed fluids were consumed (as previously described). Duplicate capillary blood samples and 5 min expired gas samples were collected at 60 min intervals and venous blood samples at 80 min intervals during recovery. Urine was collected throughout the 4-h REC period and subjects were asked to finally void their bladder upon entering the environmental chamber for a second time. Following the assessment of nude body mass subjects were dressed and instrumented as before. After the collection of expired gas, venous and a capillary blood samples, subjects stood in position on the treadmill. As before the treadmill velocity was increased to elicit 60% $\dot{V}O_2, max$. Subjects were required to run until volitional fatigue or until T_{aur} reached 39°C, whichever was reached first (T2). The frequency of data

collection during T2 was identical to T1 and subjects were again permitted to consume 2 ml.kg⁻¹ body weight of water every 15 min. At the end of the run, the subjects dried themselves before being weighed in the nude: urine samples were then collected and final venous (15 ml) and capillary (50 µl) blood samples were drawn.

6.2.5 Analytical methods

Venous blood samples were collected into a lithium-heparin tubes except for a 5-ml aliquot which was placed into a non-heparinised tube and left to clot for 1-h. Serum was subsequently removed after centrifugation for 15 min at 6000 rpm and was stored at -40°C before being analysed for insulin concentration (Soeldner and Sloane 1965) using a commercially available kit (¹²⁵I radioimmunoassay; Coat-A-Count Insulin, DPC Kit). Plasma obtained by centrifugation for 15 min at 6000 rpm at temperatures of between 3 and 5°C, was stored at -40°C and later analysed for free fatty acids (FFA) using a commercially available kit (NEFA-C test, Wako, Japan) and glycerol (Laurel and Tibbling 1966). Whole capillary blood samples were collected in capillary tubes containing heparin, fluoride and nitrate and mixed for approximately 3 min, before duplicate glucose and lactate concentrations were determined (Analox P-GM7, Analox Instruments UK).

Rates of total CHO (CHO_{tot}) and fat (FAT_{tot}) oxidation were calculated from $\dot{V}O_2$ and $\dot{V}CO_2$, using previously described methods (Péronnet and Massicotte 1991). Expired gas samples were collected into evacuated 10-ml tubes (Exetainers) and later analysed for ¹³C/¹²C ratio of expired CO₂ by isotope ratio mass spectrometry (IRMS, Europa Scientific Ltd, Crewe UK). The isotopic enrichment of expired CO₂ was expressed as the delta per mil (mil = 1000) difference between the ¹³C/¹²C ratio of the sample and a known laboratory reference standard according to the formula:

$$\delta^{13}C = \left(\left(\frac{{}^{13}C/{}^{12}C \text{ sample}}{{}^{13}C/{}^{12}C \text{ standard}} \right) - 1 \right) \cdot 10^3 \quad (6.1)$$

The amount of orally ingested ¹³C-glucose oxidised (CHO_{ing} : g.min⁻¹) was calculated according to the formula:

$$CHO_{ing} = \dot{V}CO_2 \cdot \left(\frac{\delta_{exp} - \delta_{ref}}{\delta_{ing} - \delta_{ref}} \right) \left(\frac{1}{k} \right) \quad (6.2)$$

In which, δ_{ref} is the ^{13}C enrichment of expired air at the same time point during T1 (background enrichment), δ_{exp} is the ^{13}C enrichment of expired CO_2 during exercise at different time points, δ_{ing} is the ^{13}C enrichment of the glucose in the CES and k is the amount of CO_2 (litres) produced via oxidation of 1 gram glucose ($k = 0.7466$ l per gram glucose). To correct for changes in background $^{13}C/^{12}C$ ratio of expired CO_2 , which occur from rest to exercise, T2 exercise values were corrected using values from equivalent time points during the T1 exercise periods (Figure 6.1). Not correcting for this background enrichment would have led to a consistent overestimation of CHO_{ing} oxidation rates, by a mean \pm SEM of $6.3 \pm 0.7\%$ and $34.3 \pm 0.5\%$, during the C220 and C55 trials, respectively. In addition, the 0.8 recovery factor proposed by a number of studies for resting individuals in the continuously fed or postabsorptive state, was applied to the recovery data (Yang *et al.* 1983; Irving *et al.* 1984; Hoerr *et al.* 1989). This correction factor allows for the incomplete recovery of $^{13}CO_2$ on the breath because of the loss of some carbon atoms in or around the TCA cycle and in the large pool of bone carbonates. The rate of endogenous (i.e. not ingested) CHO oxidation (CHO_{end}) was subsequently estimated according to the formula:

$$CHO_{end} = CHO_{tot} - CHO_{ing} \quad (6.3)$$

Glycogen synthesis (GS) during the REC was then estimated (method adapted from Schneiter *et al.* 1995) as the difference between the amount of $[U-^{13}C]$ -glucose ingested ($CHO_{ingested}$) and the amount of $[U-^{13}C]$ -glucose oxidised (CHO_{ing}). This estimate is based on the assumption that all of the glucose ingested was absorbed and available for storage/oxidation at the end of the 4-h recovery period. Similarly, glycogen breakdown (GB) was estimated as the difference between total CHO oxidation (CHO_{tot}) and $[U-^{13}C]$ -glucose oxidation (CHO_{ing}). Net glycogen balance (NGB) was therefore estimated as the difference between GS and GB over the 4-h recovery period.

Total body sweat loss during the T1 and T2 exercise periods was calculated from the difference in nude body mass from pre to post-exercise, and was adjusted for fluid intake, urinary excretion and respiratory water loss (Mitchell *et al.* 1972). Values were then

expressed as a rate, using the respective exercise duration. Similarly, fluid balance over the 4-h recovery period (REC) was assessed from the change in body mass, corrected for fluid intake, urinary and respiratory water vapour losses.

6.2.5 *Statistical analysis*

Data for the T1-end and T2-end time points refer to the final measurement for each data variable, for each subject prior to cessation of exercise. Statistical comparisons were made after establishing the normality or otherwise of the data distribution using the Anderson-Darling Goodness of Fit test (Anderson and Darling 1954). A two-way analysis of variance (ANOVA) with repeated measures was used to compare results between treatments and across time. Where significant *F*-ratios were found, a Tukey post-hoc test was used to determine the cause of the variance. When there were only single comparisons, a Student's *t*-test for correlated data was used to determine whether differences between treatments existed. Where normality of the data distribution could not be assumed, individual comparisons were made by Wilcoxon's matched pairs signed ranks test. Descriptive data are presented as mean \pm standard error of the mean (SEM). Differences between and within trials were considered significant when $P < 0.05$.

6.3 Results

6.3.1 *Exercise tolerance time*

The mean \pm SEM run times during T1 did not differ between the C55 (59.9 ± 5.8 min, range 37 to 90-min) and C220 trials (59.5 ± 5.8 min, range 39 to 90-min). The coefficients of variation for the T1 runs of the C220 (17.3%) and C55 (18.8%) trials were similar and there was a strong correlation between these two sets of data ($r=0.87$, $P < 0.01$). This provides an indication that subjects ran the same approximate time during T1 of both trials. Exercise tolerance time during T2 was also similar in the C220 (42.0 ± 2.9 min, range 32.0 to 59.0-min) and C55 trials (41.4 ± 2.4 min, range 32.5 to 60.0-min). Exercise tolerance time was greater during the first (T1) compared to the second (T2) exercise period in both conditions ($P < 0.01$). Eighteen of the T1 and all ($n = 22$) of the T2 exercise periods were curtailed because subjects reached the pre-determined thermoregulatory end-point ($T_{\text{aur}} > 39^{\circ}\text{C}$).

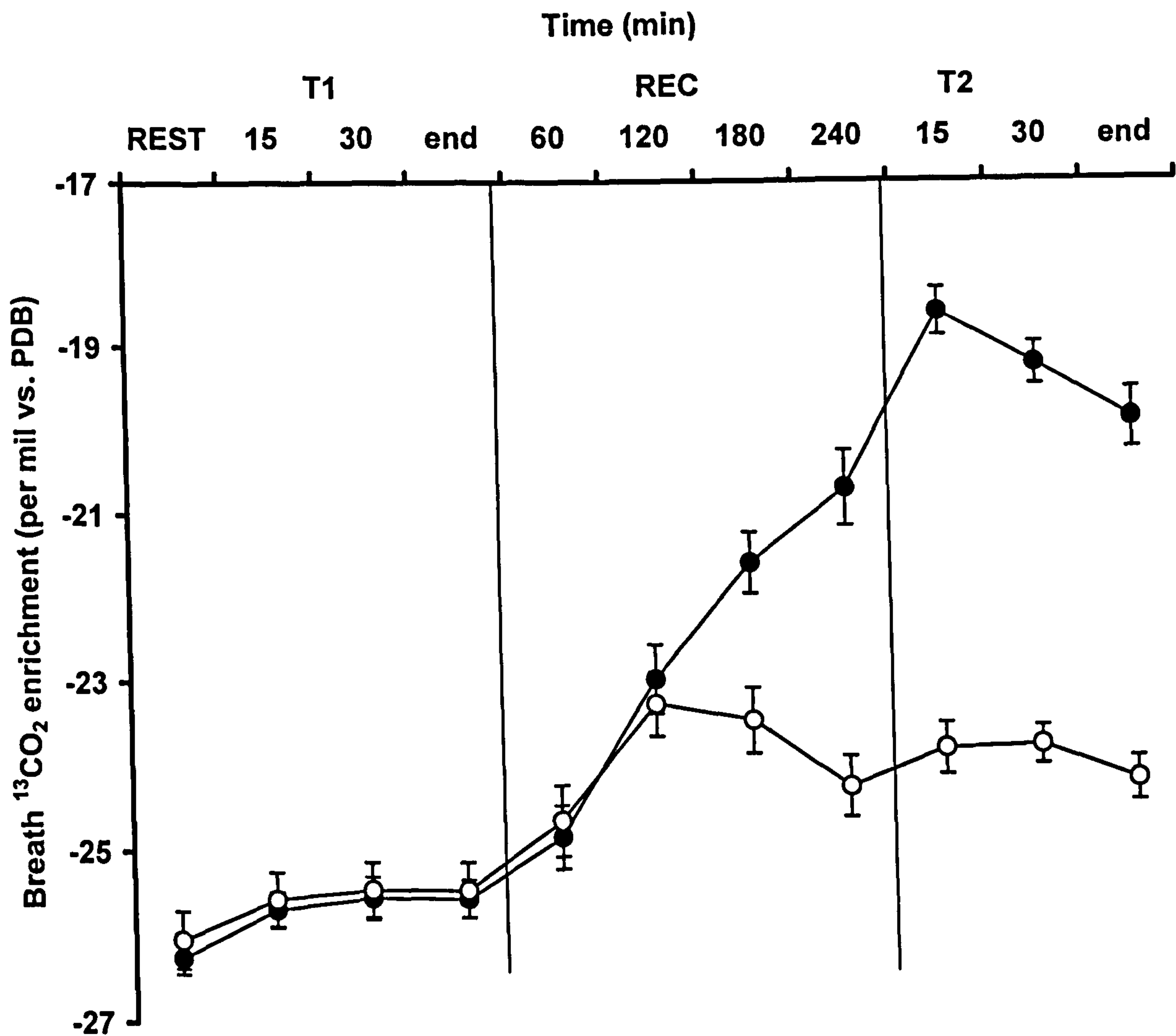


Figure 6.1 Breath $^{13}\text{CO}_2$ -enrichment (δ per mil vs. PDB) at each time point during T1, REC and T2 of the C220 (●) and C55 (○) trials. Mean \pm SEM enrichment of resting samples was -26.14 ± 0.19 δ per mil vs. PDB. Values are mean \pm SEM ($n = 11$).

6.3.2 Fluid balance

Body mass prior to T1 did not differ between the two trials (Table 6.1). Sweat losses equivalent to 2.8 ± 0.3 % and 2.7 ± 0.3 % of pre-exercise body mass were incurred during the T1 exercise periods of the C220 and C55 trials, respectively. Sweat rates were therefore similar in the C220 (33.8 ± 1.8 g.min $^{-1}$) and C55 exercise trials (33.1 ± 2.4 g.min $^{-1}$). Similar quantities of fluid were ingested in the T1 exercise periods of both trials and subjects were therefore in net negative fluid balance (dehydrated) equivalent to 2.1 ± 0.3 % and 2.0 ± 0.3 % of pre-exercise body mass in the C220 and C55 trials, respectively. During the subsequent 4-h recovery period subjects ingested 2668 g of the appropriate solution and subsequent urine production was equivalent to 54 (C220) and 55% (C55) of the ingested fluid volume.

	C220	C55
	mean ± SEM	mean ± SEM
T1		
Pre-exercise body mass (kg)	74.82 ± 3.04	74.60 ± 3.14
Post-exercise body mass (kg)	73.25 ± 3.02	73.09 ± 3.09
Fluid intake during exercise (g)	470 ± 52	458 ± 56
Sweat loss (g)	2045 ± 246	1972 ± 222
Sweat rate (g.min ⁻¹)	33.8 ± 1.8	33.1 ± 2.4
Net fluid balance (g)	-1575 ± 199	-1514 ± 180
REC		
Post-recovery body mass (kg)	74.47 ± 3.01	74.29 ± 3.10
Fluid intake (g)	2668 ± 0	2668 ± 0
Urine production (g)	1441 ± 168	1467 ± 163
Net fluid balance (g)	-348 ± 67	-313 ± 75
T2		
Post-exercise body mass (kg)	73.34 ± 3.00	73.26 ± 3.08
Fluid intake during exercise (g)	347 ± 27	319 ± 25
Sweat loss (g)	1479 ± 153	1350 ± 92
Sweat rate (g.min ⁻¹)	33.9 ± 1.9	32.8 ± 1.7
Net fluid balance (g)	-1480 ± 176	-1343 ± 124

Table 6.1 Data relating to changes in net fluid balance throughout the experimental period during the C220 and C55 trials. Values are means ± SEM ($n = 11$).

Subjects were still in net negative fluid balance at the end of the recovery period, equivalent to 0.5 ± 0.1 % and 0.4 ± 0.1 % of pre-exercise body mass during C220 and C55, respectively. Sweat rates were similar to T1 during the T2 exercise period and there were no differences between the C220 (33.9 ± 1.9 g.min⁻¹) and C55 (32.8 ± 1.7 g.min⁻¹) trials.

6.3.3 *Metabolic responses*

There was a progressive increase in $\dot{V}O_2$ over time (Table 6.2) during each of the exercise periods ($P < 0.05$). Consequently, after 15-min of exercise during T1 subjects were exercising at 58.5 ± 2.0 % and 59.6 ± 1.9 % $\dot{V}O_{2,max}$, which increased to 61.5 ± 2.4 % and 61.2 ± 2.0 % $\dot{V}O_{2,max}$ during the C220 and C55 trials, respectively. Similar changes took place between 15-min and the end of the T2 exercise periods in both trials. $\dot{V}O_2$ was similar during T1, REC and T2 of the C220 and C55 trials, respectively. $\dot{V}CO_2$ did not change over time, and whilst values were similar during the T1 exercise periods, values were greater during REC and T2 of the C220 compared to the C55 trial. Although, there was no difference in RER between trials during T1, values were greater after 180-min of REC and at each time point during REC and T2 of the C220 compared to the C55 trial, respectively (Table 6.2).

Substrate metabolism did not differ between the two trials during T1, but the relative contribution of carbohydrate (CHO_{tot}) decreased ($P < 0.01$) and fat (FAT_{tot}) oxidation increased over time ($P < 0.01$), in both trials. Carbohydrate oxidation contributed 72 ± 3 % and 71 ± 4 % of the total energy requirement during T1 of the C220 and C55 trials, respectively. CHO_{tot} was higher and FAT_{tot} was lower after 180 min and 240 min of REC and at each subsequent time point during T2 of the C220 compared to the C55 trial (Table 6.3).

Estimated glycogen synthesis over the 4-h recovery period was estimated to be approximately 5-fold greater ($P < 0.01$) in the C220 (177 ± 4 g) compared to the C55 (33 ± 3 g) trial (Table 6.4). However, glycogen breakdown was estimated to be similar in the two trials (C220, 42 ± 7 g; C55, 35 ± 6 g). As such, net glycogen balance was significantly greater ($P < 0.01$) at the end of the recovery period in the C220 (135 ± 8 g) compared to the C55 trial, which was negligible (-2 ± 8 g).

	T1					T2				
	15 min	30 min	45 min	end		15 min	30 min	end		
	Mean \pm SEM	Mean \pm SEM	mean \pm SEM	mean \pm SEM		mean \pm SEM	mean \pm SEM	mean \pm SEM		
$\dot{V}O_2$ (l.min ⁻¹)										
C220	2.60 \pm 0.11	2.66 \pm 0.11	2.74 \pm 0.13	2.77 \pm 0.12		2.57 \pm 0.09	2.74 \pm 0.11	2.84 \pm 0.11		
C55	2.65 \pm 0.10	2.69 \pm 0.10	2.72 \pm 0.09	2.71 \pm 0.09		†2.77 \pm 0.10	2.82 \pm 0.11	2.87 \pm 0.10		
$\dot{V}CO_2$ (l.min ⁻¹)										
C220	2.44 \pm 0.09	2.45 \pm 0.10	2.47 \pm 0.12	2.38 \pm 0.11		2.49 \pm 0.09	2.63 \pm 0.11	2.62 \pm 0.10		
C55	2.48 \pm 0.09	2.49 \pm 0.09	2.43 \pm 0.08	2.32 \pm 0.08		2.41 \pm 0.09	†2.39 \pm 0.10	†2.35 \pm 0.10		
RER										
C220	0.94 \pm 0.03	0.92 \pm 0.04	0.90 \pm 0.04	0.86 \pm 0.04		0.97 \pm 0.02	0.96 \pm 0.04	0.92 \pm 0.04		
C55	0.93 \pm 0.04	0.93 \pm 0.04	0.90 \pm 0.03	0.86 \pm 0.05		†0.87 \pm 0.04	†0.85 \pm 0.05	†0.81 \pm 0.04		

Table 6.2 Oxygen uptake ($\dot{V}O_2$, l.min⁻¹), carbon dioxide production ($\dot{V}CO_2$, l.min⁻¹), respiratory exchange ratio (RER) at each time point during the T1 and T2 exercise periods of the C220 and C55 trials. Values are mean \pm SEM ($n = 11$). Symbols denote that values are significantly different from C220: † ($P < 0.05$); ‡ ($P < 0.01$).

	REC				T2			
	60 min	120 min	180 min	240 min	15 min	30 min	end	
	mean ± SEM	Mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM	mean ± SEM	
CHO_{tot} (g.min ⁻¹)								
C220	0.34 ± 0.03	0.33 ± 0.03	0.38 ± 0.04	0.37 ± 0.06	3.05 ± 0.13	3.11 ± 0.16	2.78 ± 0.17	
C55	0.26 ± 0.03	0.26 ± 0.03	†0.21 ± 0.05	†0.21 ± 0.04	‡2.04 ± 0.16	‡1.80 ± 0.17	‡1.39 ± 0.18	
CHO_{ing} (g.min ⁻¹)								
C220	0.07 ± 0.01	0.15 ± 0.01	0.22 ± 0.02	0.28 ± 0.02	1.48 ± 0.07	1.43 ± 0.06	1.32 ± 0.06	
C55	0.06 ± 0.01	0.12 ± 0.01	‡0.11 ± 0.01	‡0.08 ± 0.01	‡0.42 ± 0.05	‡0.43 ± 0.05	‡0.34 ± 0.05	
CHO_{end} (g.min ⁻¹)								
C220	0.27 ± 0.03	0.18 ± 0.03	0.16 ± 0.04	0.09 ± 0.05	1.57 ± 0.10	1.68 ± 0.13	1.46 ± 0.16	
C55	0.20 ± 0.03	0.14 ± 0.02	0.10 ± 0.04	0.13 ± 0.03	1.62 ± 0.16	1.37 ± 0.15	†1.05 ± 0.15	
FAT_{tot} (g.min ⁻¹)								
C220	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.05 ± 0.02	0.13 ± 0.02	0.20 ± 0.04	0.34 ± 0.08	
C55	0.06 ± 0.01	0.05 ± 0.01	†0.08 ± 0.01	0.08 ± 0.01	‡0.60 ± 0.06	‡0.71 ± 0.07	‡0.90 ± 0.07	

Table 6.3 Rates of total carbohydrate oxidation (CHO_{tot} ; g.min⁻¹), ingested ¹³C-glucose oxidation (CHO_{ing} ; g.min⁻¹), endogenous CHO oxidation (CHO_{end} ; g.min⁻¹) and fat oxidation (FAT_{tot} ; g.min⁻¹) at each time point during REC and T2 of the C220 and C55 trials.

Values are mean ± SEM ($n = 11$). Symbols denote that values are significantly different from C220: † ($P < 0.05$); ‡ ($P < 0.01$).

		C220		C55	
		Mean	± SEM	mean	± SEM
	<i>CHO_{ingested}</i> (g)	220.0	± 0.0	55.0	± 0.0
	<i>CHO_{tot}</i> (g)	85.4	± 8.2	‡56.7	± 7.6
Recovery	<i>FAT_{tot}</i> (g)	8.7	± 2.2	†16.3	± 2.3
(4-h)	<i>CHO_{ing}</i> (g)	43.2	± 3.6	†22.0	± 2.7
	<i>GS</i> (g)	176.8	± 3.6	†33.0	± 2.7
	<i>GB</i> (g)	42.1	± 7.4	34.7	± 6.1
	<i>NGB</i> (g)	134.6	± 8.2	†-1.7	± 7.6
	<i>CHO_{tot}</i> (g)	134.2	± 6.6	†78.5	± 7.2
T2	<i>FAT_{tot}</i> (g)	10.0	± 2.0	†33.1	± 2.7
Exercise	<i>CHO_{ing}</i> (g)	63.6	± 2.6	†17.8	± 1.9
(45-min)	<i>CHO_{end}</i> (g)	70.6	± 5.6	60.7	± 6.4

Table 6.4 Total amounts of ¹³C-glucose ingested (*CHO_{ingested}*), total CHO (*CHO_{tot}*) and fat (*FAT_{tot}*) oxidised, ingested (*CHO_{ing}*) and endogenous (*CHO_{end}*) CHO oxidised, and estimated glycogen synthesis (*GS*), breakdown (*GB*) and net balance (*NGB*) during the 4-h of REC and 45-min of T2 in the C220 and C55 trials. Values are mean ± SEM (*n* = 11). Symbols denote that values are significantly different from C220: † (*P* < 0.05); ‡ (*P* < 0.01).

Consequently, the contribution of CHO to the total energy requirement was greater (*P* < 0.01) during T2 (86 ± 3 %) compared to T1 of the C220 trial, but lower (*P* < 0.01) than in the C55 trial (51 ± 4 %). Despite this difference in substrate metabolism during T2, *CHO_{end}* was similar at all time points, except for the end of exercise during the two trials (Table 6.3). As such, the increase in *CHO_{tot}* appeared to be met from ingested sources, with *CHO_{ing}* being more than threefold greater (*P* < 0.01) at all time points during T2 of the C220 compared to the C55 trial. However, more of the CHO consumed remained unoxidised on completion of T2 during the C220 (113.2 ± 3.1 g) compared to C55 (15.2 ± 2.3 g) trial, respectively (*P* < 0.01).

6.3.4 *Biochemical responses*

Expressed as percentage change from rest, plasma volume ($\% \Delta PV$) was reduced by $-7.4 \pm 1.1\%$ and $-7.1 \pm 1.1\%$ at the end of the C220 and C55 trials, respectively. There were no differences between trials in the magnitude of the calculated decrease in plasma volume (Figure 6.2). Plasma volume was subsequently restored to $-2.3 \pm 1.1\%$ and $-1.4 \pm 1.0\%$ after the first 80 min of REC during the C220 and C55 trials, respectively. Plasma volume remained relatively constant for the remainder of the REC period during the C55 trial, and subjects remained slightly hypovolaemic at the end of REC ($-0.7 \pm 1.3\%$). In contrast, plasma volume continued to increase and subjects were hypervolaemic at the end of REC ($P < 0.05$) in the C220 trial ($2.1 \pm 0.8\%$). Plasma volume was subsequently reduced ($P < 0.01$) to a similar extent during T2 of the C220 ($-5.8 \pm 1.0\%$) and C55 ($-7.3 \pm 1.3\%$) trials, respectively.

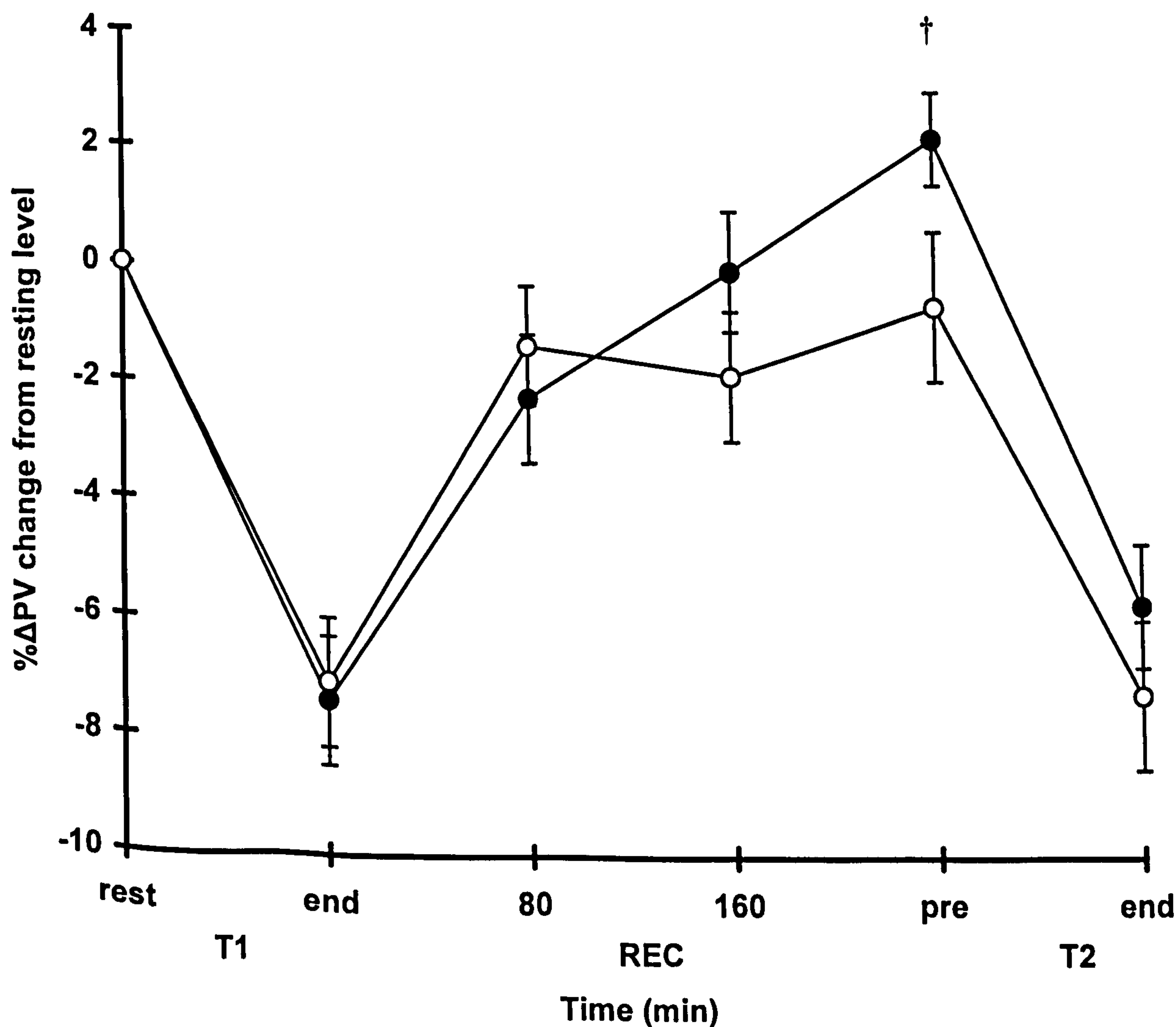


Figure 6.2 Percentage change in plasma volume ($\% \Delta PV$) during T1, REC and T2 of the C220 (●) and C55 (○) trials. Values are means \pm SEM ($n = 11$). † Denotes a significant difference between the C220 and C55 trials ($P < 0.05$).

Although blood glucose concentrations were lower after 30 min ($P<0.05$) and at the end ($P<0.05$) of the T1 exercise period compared to rest, there were no differences between trials (Figure 6.3). Subjects were hyperglycaemic relative to resting, after 60 min of REC in the C220 ($7.8 \pm 0.6 \text{ mmol.l}^{-1}$) and C55 ($7.6 \pm 0.4 \text{ mmol.l}^{-1}$) trials, respectively. Blood glucose was maintained at a higher concentration at 120 min ($P<0.01$), 180 min ($P<0.01$) and 240 min ($P<0.05$) during REC of the C220 compared to the C55 trial. However, subjects became hypoglycaemic at the end of T2 during C220 compared to C55 ($P<0.01$).

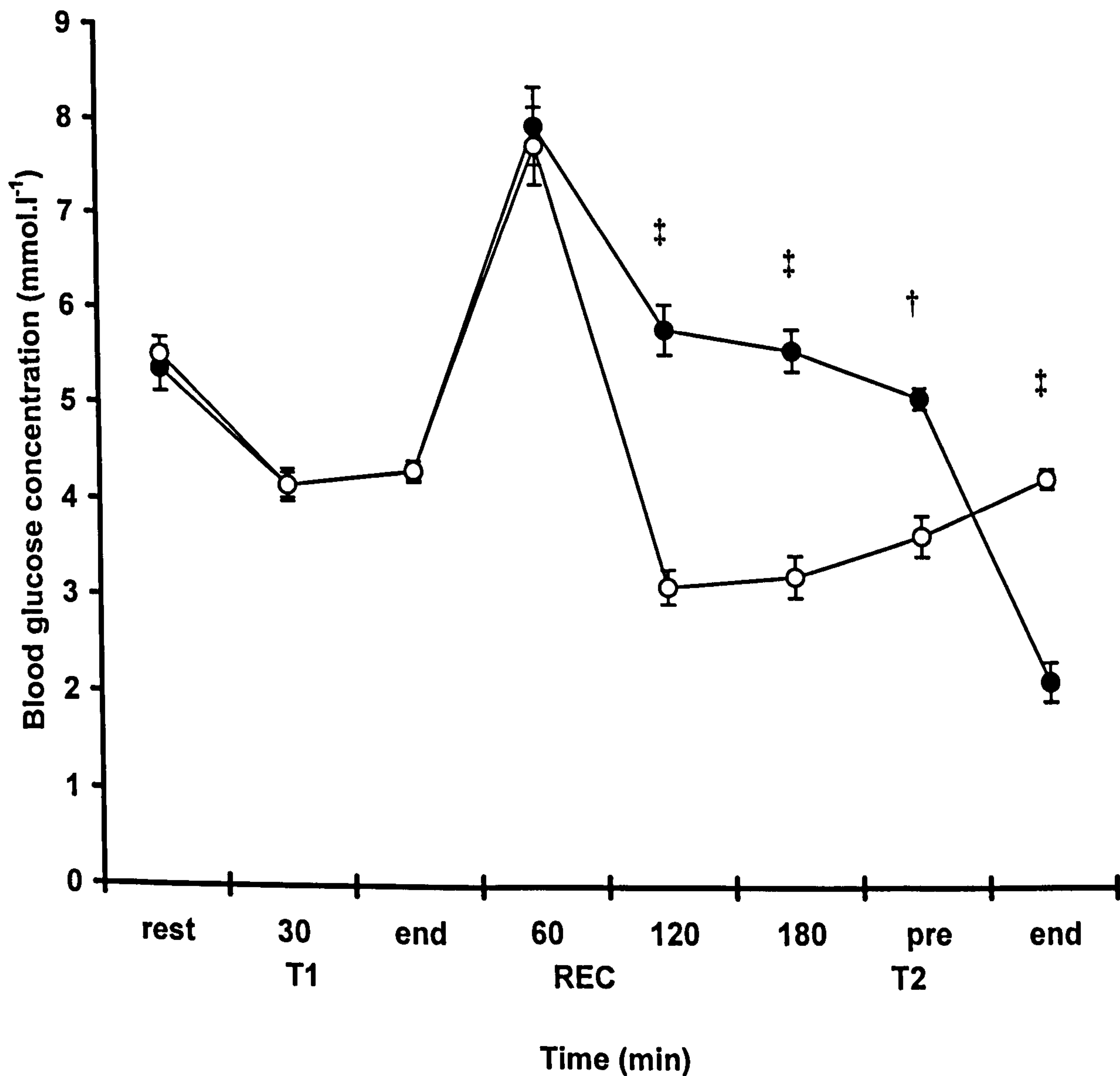


Figure 6.3 Plasma glucose concentrations (mmol.l^{-1}) during T1, REC and T2 of the C220 (●) and C55 (○) trials. Values are means \pm SEM ($n = 11$). Symbols denote a significant difference between the C220 and C55 trials: † ($P<0.05$); ‡ ($P<0.01$).

Serum insulin concentrations (Figure 6.4) revealed a similar pattern of change to blood glucose, being greater at 160 min ($P<0.01$) and 240 min of REC ($P<0.01$) during C220 compared to C55. Subjects were therefore hyperinsulinaemic at the onset of T2 during the C220 but not during the C55 trial.

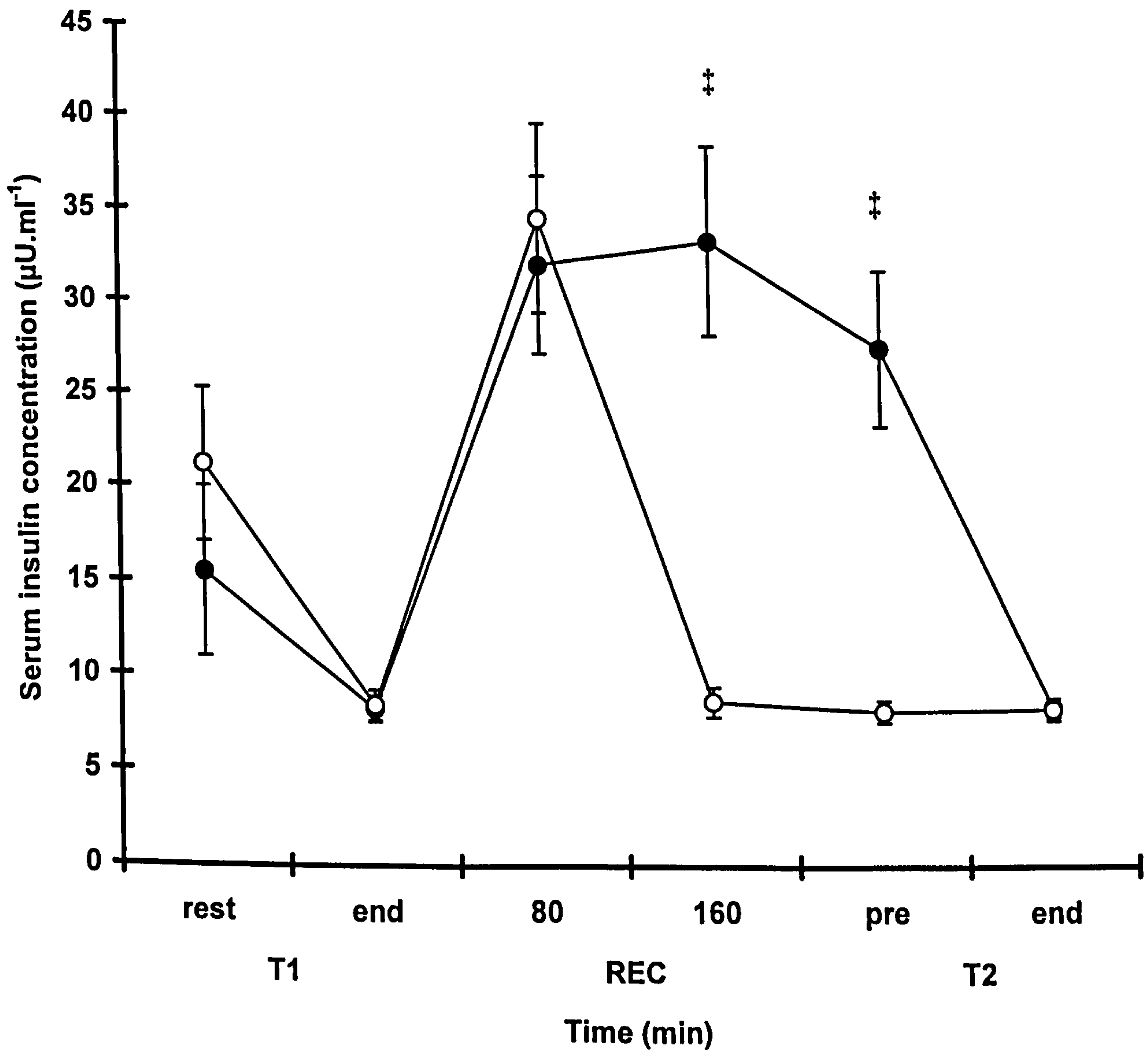


Figure 6.4 Serum insulin concentrations ($\mu\text{U}\cdot\text{ml}^{-1}$) during T1, REC and T2 of the C220 (\bullet) and C55 (\circ) trials. Values are means \pm SEM ($n = 11$). ‡ Denotes a significant difference between the C220 and C55 trials ($P<0.01$).

Plasma FFA concentrations (Figure 6.5) increased fourfold ($P<0.01$) and plasma glycerol concentrations (Figure 6.6) increased approximately eightfold ($P<0.01$) from rest to exercise, during T1 of both trials. Although plasma FFA and glycerol concentrations were reduced after 80-min of REC, both remained elevated throughout the REC period during the C55 compared to the C220 trial ($P<0.01$). As such, plasma glycerol and FFA concentrations were elevated in the C55 compared to the C220 trial at the start and end of the T2 exercise periods ($P<0.01$).

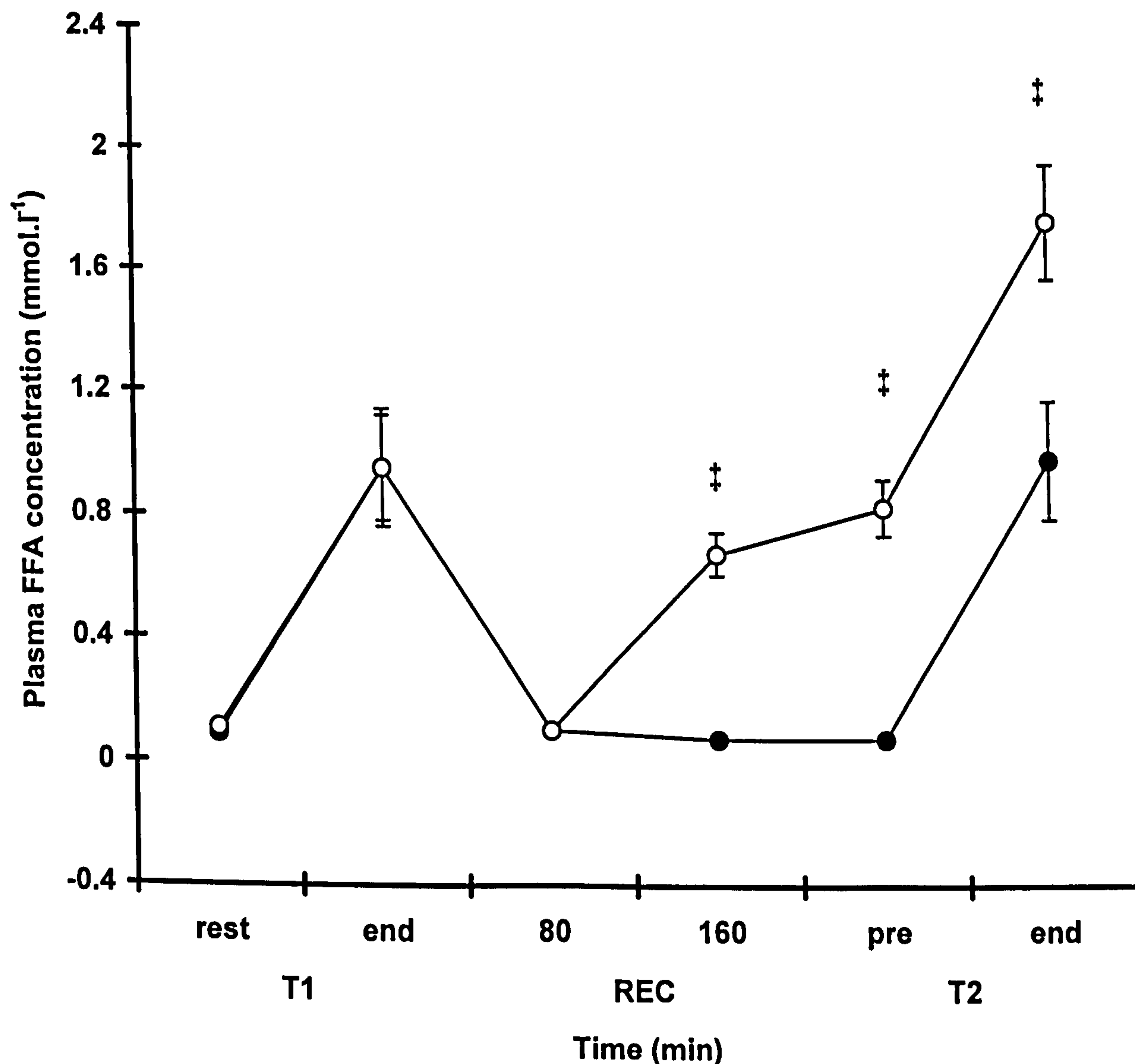


Figure 6.5 Plasma free-fatty acid (FFA) concentrations (mmol.l^{-1}) during T1, REC and T2 of the C220 (●) and C55 (○) trials. Values are means \pm SEM ($n = 11$). ‡ Denotes a significant difference between the C220 and C55 trials ($P<0.01$).

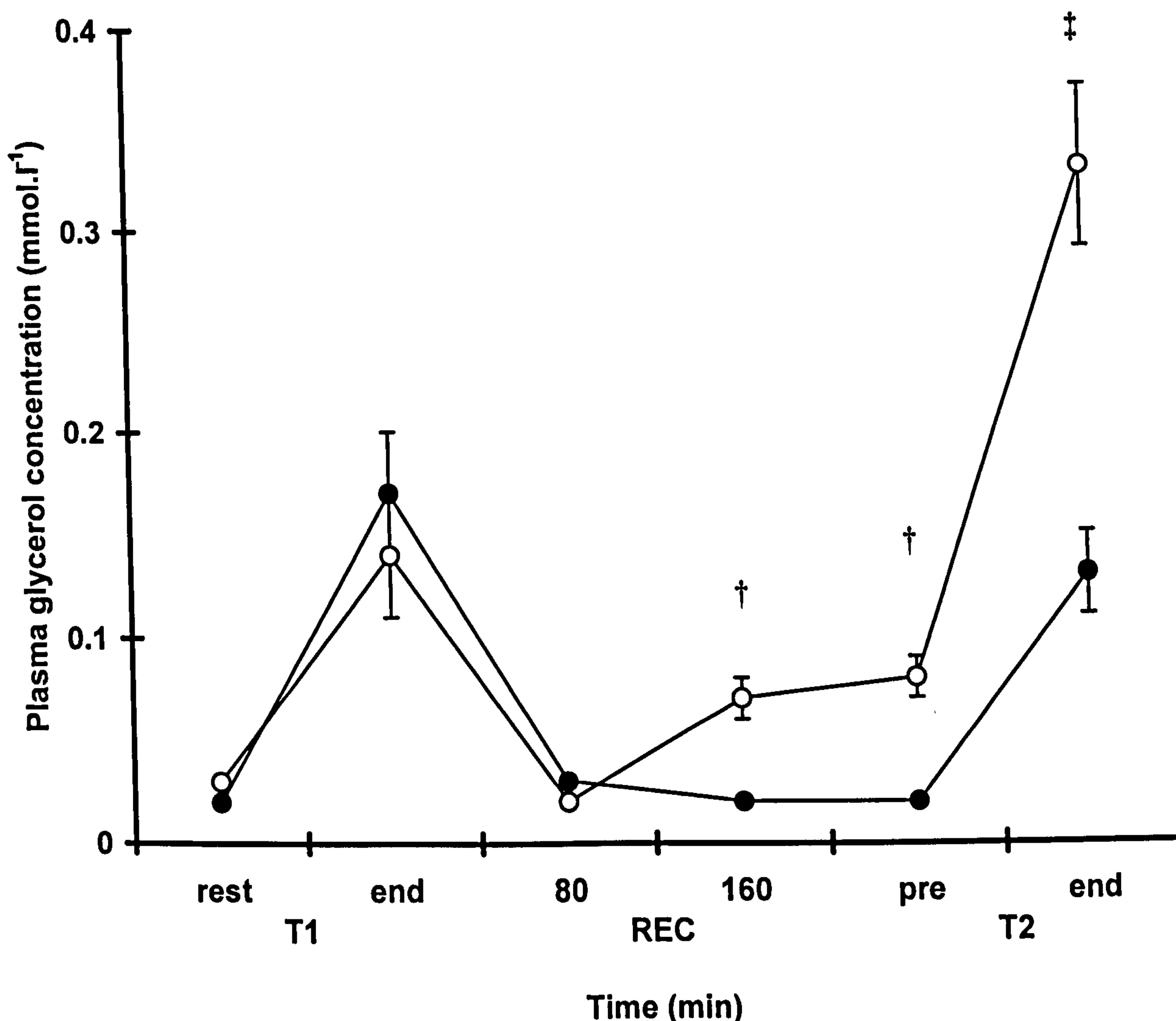


Figure 6.6 Plasma glycerol concentrations (mmol.l^{-1}) during T1, REC and T2 of the C220 (●) and C55 (○) trials. Values are means \pm SEM ($n = 11$). Symbols denote a significant difference between the C220 and C55 trials: † ($P < 0.05$); ‡ ($P < 0.01$).

Serum aldosterone concentrations were similar at rest ($\sim 430 \text{ pmol.l}^{-1}$), prior to T1, in the C220 and C55 trials (Figure 6.7). Concentrations peaked at $\sim 750 \text{ pmol.l}^{-1}$ at the end of the T1 exercise periods and there were no differences between the two trials. Concentrations gradually fell over the 4-h recovery period and were lower ($P < 0.01$) prior to T2 ($\sim 330 \text{ pmol.l}^{-1}$) compared with T1. Concentrations increased during T2 and were higher ($P < 0.05$) at the end of exercise during the C220 ($845 \pm 64 \text{ pmol.l}^{-1}$) compared to the C55 trial ($702 \pm 72 \text{ pmol.l}^{-1}$). Serum cortisol concentrations showed a similar pattern of change to aldosterone, increasing from $\sim 200 \text{ nmol.l}^{-1}$ prior to T1 and peaking at $\sim 700 \text{ nmol.l}^{-1}$ at the end of T1 (Figure 6.7). Concentrations gradually fell during REC before increasing during T2. There were no differences between trials.

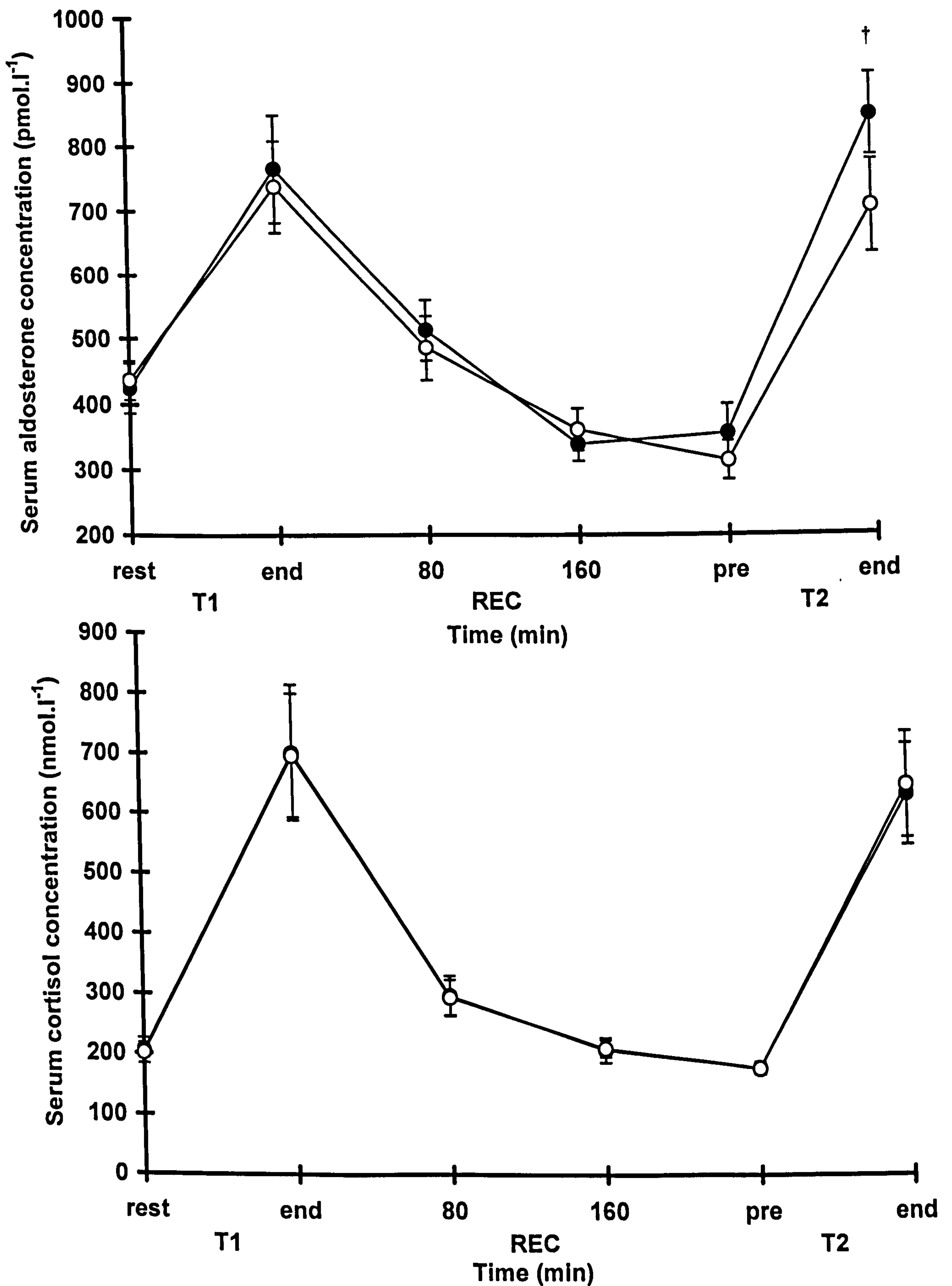


Figure 6.7 Serum aldosterone (pmol.l⁻¹) and cortisol (nmol.l⁻¹) concentrations during T1, REC and T2 of the C220 (●) and C55 (○) trials. Values are means \pm SEM ($n = 11$). † Denotes a significant difference between the C220 and C55 trials ($P < 0.05$).

5.3.5 *Thermal, cardiovascular and subjective responses*

Heart rate (HR) increased from approximately 62 beats.min⁻¹ at rest, to approximately 154 beats.min⁻¹ after 15-min of exercise during T1 of the C220 and C55 trials, respectively (Table 6.5). Heart rate continued to increase over time ($P<0.01$), reaching peak values of ~178 beats.min⁻¹ at the end of exercise in both trials. During T2, HR increased similarly over time ($P<0.01$), being elevated by 4-6 beats.min⁻¹ in comparison to equivalent time points during T1 in both trials. There were no differences between the C220 and C55 trials. Ratings of perceived exertion (RPE) showed a similar pattern of change to HR, increasing over time ($P<0.01$) and reaching peak values (RPE ~16) at the end of exercise. Again, values were higher at equivalent time points during T2 compared to T1 ($P<0.01$), and there were no differences between trials.

Aural temperature (T_{aur}) increased over time ($P<0.01$) from approximately 37.1 °C at rest to approximately 38.9 °C at the end of exercise in both trials (Table 6.5). Similar changes took place over time ($P<0.01$) during the T2 exercise periods. There were no differences in T_{aur} between the C220 and C55 trials at any time point during the T1 or T2 exercise periods. Similarly, perceived ratings of thermal discomfort (TD) gradually increased from rest (~1.3) through to the end of exercise (~9.6) during T1 ($P<0.01$) and T2 ($P<0.01$) of both trials. There were no differences between trials.

	T1				T2			
	15 min	30 min	45 min	End	15 min	30 min	45 min	end
	Mean \pm SEM	mean \pm SEM	Mean \pm SEM	mean \pm SEM	mean \pm SEM	mean \pm SEM	mean \pm SEM	mean \pm SEM
HR (beats.min⁻¹)								
C220	154 \pm 2	164 \pm 3	171 \pm 3	178 \pm 3	158 \pm 3	168 \pm 3	176 \pm 3	176 \pm 3
C55	154 \pm 3	164 \pm 3	172 \pm 3	177 \pm 3	159 \pm 3	170 \pm 3	176 \pm 3	176 \pm 3
RPE								
C220	10.1 \pm 0.3	11.7 \pm 0.3	13.7 \pm 0.4	15.7 \pm 0.4	11.3 \pm 0.5	13.5 \pm 0.5	15.7 \pm 0.4	15.7 \pm 0.4
C55	10.2 \pm 0.4	12.2 \pm 0.4	14.1 \pm 0.4	16.0 \pm 0.3	11.6 \pm 0.3	13.7 \pm 0.4	16.1 \pm 0.3	16.1 \pm 0.3
T_{aur} (°C)								
C220	37.8 \pm 0.1	38.3 \pm 0.1	38.7 \pm 0.1	38.9 \pm 0.1	37.9 \pm 0.1	38.4 \pm 0.1	38.8 \pm 0.1	38.8 \pm 0.1
C55	37.7 \pm 0.1	38.2 \pm 0.1	38.6 \pm 0.1	38.8 \pm 0.1	37.9 \pm 0.1	38.4 \pm 0.1	38.8 \pm 0.1	38.8 \pm 0.1
TD								
C220	4.6 \pm 0.3	6.5 \pm 0.3	8.1 \pm 0.3	9.6 \pm 0.2	5.2 \pm 0.4	7.5 \pm 0.4	9.2 \pm 0.3	9.2 \pm 0.3
C55	4.1 \pm 0.3	6.3 \pm 0.4	8.3 \pm 0.3	9.7 \pm 0.2	5.1 \pm 0.4	7.3 \pm 0.4	9.6 \pm 0.2	9.6 \pm 0.2

Table 6.5 Heart rate (HR, beats.min⁻¹), ratings of perceived exertion (RPE, Borg 1973), aural temperature (T_{aur}, °C) and thermal discomfort (TD) at each time point during T1 and T2 of the C220 and C55 trials. Values are mean \pm SEM ($n = 11$).

6.4 Discussion

The main finding of this study was that, despite a marked increase in estimated glycogen resynthesis during recovery and the maintenance of a higher rate of total carbohydrate (CHO_{tot}) oxidation during subsequent exercise (T2) after ingesting 220 g compared to 55 g of ^{13}C -glucose, the durations of the subsequent exercise were similar in the two conditions. The main reason for this appears to be that, in contrast to a previous investigation (Chapter 5), all T2 exercise periods were curtailed by subjects reaching the predetermined thermoregulatory end-point ($T_{aur} >39^{\circ}C$), showing signs (e.g. hyperventilation, disorientation) and feeling symptoms (e.g. nausea, thermal discomfort) indicative of heat exhaustion. Thermoregulatory incapacity and ensuing hyperthermia, rather than substrate availability *per se*, would therefore seem to be the primary factor limiting exercise tolerance in a warm environment. Furthermore, whilst ingesting 220 g of glucose did not spare endogenous (existing) reserves (CHO_{end}) compared to ingesting 55 g, more of the ingested glucose remained unoxidised on completion of T2 during the C220 condition. Whilst this may not be of benefit to post-recovery exercise capacity in a warm environment, it remains unclear how this may have influenced exercise capacity in a temperate environment.

The fact that T_{aur} continued to rise throughout the T1 and T2 exercise periods, despite the fact that subjects were producing theoretically near maximal sweat rates ($\sim 1.8 \text{ l}\cdot\text{h}^{-1}$), is indicative of uncompensable heat stress (Montain *et al.* 1994). These responses occurred despite the fact that subjects were well hydrated prior to each experimental condition, were permitted to ingest water during exercise and were only slightly dehydrated prior to the T2 exercise periods (Table 6.1). Clearly, the evaporative cooling capacity of the environment was less than that required to achieve thermal equilibrium during exercise. The ensuing hyperthermia appeared debilitating and would have undoubtedly lead to an early onset of fatigue or heat-related illness if exercise periods had not been prematurely curtailed at the predetermined thermoregulatory end-point ($T_{aur} >39^{\circ}C$).

Previous research has demonstrated that prolonged exercise capacity is limited by reduced CHO availability and is characterised by a reduction in muscle glycogen concentration (Ahlborg *et al.* 1967a). It was estimated from indirect respiratory calorimetry that $139 \pm 11 \text{ g}$ and $136 \pm 12 \text{ g}$ of CHO was oxidised during the T1 exercise

periods of the C220 and C55 conditions, respectively. These estimates equate to similar rates of total CHO oxidation observed in a previous investigation, at the same exercise intensity in a warm environment (Chapter 5). The quantities of ^{13}C -glucose ingested during the 4-h recovery period were sufficient to replace 40% and 158% of that estimated to have been utilised during T1 of the C55 and C220 conditions, respectively. However, since endogenous and ingested glucose was used during REC, net glycogen balance at the end of the recovery period was approximately equal to glycogen utilisation during T1 of the C220 condition [135 ± 8 g] and was virtually zero in the C55 condition. The negligible amount of glycogen resynthesis observed in the C55 condition is not dissimilar from that reported by Casey *et al.* (1999) when subjects ingested $1 \text{ g}\cdot\text{kg}^{-1}$ body weight of glucose during a 4-h recovery period.

Whilst it was not possible to independently quantify liver and muscle glycogen resynthesis during REC, as indicated above, total glycogen resynthesis was estimated. As the amount of glucose ingested in the C220 trial was approximately three times greater than in the study reported by Casey *et al.* (1999), it is speculated that 20 to 30 g of the ingested glucose would have been incorporated into the liver glycogen store. Assuming that the remainder of the 135 g was stored as skeletal muscle glycogen and that subjects had a mean dry muscle mass of ~ 10 kg, this equates to an estimated muscle glycogen resynthesis of $\sim 79 \text{ mmol glucosyl units}\cdot(\text{kg DM})^{-1}$. This value is not dissimilar from that reported by Tsintzas *et al.* (1999), for subjects ingesting repeated CHO solution feedings (175-g CHO) during a 4-h recovery period from exhaustive exercise [$75 \pm 20 \text{ mmol glucosyl units}\cdot(\text{kg DM})^{-1}$].

Although, the relative importance of the timing and quantity of CHO feedings for muscle glycogen resynthesis have been explored (Ivy *et al.* 1988a, 1988b), the influence of these two factors on substrate utilisation during subsequent exercise are less clear. The ingestion of 220 g of ^{13}C -glucose increased the availability and utilisation of CHO during T2 of the present trial, such that subjects were less reliant on CHO as a fuel for exercise during C55, metabolising only 78 ± 7 g compared to 134 ± 7 g during the C220 condition. Such a finding has been previously reported (Fallowfield *et al.* 1995; Wong *et al.* 1996b), although the use of CHO with a high natural ^{13}C -abundance has allowed us to partition the relative contributions from ingested and endogenous (existing) CHO sources. Although, the contribution of CHO to the metabolic demands of the T2 exercise

periods was increased by continuous feedings (C220), this was achieved by a fourfold increase in ^{13}C -glucose oxidation (CHO_{ing} : C220, 64 ± 3 g; C55, 18 ± 2 g), whilst sparing of the existing CHO stores was not apparent (CHO_{end} : C220, 71 ± 6 g; C55, 61 ± 6 g). Furthermore, the greater oxidation of CHO_{tot} and CHO_{ing} resulted in a reduced rate of fat oxidation (FAT_{tot}) during C220 (10 ± 2 g) compared to C55 (33 ± 3 g). These results are in agreement with those of Tsintzas *et al.* (1999), who also showed that fat oxidation was suppressed and endogenous muscle glycogen was not 'spared' during exercise following ingestion of 175 g compared to 50 g of CHO during a 4-h recovery period.

Despite the apparent physiological advantages of the continuous CHO feeding regimen, subjects were hyperinsulinaemic at the onset of the T2 exercise period during C220 but not during C55. Physiological hyperinsulinaemia has previously been associated with elevated muscle glucose uptake, CHO oxidation, and blood lactate concentrations (Weltan *et al.* 1998), whereas plasma FFA concentrations, glycerol concentrations and fat oxidation are suppressed (Wasserman *et al.* 1991). Consequently, hyperinsulinaemia is reported to decrease hepatic glucose production and lead to hypoglycaemia during exercise, a phenomenon known as 'rebound hypoglycaemia' (Arogyasami *et al.* 1992). Although subjects were hypoglycaemic at the end of T2 during the C220 compared to the C55 condition, this did not appear to limit exercise capacity during the current exercise periods. However, the hypoglycaemic response and decreased concentrations of lipolytic intermediates during the C220 trial suggest that there was a decrease in lipolysis and endogenous glucose production.

These biochemical responses provide evidence that subjects were less able to rely on blood borne FFA and glucose as a fuel source during T2 of the C220 compared to the C55 trial. As such, it is hypothesised that subjects became more dependent on muscle glycogen as a source of fuel for exercise, diminishing any previous advantages gained from increased glycogen storage. This may explain why Wong *et al.* (1996) did not observe an increase in exercise capacity with further exogenous CHO feedings compared to ingesting only 50-g at the beginning of recovery. The ^{13}C -glucose oxidation data from this trial clearly demonstrate that, despite a fourfold increase in CHO_{ing} oxidation during C220 there was no sparing of the existing (endogenous) CHO stores. Whether this was due to the quantity of substrate ingested or the timing of ingestion and subsequent hyperinsulinaemic response remains unclear.

In conclusion, deep body temperature (T_{aur}) continued to rise throughout exercise, with subjects reaching the predetermined thermoregulatory end-point at the same time during T2 of both conditions. As such, exercise tolerance appears to be limited by thermoregulatory incapacity rather than substrate availability when running at 60% $\dot{V}O_2max$ in a warm environment. Whilst glycogen resynthesis was greater during recovery and total CHO oxidation during T2 was greater after ingesting 220 compared to 55-g of glucose, fat oxidation was suppressed and endogenous (existing) glycogen stores were not 'spared' during C220. Whilst the increase in total CHO oxidation was met from ingested sources, more of the ingested substrate remained unoxidised on completion of T2 during C220. Glucose ingestion during prolonged running in the heat may 'spare' endogenous glycogen reserves and improve exercise tolerance, and therefore warrants further investigation.

CHAPTER SEVEN

INFLUENCE OF HEAT STRESS AND GLUCOSE INGESTION
ON GLUCOSE KINETICS AND EXERCISE TOLERANCE
DURING PROLONGED RUNNING IN MAN**7.1 Introduction**

Fatigue occurs earlier when prolonged exercise is performed in high ambient temperatures (Saltin *et al.* 1972). It has been suggested that this earlier onset of volitional fatigue is directly related to thermoregulatory incapacity and the hyperthermia which ensues (Fink *et al.* 1975; Brück and Olshewski 1987; Nielsen *et al.* 1990). Indeed, the effects of environmental temperature have been extensively studied with respect to changes in body fluid spaces, temperature regulation and cardiovascular responses (Rowell *et al.* 1968; Bell *et al.* 1983; Nielsen *et al.* 1990). Until more recently, relatively little was known regarding the effects of environmental temperature on substrate metabolism during exercise.

A number of studies have since demonstrated that endogenous heat stress increases the rate of total carbohydrate (CHO) oxidation (Chapter 4) and muscle glycogen utilisation (Febbraio *et al.* 1994a, 1994b). In addition, exercising in the heat increases muscle and blood lactate accumulation (Fink *et al.* 1975; Young *et al.* 1985; Febbraio *et al.* 1994a) and blood glucose concentrations (Yaspelkis *et al.* 1993). The exaggerated hyperglycaemia may occur either as a direct response to an increase in hepatic glucose production (HGP) (Rowell *et al.* 1968) or as a result of reduced muscle glucose uptake secondary to a reduction in muscle blood flow or an accelerated muscle glycogenolysis *per se* (Jansson *et al.* 1986).

There is increasing evidence that these metabolic alterations occur in response to hyperthermia and the associated increase in circulating catecholamine concentrations (Kozlowski *et al.* 1985; Febbraio *et al.* 1994a). Similar increases in plasma catecholamine concentrations and muscle glycogenolysis are observed when thermoregulatory function is impaired by progressive dehydration without adequate fluid replacement (Hargreaves *et al.* 1996a). Furthermore, adrenaline infusion has been shown

to increase skeletal muscle glycogenolysis, predominantly in type I muscle fibres, during electrical stimulation (Greenhaff *et al.* 1991). It is thought that these metabolic responses may, at least in part, explain the earlier onset of fatigue observed during exercise and heat stress. Indeed, ingestion of glucose during recovery from exercise in the heat increases glycogen synthesis (Chapter 6) and may improve subsequent exercise tolerance time in the heat (Chapter 5).

While the effects of heat stress on muscle glycogenolysis during cycling exercise are well documented, the effects on plasma glucose kinetics and muscle metabolism during running are less clear. Only one study has attempted to quantify the rates of appearance (Ra) and disappearance (Rd) of plasma glucose during exercise and heat stress and the mode of exercise adopted was cycling (Hargreaves *et al.* 1996b). To our knowledge no other studies have simultaneously attempted to quantify the effects of heat stress on glucose kinetics and muscle metabolism during prolonged running. Furthermore, the potential influence of glucose feedings on glucose kinetics and metabolism during running in the heat has not been investigated. As observed during running in thermoneutral environments (Tsintzas *et al.* 1995, 1996a), glucose feedings during exercise in the heat may attenuate the increased reliance on the limited endogenous glycogen stores by increasing muscle glucose uptake.

Recently, dual isotope tracer techniques have been adopted, permitting the simultaneous measurement of glucose kinetics and oxidation rates from various body pools (Jeukendrup *et al.* 1999a). The primary purpose of this study was to use a similar methodology to test the hypotheses that: (1) heat stress increases the rate of muscle glycogen utilisation during prolonged running; and (2) glucose feedings increase the Ra and Rd and rate of oxidation (Rox) of plasma glucose, thus 'sparing' endogenous glycogen reserves. A secondary aim of the study was to investigate how much of the glucose disappearing from the plasma is oxidised during exercise in a hot and in a temperate environment in the presence and absence of glucose ingestion.

7.2 Methods

7.2.1 Subjects

Nine healthy male volunteers participated in this study ($n = 9$), which was carried out with the approval of the Ministry of Defence (Navy) Personnel Research Ethics Committee (MOD(N) PREC). Written consent to participate was obtained from all subjects after the nature of the study had been explained to them. All were involved in various training programmes, of which submaximal running was a central feature. Their mean \pm standard error of the mean (SEM) physical characteristics were: age, 30 ± 1 years; height, 176 ± 2 cm; body mass, 80 ± 2 kg; body fat, 16 ± 2 %.

7.2.2 Preliminary measurements

Following a full medical examination, including 12-lead ECG, and the estimation of percentage body fat from skinfold thickness at four sites (Durnin and Womersley 1974), subjects were familiarised with treadmill running and with the experimental procedures. Each subject then completed two preliminary tests at least 7-d before the main experimental conditions. The first test consisted of 16-min of continuous running on a level treadmill to determine the oxygen cost ($\dot{V}O_2$) of running over a range of submaximal speeds (7 to 13-kph). The second of these tests was to determine each subject's maximal oxygen uptake ($\dot{V}O_{2,max}$) during uphill treadmill running (Taylor *et al.* 1955) in a warm environment (see procedures): mean \pm SEM $\dot{V}O_{2,max}$ was 60.1 ± 2.2 ml.kg⁻¹.min⁻¹. The results of these tests were used to individually determine a treadmill speed which would elicit a metabolic demand of 60% $\dot{V}O_{2,max}$ during the experimental conditions.

7.2.3 Experimental conditions

Each subject performed five experimental conditions, each separated by at least seven days. The first trial was performed to determine the background ¹³C-enrichment of expired CO₂ and plasma glucose. The order of the four remaining experimental conditions was determined by a latin-square design. Each experiment consisted of running on a level treadmill at 60% $\dot{V}O_{2,max}$ for 120-min or until rectal temperature (T_{rec}) reached 39.5°C. Subjects ingested either water (CON) or a 12.5% (w/v) glucose solution (GLU). Each condition was completed in an environment controlled at $19.3 \pm$

0.2 °C (CON19 and GLU19) and 33.9 ± 0.1 °C (CON34 and GLU34). Relative humidity and air velocity was controlled at 40% and $3.1 \text{ m}\cdot\text{s}^{-1}$, respectively in all conditions.

7.2.4 Glucose solutions

During the GLU trials subjects ingested a 12.5% (w/v) glucose solution prepared from corn-derived glucose (Amylum, Belgium), which has a high natural abundance of ^{13}C [-10.5 ‰ vs. Pee Dee Bellemnitella (PDB)]. Glucose solutions were further enriched with [U- ^{13}C]-glucose to achieve a mean \pm SEM final ^{13}C -enrichment of $+51.5 \pm 0.3 \text{ ‰}$ vs. PDB. The ^{13}C -enrichment of glucose was determined by elemental analyser isotope ratio mass spectrometry (Carlo Erba-Finnigan MAT 252, Bremen, Germany). This high ^{13}C -enrichment of exogenous glucose provides a very strong signal in plasma glucose as well as in expired CO_2 (Péronnet *et al.* 1998). To minimise possible shifts in background enrichment as a result of a change in endogenous substrate utilisation and differences in background enrichment of the different fuel stores, subjects were instructed not to consume products with a high natural abundance of ^{13}C throughout the entire experimental period. The magnitude of shifts in background ^{13}C -enrichment reported in subjects on a European diet (Wagenmakers *et al.* 1993b) is $<1 \text{ ‰}$ per mil, compared with the enrichment of the glucose solutions used here, which obviates the need for background correction (Péronnet *et al.* 1990). Subjects were further instructed to refrain from heavy physical training and to keep their diet as consistent as possible during the 48-h period prior to the experimental conditions.

7.2.5 Procedures

Subjects reported to the laboratory at 0800 after an overnight fast. An 18 gauge teflon cannula was inserted into an antecubital vein of one arm. In the contralateral antecubital vein a second teflon catheter was inserted for isotope infusion. At 0900 a resting blood sample (20-ml) was drawn. Subjects were then asked to void their bladder and nude body mass was recorded to the nearest 1-g (Sartorius AG, Göttingen, Germany). Each was then instrumented with a rectal thermistor (inserted to a depth of 10-cm), four skin thermistors (Ramanathan 1964) and three ECG chest electrodes. Resting breath gases were collected over a 5-min period (Sensormedics Vmax29, CA, USA) and 15-ml vacutainers (Becton-Dickinson, Maylan, France) were

filled in triplicate from a 1 litre expired gas sample bag, to determine the $^{13}\text{C}/^{12}\text{C}$ ratio in expired CO_2 . At 0955 subjects stood in position on the treadmill and a sodium bicarbonate prime was administered ($5.46 \pm 0.04 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ $\text{NaH}^{13}\text{CO}_3$, Cambridge Isotope Laboratories, USA), followed by a $[6,6\text{-}^2\text{H}_2]$ -glucose (Cambridge Isotope Laboratories, USA) prime (dose equal to one hour infusion). In the CON conditions a $[\text{U-}^{13}\text{C}]$ -glucose (Cambridge Isotope Laboratories, USA) prime was also administered (dose equal to 1-h infusion). Thereafter a continuous infusion of sterile pyrogen free $[6,6\text{-}^2\text{H}_2]$ -glucose was started via a calibrated IVAC 3000 pump (San Diego, CA). In the CON conditions a continuous infusion of sterile pyrogen free $[\text{U-}^{13}\text{C}]$ -glucose was also started. The concentration of isotopes in the infusate was estimated and the infusion rate was calculated to deliver approximately 1.6 and 0.06 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ of $[6,6\text{-}^2\text{H}_2]$ -glucose and $[\text{U-}^{13}\text{C}]$ -glucose, respectively. The concentration of isotopes in the infusate was subsequently measured and the precise mean \pm SEM infusion rates were calculated as 1.589 ± 0.012 and $0.062 \pm 0.001 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for $[6,6\text{-}^2\text{H}_2]$ -glucose and $[\text{U-}^{13}\text{C}]$ -glucose, respectively.

At 1000 subjects consumed an initial bolus (400-ml) of either water or a 12.5% glucose solution (50-g glucose during GLU conditions) and the treadmill speed was increased to 60% $\dot{V}O_2 \text{ max}$. Thereafter every 15-min a beverage volume of 120-ml was provided (15-g glucose during GLU conditions). Similar, feeding patterns have been shown to result in high rates of gastric emptying (Rehrer *et al.* 1990b). The mean amount of glucose provided during the 120-min of exercise was therefore 155-g during the GLU conditions and 0-g during the CON conditions. Blood samples (20-ml) were drawn at 15-min intervals until the end of exercise. Similarly, breath gases were collected over a 2-min period, 15-ml vacutainers were filled, rectal temperature (T_{rec}), skin temperature (T_{skin}), heart rate (f_c) and ratings of perceived exertion (RPE; Borg 1973) were recorded every 15-min.

On completion of each exercise condition, subjects were towel dried and nude body mass recorded. Total sweat loss was calculated from the change in nude body mass (corrected for fluid intake and respiratory water vapour loss) over the exercise period.

7.2.6 Analytical methods

Part (2-ml) of each blood sample was decanted into a vacutainer tube containing anticoagulant (EDTA) and used for the determination of haemoglobin (cyanmethaemoglobin method: Boehringer Mannheim, UK) and spun microhaematocrit (packed-cell volume, in triplicate). These results were used for the estimation of change in plasma volume relative to pre-exercise (Dill and Costill 1974). A further 4-ml of each sample was left to clot for 1-h in a domestic refrigerator (10-12°C) and serum separated by centrifugation for 15-min at 6000 rpm and 4°C. Serum samples were stored at -70°C and later analysed for insulin concentration (^{125}I radioimmunoassay; Coat -A-Count Insulin, DPC kit) using a gamma counter (Packard, Cobra 5000).

The remainder was decanted into lithium heparin tubes and plasma obtained by centrifugation. Plasma was stored at -70°C and subsequently analysed for free fatty acid (FFA, Wako chemicals GmbH kit), glucose, lactate (ABX Diagnostics, UK), and catecholamine (Bio-Rad Laboratories, CA, USA) concentrations.

For determination of $^{13}\text{C}/^{12}\text{C}$ ratio in plasma glucose, glucose was first extracted with chloroform-methanol-water and derivatisation was performed with butyl-boronic acid and acetic anhydride as described previously (Pickert *et al.* 1991). The measured $^{13}\text{C}/^{12}\text{C}$ ratios in the derivative (GC combustion-IRMS) were corrected for the isotopic carbon dilution. This was done by measuring a series of glucose standards both in the derivatised form (GC combustion-IRMS) and by direct combustion of underivatised glucose (elemental analyser-IRMS). The standard curve was linear over a range from 0 to 500‰ vs. PDB. Plasma [^2H]-glucose enrichment was determined by gas chromatography mass spectrometry (GC-MS) analysis of the derivatives on a Finningan INCOS-XL (Bremen, Germany). For [^2H]-glucose enrichment, ion masses of 200 and 202 were selectively monitored.

7.2.7 Calculations (general)

From measurements of $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$, total carbohydrate (CHO_{tot}) and fat (FAT_{tot}) oxidation rates were calculated using the non-protein respiratory quotient (Péronnet and Massicotte 1991).

$$CHO_{tot} = 4.585 \dot{V}CO_2 - 3.226 \dot{V}O_2 \quad (7.1)$$

$$FAT_{tot} = 1.695 \dot{V}O_2 - 1.701 \dot{V}CO_2 \quad (7.2)$$

The total rate of appearance (Ra) and rate of disappearance (Rd) of glucose was calculated using the single-pool non-steady state equations of Steele (1959) as described elsewhere (Wolfe 1992). Total Ra represents the splanchnic Ra from ingested CHO (during GLU conditions only), hepatic glycogenolysis and gluconeogenesis.

$$total\ Ra = \frac{\dot{F} - V [(C_2 + C_1) / 2] [(E_2 - E_1) / (t_2 - t_1)]}{(E_2 + E_1) / 2} \quad (7.3)$$

$$total\ Rd = R_a - V \left(\frac{C_2 - C_1}{t_2 - t_1} \right) \quad (7.4)$$

where F is the infusion rate; V is the plasma volume of distribution (160 ml.kg⁻¹); C_2 and C_1 are the plasma glucose concentrations at time 2 and 1 (i.e. t_2 and t_1), respectively; and E_2 and E_1 are the plasma glucose enrichments at time 2 and 1, respectively.

The isotopic enrichment was expressed as the delta per mil difference between the ¹³C/¹²C ratio of the sample and a known laboratory reference standard according to the formula of Craig (1957):

$$\delta^{13}C = \left(\left(\frac{^{13}C/^{12}C\ sample}{^{13}C/^{12}C\ standard} \right) - 1 \right) \times 10^3\text{‰} \quad (7.5)$$

The $\delta^{13}C$ was then related to the international standard PDB.

7.2.8 Calculations (GLU condition)

Exogenous glucose oxidation (EGO) was calculated according to the formula:

$$EGO = VCO_2 \cdot \left(\frac{\delta Exp - \delta Exp_{bkg}}{\delta Ing - \delta Exp_{bkg}} \right) \left(\frac{1}{k} \right) \quad (7.6)$$

in which δExp is the ^{13}C -enrichment of expired CO_2 during exercise at the relevant time point, δIng is the ^{13}C -enrichment of ingested glucose, δExp_{bkg} is the ^{13}C enrichment of expired CO_2 before exercise (background), and k (0.7467) is the amount of CO_2 (in litres) produced by the oxidation of 1 g glucose. Following the assessment of blood glucose enrichment, the following formula was used to calculate plasma glucose oxidation (PGO):

$$PGO = VCO_2 \cdot \left(\frac{\delta Exp - \delta Exp_{bkg}}{\delta PG - \delta PG_{bkg}} \right) \left(\frac{1}{k} \right) \quad (7.7)$$

in which δPG is the plasma glucose ^{13}C -enrichment, δPG_{bkg} is the plasma glucose ^{13}C -enrichment before exercise (background), and k is the same as in Eq. 7.6.

Because PGO represents the oxidation of both glucose appearing from the gut (exogenous glucose) and the contribution of the liver (glycogenolysis/gluconeogenesis), the rate of liver-derived glucose oxidation (LGO) was calculated from the following formula:

$$LGO = PGO - EGO \quad (7.8)$$

Liver-derived glucose equals the sum of glucose that originates from liver glycogen breakdown and from gluconeogenesis. It was previously demonstrated that 90-95% of glucose released by the liver is oxidised during exercise at 50% $\dot{V}O_2, max$ (Jeukendrup *et al.* 1999a). Muscle glycogen oxidation (MGO) can be estimated using the formula:

$$MGO = CHO_{tot} - PGO \quad (7.9)$$

7.2.9 Calculations (CON trial)

The $\dot{V}^{13}CO_2$ production from the tracer infusion was calculated using the formula:

$$\dot{V}^{13}CO_2 (\mu mol.kg^{-1}.min^{-1}) = ECO_2 \cdot \dot{V}CO_2 \quad (7.10)$$

where ECO_2 is the breath $^{13}C/^{12}C$ ratio at a given time, using a conversion factor of 1 mol $CO_2 = 22.4$ litres. The percentage of infused $[U-^{13}C]$ -glucose tracer oxidised was then calculated as:

$$\% \text{ tracer oxidised} = (\dot{V}^{13}CO_2/k) / [U-^{13}C]\text{-tracer infusion rate} \cdot 100 \quad (7.11)$$

Plasma glucose oxidation (PGO) was calculated as:

$$PGO = Rd \text{ glucose} \cdot \% \text{ tracer oxidised} \quad (7.12)$$

As there was no exogenous glucose feedings during the CON trials, exogenous glucose oxidation (EGO) was assumed to be zero and, assuming a 'steady-state', liver-derived glucose oxidation (glycogenolysis and gluconeogenesis) equals PGO . Muscle glycogen oxidation (MGO) will be estimated using *Eq. 7.9*.

Glucose carbon recycling rate (GRR) was calculated as the difference between Ra measured with a $[6,6-^2H_2]$ -glucose tracer and a $[U-^{13}C]$ -glucose tracer:

$$GRR = Ra ([6,6-^2H_2] \text{ glucose}) - Ra ([U-^{13}C] \text{ glucose}) \quad (7.13)$$

Metabolic clearance rate (MCR) was calculated as the Rd glucose divided by the mean glucose concentration over that time period.

$$MCR = Rd / [(C_1 + C_2) / 2] \quad (7.14)$$

7.2.10 Statistical analysis

When experiments were performed in the hot environment ($34^\circ C$), some subjects were unable to complete the full 120-min of exercise and statistical comparisons of group mean data is therefore restricted to the first 90-min of exercise. Statistical comparisons were made after establishing the normality or otherwise of the data distribution using the Anderson-Darling Goodness of Fit test (Anderson and Darling 1954). Where normality of the data distribution could not be assumed, a Wilcoxon signed ranks test was used to compare group means. Analysis of variance (ANOVA)

for repeated measures techniques were applied to normally distributed data, to study interactions and differences between feeding (CON versus GLU) and temperature (19 versus 34°C) conditions across time. In the case of a significant F -ratio, a Tukey post-hoc test was applied to identify differences. Descriptive data are presented as mean \pm standard error of the mean (SEM). Differences between and within conditions were considered significant when $P < 0.05$.

7.3 Results

7.3.1 *Exercise tolerance time*

All subjects were able to run for the full 120-min when exercise was performed in a temperate environment (CON19 and GLU19 conditions). When exercise was performed in the heat, individual exercise times ranged from 90 to 120-min and subjects were able to run for longer ($P < 0.05$) during the CON34 (112 ± 4 min) compared to the GLU34 (100 ± 5 min) condition. In addition, six of the nine subjects complained of gastric discomfort during the GLU34, but not during the CON34 condition.

7.3.2 *Sweat production*

Mean sweat rates were similar during the CON19 (16 ± 2 ml.min⁻¹) and GLU19 (16 ± 2 ml.min⁻¹) conditions, both of which were lower ($P < 0.01$) than in the CON34 (27 ± 2 ml.min⁻¹) and GLU34 (28 ± 2 ml.min⁻¹) conditions. Despite the fact that exercise time was shorter, fluid deficit was therefore greater ($P < 0.01$) at the end of the hot (CON34, -1605 ± 198 ml; GLU34, -1446 ± 239 ml) compared to the temperate (CON19, -426 ± 175 ; GLU19, -371 ± 180 ml) conditions.

7.3.3 *Whole body fat and carbohydrate oxidation*

Mean oxygen uptake was similar during the four experimental conditions (35 - 37 ml.kg⁻¹.min⁻¹) and elicited approximately 60 ± 2 % $\dot{V}O_2$ max (Table 7.1). RER remained fairly constant during exercise but was elevated at each time point with glucose ingestion compared to water ingestion in the temperate (CON19, 0.83 ± 0.01 ; GLU19, 0.89 ± 0.01 ; $P < 0.01$) and hot environments (CON34, 0.85 ± 0.01 ; GLU34, 0.88 ± 0.02 ; $P < 0.05$).

Trial	Time (min)	$\dot{V}O_2$ (ml.kg ⁻¹ .min ⁻¹)	Respiratory exchange ratio	Carbohydrate Oxidation ($\mu\text{mol.kg}^{-1}.\text{min}^{-1}$)	Fat Oxidation ($\mu\text{mol.kg}^{-1}.\text{min}^{-1}$)
CON19	60-75	35.7 \pm 0.9	0.84 \pm 0.01	129 \pm 12	34 \pm 3
	75-90	35.7 \pm 1.0	0.83 \pm 0.01	119 \pm 10	36 \pm 2
	90-105	36.3 \pm 1.0	0.84 \pm 0.01	124 \pm 12	36 \pm 3
	105-120	36.2 \pm 1.0	0.83 \pm 0.02	115 \pm 14	38 \pm 4
CON34	60-75	36.0 \pm 0.9	0.85 \pm 0.02	137 \pm 16	32 \pm 4
	75-90	36.5 \pm 1.0	0.85 \pm 0.01	132 \pm 13	34 \pm 3
GLU19	60-75	34.9 \pm 1.0	0.89 \pm 0.01 <i>f</i>	164 \pm 11 <i>f</i>	23 \pm 3 <i>f</i>
	75-90	35.0 \pm 0.9	0.89 \pm 0.01 <i>f</i>	163 \pm 10 <i>f</i>	24 \pm 2 <i>f</i>
	90-105	35.3 \pm 0.8	0.89 \pm 0.01 <i>f</i>	165 \pm 10 <i>f</i>	24 \pm 2 <i>f</i>
	105-120	35.3 \pm 0.9	0.87 \pm 0.01 <i>f</i>	152 \pm 13 <i>f</i>	27 \pm 3 <i>f</i>
GLU34	60-75	36.4 \pm 1.0	0.88 \pm 0.02§	158 \pm 14§	27 \pm 5§
	75-90	36.6 \pm 1.0	0.87 \pm 0.02§	154 \pm 14§	28 \pm 4§

Table 7.1 Oxygen uptake ($\dot{V}O_2$, ml.kg⁻¹.min⁻¹), respiratory exchange ratio (RER), carbohydrate and fat oxidation rates ($\mu\text{mol.kg}^{-1}.\text{min}^{-1}$) during the 60-120 min exercise period of the CON19, CON34, GLU19 and GLU34 conditions. Values are mean \pm SEM ($n = 9$). Symbols denote that values are significantly different: *f* from CON19 ($P < 0.01$); § from CON34 ($P < 0.05$).

Total carbohydrate oxidation rates were lowest during the CON19 condition ($\sim 122 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) and highest during the GLU19 condition ($\sim 161 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). Glucose ingestion elevated total carbohydrate oxidation rates at each time point in the temperate ($P < 0.01$) and hot ($P < 0.05$) environments (Table 7.1). After 90-min of exercise, total CHO oxidation rates were 119 ± 10 , 132 ± 13 , 163 ± 10 and $154 \pm 14 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for the CON19, CON34, GLU19 and GLU34 conditions, respectively. Total fat oxidation rates were suppressed by glucose ingestion at each time point in the temperate ($P < 0.01$) and hot ($P < 0.05$) environments. After 90-min total fat oxidation rates were 36 ± 2 , 34 ± 3 , 24 ± 2 and $28 \pm 4 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for the CON19, CON34, GLU19 and GLU34 conditions, respectively.

7.3.4 *Breath $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio*

In the experimental condition without tracer infusion or ingestion, used to measure shifts in background or endogenous $^{13}\text{CO}_2$ production, there was a very slight non-significant elevation in the $^{13}\text{C}/^{12}\text{C}$ ratio of expired CO_2 (Figure 7.1). The expired CO_2 ratios with [U- ^{13}C]-glucose tracer infusion (CON19 and CON34 conditions) and ingestion (GLU19 and GLU34 conditions) are also shown in Figure 7.1. There were no differences in the $^{13}\text{C}/^{12}\text{C}$ ratio of expired CO_2 between the CON19 and CON34 conditions with isotope infusion. When ^{13}C -glucose was ingested, the $^{13}\text{C}/^{12}\text{C}$ ratio of expired CO_2 was greater ($P < 0.01$) from 15-min onwards during the GLU19 compared to the GLU34 condition. The $^{13}\text{C}/^{12}\text{C}$ ratio of expired CO_2 reached a 'steady-state' after approximately 60-min of exercise in all conditions.

7.3.5 *Plasma glucose enrichment*

In the experimental condition without tracer infusion or ingestion, used to measure shifts in background or endogenous plasma glucose $^{13}\text{C}/^{12}\text{C}$ ratio, there was no change in $^{13}\text{C}/^{12}\text{C}$ ratio (Figure 7.2). The plasma glucose $^{13}\text{C}/^{12}\text{C}$ ratios with [U- ^{13}C]-glucose tracer infusion (CON19 and CON34 conditions) and ingestion (GLU19 and GLU34 conditions) are also shown in Figure 7.2. With isotope infusion, plasma glucose $^{13}\text{C}/^{12}\text{C}$ ratios peaked at ~ 0.012 after 15-min of exercise in the CON19 and CON34 conditions. Values decreased over time in both conditions, but more rapidly in the CON34 condition, values being lower than in the CON19 condition after 75 ($P < 0.05$) and 90-min ($P < 0.01$) of exercise. When ^{13}C -glucose was ingested, plasma glucose $^{13}\text{C}/^{12}\text{C}$ ratios continued to rise during the first 60-min of exercise before reaching a

plateau. Values were greater at each time point during the GLU19 ($P < 0.01$) compared to the GLU34 condition.

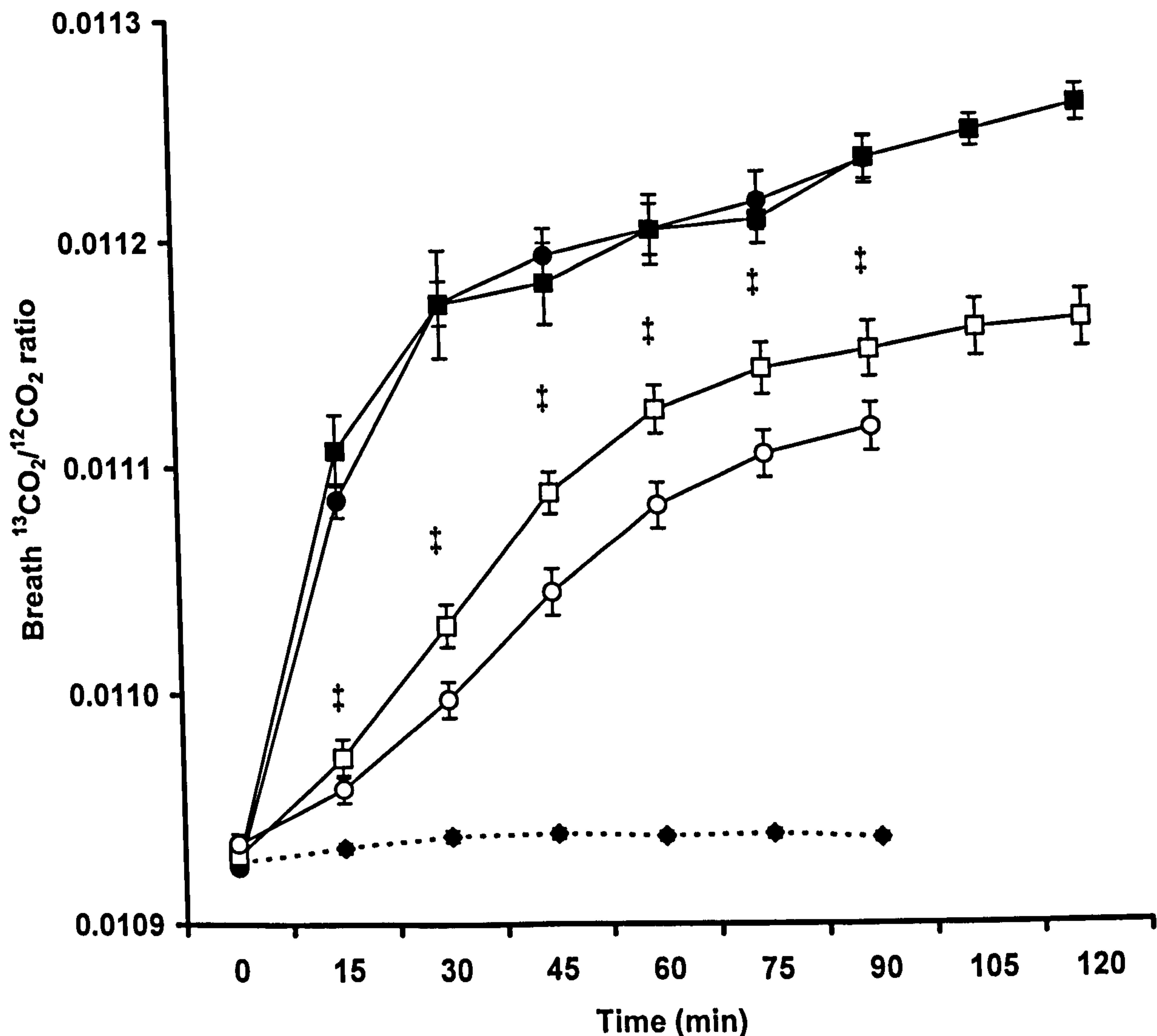


Figure 7.1 Breath $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio at each time point during the CON19 (■), CON34 (●), GLU19 (□), GLU34 (○) and background correction (---) conditions. Values are mean \pm SEM ($n = 9$). ‡ Denotes a significant difference between the GLU19 and GLU34 conditions ($P < 0.05$).

When water was ingested during exercise (CON19 and CON34), plasma [$^2\text{H}_2$]-glucose enrichment peaked at ~ 8 APE after 15-min of exercise in both conditions. Values subsequently fell in both conditions, but to a greater extent in the CON34 condition, values being lower than in the CON19 condition after 75 ($P < 0.05$) and 90-min ($P < 0.01$) of exercise (Figure 7.3). When glucose was ingested during exercise (GLU19 and GLU34), the peak plasma [$^2\text{H}_2$]-glucose enrichment (~ 6 APE) was lower than in the CON conditions. Enrichments were lower at each subsequent time point in

the GLU compared to the CON conditions. Plasma [$^2\text{H}_2$]-glucose enrichments were also lower from 30 to 75-min in the GLU19 compared to the GLU34 condition.

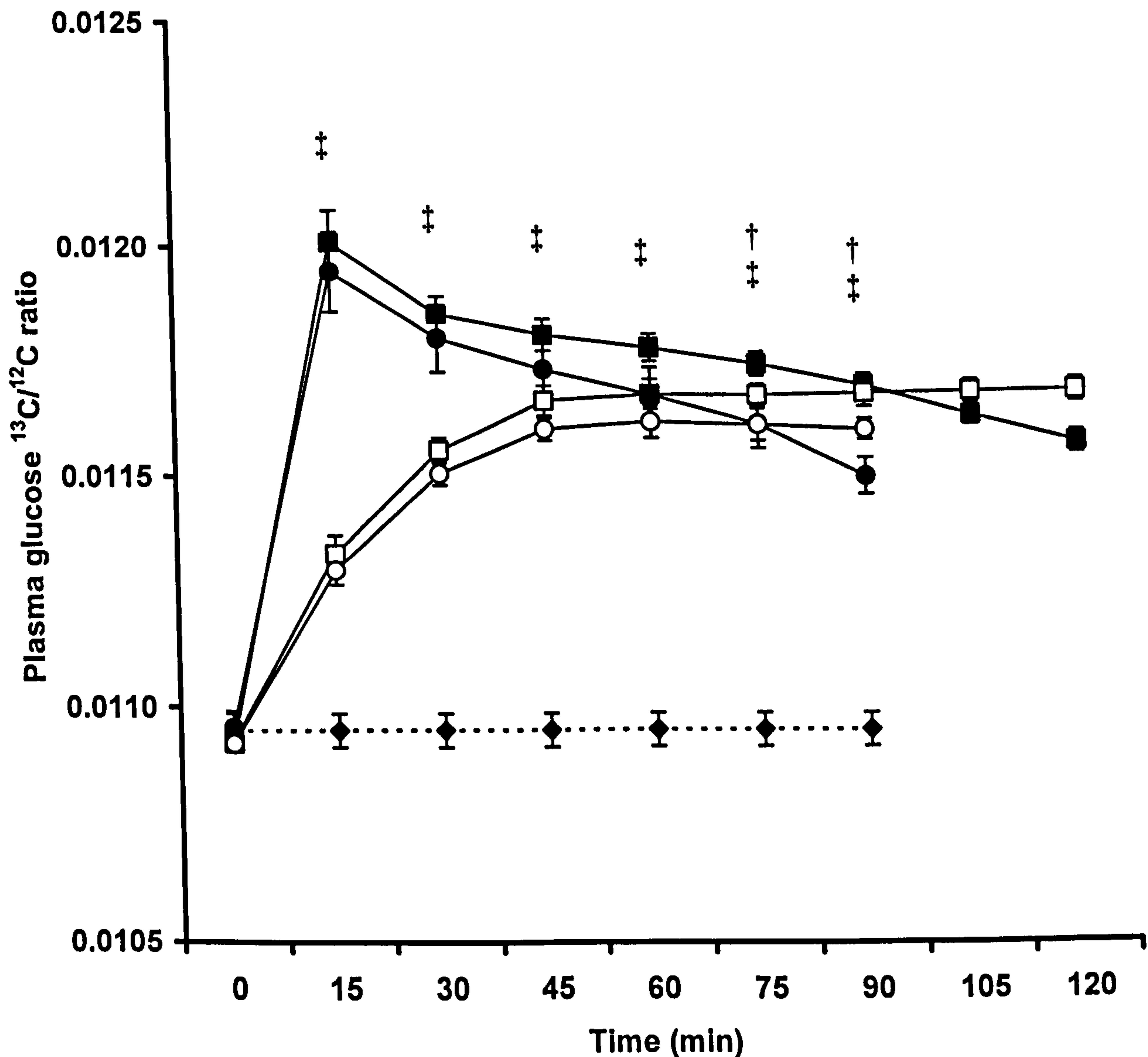


Figure 7.2 Plasma glucose $^{13}\text{C}/^{12}\text{C}$ ratio at each time point during the CON19 (■), CON34 (●), GLU19 (□), GLU34 (○) and background correction (---) conditions. Values are mean \pm SEM ($n = 9$). Symbols denote a significant difference: † between CON19 and CON34 ($P < 0.05$); ‡ between GLU19 and GLU34 conditions ($P < 0.05$).

7.3.6 Rate of appearance (R_a) and disappearance (R_d) of plasma glucose

The rate of appearance (R_a) and disappearance (R_d) of plasma glucose was calculated using the [$6,6\text{-}^2\text{H}_2$]-glucose tracer in all conditions and using the [$\text{U-}^{13}\text{C}$]-glucose tracer during the CON conditions. Although values were slightly lower when using the [$\text{U-}^{13}\text{C}$]-glucose enrichment data, the differences were negligible (Figure 7.4 and Table 7.3). R_a was virtually identical to R_d at all time points and in all conditions

(Table 7.2). Values for Ra (28-35 $\mu\text{mol.kg}^{-1}.\text{min}^{-1}$) and Rd (30-36 $\mu\text{mol.kg}^{-1}.\text{min}^{-1}$) were lowest in the CON19 condition. Ra and Rd were elevated during the 60-75 and 75-90 min periods during the CON34 compared to the CON19 conditions. Both Ra and Rd glucose were markedly elevated with glucose ingestion in the thermoneutral (by ~209%) and hot environments (by ~150%) at the 60-90 min time points.

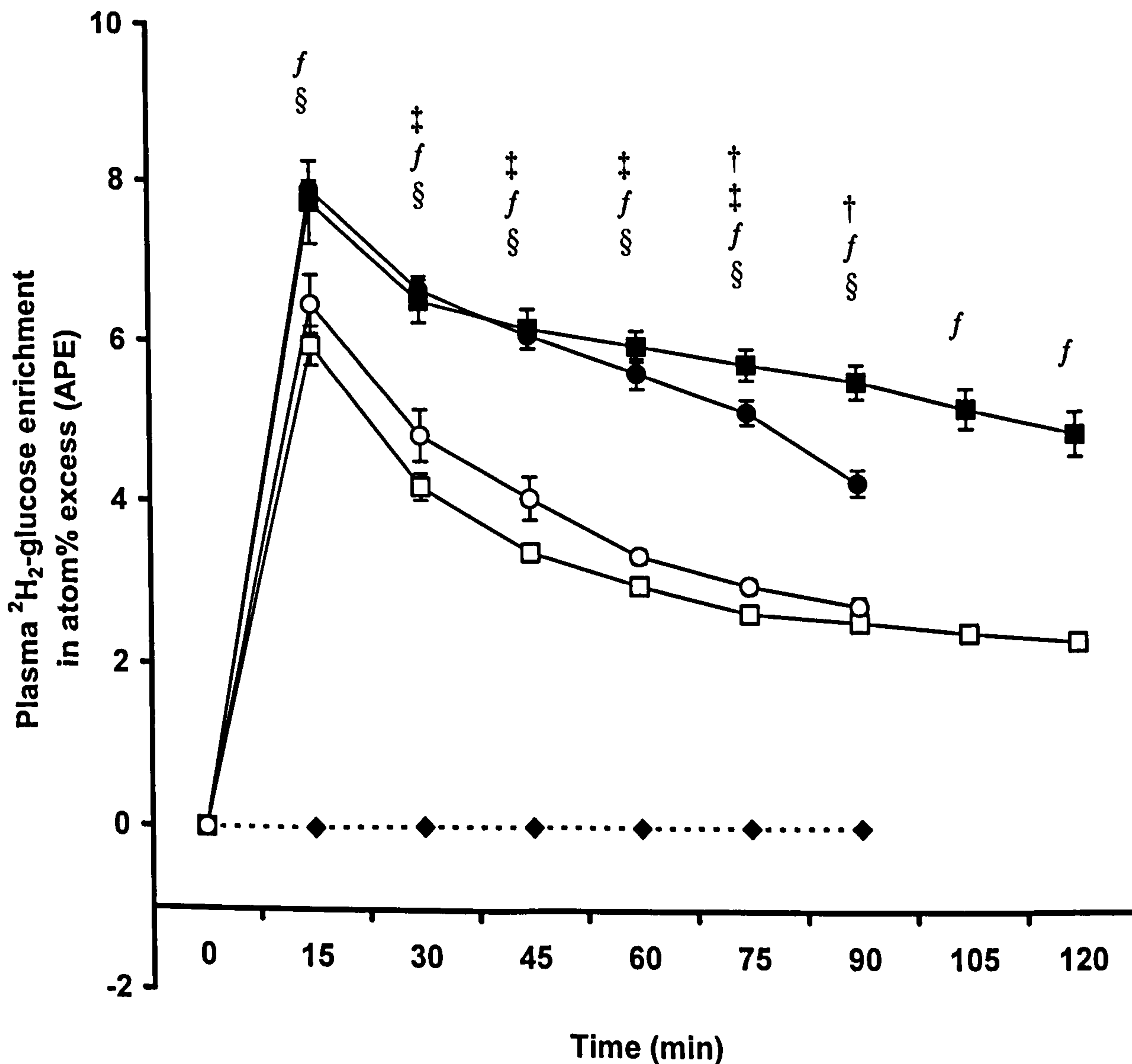


Figure 7.3 Plasma $^2\text{H}_2$ -glucose enrichment at each time point during the CON19 (■), CON34 (●), GLU19 (□), GLU34 (○) and background correction (---) conditions. Values are mean \pm SEM ($n = 9$). Symbols denote a significant difference: † between CON19 and CON34 ($P < 0.05$); ‡ between GLU19 and GLU34 ($P < 0.05$); f between CON19 and GLU19 ($P < 0.01$); § between CON34 and GLU34 ($P < 0.01$).

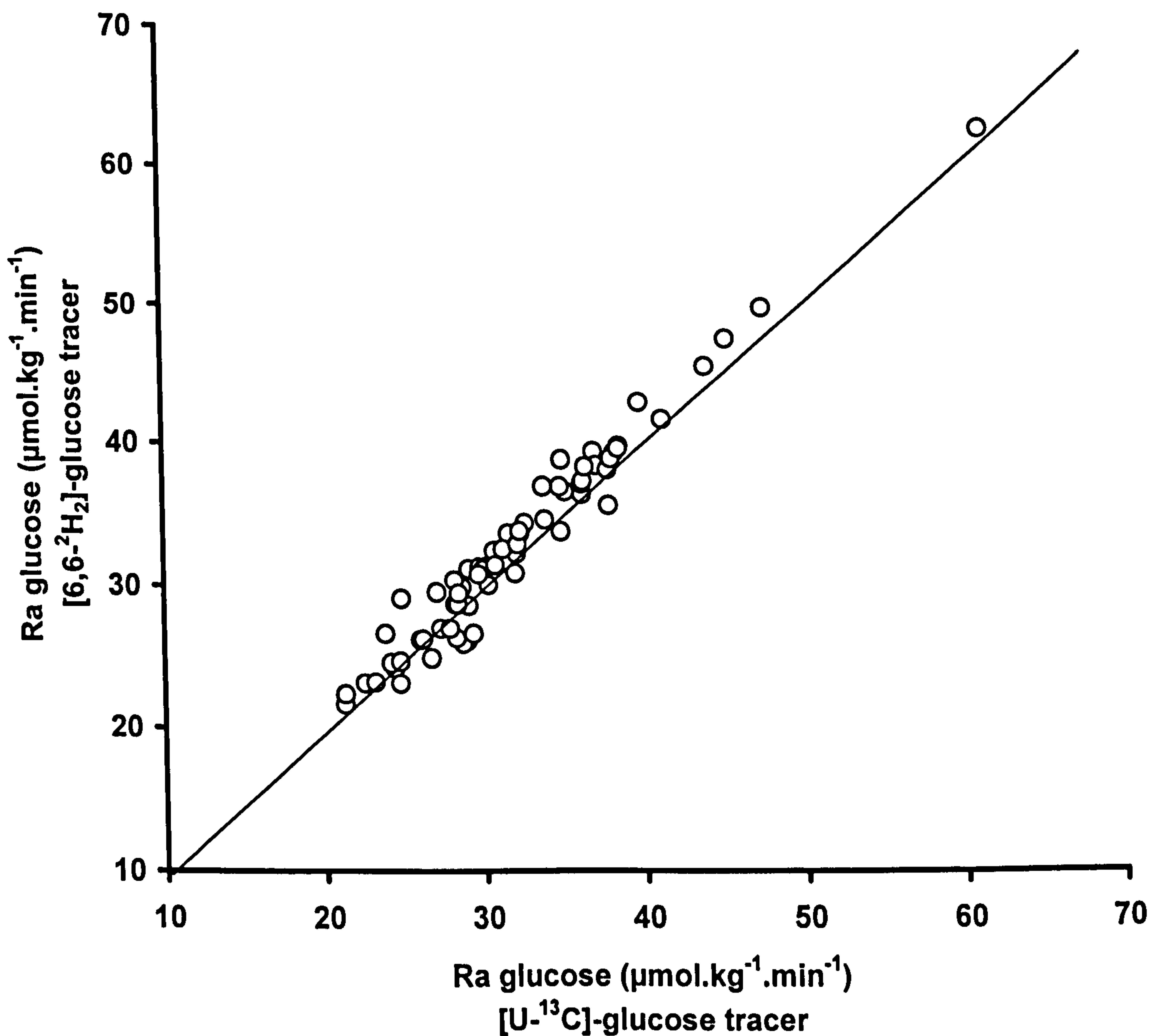


Figure 7.4 Comparison of the [6,6-²H₂]-glucose and [U-¹³C]-glucose tracers to measure the rate of appearance (*Ra*) of plasma glucose during the CON19 and CON34 conditions. The line represents the line of identity.

7.3.7 Plasma glucose and muscle glycogen oxidation

The *Rd* and *Rox* of plasma glucose were similar at all time points and in all conditions (Figure 7.5). The rate of plasma glucose oxidation was lowest in the CON19 condition (28-35 μmol.kg⁻¹.min⁻¹) and was observed to gradually increase over time in all conditions (Table 7.2). Plasma glucose oxidation was elevated with heat stress in the CON34 condition (34-46 μmol.kg⁻¹.min⁻¹) during the 60-75 and 75-90 min periods of exercise. Oxidation rates were further elevated with glucose ingestion and peak plasma glucose oxidation rates of 71 and 63 μmol.kg⁻¹.min⁻¹ were observed during the final 15-min of exercise in the GLU19 and GLU34 conditions, respectively.

Trial	Time (min)	Total CHO Oxidation	Ra glucose	Rd glucose	Exogenous glucose oxidation	Plasma glucose oxidation	Muscle glycogen and/or lactate oxidation
		($\mu\text{mol.kg}^{-1}.\text{min}^{-1}$) mean \pm SEM	($\mu\text{mol.kg}^{-1}.\text{min}^{-1}$) mean \pm SEM	($\mu\text{mol.kg}^{-1}.\text{min}^{-1}$) mean \pm SEM	($\mu\text{mol.kg}^{-1}.\text{min}^{-1}$) mean \pm SEM	($\mu\text{mol.kg}^{-1}.\text{min}^{-1}$) mean \pm SEM	($\mu\text{mol.kg}^{-1}.\text{min}^{-1}$) mean \pm SEM
CON19	60-75	129 \pm 12	28 \pm 2	30 \pm 3	0	28 \pm 2	101 \pm 11
	75-90	119 \pm 10	29 \pm 2	31 \pm 2	0	29 \pm 2	90 \pm 9
	90-105	124 \pm 12	33 \pm 2	32 \pm 2	0	32 \pm 2	93 \pm 12
	105-120	115 \pm 14	35 \pm 2	36 \pm 3	0	35 \pm 3	80 \pm 14
CON34	60-75	137 \pm 16	34 \pm 1 <i>f</i>	35 \pm 2 <i>f</i>	0	34 \pm 2 <i>f</i>	103 \pm 15
	75-90	132 \pm 13	44 \pm 3 <i>f</i>	47 \pm 3 <i>f</i>	0	46 \pm 4 <i>f</i>	86 \pm 12
GLU19	60-75	164 \pm 11 <i>f</i>	64 \pm 3 <i>f</i>	65 \pm 4 <i>f</i>	55 \pm 2	66 \pm 3 <i>f</i>	99 \pm 11
	75-90	163 \pm 10 <i>f</i>	64 \pm 3 <i>f</i>	64 \pm 3 <i>f</i>	57 \pm 2	68 \pm 3 <i>f</i>	95 \pm 10
	90-105	165 \pm 10 <i>f</i>	67 \pm 3 <i>f</i>	66 \pm 3 <i>f</i>	60 \pm 2	71 \pm 2 <i>f</i>	94 \pm 10
	105-120	152 \pm 13 <i>f</i>	70 \pm 3 <i>f</i>	71 \pm 3 <i>f</i>	60 \pm 2	71 \pm 2 <i>f</i>	82 \pm 13
GLU34	60-75	158 \pm 14§	58 \pm 2§	60 \pm 3§	45 \pm 3‡	58 \pm 4§	101 \pm 12
	75-90	154 \pm 14§	62 \pm 3§	63 \pm 4§	48 \pm 4‡	63 \pm 5§	85 \pm 10

Table 7.2 Total CHO oxidation, Ra and Rd glucose, exogenous glucose, plasma glucose and muscle glycogen oxidation during the 60-120 min exercise period of the CON19, CON34, GLU19 and GLU34 conditions. Values are mean \pm SEM ($n = 9$). Symbols denote that values are significantly different: ‡ from GLU19 ($P < 0.01$); § from CON19 ($P < 0.01$); § from CON34 ($P < 0.01$).

Trial	Time (min)	<i>Ra</i> glucose ($\mu\text{mol kg}^{-1} \text{min}^{-1}$) [6,6- ² H ₂]-glucose mean \pm SEM	<i>Ra</i> glucose ($\mu\text{mol kg}^{-1} \text{min}^{-1}$) [U- ¹³ C]-glucose Mean \pm SEM	<i>Rd</i> glucose ($\mu\text{mol kg}^{-1} \text{min}^{-1}$) [6,6- ² H ₂]-glucose mean \pm SEM	<i>Rd</i> glucose ($\mu\text{mol kg}^{-1} \text{min}^{-1}$) [U- ¹³ C]-glucose mean \pm SEM
CON19	60-75	28 \pm 2	28 \pm 1	30 \pm 3	29 \pm 2
	75-90	29 \pm 2	30 \pm 2	31 \pm 2	31 \pm 2
	90-105	33 \pm 2	32 \pm 2	32 \pm 2	32 \pm 2
	105-120	35 \pm 2	34 \pm 2	36 \pm 3	35 \pm 3
CON34	60-75	34 \pm 1	33 \pm 1	35 \pm 2	34 \pm 2
	75-90	44 \pm 3	43 \pm 3	47 \pm 3	45 \pm 3

Table 7.3 The rate of appearance (*Ra* glucose) and disappearance (*Rd* glucose) of plasma glucose at each time point during the CON19 and CON34 conditions, calculated from the [6,6-²H₂]-glucose tracer compared with the [U-¹³C]-glucose tracer. Values are mean \pm SEM ($n = 9$).

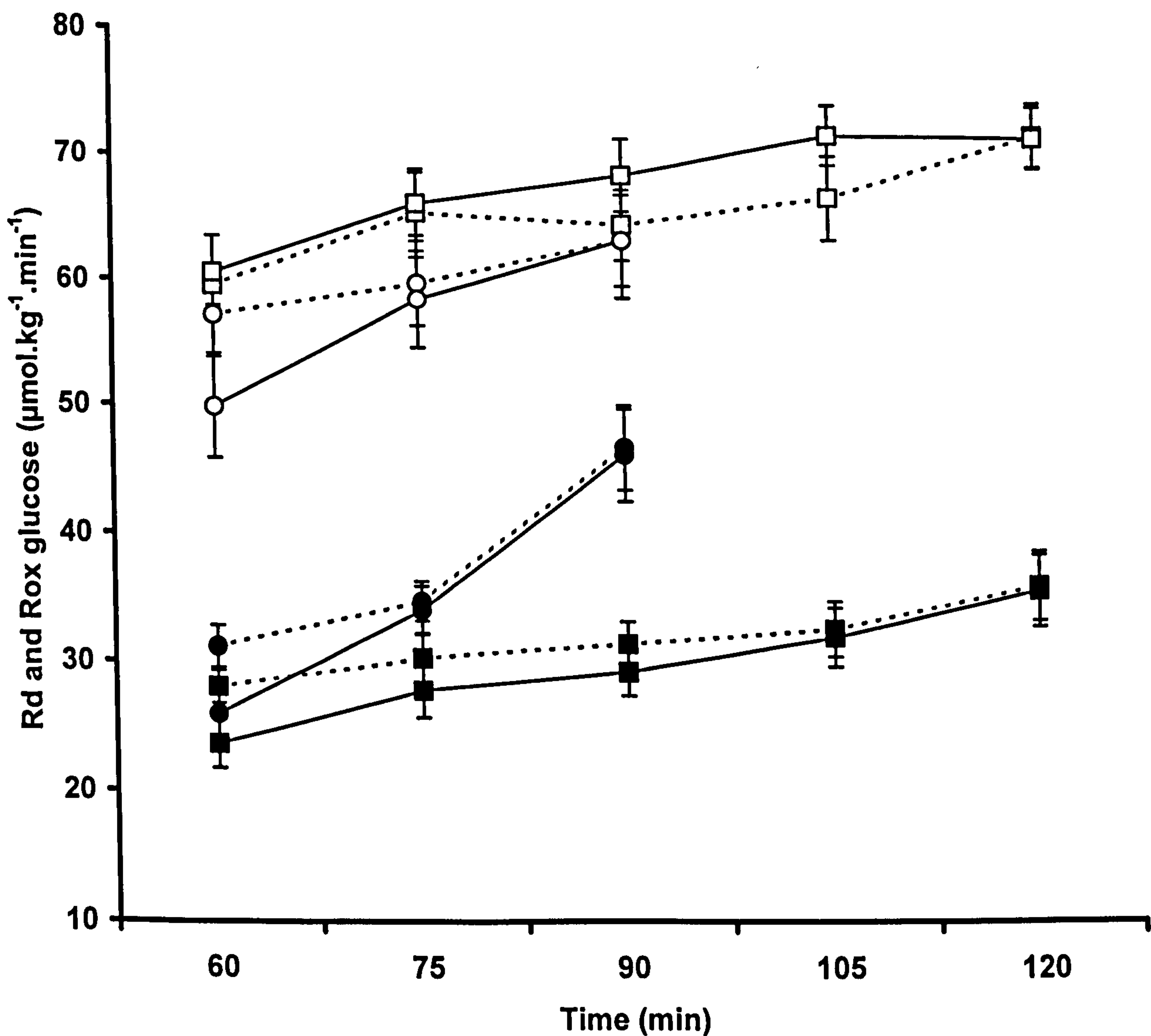


Figure 7.5 The rate of disappearance (*Rd* glucose \cdots) and rate of oxidation (*Rox* glucose —) of plasma glucose during the 60-120 minute exercise period of the CON19 (■), CON34 (●), GLU19 (□), GLU34 (○) conditions. Values are mean \pm SEM ($n = 9$).

7.3.8 Metabolic Clearance Rate (MCR) of glucose

The metabolic clearance rate (MCR) of plasma glucose increased with exercise duration between 60 and 120 min of exercise ($P < 0.01$). At all time points, values were approximately 2-fold higher during GLU19 compared to CON19 (Figure 7.6). Interestingly, MCR was greater ($P < 0.01$) during the 75-90 min time period during CON34 compared to CON19. In fact, at this time point the CON34 values were similar to the GLU34 values. The MCR of glucose was lower at both the 60-75 and 75-90 min time points during the GLU34 compared to the GLU19 condition ($P < 0.01$).

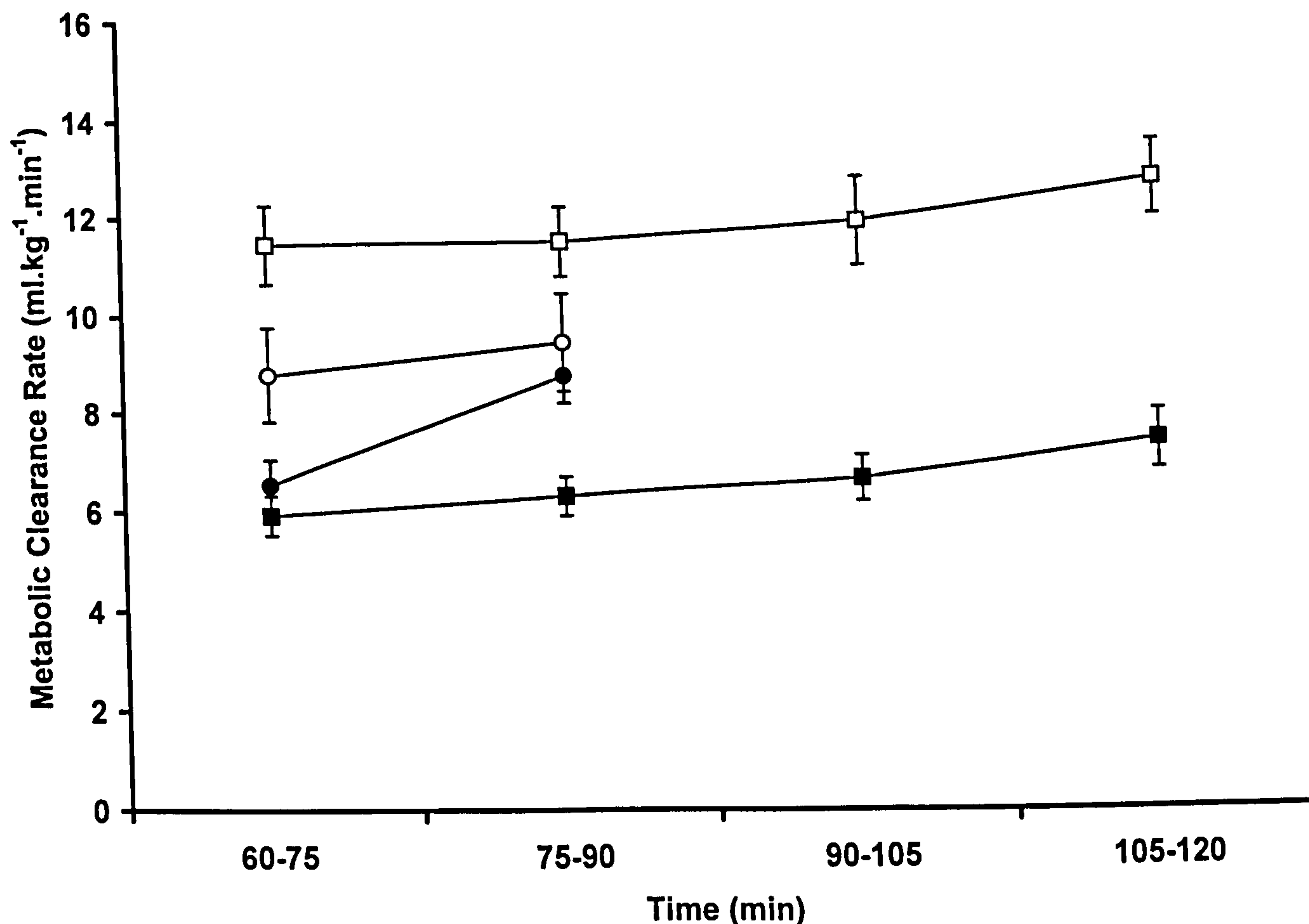


Figure 7.6 The metabolic clearance rate (MCR) of plasma glucose ($\text{ml.kg}^{-1}.\text{min}^{-1}$) during the 60-120 min exercise period of the CON19 (■), CON34 (●), GLU19 (□), GLU34 (○) conditions. Values are mean \pm SEM ($n = 9$).

Glucose ingestion was observed to reduce liver-derived glucose oxidation from ~ 31 and $\sim 40 \mu\text{mol.kg}^{-1}.\text{min}^{-1}$ during the CON19 and CON34 conditions, to ~ 11 ($P < 0.01$) and $\sim 14 \mu\text{mol.kg}^{-1}.\text{min}^{-1}$ ($P < 0.01$) during the GLU19 and GLU34 conditions, respectively. However, liver-derived glucose oxidation was greater during the 75-90 min exercise period in the GLU34 ($15 \pm 1 \mu\text{mol.kg}^{-1}.\text{min}^{-1}$) compared to the GLU19 condition ($11 \pm 2 \mu\text{mol.kg}^{-1}.\text{min}^{-1}$). Furthermore, calculated muscle glycogen oxidation rates (total CHO oxidation minus plasma glucose oxidation) tended to decrease over time in all conditions ($P < 0.01$), but were not influenced by heat stress or glucose ingestion. Figure 7.7 describes the shifts in substrate utilisation as a result of heat stress and glucose ingestion. It should be noted that glucose feedings, markedly reduced fat oxidation and increased plasma glucose oxidation whilst reducing liver-derived glucose oxidation, but did not alter the rate of muscle and/or lactate oxidation. Furthermore, heat stress increased plasma glucose oxidation in the

CON conditions and reduced exogenous glucose oxidation in the GLU conditions, but again, did not alter the rate of muscle and/or lactate oxidation.

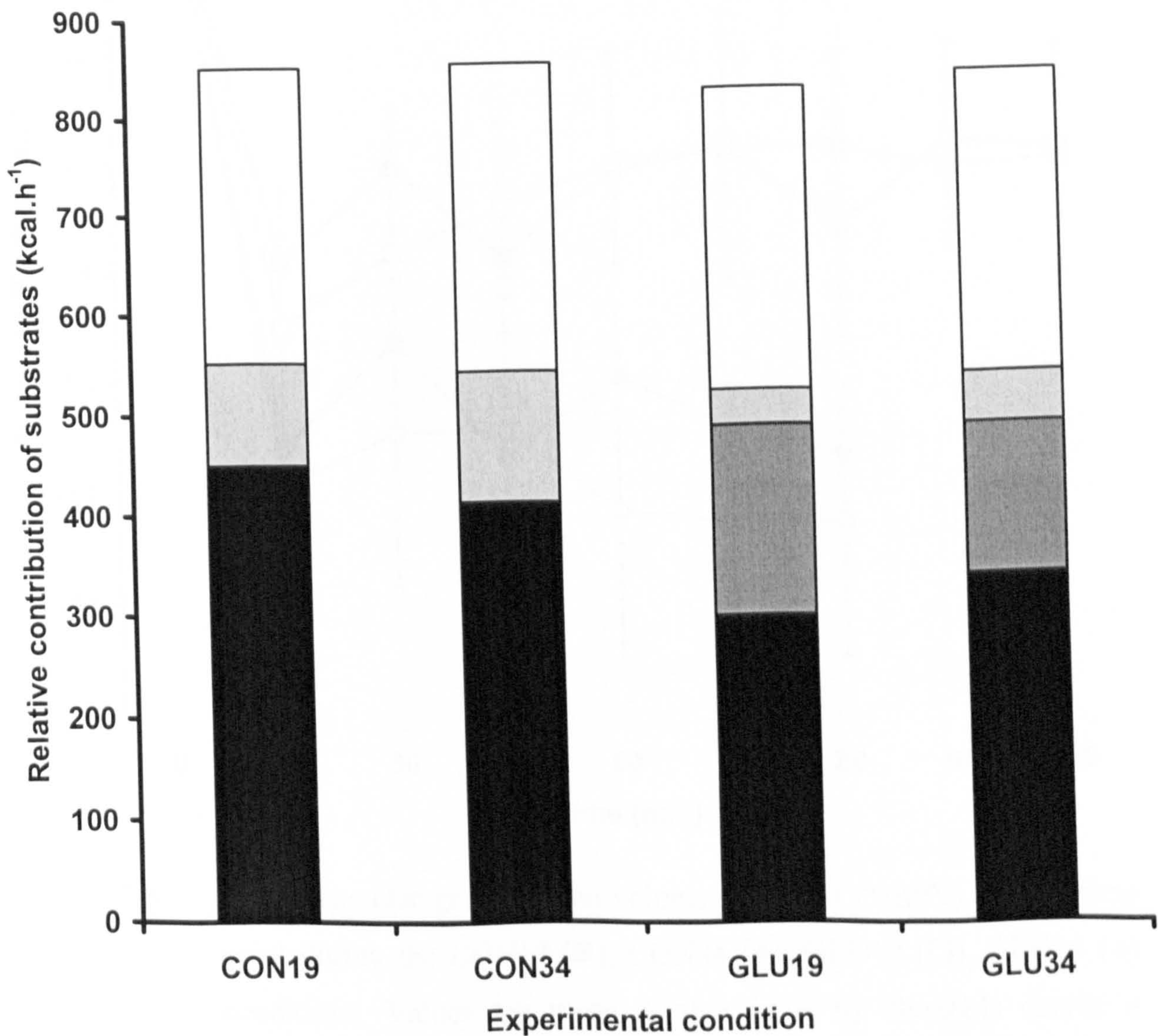


Figure 7.7 The energetic contribution ($\text{kcal}\cdot\text{h}^{-1}$) of fat (■), exogenous glucose (▣), liver-derived glucose (□) and muscle glycogen (□) during the 60-120 min period of exercise during the CON19, CON34, GLU19 and GLU34 conditions. Values are mean ($n = 9$).

7.3.9 Biochemical responses

Plasma volume was reduced in all conditions after 15-min of exercise (Figure 7.8). There was no effect of glucose feedings on percentage change in plasma volume. Plasma volume was reduced to a greater extent ($P < 0.05$) in the hot (CON34 and GLU34) compared to the temperate conditions (CON19 and GLU19) from 60-min onwards during exercise.

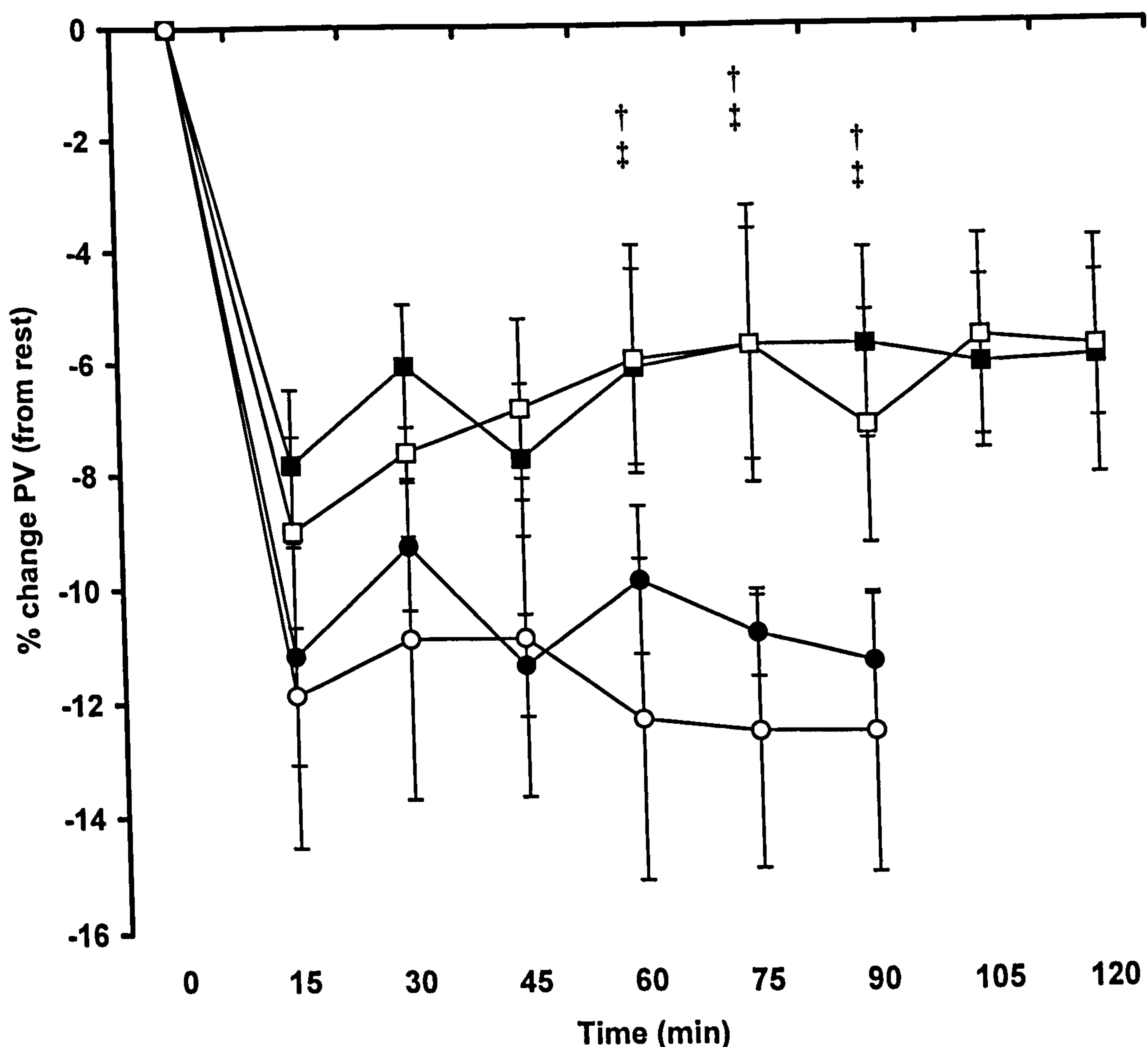


Figure 7.8 Percentage change in plasma volume from rest ($\% \Delta PV$) at each time point during the CON19 (■), CON34 (●), GLU19 (□), GLU34 (○) conditions. Values are mean \pm SEM ($n = 9$). Symbols denote a significant difference: † between CON19 and CON34 ($P < 0.05$); ‡ between GLU19 and GLU34 conditions ($P < 0.05$).

In the CON19 condition, plasma glucose concentrations were in the range of 4.8-5.2 mmol.l^{-1} at rest and throughout the exercise period (Figure 7.9). In comparison, plasma glucose concentrations appeared to be elevated from 15-min onwards during the exercise period of the CON34 condition (5.2-5.5 mmol.l^{-1}), but were not statistically different from CON19. With glucose ingestion in the GLU19 condition, plasma glucose concentrations peaked at $6.5 \pm 0.2 \text{ mmol.l}^{-1}$ after 15-min of exercise and remained elevated compared to the CON19 ($P < 0.01$) condition. In the GLU34 condition, plasma glucose concentrations peaked at $7.8 \pm 0.4 \text{ mmol.l}^{-1}$ after 30-min

and remained elevated (>7.0 mmol.l⁻¹) for the remainder of the exercise period, compared to the GLU19 ($P<0.01$) and CON34 ($P<0.01$) conditions.

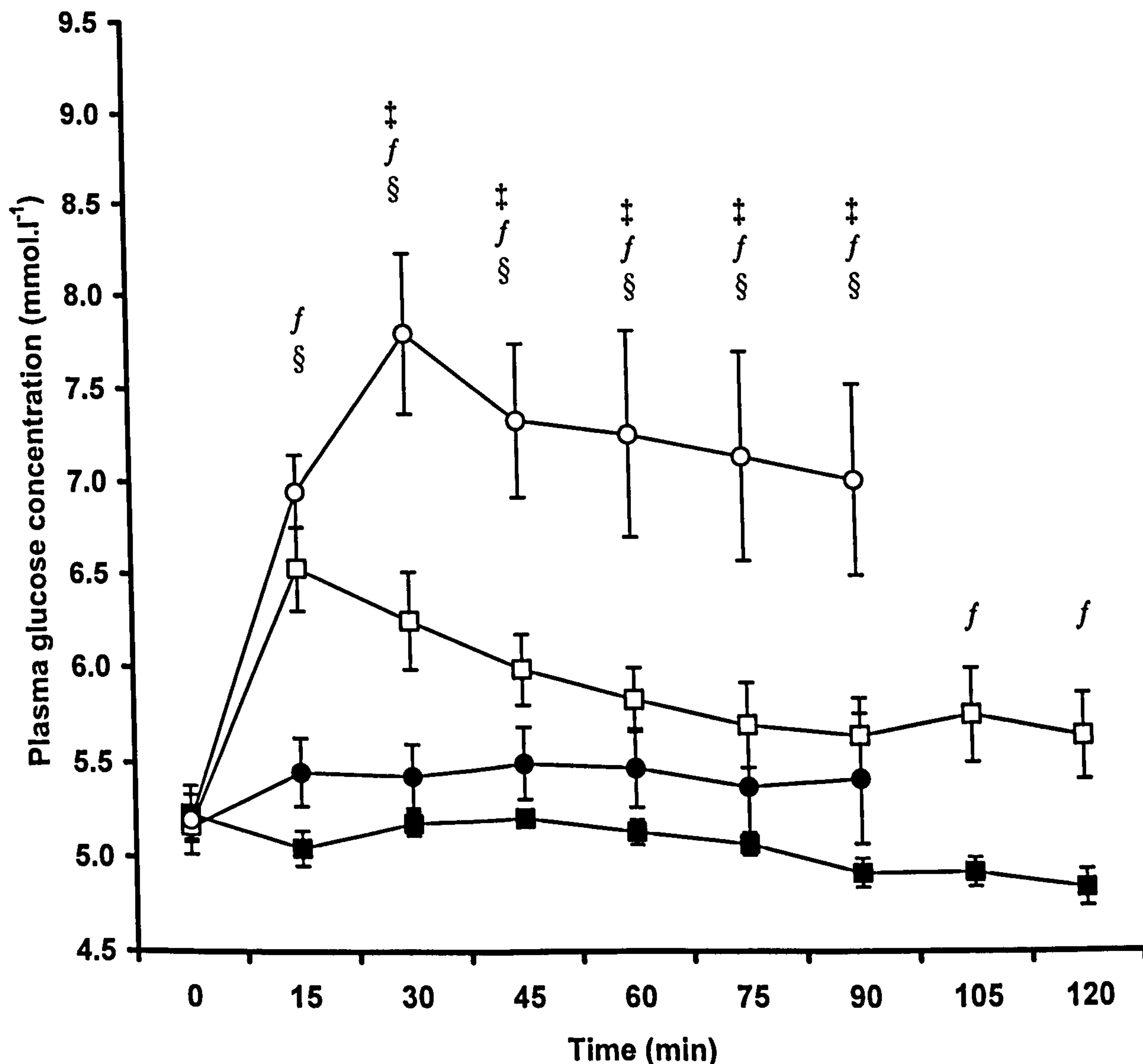


Figure 7.9 Plasma glucose concentrations (mmol.l⁻¹) at each time point during the CON19 (■), CON34 (●), GLU19 (□), GLU34 (○) conditions. Values are mean \pm SEM ($n = 9$). Symbols denote a significant difference: ‡ between GLU19 and GLU34 ($P<0.01$); *f* between CON19 and GLU19 ($P<0.01$); § between CON34 and GLU34 ($P<0.01$).

Serum insulin concentrations were similar at rest (10 ± 1 μ IU.ml⁻¹) in all conditions (Figure 7.10). When water was ingested, insulin concentrations gradually declined throughout exercise and were 5 ± 1 and 6 ± 1 μ IU.ml⁻¹ at the end of exercise during the CON19 and CON34 conditions, respectively. There were no differences between the CON19 and CON34 conditions. When glucose was ingested, insulin

concentrations increased markedly and peaked at 18 ± 2 and 17 ± 2 $\mu\text{IU}\cdot\text{ml}^{-1}$ after 15-min of exercise during the GLU19 and GLU34 conditions, respectively. Whilst there were no differences between the GLU19 and GLU34 conditions, serum insulin concentrations were greater at all time points ($P < 0.01$) in the GLU34 compared to the CON34 and in the GLU19 compared to the CON19 conditions.

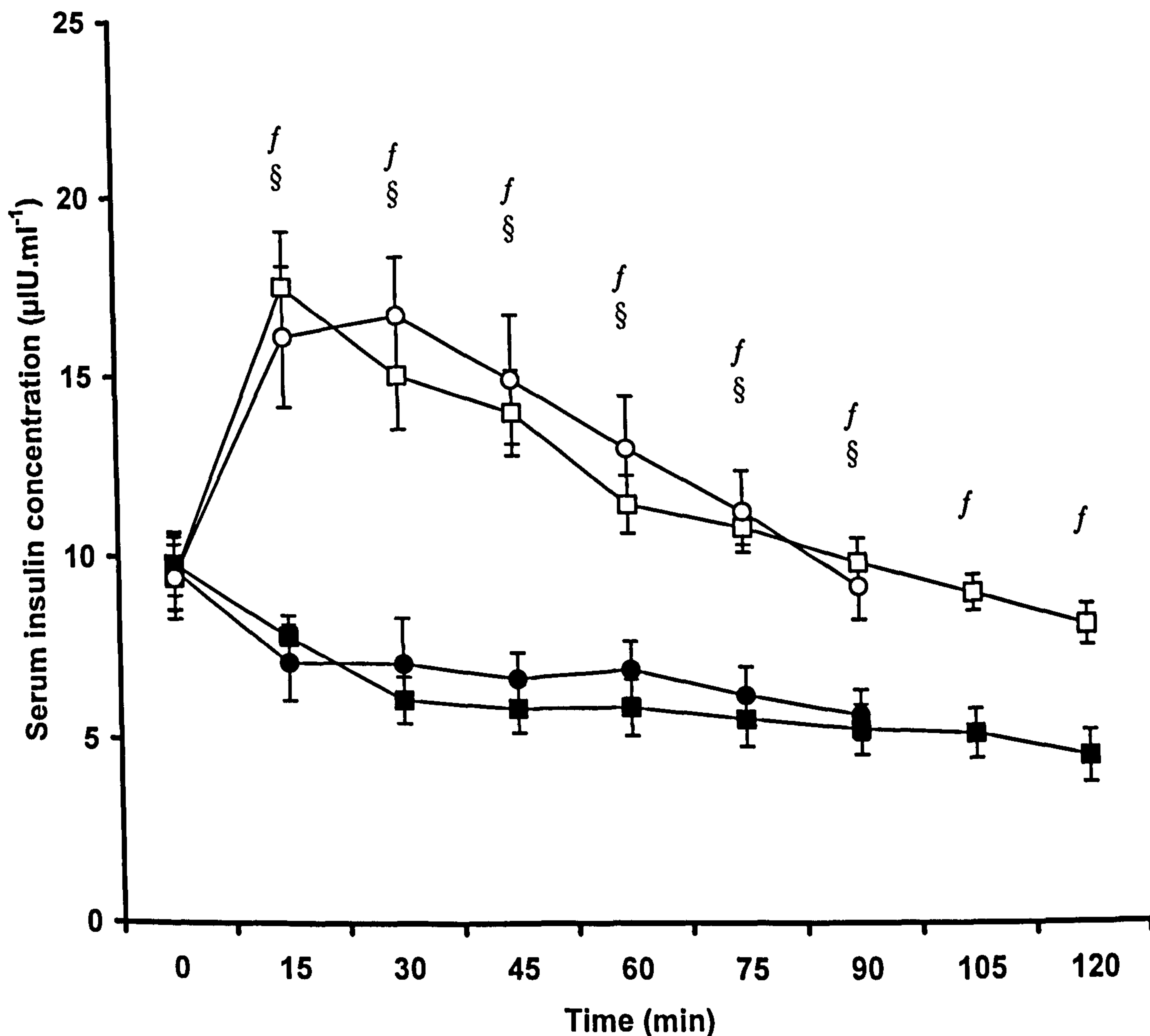


Figure 7.10 Serum insulin concentrations ($\mu\text{IU}\cdot\text{ml}^{-1}$) at each time point during the CON19 (■), CON34 (●), GLU19 (□), GLU34 (○) conditions. Values are mean \pm SEM ($n = 9$). Symbols denote a significant difference: *f* between CON19 and GLU19 ($P < 0.01$); § between CON34 and GLU34 ($P < 0.01$).

Plasma free fatty acid (FFA) concentrations were similar at rest (0.44 ± 0.05 $\text{mmol}\cdot\text{l}^{-1}$) in all conditions (Figure 7.11), and declined similarly ($P < 0.01$) over the first 15-min of exercise (0.24 ± 0.03 $\text{mmol}\cdot\text{l}^{-1}$). Thereafter, plasma FFA concentrations continued

to rise throughout exercise in the CON19 and CON34 conditions, reaching peak values of 0.72 ± 0.06 and 0.73 ± 0.10 mmol.l^{-1} at the end of exercise, respectively. Values were greater after 75 ($P < 0.05$) and 90-min ($P < 0.01$) of exercise during the CON34 compared to the CON19 condition. In comparison, FFA concentrations remained suppressed throughout exercise with glucose ingestion. There were no differences between the GLU19 and GLU34 conditions during exercise.

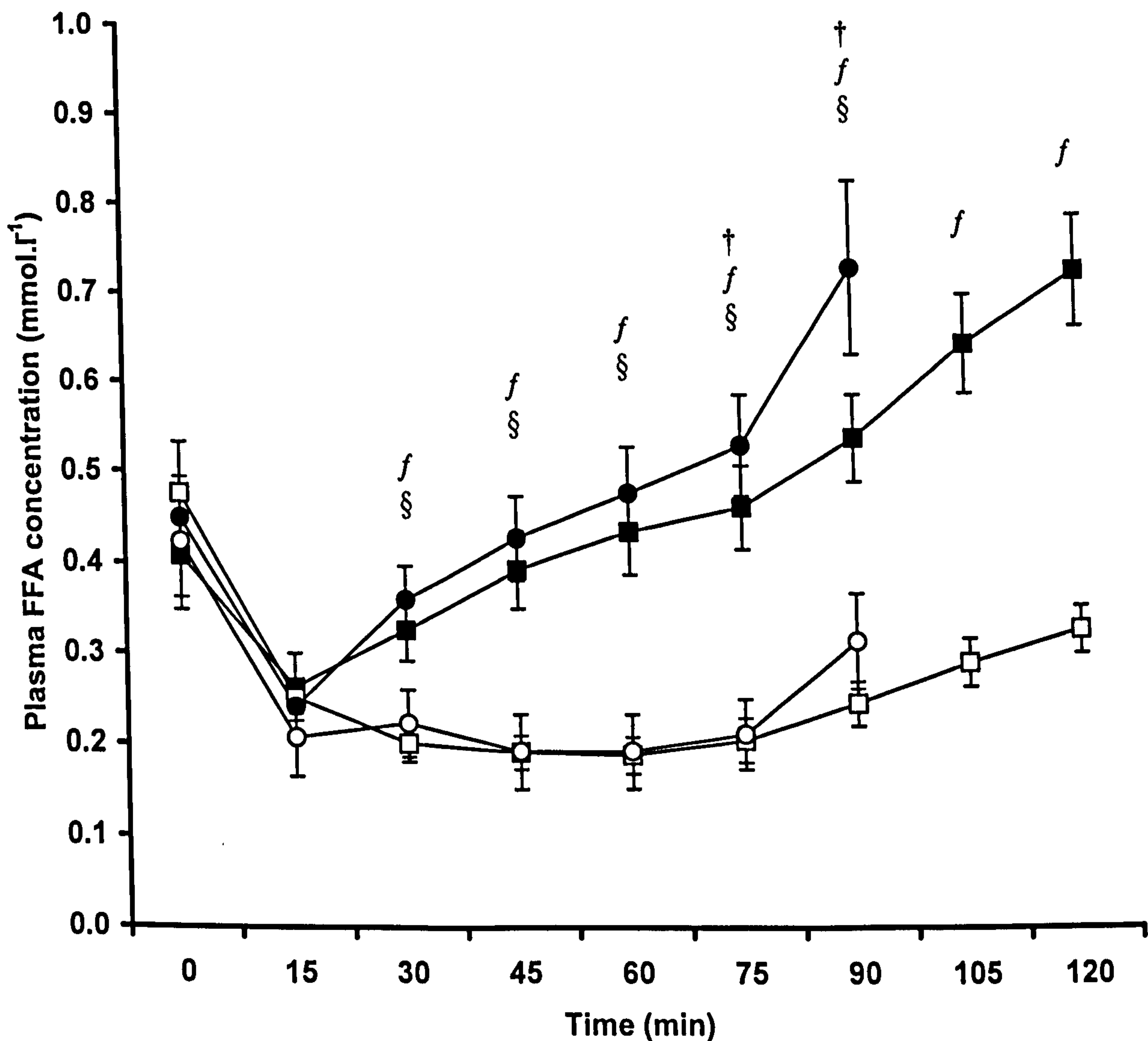


Figure 7.11 Plasma FFA concentrations (mmol.l^{-1}) at each time point during the CON19 (■), CON34 (●), GLU19 (□), GLU34 (○) conditions. Values are mean \pm SEM ($n = 9$). Symbols denote a significant difference: † between CON19 and CON34 ($P < 0.05$); *f* between CON19 and GLU19 ($P < 0.01$); § between CON34 and GLU34 ($P < 0.01$).

In the temperate environment in the absence of glucose ingestion (CON19), plasma lactate concentrations peaked at 1.4 mmol.l^{-1} after 15-min of exercise and remained

between 1.1 and 1.3 mmol.l⁻¹ for the remainder of the exercise period (Figure 7.12). Plasma lactate concentrations were similar in the GLU19 condition. When exercise was performed in a hot environment in the absence of glucose ingestion (CON34), plasma lactate concentrations were greater than in the temperate conditions from 60-min onwards ($P<0.05$), and peaked at 2.1 mmol.l⁻¹ at the end of exercise. When glucose was ingested during exercise in the heat (GLU34), plasma lactate concentrations were higher than in the other three conditions and peaked at 2.8 mmol.l⁻¹ at the end of exercise.

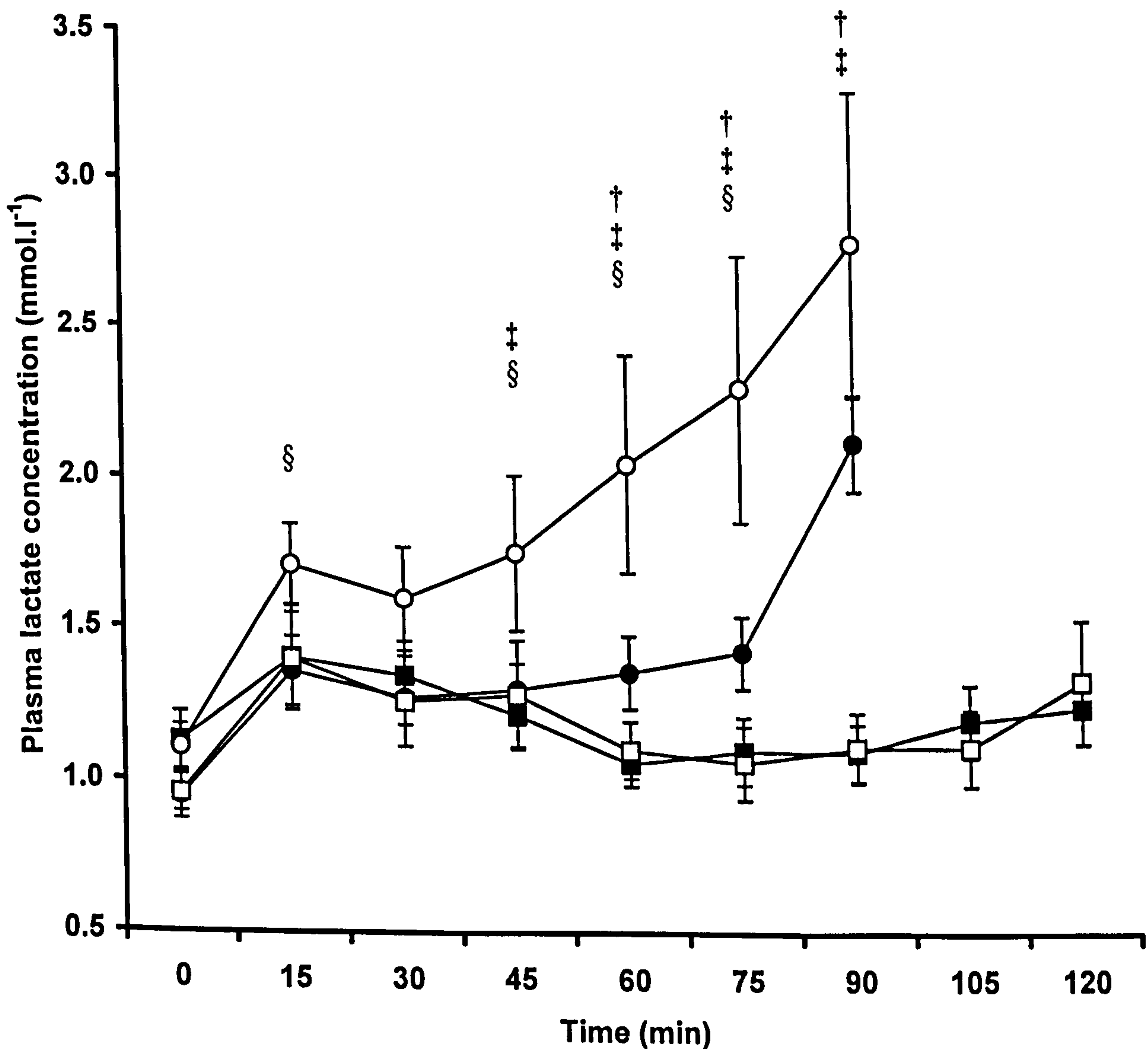


Figure 7.12 Plasma lactate concentrations (mmol.l⁻¹) at each time point during the CON19 (■), CON34 (●), GLU19 (□), GLU34 (○) conditions. Values are mean \pm SEM ($n = 9$). Symbols denote a significant difference: † between CON19 and CON34 ($P<0.01$); ‡ between GLU19 and GLU34 ($P<0.01$); § between CON34 and GLU34 ($P<0.01$).

Exercise in the heat elevated plasma adrenaline concentrations compared to exercise in the temperate conditions (Figure 7.13). Values were greater from 60-min of exercise onwards during the CON34 compared to the CON19 condition and from 15-min onwards during the GLU34 compared to the GLU19 condition. Glucose ingestion appeared to attenuate the rise in plasma adrenaline concentration in the temperate environment, values being lower from 30-min of exercise onwards during the GLU19 compared to the CON19 condition.

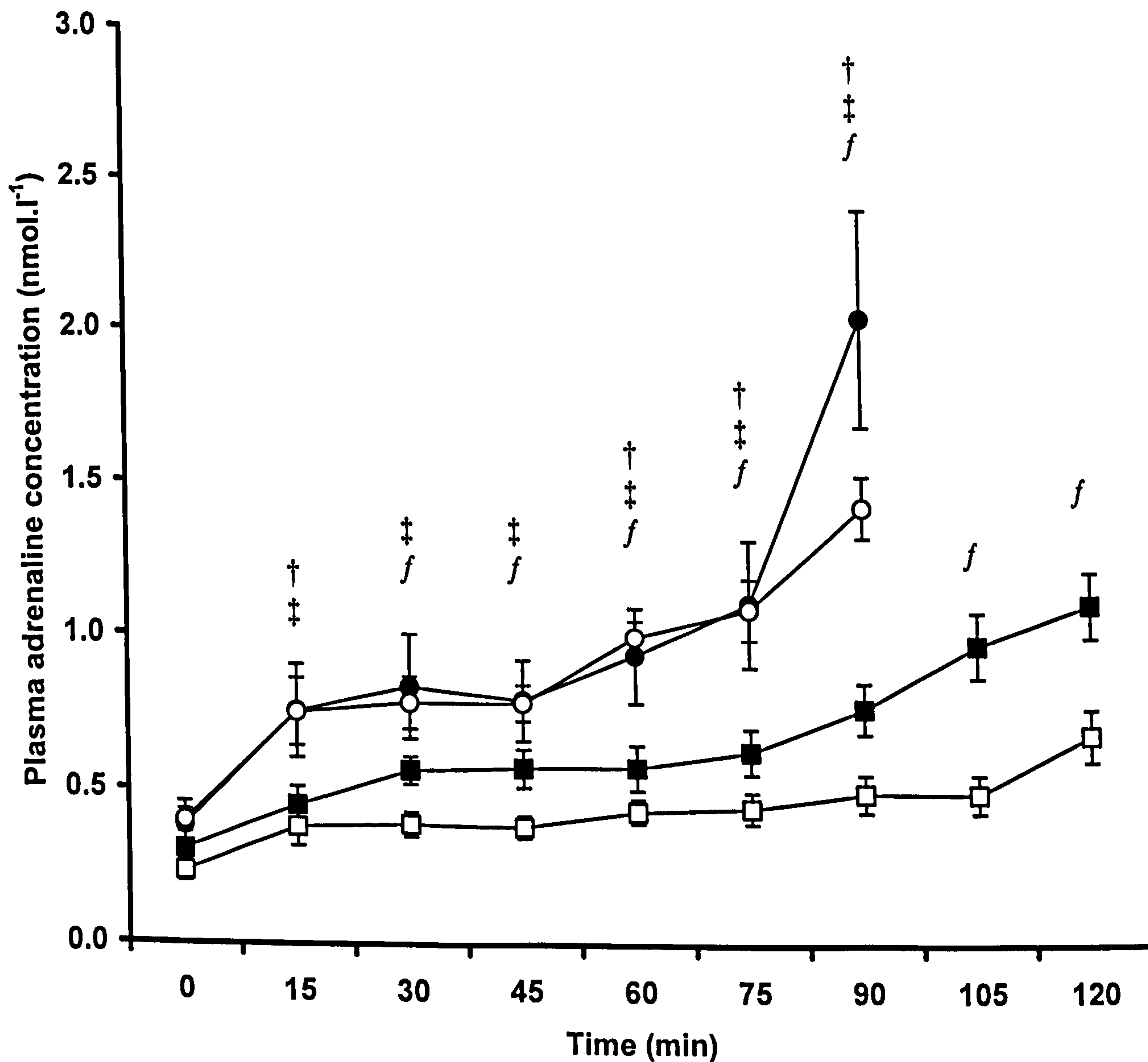


Figure 7.13 Plasma adrenaline concentrations (nmol.l^{-1}) at each time point during the CON19 (■), CON34 (●), GLU19 (□), GLU34 (○) conditions. Values are mean \pm SEM ($n = 9$). Symbols denote a significant difference: † between CON19 and CON34 ($P < 0.01$); ‡ between GLU19 and GLU34 ($P < 0.01$); f between CON19 and GLU19 ($P < 0.05$).

Exercise in the heat also elevated plasma noradrenaline concentrations compared to exercise in the temperate environment (Figure 7.14). Noradrenaline concentrations were greater from 45-min until the end of exercise during the CON34 compared to the CON19 condition and from 15-min until the end of exercise during the GLU34 compared to the GLU19 condition. Glucose feedings did not appear to influence plasma noradrenaline concentrations in the hot or temperate environments.

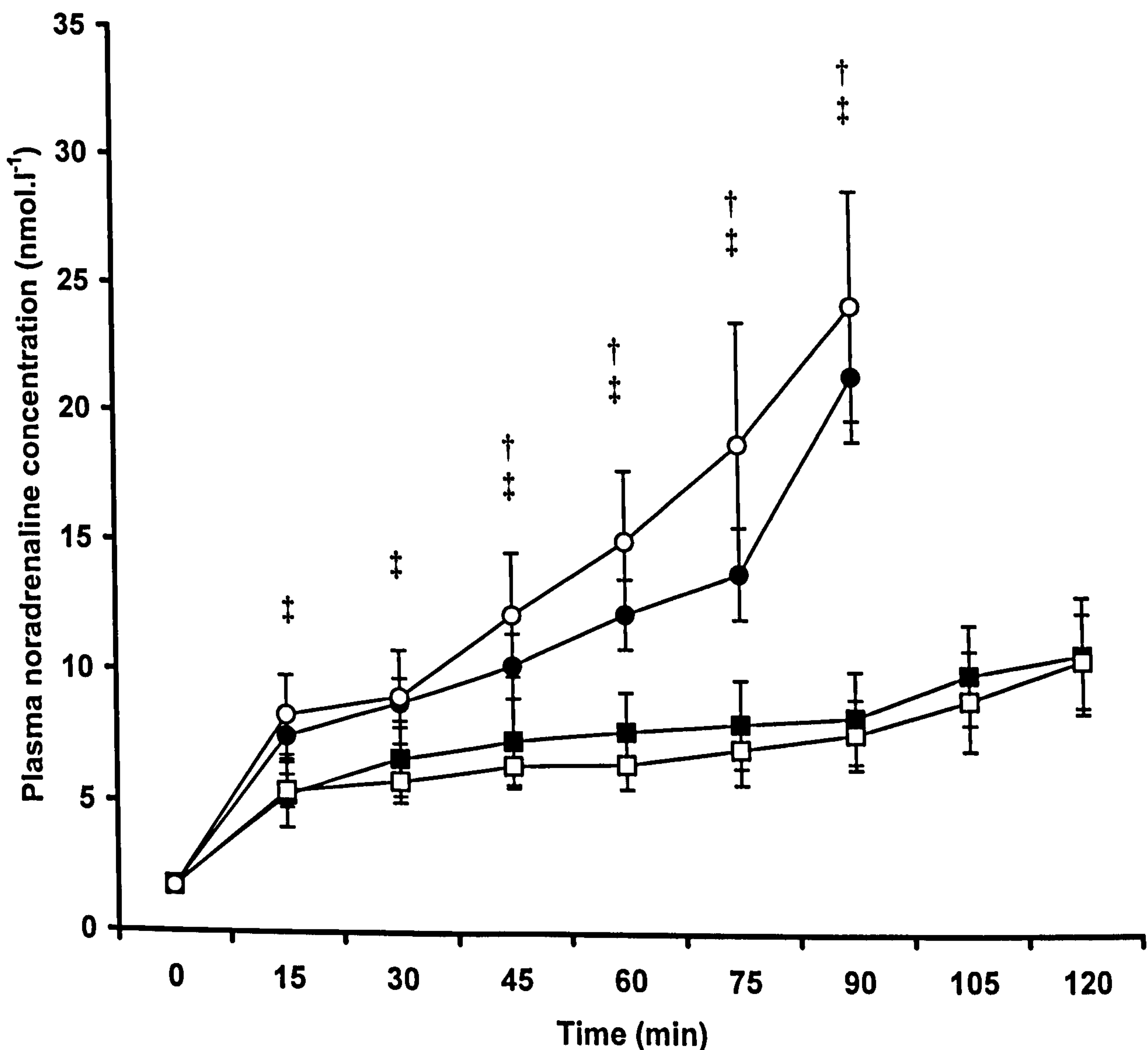


Figure 7.14 Plasma noradrenaline concentrations (nmol.l^{-1}) at each time point during the CON19 (■), CON34 (●), GLU19 (□), GLU34 (○) conditions. Values are mean \pm SEM ($n = 9$). Symbols denote a significant difference: † between CON19 and CON34 ($P < 0.01$); ‡ between GLU19 and GLU34 ($P < 0.01$).

7.3.10 *Thermal, cardiovascular and subjective responses*

From the onset of exercise rectal temperature (T_{rec}) increased in all conditions until 45-min (Table 7.4). There was an effect of glucose feedings and environmental temperature on T_{rec} ($P < 0.01$). From 45-min onwards, T_{rec} was relatively constant in the CON19 ($38.4 \pm 0.1^{\circ}\text{C}$) and GLU19 ($38.5 \pm 0.1^{\circ}\text{C}$) conditions, but continued to rise until the end of exercise in the CON34 ($39.3 \pm 0.1^{\circ}\text{C}$) and GLU34 ($39.3 \pm 0.1^{\circ}\text{C}$) conditions. These maximum values were reached on average 12-min later in the CON34 trial. Mean skin temperature was relatively constant in all conditions and was lower ($P < 0.01$) in the temperate ($26.6 \pm 0.5^{\circ}\text{C}$) compared to the hot ($34.0 \pm 0.5^{\circ}\text{C}$) conditions. Both heart rate and ratings of perceived exertion (RPE) continued to rise throughout exercise in all conditions (Table 7.4). The rate of rise of both parameters was greater in the hot (34°C) compared to the temperate (19°C) environment. Whilst there was no effect of glucose feeding on these parameters in the temperate environment, heart rate and RPE were greater in the GLU34 compared to the CON34 condition during the latter stages of exercise.

	CON19	CON34	GLU19	GLU34
Time (min)	mean \pm SEM	mean \pm SEM	mean \pm SEM	mean \pm SEM
Rectal temperature (T_{rec} , °C)				
REST	37.10 \pm 0.15	37.10 \pm 0.05	37.05 \pm 0.10	37.15 \pm 0.10
15	37.70 \pm 0.15	37.60 \pm 0.05	37.60 \pm 0.10	37.70 \pm 0.05
30	38.10 \pm 0.15	38.10 \pm 0.05	38.10 \pm 0.10	38.25 \pm 0.05
45	38.25 \pm 0.05	38.45 \pm 0.10†	38.20 \pm 0.15	38.50 \pm 0.05‡
60	38.30 \pm 0.10	38.65 \pm 0.10†	38.30 \pm 0.15	38.75 \pm 0.10‡
75	38.35 \pm 0.10	38.85 \pm 0.15†	38.35 \pm 0.15	39.05 \pm 0.15‡
90	38.40 \pm 0.10	39.00 \pm 0.10†	38.45 \pm 0.15	39.35 \pm 0.10‡§
105	38.40 \pm 0.10	39.30 \pm 0.10†	38.50 \pm 0.15	
120	38.45 \pm 0.15		38.60 \pm 0.15	
Heart rate (beats.min ⁻¹)				
REST	59 \pm 3	67 \pm 2	56 \pm 3	66 \pm 3
15	136 \pm 2	146 \pm 2†	138 \pm 2	151 \pm 2‡
30	138 \pm 1	152 \pm 1†	142 \pm 2	158 \pm 3‡
45	143 \pm 1	159 \pm 2†	146 \pm 3	166 \pm 3‡
60	145 \pm 1	165 \pm 3†	147 \pm 3	170 \pm 3‡
75	148 \pm 1	168 \pm 3†	148 \pm 3	177 \pm 4‡§
90	148 \pm 1	176 \pm 2†	150 \pm 3	184 \pm 3‡§
105	152 \pm 2	184 \pm 3†	153 \pm 3	
120	154 \pm 2		154 \pm 3	
Rating of perceived exertion (RPE, Borg 1982)				
REST	6.0 \pm 0.0	6.0 \pm 0.0	6.0 \pm 0.0	6.0 \pm 0.0
15	6.7 \pm 0.2	6.9 \pm 0.3	6.4 \pm 0.2	6.9 \pm 0.3
30	7.1 \pm 0.4	7.9 \pm 0.4	7.3 \pm 0.3	8.3 \pm 0.4
45	8.1 \pm 0.6	8.9 \pm 0.6	8.6 \pm 0.4	9.7 \pm 0.6
60	9.0 \pm 0.8	10.1 \pm 0.7†	9.1 \pm 0.5	11.4 \pm 0.6‡§
75	10.0 \pm 0.8	11.4 \pm 0.7†	9.9 \pm 0.6	13.2 \pm 0.6‡§
90	10.8 \pm 0.8	13.8 \pm 0.7†	10.6 \pm 0.7	15.6 \pm 0.6‡§
105	11.0 \pm 0.8	15.2 \pm 0.7†	11.0 \pm 0.7	
120	11.3 \pm 0.8		11.0 \pm 0.7	

Table 7.4 Rectal temperature, heart rate and ratings of perceived exertion at each time point during the CON19, CON34, GLU19 and GLU34 conditions. Values are mean \pm SEM ($n = 9$). Symbols denote that values are significantly different: † from CON19 ($P < 0.01$); ‡ from GLU19 ($P < 0.01$); § from CON34 ($P < 0.05$).

7.4 Discussion

The main finding of this study was that, despite 'sparing' liver glycogen and maintaining high rates of CHO oxidation, the ingestion of a 12.5% glucose solution during exercise in the heat (GLU34) impaired exercise tolerance time when compared to water (CON34). Whilst the exact cause of this impairment of physiological function is unknown, six of the nine subjects reported feelings of gastric discomfort and nausea during the GLU34 condition. Perceptions of gastric discomfort have previously been reported to impair exercise tolerance when a 14% glucose solution was ingested during exercise in a temperate environment (Febbraio *et al.* 1996a). This response may have been exacerbated during exercise in the heat because of reduced splanchnic blood flow (Rowell *et al.* 1968) and reduced gastric emptying of the ingested hypertonic solution (Leiper and Maughan 1986). This notion is supported by the current data, as the rate of appearance (Ra) and oxidation (Rox) of ingested (exogenous) glucose was reduced by ~36% in the GLU34 compared to the GLU19 condition. In addition, a reduction in the availability of the ingested solution may explain why heart rate, thermal strain and ratings of perceived exertion were greater during the latter stages of exercise in the heat when ingesting the glucose solution compared to water. As such, ingestion of a 12.5% glucose solution during exercise may impair exercise tolerance when exercising at 60% $\dot{V}O_2, max$ in hot climate.

The results of this study also demonstrate that, when water is ingested (CON conditions), heat stress does not influence total CHO or fat oxidation during prolonged running at 60% $\dot{V}O_2, max$. However, heat stress markedly increases the Ra , Rd and Rox of plasma glucose, but does not appear to influence skeletal muscle glycogen metabolism, at least during the last 30-60 min of prolonged running. These findings are in direct contrast to those previously observed during cycling exercise at 70% $\dot{V}O_2, max$, where heat stress has been observed to increase muscle glycogen metabolism (Febbraio *et al.* 1994a, 1994b), but not alter the Rd and Rox of plasma glucose (Hargreaves *et al.* 1996b). This contrast may be attributable to experimental differences in exercise intensity, modality, duration and/or the method of assessing muscle glycogen utilisation. This is an important finding because an increased utilisation of the limited liver glycogen stores may lead to hypoglycaemia and an

earlier onset of fatigue during prolonged running. Whilst glucose ingestion appeared to 'spare' liver glycogen in the temperate condition, it did not 'spare' muscle glycogen, as previously observed during prolonged running at 70% $\dot{V}O_2, max$ (Tsintzas *et al.* 1995, 1996a). It may be that at this lower exercise intensity (60% $\dot{V}O_2, max$), the body is more reliant on a constant supply of plasma glucose than muscle glycogen *per se*.

7.4.1 Influence of heat stress (CON19 versus CON34)

When exercise was performed in the heat, with water ingestion, T_{rec} continued to rise throughout exercise, reaching maximal values of 39.4 ± 0.1 °C at the end of exercise, when the majority of subjects stopped exercising of their own volition. These values are similar to those (39.3-39.4 °C) previously reported to coincide with volitional fatigue during prolonged exercise (Chapter 4, Nielsen 1994, Nielsen *et al.* 1993). These thermal responses occurred with subjects producing near maximal sweat rates (~ 1.62 l.h⁻¹), and are therefore indicative of uncompensable heat stress (Montain *et al.* 1994). In contrast, T_{rec} reached a plateau (38.4 ± 0.1 °C) after ~ 45 min of exercise in the temperate environment, with lower sweat rates (~ 0.96 l.h⁻¹). Consequently, whilst subjects were able to run for the full 120-min during the CON19 condition, mean \pm SEM exercise tolerance time was reduced to 112 ± 4 min in the CON34 condition. This earlier onset of fatigue during exercise in the heat has previously been reported (Saltin *et al.* 1972).

Whole body carbohydrate (CHO) oxidation contributed 47% and 52% to the metabolic demand of the last 60-min of exercise during the CON19 and CON34 conditions, respectively. Whilst these values were not statistically different, previous investigations have observed increases in whole body CHO oxidation when hyperthermia is induced by exercising in a hot environment (Febbraio *et al.* 1994a, 1994b), when dehydrated (Hargreaves *et al.* 1996a) and when wearing protective clothing (Chapter 4). That differences were not observed in the present experiment may be explained by the fact that the exercise intensity (60% $\dot{V}O_2, max$), and therefore the reliance on CHO as a fuel, was lower during the present experiment compared to those previously reported (70% $\dot{V}O_2, max$).

Although total CHO and muscle glycogen oxidation rates were not different between the CON19 and CON34 conditions, the rate of appearance (Ra), disappearance (Rd) and oxidation (Rox) of plasma glucose was increased by heat stress during the 60-90 min exercise period. This time period coincides with the maximum differences in T_{rec} and circulating plasma adrenaline concentrations between the CON19 and CON34 conditions. Whilst Ra has previously been reported to increase during exercise in the heat, increases in Rd and Rox have not (Hargreaves *et al.* 1996b). Numerous studies have reported that the imbalance between glucose production and utilisation results in a relative hyperglycaemic response during exercise in the heat (Fink *et al.* 1975; Yaspelkis *et al.* 1993; Hargreaves *et al.* 1996b), and is caused by an increase in HGP (Rowell *et al.* 1968; Angus *et al.* 2001). Whilst such a marked hyperglycaemic response was not observed during the CON34 condition, this is because Rd and Rox were similarly increased to match Ra . There is some evidence that this increase in HGP during exercise and heat stress is mediated by an augmented sympatho-adrenal response (Howlett *et al.* 1999). The current data support the notion that the hyperthermia-induced alterations in liver-derived plasma glucose oxidation are mediated by an enhanced β -adrenergic receptor stimulation, secondary to an increase in circulating adrenaline concentrations. Furthermore, given the similar serum insulin concentrations in the CON19 and CON34 conditions, it appears that insulin plays less of a role in mediating HGP during exercise in the heat, probably because insulin secretion from the pancreas is attenuated by adrenaline.

7.4.2 Influence of glucose ingestion (CON19 versus GLU19)

With glucose ingestion (GLU19), plasma glucose concentrations remained elevated and FFA concentrations suppressed throughout the 120-min of exercise compared to the CON19 condition. Such responses to glucose ingestion during prolonged submaximal exercise are frequently observed (Tsintzas *et al.* 1995, 1996a, Jeukendrup *et al.* 1999a). In addition, total CHO oxidation rates remained elevated throughout exercise in the GLU19 compared to the CON19 condition, contributing 64% and 47% to the total metabolic demand of the last 60-min of exercise, respectively. Conversely, fat oxidation rates were suppressed throughout exercise following glucose ingestion, probably because circulating FFA concentrations were reduced by insulin secretion. Similar responses are observed when glucose is ingested during cycling exercise at

50% $\dot{V}O_2, max$ (Jeukendrup *et al.* 1999a), but are not apparent during prolonged running at 70% $\dot{V}O_2, max$ (Tsintzas *et al.* 1995, 1996a). It may be that at higher exercise intensities, CHO oxidation rates are already sufficiently high to negate any additional influence of glucose feedings on total rates of CHO oxidation.

There is little doubt that glucose feedings exert an ergogenic effect on prolonged exercise performance (Coggan and Coyle 1987, 1989, Tsintzas *et al.* 199b), but some controversy exists over the mechanism(s) by which this occurs. During cycling, glucose ingestion exerts its ergogenic effect by 'sparing' liver glycogen, maintaining blood glucose and high rates of CHO oxidation (Coyle *et al.* 1986, Jeukendrup *et al.* 1999b). However, during prolonged running glucose feedings have been shown to 'spare' muscle glycogen, particularly in type I muscle fibres (Tsintzas *et al.* 1995, 1996a). In the present investigation, differences in total muscle glycogen oxidation were not observed between the CON19 and GLU19 conditions. Muscle glycogen oxidation contributed on average 91 and 93 $\mu\text{mol.kg}^{-1}.\text{min}^{-1}$ to the metabolic demand of the last 60 min of exercise, during the CON19 and GLU19 conditions, respectively. However, glucose ingestion did appear to 'spare' liver glycogen, reducing its contribution from $32 \pm 2 \mu\text{mol.kg}^{-1}.\text{min}^{-1}$ (CON19) to $11 \pm 2 \mu\text{mol.kg}^{-1}.\text{min}^{-1}$ (GLU19). This occurred despite the fact that glucose ingestion increased total plasma glucose oxidation by ~200%. Exogenous glucose oxidation peaked at $\sim 0.86 \text{ g.min}^{-1}$ during the last 30-min of exercise in the GLU19 condition, which is similar to the theoretical maximum value (1 g.min^{-1}) reported in many studies (Hawley *et al.* 1992; Rehrer *et al.* 1992; Wagenmakers *et al.* 1993b).

7.4.3 *Influence of heat stress and glucose ingestion*

Plasma glucose concentrations were elevated with glucose ingestion in the temperate environment (GLU19), but returned to $\sim 5.6 \text{ mmol.l}^{-1}$ during the last 60-min of exercise. However, with glucose ingestion and heat stress (GLU34), plasma glucose concentrations remained elevated ($>7 \text{ mmol.l}^{-1}$) throughout the exercise period. To our knowledge this hyperglycaemic response to glucose ingestion and heat stress has not been reported previously. These data provide further support to the notion that during the earlier stages of exercise and heat stress (first 60-min), HGP is augmented despite high rates of exogenous glucose absorption, leading to hyperglycaemia.

Whilst the current data support this, it is also interesting that the metabolic clearance rate of plasma glucose was lower at all time points during GLU34 compared to GLU19, suggesting that hyperthermia impairs the maximum rate of skeletal muscle glucose uptake. Again, this is most probably attributable to the hyperthermia-induced increase in circulating adrenaline concentrations, which stimulate the β -adrenergic receptors. During the latter stages of exercise (last 60-min), this increase in Ra glucose is matched by a concomitant increase in Rd and Rox glucose, and plasma glucose remains in equilibrium, albeit at an elevated concentration.

Whilst glucose ingestion in the heat elevated total CHO oxidation rates, no differences in muscle glycogen oxidation were observed between any of the experimental conditions. As in the temperate conditions, glucose ingestion in the heat markedly increased plasma glucose Ra , Rd and Rox . Plasma glucose oxidation rates reached a maximum of $0.91 \text{ g}\cdot\text{min}^{-1}$ at the end of exercise in the GLU34 condition, compared to $0.66 \text{ g}\cdot\text{min}^{-1}$ in the CON34 condition and $1.02 \text{ g}\cdot\text{min}^{-1}$ in the GLU19 condition. Whilst there were no differences in total plasma glucose oxidation between the GLU19 and GLU34 conditions, differences in exogenous and liver-derived glucose were apparent. Exogenous glucose oxidation was lower in the heat (GLU34) compared to the temperate environment (GLU19), reaching a maximum value equivalent to $0.69 \text{ g}\cdot\text{min}^{-1}$, compared to $0.86 \text{ g}\cdot\text{min}^{-1}$ in the GLU19 condition. This lower rate of exogenous glucose oxidation appears to be due to a reduced absorption of glucose from the gut when exercise was performed in the heat, and may be as a result of impaired splanchnic blood flow (Rowell *et al.* 1968). It is unlikely to be as a result of reduced muscle blood flow (and therefore substrate delivery and oxidation) as the ^{13}C enrichment of the plasma glucose was lower in the GLU34 compared to the GLU19 condition throughout exercise (see Fig. 7.2). As such, liver-derived glucose was 'spared' to less of an extent with glucose ingestion in the heat ($0.22 \text{ g}\cdot\text{min}^{-1}$), compared to in the temperate environment ($0.16 \text{ g}\cdot\text{min}^{-1}$).

The results of this study demonstrate, for the first time, that muscle glycogen oxidation is not 'spared' by glucose ingestion and is not increased by thermal stress during prolonged running at $60\% \dot{V}O_2 \text{ max}$. Furthermore, the rates of appearance (Ra) and oxidation (Rox) of liver-derived glucose are augmented when exercise is

performed in the heat, and this response occurs with or without exogenous glucose ingestion. However, the absorption and oxidation of exogenous glucose is impaired by thermal stress and may lead to gastric discomfort, increased cardiovascular and thermal strain and feelings of perceived exertion during exercise in the heat. As such, exercise tolerance time during exercise in the heat is reduced by the ingestion of a 12.5% glucose solution compared to water. Carbohydrate-electrolyte solutions of lower concentration (6-8% w/v) and those composed from maltodextrins as opposed to glucose, will have a lower osmolality and may be more readily absorbed during exercise in the heat. As such, the associated problems with gastric discomfort may be avoided, ratings of perceived exertion reduced and exercise tolerance time not impaired compared to ingesting water.

CHAPTER EIGHT

GENERAL DISCUSSION

It has been known for many years that fatigue occurs earlier when prolonged exercise is performed in hot climates (Saltin *et al.* 1972). Moreover, in highly motivated athletes and soldiers the combined stresses of exercise and hyperthermia can present life-threatening challenges (Rowell 1986). This impairment of physiological function is thought to be directly related to thermoregulatory incapacity and the hyperthermia which ensues (Fink *et al.* 1975; Brück and Olszewski 1987; Nielsen *et al.* 1990). Whilst this may be true, the precise physiological mechanism(s) by which hyperthermia results in muscular fatigue during prolonged exercise are not fully understood. As such, scientists have become increasingly interested in exploring the compensatory metabolic responses to hyperthermia during prolonged exercise. Early investigations demonstrated that environmental temperature affects intramuscular substrate utilisation during submaximal (Fink *et al.* 1975) and isometric exercise (Edwards *et al.* 1972). Furthermore, skeletal muscle energy depletion has been implicated in the pathophysiology of heat stroke (Hubbard *et al.* 1987). Consistent with these findings, several experiments have observed an augmented intramuscular CHO (glycogen) utilisation during prolonged cycling in the heat (Febbraio *et al.* 1994a, 1994b; Hargreaves *et al.* 1996a). Whilst a number of studies have attempted to investigate the influence of CHO ingestion during exercise in the heat, the findings were equivocal (Millard-Stafford *et al.* 1992; Febbraio *et al.* 1996a; Carter *et al.* 2002, 2003). The studies reported in this thesis make a contribution towards greater understanding of the effects of heat stress and CHO ingestion (during exercise and recovery) on energy metabolism and exercise tolerance during prolonged running.

Investigators in the past have principally used prolonged cycling (Febbraio *et al.* 1994a, 1994b; Hargreaves *et al.* 1996a) or intermittent isometric exercise (Edwards *et al.* 1972) to study the effects of heat stress on muscle metabolism. However, it was the intention at the outset of this thesis to ensure that the exercise model was representative of the activities undertaken by military personnel during sustained operations and training. For this reason, and the paucity of research on the effects of heat stress and CHO ingestion on energy metabolism during prolonged running,

treadmill running was used during the four experiments reported in this thesis. Furthermore, even when training in temperate environmental conditions, military personnel have the additional thermal burden of exercising whilst wearing personal protective clothing. For this reason, the study reported in Chapter 4 was used to assess the influence of a military protective clothing ensemble on the thermal and metabolic responses to a simulated military task. In doing so, it was also possible to quantify the relative metabolic demand of such activities for simulation during the subsequent controlled experiments.

In the experiment reported in Chapter 4, the mean relative metabolic demand of exercise was equivalent to $69 \pm 2 \% \dot{V}O_2 \text{ max}$ (range 60 to $80 \% \dot{V}O_2 \text{ max}$) during the CON and CFO conditions. However, the military protective clothing ensemble (CFO condition) imposed a significant heat strain on the subjects, which increased both thermal and cardiovascular strain compared to CON. Such responses to exercise in various clothing ensembles have previously been observed (Fox *et al.* 1966; Givoni and Goldman 1972, 1973). Whilst volitional fatigue occurred at approximately the same rectal temperature (T_{rec}) of 39.4°C , it occurred 26-min earlier in the CFO compared to the CON condition. These results therefore support the earlier findings of Nielsen *et al.* (1990), that fatigue occurs at the same rectal temperature during exercise in the heat. This appeared to be largely as a result of reduced sweat evaporation and heat dissipation via this mechanism, despite a higher total sweat rate when wearing the CFO clothing ensemble. Indeed, the results of all of the studies reported in this thesis support the notion that fatigue occurs at the same deep body temperature, whether measured at the rectum (T_{rec}) or the aural canal (T_{aur}), during exercise in the heat (Figure 8.1). This appears to be true, irrespective of the relative metabolic demand of exercise, which was higher in the experiment reported in Chapter 4, than in the other three experiments ($60 \% \dot{V}O_2 \text{ max}$). However, it should be noted that during exercise in the heat, deep body temperature was generally lower at the point of fatigue, when measured at the aural canal compared to the rectum. This is probably because aural canal temperature can be influenced by environmental and facial skin temperatures and therefore underestimate the degree of hyperthermia. These findings are therefore consistent with previously published observations (Deschamps *et al.* 1992; Roberts 1994).

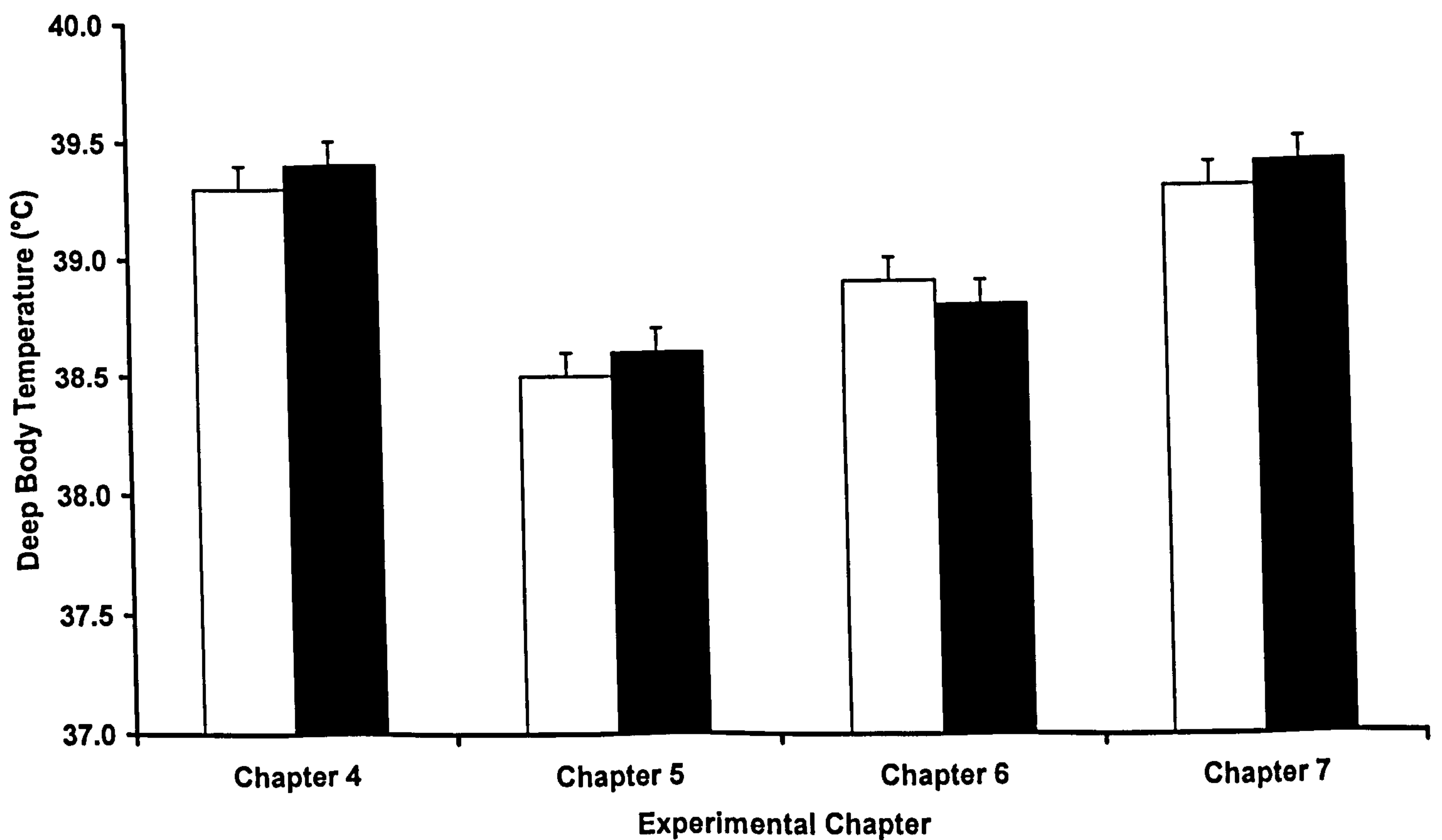


Figure 8.1 Deep body temperature ($^{\circ}\text{C}$) at the end of exercise, measured at the rectum (Chapters 4 and 7) and at the aural canal (Chapters 5 and 6). Values are mean \pm SEM and relate to: \square the CFO, CES, C220 and CON34 conditions; and \blacksquare the CON, P, C55 and GLU34 conditions, respectively.

Whilst fatigue occurred at the same T_{rec} in the experiment reported in Chapter 4, the results support the experimental hypothesis that the heat stress imposed by a clothing ensemble increase the rate of total CHO oxidation. These results therefore demonstrate, for the first time, that the metabolic responses to the heat stress imposed by clothing during prolonged running, are similar to those imposed by environmental heat stress during other modes of exercise (Fink *et al.* 1975; Febbraio *et al.* 1994a, 1994b; Hargreaves *et al.* 1996a). This is an important finding, as numerous occupational groups and sports performers, including the military, are required to perform arduous physical tasks whilst wearing protective clothing. That the rate of CHO oxidation was lower when the clothing was not worn during exercise (CON condition) is consistent with the suggestion that when a rise in deep body temperature is attenuated the rate of CHO oxidation is reduced. Previous studies have demonstrated this effect by prior heat acclimation (King *et al.* 1985; Young *et al.*

1985; Febbraio *et al.* 1994a), preventing dehydration (Hargreaves *et al.* 1996b; Gonzalez-Alonso *et al.* 1999), reducing ambient temperature (Febbraio *et al.* 1996b; Parkin *et al.* 1999), or providing external cooling during exercise (Kozlowski *et al.* 1985).

Whilst it was not possible to partition the contribution of CHO from the various body pools (i.e. liver, plasma, muscle) during the experiment reported in Chapter 4, other studies have demonstrated that the increase in CHO utilisation during exercise in the heat, results predominantly from an increase in skeletal muscle glycogen utilisation (Fink *et al.* 1975; Febbraio *et al.* 1994a, 1994b). Indeed, the consistent observation of an increased respiratory exchange ratio (RER) suggests that CHO, in particular muscle glycogen, is oxidised to a greater extent during exercise and heat stress, possibly at the expense of lipid oxidation. Furthermore, the increase in glycogen utilisation observed during exercise and heat stress appears to involve both oxidative and non-oxidative pathways. The findings in Chapters 4 and 7 support this notion, particularly as circulating plasma lactate concentrations were greater when heat stress was imposed by clothing (Figure 4.3) and an increase in environmental temperature (Figure 7.11). These data are further supported by the observation that muscle lactate accumulation is augmented in both humans (Young *et al.* 1985; Febbraio *et al.* 1994a, 1994b; Hargreaves *et al.* 1996a; Gonzalez-Alonso *et al.* 1999; Parkin *et al.* 1999) and dogs (Kozlowski *et al.* 1985) during exercise and heat stress. Whilst these studies suggest that flux through anaerobic glycolysis is augmented during exercise and heat stress, they do not allow for precise measurement of glycolytic rate. However, researchers have recently demonstrated a higher post-exercise muscle lactate content in the presence of an augmented lactate release, when hyperthermia was induced by dehydration during exercise (Gonzalez-Alonso *et al.* 1999).

Whilst the findings in Chapter 7 suggest that total CHO oxidation may be slightly elevated (not significantly) during exercise in the heat, there was no evidence of an increase in estimated skeletal muscle glycogen utilisation. However, there was a marked increase in the rate of appearance (R_a), disappearance (R_d) and oxidation (R_{ox}) of plasma (liver-derived) glucose as the subjects became progressively hyperthermic during exercise in the heat. These findings are in stark contrast to evidence from other research groups (Hargreaves *et al.* 1996a; Gonzalez-Alonso *et al.*

1999). Using similar isotopic tracer methods Hargreaves *et al.* (1996a) demonstrated that, despite an increase in hepatic glucose production (Ra), Rd was not different when comparing the metabolic responses to exercise in a 40°C and 20°C environment. However, glucose Rox was not measured in this study, and it was assumed that all of the glucose disappearing from the plasma pool was fully oxidised within contracting skeletal muscle. This assumption appears to be valid during moderate intensity exercise in temperate (Jeukendrup *et al.* 1999) and hot environments (Chapter 7). The findings of Hargreaves *et al.* (1996a) are also supported by Gonzalez-Alonso *et al.* (1999) who demonstrated that contracting limb respiratory quotient (RQ) was higher when hyperthermia was induced by dehydration during exercise. These authors observed no difference in contracting skeletal muscle glucose uptake (measured by arteriovenous difference), suggesting that glycogen oxidation was augmented.

As the exercise intensity used in the study reported in Chapter 7 ($60 \pm 2 \text{ \%}\dot{V}O_2, \text{max}$) was only slightly lower than that used by Gonzalez-Alonso *et al.* ($61 \pm 2 \text{ \%}\dot{V}O_2, \text{max}$) and Hargreaves *et al.* ($65 \pm 2 \text{ \%}\dot{V}O_2, \text{max}$), this is unlikely to explain the observed differences in results. The main methodological differences between the present study and those previously reported are the modality of exercise, the method of inducing hyperthermia and the method of assessing substrate utilisation. The differences in modality of exercise are worthy of mention, particularly as previous studies have suggested that there may be differences in muscle energy metabolism during cycling and running. Fatigue during prolonged cycling appears to be related to a reduction in plasma glucose availability and the hypoglycaemia that ensues (Coyle *et al.* 1986), whereas during prolonged running fatigue appears to be associated with a reduction in skeletal muscle glycogen concentration (Tsintzas *et al.* 1996a). Furthermore, ingestion of carbohydrate-electrolyte solutions during prolonged cycling appear to 'spare' predominantly liver glycogen reserves (Coyle *et al.* 1986; Hargreaves and Briggs 1988), whereas during prolonged running they also appear to 'spare' muscle glycogen reserves (Tsintzas *et al.* 1995, 1996a).

More recent evidence suggests that humans are also able to mobilise and oxidise free-fatty acids (FFA) at a higher rate during prolonged running compared to cycling

(Achten *et al.* 2003). It remains to be determined whether it is also possible to utilise plasma glucose at a higher rate during prolonged running. This, however, seems unlikely, as there is a smaller muscle mass involved in cycling compared to running, which would lend itself to a greater plasma glucose uptake during cycling (Richter *et al.* 1988). Whilst these data do not directly explain the observed differences in the effects of heat stress on muscle energy metabolism, they do highlight potential differences in substrate metabolism which may be accounted for by differences in exercise modality. Whilst it was beyond the scope of this thesis, further research should attempt to directly compare the effects of heat stress on muscle energy metabolism during running and cycling, with and without CHO ingestion. In addition, it would aid in comparing the results of various studies if the methods of indirectly estimating muscle glycogen utilisation from isotopic tracer techniques were compared with the more direct muscle biopsy technique.

A number of mechanisms have been proposed to account for the shift towards increased CHO utilisation during exercise in the heat (Rowell 1974; Kozlowski *et al.* 1985; Young *et al.* 1985). The data reported in Chapters 4 and 7 support the notion that the increases in circulating catecholamine concentrations (particularly adrenaline) observed during exercise and heat stress may be responsible (Figure 8.2). It is well established that glycogen phosphorylase activity is enhanced by β -adrenergic receptor stimulation (Richter *et al.* 1982) and any increase in circulating adrenaline levels may result in a concomitant increase in intramuscular glycogen utilisation. Indeed, intramuscular glycogen utilisation often closely matches the plasma adrenaline response during exercise and heat stress (Febbraio *et al.* 1994a, 1996b; Hargreaves *et al.* 1996b; Gonzalez-Alonso *et al.* 1997). Further support is provided from animal studies where adrenaline infusion increases glycogen utilisation during voluntary submaximal exercise or electrical stimulation in both rats (Richter *et al.* 1981, 1982) and dogs (Issekutz 1984). Whilst these experiments provide a strong case, there was no evidence of an increase in estimated muscle glycogen utilisation, despite marked increases in circulating catecholamine and lactate concentrations, during exercise in the heat in Chapter 7. Application of the more direct muscle biopsy technique and subsequent analysis of single-fibre glycogen utilisation, may provide more insight within such an experimental design.

In addition to the effects of adrenaline on muscle glycogen utilisation, increases in circulating catecholamine concentrations may also play a role in stimulating hepatic glucose production (Rowell *et al.* 1968; Hargreaves *et al.* 1996a). Indeed, a number of studies, including those reported in Chapters 4 and 7 (Figure 8.3), have observed a relative hyperglycaemic response during exercise and heat stress (Fink *et al.* 1975; Febbraio *et al.* 1994a, 1996b; Yaspelkis *et al.* 1993; Hargreaves *et al.* 1996a). Using a dye infusion technique, Rowell *et al.* (1968) were probably the first to demonstrate that this hyperglycaemia was caused by an increase in hepatic glucose production in heat stressed humans. This response was confirmed more recently using both isotopic tracer dilution (Hargreaves *et al.* 1996a) and arteriovenous balance techniques (Gonzalez-Alonso *et al.* 1999).

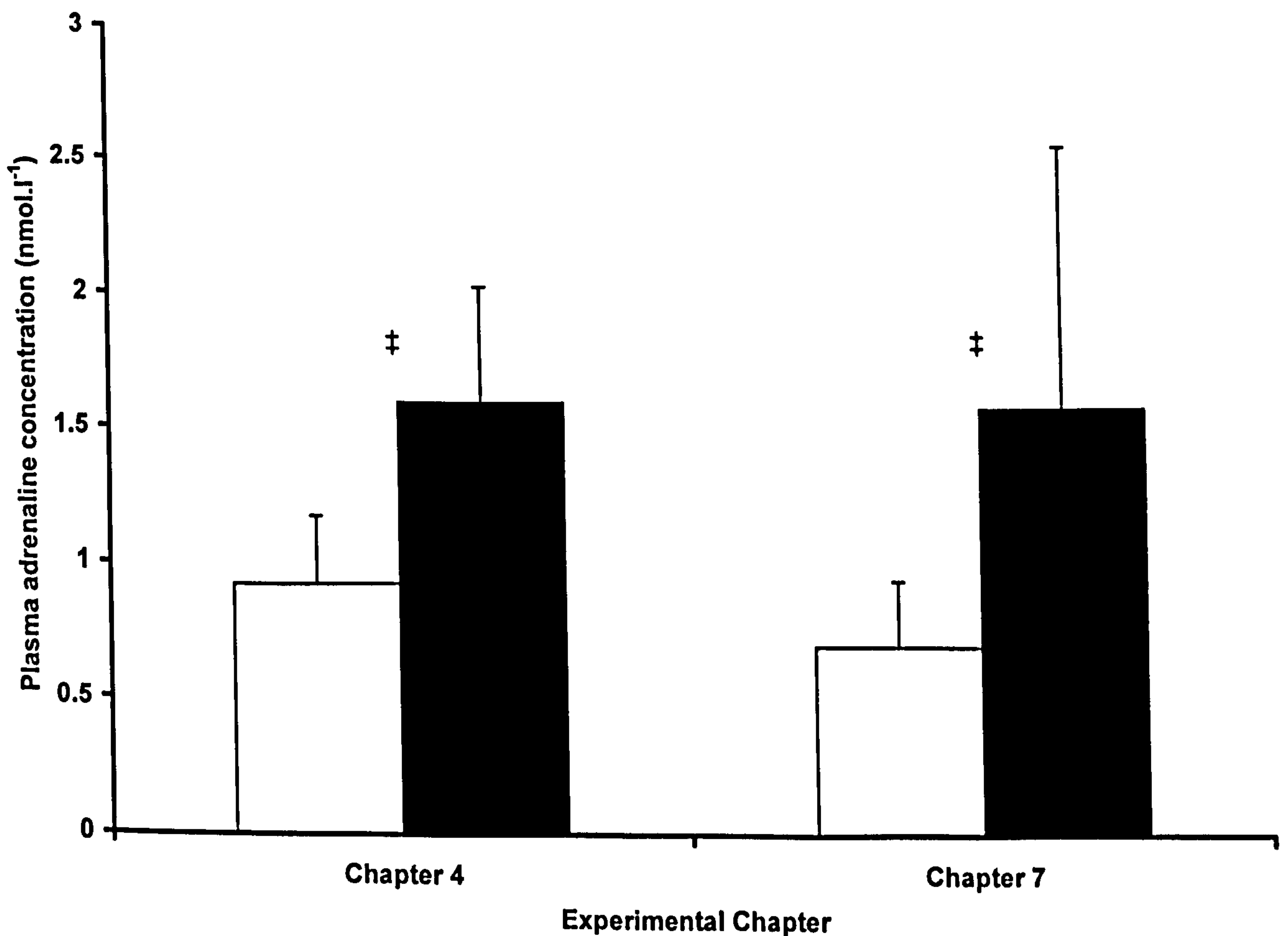


Figure 8.2 Mean (SEM) plasma adrenaline concentrations (nmol.l⁻¹) during the 15 to 45-min exercise period of the CON (□) and CFO (■) conditions (Chapter 4) and 60 to 90-min exercise period of the CON19 (□) and CON34 (■) conditions (Chapter 7). ‡ Symbol denotes a significant difference between conditions ($P < 0.01$).

Whilst ingestion of glucose solutions attenuates the increase in hepatic glucose production during exercise in temperate conditions (Jeukendrup *et al.* 1999; McConnell *et al.* 1994), hepatic glucose production appears to be unaffected by glucose ingestion during exercise in the heat (Angus *et al.* 2001; Jentjens *et al.* 2002). Whilst the data reported in Chapter 7 support the notion of an increase in hepatic glucose production (Ra) during exercise and heat stress, the results suggest that there was a concomitant increase in Rd and Rox of plasma glucose. Whilst glucose ingestion did not appear to influence hepatic glucose production during the first 30-min of exercise, resulting in a relative hyperglycaemia, thereafter hepatic glucose production was attenuated and plasma glucose concentrations remained constant albeit at an elevated concentration. The results therefore suggest that with a high rate of glucose ingestion ($\sim 1 \text{ g}\cdot\text{min}^{-1}$), hepatic glucose production may be attenuated during exercise in the heat.

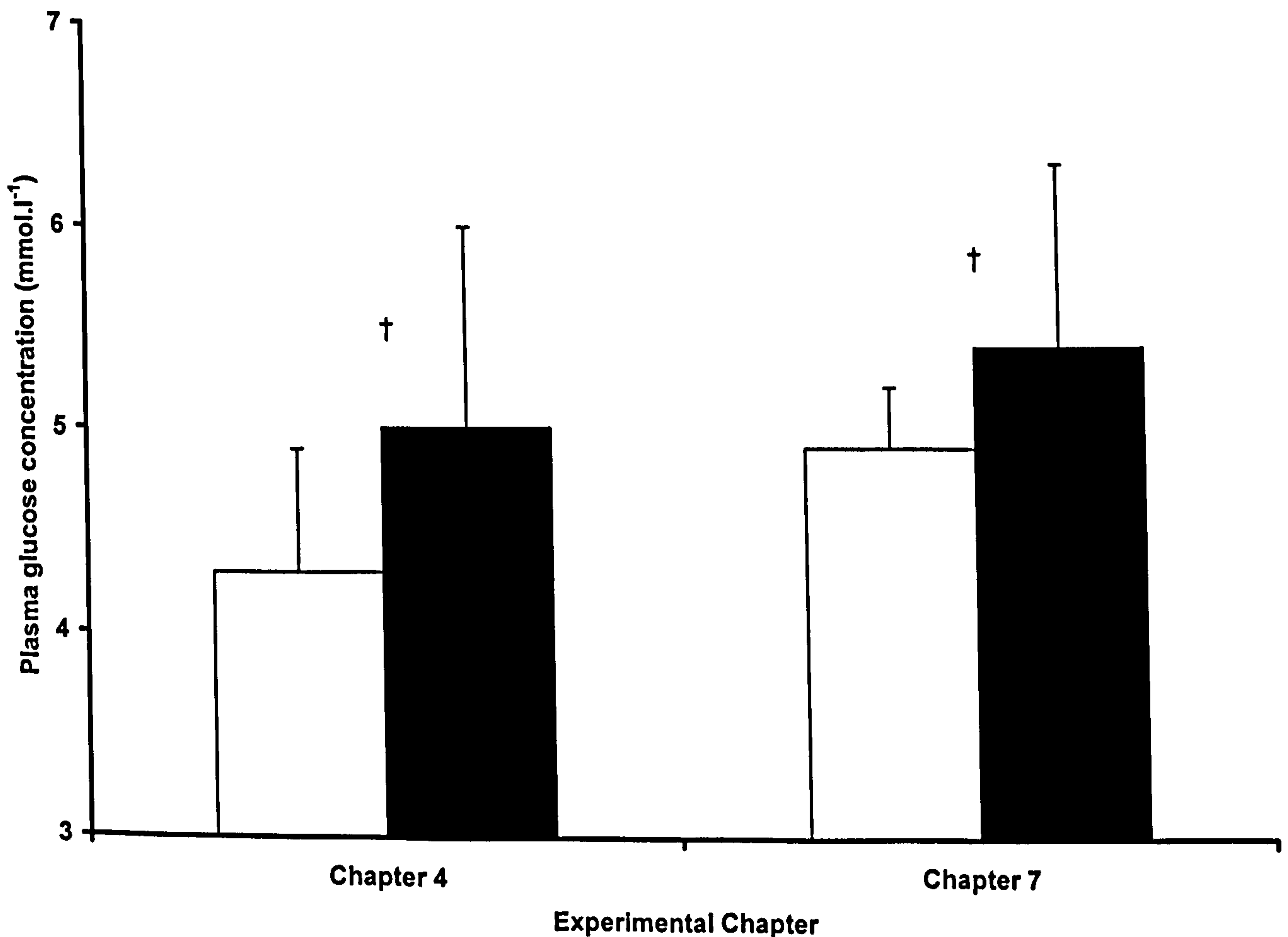


Figure 8.3 Mean (SEM) plasma glucose concentrations ($\text{mmol}\cdot\text{l}^{-1}$) during the 15 to 45-min exercise period of the CON (□) and CFO (■) conditions (Chapter 4) and 60 to 90-min exercise period of the CON19 (□) and CON34 (■) conditions (Chapter 7). † Symbol denotes a significant difference between conditions ($P < 0.05$).

Whilst there remains some conflicting evidence over the mechanisms involved in the effects of heat stress on muscle energy metabolism during prolonged exercise, there is a general consensus that the ensuing hyperthermia will increase circulating adrenaline concentrations, which results in an increased reliance on CHO. In addition to the substantial losses of water and electrolytes through sweating during prolonged exercise in the heat (Adolph *et al.* 1947; Lentner 1981), depletion of the endogenous CHO reserves will therefore ensue more rapidly. Inclusion of CHO within a rehydration beverage may therefore be essential when repeated bouts of exercise are performed in a warm environment, on the same or successive days. A number of studies have clearly demonstrated that inclusion of CHO in a rehydration solution, ingested during relatively short (4-h) and long-term (22.5-h) recovery periods, enhance subsequent exercise capacity in a temperate environment (Fallowfield and Williams 1993; Fallowfield *et al.* 1995). However, the influence of including CHO within a rehydration solution on post-recovery exercise capacity and muscle metabolism during exercise in the heat had not previously been investigated. The experiments that are reported in Chapters 5 and 6 were therefore designed to address this question.

The experiment that is reported in Chapter 5 clearly demonstrated that post-recovery (4-h) exercise capacity in the heat (35°C) was enhanced following rehydration with a 6.9% carbohydrate-electrolyte solution (CES) compared to a sweetened placebo. This occurred despite the fact that subjects ingested a breakfast containing 114-g of CHO 90-min prior to the first exercise period (T1). Whilst this was included as part of the protocol in order to simulate a military operational scenario, it was previously demonstrated that such pre-exercise feedings negate the impact of subsequent CHO feedings (Widrick *et al.* 1993). As the subjects were similarly rehydrated at the end of the 4-h recovery period, however, the observed improvement in exercise capacity appears to be related to the provision of CHO during recovery. In contrast, there was no difference in post-recovery endurance capacity time after ingesting 55 or 220-g of CHO during recovery (Chapter 6). In order to allow for clearer comparison between studies, the T2 run times from these two experiments have been expressed as a percentage of the T1 run times and the mean values plotted against CHO intake during recovery (Figure 8.4).

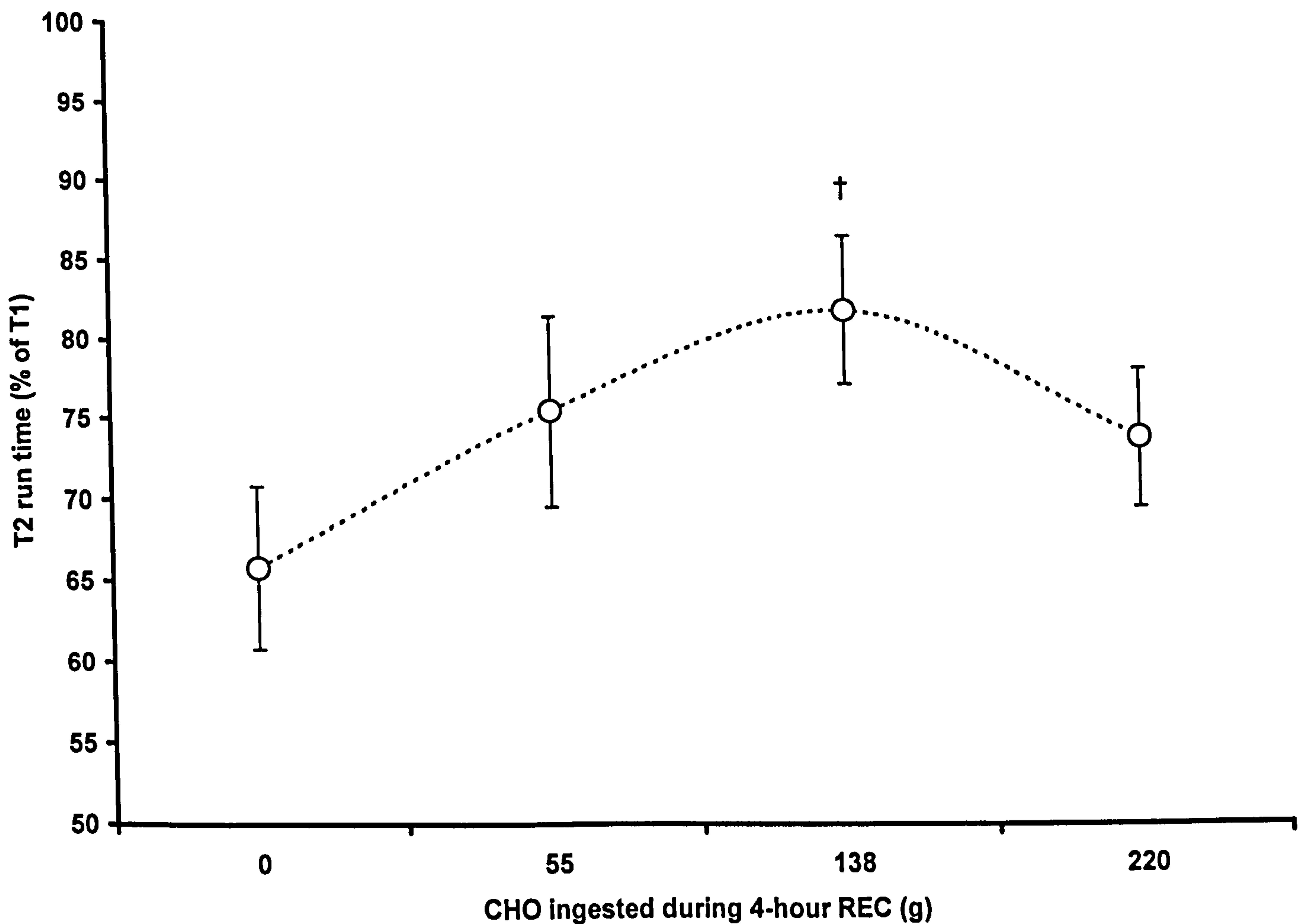


Figure 8.4 Mean (SEM) T2 run times (expressed as % of T1 run times) following various levels of CHO ingestion during a 4-hour recovery period (data from Chapters 5 and 6). † Symbol denotes that value was significantly different from 0-g CHO trial ($P < 0.05$).

These data suggest that, whilst there is not a clear relationship between CHO intake during recovery and the restoration of endurance running capacity, ingestion of some CHO within a CES appears to be beneficial. However, there was no difference in the restoration of endurance running capacity after ingesting 55-g, 138-g or 220-g of CHO. As previously observed during exercise in a temperate environment (Wong *et al.* 1996a), it may be that ingestion of 50-g of CHO immediately after exercise, is sufficient to produce a maximal improvement in endurance capacity after a 4-hour recovery period. It therefore appears that there may be some ergogenic effect of CHO ingestion on post-recovery endurance running capacity, but the possible mechanism remains unclear.

The application of stable isotope techniques in the experiment reported in Chapter 6, allowed the estimation of total glycogen synthesis and breakdown during recovery. It was estimated that total glycogen synthesis (liver and muscle) over the 4-hour recovery period was approximately five-fold greater after ingesting 220-g versus 55-g of CHO. These results are not dissimilar to those reported by Tsintzas *et al.* (1999), when subjects ingested 175-g compared to 50-g over the same 4-hour post-exercise recovery period. These high rates of CHO ingestion and associated increases in endogenous glycogen storage helped maintain high rate of CHO oxidation during the subsequent T2 exercise periods. Indeed, there appears to be a strong association between the quantity of CHO ingested during recovery and the relative rate of total CHO oxidation ($\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) after 15-min of the T2 exercise periods (Figure 8.5).

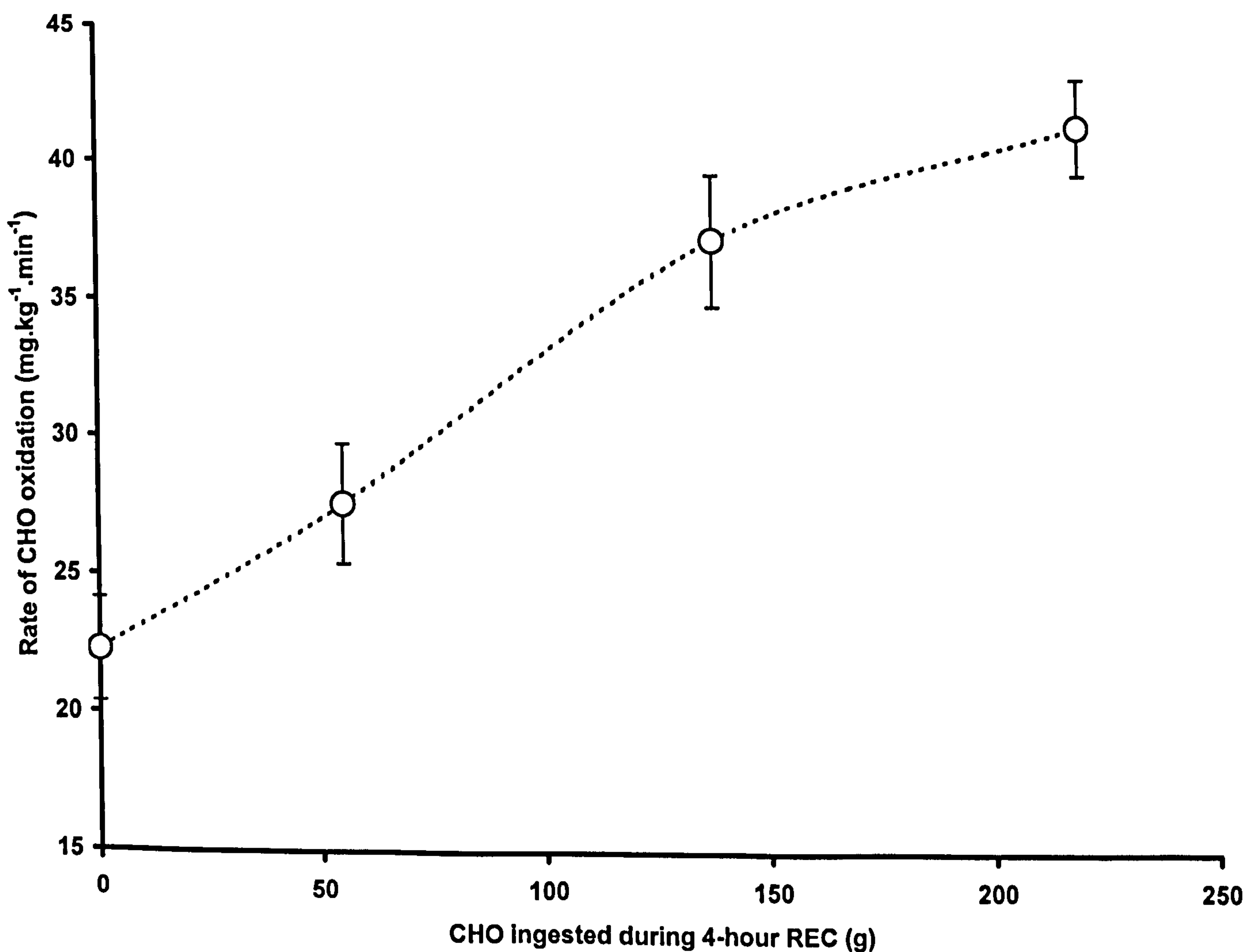


Figure 8.5 Relationship between CHO intake during REC (g) and the mean (SEM) relative rate of CHO oxidation ($\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) after 15-min of the subsequent T2 exercise periods (Chapters 5 and 6).

It is highly unlikely that the onset of fatigue was associated with endogenous CHO depletion in the trials where subjects were fed ~138-g or 220-g of CHO during REC, as total CHO oxidation rates were still high (31-38 mg.kg⁻¹.min⁻¹) at the point of fatigue. However, very low CHO oxidation rates (~19 mg.kg⁻¹.min⁻¹) were observed at the point of fatigue after subjects had ingested either 0-g or 55-g of CHO during REC, and this may be indicative of significantly depleted endogenous CHO reserves. It is difficult to establish whether CHO depletion contributed to the onset of fatigue, as there was no indication of subjects becoming hypoglycaemic during the 0-g or 55-g trials, and muscle biopsy samples were not taken for analysis of muscle glycogen content at the end of these experiments.

Whilst the improvement in exercise capacity was related to the increased storage and subsequent availability and utilisation of CHO *per se*, the data reported in Chapter 5 also suggest an alteration in thermoregulatory response. Whilst, aural canal temperature was the same at the end of the T2 exercise periods in the CES and P conditions (~38.5°C), this occurred 16-min later in the CES condition and values were significantly different after the first 30-min of exercise. It would appear that such thermoregulatory responses to exercise following CHO ingestion have not previously been reported. Interestingly, this response may be related to the provision of CHO and the consequent biochemical responses to the T2 exercise period. Whilst plasma insulin concentrations were not measured during this experiment, it has been assumed that in response to the relative hyperglycaemia, subjects were hyperinsulinaemic at the onset of the T2 exercise period of the CES trial. Thus, in response to exercise, plasma glucose uptake would have increased at a time when hepatic glucose production was partially attenuated by high insulin concentrations (Ahlborg and Bjorkman 1987). The resultant hypoglycaemic response at the onset of T2 may have been responsible for an earlier increase in peripheral blood flow and evaporative heat loss during the CES trial (Gale *et al.* 1981; MacDonald *et al.* 1982). Whilst this tentative explanation for the observed results may be worthy of further investigation, similar responses were not observed to hypoglycaemia during the experiment reported in Chapter 6.

The one factor that was consistently observed at the end of exercise in the heat, regardless of CHO intake during recovery or exercise, was a similarly elevated deep

body temperature (Figure 8.1). In the absence of any clear metabolic explanation for the observed ergogenic effect of CHO ingestion during recovery, it is proposed that CHO ingestion acts on a central mechanism which either increases thermal tolerance, or alters thermoregulatory function during subsequent exercise. Indeed, it has previously been proposed that hyperthermia eventually results in an inhibition of motor activity (Brück and Olszewski 1987; Nielsen *et al.* 1993; Nybo and Nielsen 2001), presumably to reduce metabolic heat production. Whilst the mechanism by which hyperthermia may inhibit motor output is not fully understood, it is likely to be controlled by the hypothalamus, as the central point of thermal integration. Further research should examine the influence of CHO ingestion/availability on this relationship between brain activity (motor output) and perceived exertion during progressive hyperthermia.

During the experiment reported in Chapter 7, it was hypothesised that high CHO ingestion during exercise in the heat would also enhance exercise capacity, either by 'sparing' liver or muscle glycogen, or similarly altering thermoregulatory function or thermal tolerance. In fact, fatigue occurred approximately 12-min earlier ($P < 0.05$) when glucose was ingested (GLU34) compared to the placebo solution (CON34) during exercise in the heat. These results are in agreement with those of Febbraio *et al.* (1996a), who observed that ingesting a 14% hypertonic CHO solution impaired exercise capacity during prolonged cycling in the heat compared to a 7% CHO solution. Indeed, it appears that endurance cycling capacity (Febbraio *et al.* 1996a; Carter *et al.* 2003) is enhanced when a CHO solution of moderate concentration (6-7% w/v) is ingested. Ingestion of a hypertonic high CHO solution appears to consistently result in gastric discomfort during exercise in the heat (Chapter 7; Febbraio *et al.* 1996a). This appears to be related to a reduction in absorption of the ingested fluid, as the appearance and oxidation of labelled glucose was lower when CHO was ingested during exercise in the heat compared to the temperate environment. This may have been due to a reduction in splanchnic blood flow (Rowell *et al.* 1968), either as a direct result of heat stress and increased peripheral blood flow, or in response to progressive dehydration which reduces blood and plasma volume to a greater extent during exercise in the heat.

It was somewhat surprising that estimated rates of skeletal muscle glycogen utilisation were not attenuated with glucose ingestion or enhanced with heat stress. However, these data should be treated with some caution. Such estimates of whole-body muscle glycogen utilisation may mask effects which are only quantifiable within single muscle fibres within specific muscle groups. Indeed, the higher circulating lactate concentrations during exercise in the heat (with and without glucose ingestion) are indicative of an increase in anaerobic glycolysis. If this were true, it is likely to have been mediated by an enhanced β -adrenergic receptor stimulation secondary to an increase in circulating adrenaline concentrations (Greenhaff *et al.* 1991). Such a response may also have increased HGP, which lead to a hyperglycaemic response to exercise in the heat (Howlett *et al.* 1999). Indeed our data are in agreement with those of Angus *et al.* (2001), that HGP is maintained in the earlier stages of exercise in the heat, even when exogenous CHO is ingested. However, after the first 30 to 45-min of exercise in the GLU34 condition, HGP appeared to be attenuated, despite increasing circulating adrenaline concentrations. It is worthy of note, however, that despite high rates of CHO ingestion, HGP was never completely suppressed even though blood glucose concentrations were above 7 mmol.l^{-1} for the whole of the GLU34 trial.

In summary, the heat stress imposed by military protective clothing ensembles and environmental heat stress during prolonged running result in a similar series of metabolic responses to those observed when prolonged cycling is performed in a hot environment, or when dehydrated. The rapidly ensuing hyperthermia increases endogenous CHO utilisation, circulating adrenaline, lactate and glucose concentrations, which may be indicative of an increase in anaerobic glycolysis. Such responses are therefore important to our understanding of fatigue during prolonged running and military training and operational activities, particularly in hot environments. Given the high rates of sweat loss and endogenous CHO utilisation during prolonged running in the heat (or in protective clothing), ingestion of CHO solutions seems a logical means of delaying fatigue during such activities. From the results of the experiments in Chapters 5 and 6, it appears that the inclusion of CHO within a rehydration beverage during short-term recovery periods may be beneficial to subsequent endurance running capacity at $60\% \dot{V}O_{2, \text{max}}$ in the heat. However, ingestion of 55-g of CHO immediately after exercise may be sufficient to produce a

maximum improvement in post-recovery endurance running capacity in the heat, provided that rehydration is similarly attained with water. Although ingestion of greater amounts of CHO will substantially increase glycogen synthesis during recovery, and may improve endurance running capacity in a temperate environment, it is likely that hyperthermia rather than CHO availability will limit endurance running capacity in a 35°C environment. Similarly, whilst the ingestion of a hypertonic 12.5% glucose solution may 'spare' liver glycogen during prolonged running, such practices will induce significant gastric discomfort and impair thermoregulatory function and endurance running capacity. Whilst there may be an effect of CHO ingestion/availability, the main factor limiting exercise capacity in the heat appears to be hyperthermia, which possibly reduces the mental drive for motor performance.

It is therefore recommended that a 7% CHO solution be ingested during recovery from prolonged running in the heat. At least 50-g of CHO should be ingested as soon after exercise as is possible, and subsequent rehydration achieved by either water or CES ingestion. Whilst ingestion of a 6-7% CHO solution may also improve endurance capacity during prolonged cycling in the heat (Febbraio *et al.* 1996a; Carter *et al.* 2003), it remains to be determined whether such interventions improve prolonged running capacity in the heat. Future research should aim to identify the mechanism by which hyperthermia reduces the mental drive for motor performance during exercise in the heat, and the impact of endogenous CHO availability and exogenous CHO feedings on this central mechanism. Applied scientists should also begin to focus their efforts on applying stable isotope techniques to study substrate kinetics and metabolism during prolonged running in a range of climatic conditions. It will then become clearer whether the apparent differences in skeletal muscle metabolism between running and cycling are indeed true, or merely a function of the techniques that have predominantly been applied within such studies. Our profession is in danger of making generic conclusions and recommendations for endurance athletes on the basis of findings predominantly from cycling studies, particularly on the effects of heat stress and exogenous glucose feedings on substrate kinetics and muscle metabolism. To my knowledge, the study reported in Chapter 7 was the first to have systematically applied stable isotope techniques to the study of glucose kinetics and muscle metabolism during prolonged running.

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APPENDIX 1• **Subject Information Sheet.**

You are asked to read this form carefully. If you consent to take part as a subject in the study being undertaken by J L J Bilzon (Project Officer Tel: 9380 68050 or 01705 768050), then you should sign the consent form. If you have any query, or are unsure or uncertain about anything, then you should not sign until your problem has been resolved and you are completely happy to volunteer.

This study is part of a larger research programme within the INM's Environmental Medicine Unit (EMU), which aims to assess the impact of environmental heat stress and glucose ingestion, on metabolism and exercise performance. Such research has wider implications for fluid and nutrient requirements during military training and operations and the potential risk of heat related illnesses associated with military activities.

In order to investigate the effects of environmental heat stress and glucose drinks on metabolism and exercise performance, volunteers will be asked to complete a prolonged exercise period to fatigue, on a motorised treadmill in our environmental chamber. The exercise intensity for each individual will be determined during an initial maximal fitness test. The exercise periods are expected to last between 45 and 120 minutes. The environmental conditions will be controlled at either 35°C or 20°C, with a relative humidity of 40%.

After the initial medical examination and fitness test (approximately 2 hours in total), each volunteer is required to attend to the Environmental Medicine Unit on four occasions (0800-1500), separated by at least 5 days. Each volunteer will then exercise to exhaustion in a 35°C environment with (GLU34) and without (CON34) glucose ingestion, or a 19°C environment with (GLU19) and without (CON19) glucose ingestion. Prior to each of the exercise periods, a catheter will be inserted into a forearm vein of each arm for blood sampling and infusions during exercise. Additionally, a rectal thermistor will be inserted, in order that our trained medical staff can continuously monitor your core temperature during exercise. Although, this

information will be used for research purposes, it is essential for your personal safety during exercise in the heat.

The only risks to you are from heat-related illnesses or blood clots formed from the cannulae. Both of these are unlikely to occur and medical staff will be in attendance at all times to monitor your heart rate and core temperature, and to ensure your safety.

We also ask that you refrain from drinking any alcohol for 48 hours or caffeine for 24 hours prior to any of the trials and also refrain from any exhaustive physical activity for 48 hours prior to the tests.

An independent medical officer will be on call during the trials. His sole function is to act independently of the study team to ensure your safety and well being. He may terminate the trial on medical grounds at any time, and you may consult with him at any time. You may at any time withdraw from the experiment. You do not have to give any reason, and no one can attempt to dissuade you. If you require further information, please do not hesitate to ask. Any information obtained during this trial will remain confidential as to your identity: if it can specifically be identified with you, your permission will be sought in writing before it will be published. Other material, which cannot be identified with you, will be published or presented at meetings with the aim of benefiting others. All information will be subject to the conditions of the Data Protection Act 1984 and subsequent statutory instruments. The trial has received ethical and scientific approval to be undertaken, in accordance with current INM regulations.

APPENDIX 2

Subject Consent Form.

1. I have read the information sheet, which provides full details of this study, and have had the opportunity to raise and discuss my questions with the project officer, with regard to the general nature, the object, potential risks and duration of the study, and understand what is expected of me.
2. I understand that in the event of my sustaining injury, illness or death as a result of participating as a volunteer in INM research, I or my dependants may enter a claim with the Ministry of Defence for compensation under the provisions of the no-fault compensation scheme, details of which are attached. Such a scheme does not require me or my dependants to establish negligence on the part of the Ministry of Defence or its employees. I also understand that should such injury, illness or death have been caused by the negligence of the Ministry of Defence or its employees either I or my dependants may have a claim in law.
3. I understand that the aim of this study is to assess the influence of environmental heat stress and glucose ingestion on metabolism and exercise performance.
4. I agree to volunteer as a subject for the study described in the information sheet. I give my full consent to my participation in this study.
5. This consent is specific to the particular experiment described in the information sheet attached, and shall not be taken to imply my consent to participate in any subsequent experiment or deviation from that detailed here.
6. I reserve the right to withdraw from this experiment at any time; I also understand that I may be withdrawn at any time, and will suffer no penalty as a result.

Project Officer: Mr J L J Bilzon Independent Medical Officer: Dr E H N Oakley

Signed	_____	Witnessed	_____
Name	_____	Name	_____
Date	_____	Date	_____

APPENDIX 3

PRE-PARTICIPATION HEALTH QUESTIONNAIRE FOR SUBJECTS PARTICIPATING IN EXERCISE AND/OR THERMAL STRESS TESTS

- | | | | |
|----|---|-------|----|
| 1. | Do you have a family history of heart disease or early death? | YES | NO |
| 2. | Are both of your parents still alive? | YES | NO |
| 3. | If either are dead, what age did they die? | ----- | |
| 4. | Do/did your parents, brothers or sisters suffer from asthma or wheezing in the chest? | YES | NO |
| 5. | Do you or have you ever suffered from: | | |
| | Chest pain | YES | NO |
| | Breathlessness on exertion | YES | NO |
| | Dizziness on exertion | YES | NO |
| | Collapse when exercising | YES | NO |
| | Palpitations | YES | NO |
| | Asthma or wheezing | YES | NO |
| | Heat illness | YES | NO |
| | Anaemia | YES | NO |

If yes to any of the above, please give details:

6. Have you ever been admitted to hospital? YES NO
If yes, please give details:
7. Do you take any medication regularly to treat any condition? YES NO
If yes, please give details:
8. Do you undertake regular exercise? YES NO
If yes, please give details:
9. If a serving member of the Armed Forces, is your current medical category P2, fit for full duties? YES NO
If no, please detail your category and any restrictions:
10. Do you have any known allergies? YES NO
If yes, please give details:

Signed _____ Name _____ Date _____