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**The Influence of Nutrition on Recovery From Prolonged,
Constant Pace Running**

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A Doctoral Thesis

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Summary

Whilst the metabolic responses to submaximal exercise are relatively well documented, little information is available relating to recovery and further exercise performance. Thus, the principal aim of this research was to investigate the influence of nutrition on recovery from prolonged, constant pace running. ← intro

In the first study (Chapter 4), the influence of increased carbohydrate intake on endurance capacity was investigated following a bout of prolonged, constant pace running and a 22.5-h recovery. Sixteen male subjects were divided into two matched groups, which were randomly assigned to either a control (CON) or a carbohydrate (CHO) condition. Both groups ran at 70% $\dot{V}O_2$ max on a level treadmill for 90 min, or until volitional fatigue, whichever came first (R_1). Subjects ran at the same % $\dot{V}O_2$ max for as long as possible 22.5-h later, as an assessment of endurance capacity (R_2). During the recovery, the carbohydrate intake of the CHO group was increased from 5.8 (± 0.5) to 8.8 (± 0.1) g·kg⁻¹body wt (mean \pm SE). An isocaloric diet was prescribed for the CON group, providing additional energy in the form of dietary fat and protein. Run times for R_1 did not differ between the groups. However, R_2 run time of the CON group was reduced by 15.6 min ($p < 0.05$), whilst the CHO group matched their R_1 performance (CON - 70.7 (± 7.2) min; CHO - 91.9 (± 9.0) min). Thus, a high carbohydrate diet restored endurance capacity within 22.5-h, whereas an isocaloric diet without additional carbohydrate did not result in the same restoration of exercise capacity. ← ?

Exercise-induced dehydration impinges upon both exercise capacity and the capacity of the body to recover. The second study (Chapter 5), investigated the influence of water ingestion on endurance capacity during constant pace running. Four men and four women completed two randomly assigned treadmill runs at 70% $\dot{V}O_2$ max to volitional fatigue. During one trial, no fluid was ingested during exercise (NF). Whereas, during the fluid replacement (FR) trial a single water bolus equivalent to 3.0 ml·kg⁻¹body wt was provided pre-exercise, followed by serial feedings equivalent to 2.0 ml·kg⁻¹body wt·15 min⁻¹ during exercise. Run time during the NF-trial was 77.7 (± 7.7) min, compared to 103.0 (± 12.4) min during the FR-trial ($p < 0.01$). Fluid ingestion during exercise was associated with increased endurance capacity, whilst the NF-trial was accompanied by enhanced carbohydrate oxidation and suppressed fat oxidation. Thus, fluid ingestion during exercise plays a role in improving performance, and may also hold benefits for optimising post-exercise recovery. X

The third study (Chapter 6), investigated the influence of ingesting either water or a carbohydrate-electrolyte solution during post-exercise recovery, on performance 4-h later. Twelve men and four women were divided into two matched groups, which were randomly assigned to either a control (P) or a carbohydrate (CHO) trial. Both groups ran at 70% $\dot{V}O_2$ max on a level treadmill for 90 min, or until volitional fatigue, whichever came first (R_1). Four hours later, subjects ran at the same % $\dot{V}O_2$ max for as long as possible (R_2). The ← !!

CHO group ingested $1.0 \text{ g} \cdot \text{CHO} \cdot \text{kg}^{-1} \cdot \text{body wt}$ (6.9% solution) immediately after R_1 , and 2-h later. Whilst the P group ingested equal volumes of a placebo solution. Run times during R_1 did not differ between the groups, whereas the CHO group ran $22.2 (\pm 3.5)$ min longer than the P group during R_2 ($p < 0.05$). Blood glucose was equally well maintained throughout exercise in both groups. Blood lactate was elevated in the CHO group at the start of R_2 ($p < 0.05$). Whilst plasma FFA and glycerol concentrations were elevated in the P group ($p < 0.01$). Neither group restored pre- R_1 body weight during the recovery. Percentage rehydration was $65.9 (\pm 6.3)\%$ in the P group and $62.6 (\pm 7.3)\%$ in the CHO group (NS). Thus, ingestion of a carbohydrate solution during 4-h recovery from prolonged, constant pace running facilitated rehydration as effectively as water, whilst endurance capacity was enhanced during subsequent exercise.

The fourth study (Chapter 7), investigated the influence of increasing carbohydrate intake from 1.0 (D-trial) to 3.0 (C-trial) $\text{g} \cdot \text{CHO} \cdot \text{kg}^{-1} \cdot \text{body wt} \cdot 2 \text{ h}^{-1}$ during 4-h recovery on subsequent exercise performance. Nine men and eight women completed two trials in a counter-balanced design. Each trial consisted of a 90 min run at $70\% \dot{V}O_{2\text{max}}$ (R_1), followed by a 4-h recovery (REC), and a further exhaustive run at $70\% \dot{V}O_{2\text{max}}$ (R_2). Two feedings were prescribed during REC as either a 6.9% glucose-polymer (GP) solution (D-trial), or as a 19.3% GP solution (C-trial). Mean R_2 run times were $58.5 (\pm 5.2)$ min and $57.6 (\pm 6.3)$ min for the D and C trials respectively (NS). There were no differences in R_2 run times of the male and female subjects. Blood glucose was equally well maintained over R_1 in both trials. Whilst more stable concentrations were maintained during REC in the C-trial, remaining elevated in comparison with the D-trial after 210 min ($p < 0.01$). This was despite higher plasma insulin concentrations in the C-trial prior to R_2 (C- $22.21 \pm 4.17 \text{ mU} \cdot \text{l}^{-1}$; D- $5.42 \pm 0.85 \text{ mU} \cdot \text{l}^{-1}$) ($p < 0.01$). There was evidence of enhanced carbohydrate oxidation and suppressed fat oxidation during REC and R_2 in the C-trial. In conclusion, excessive carbohydrate ingestion does not further improve the recovery process, or endurance capacity 4-h later. It was speculated from indirect evidence that one rate-limiting step in the repletion of cellular carbohydrate stores is the conversion of glucose to glycogen. Enhanced oxidation appears to be a major avenue of free glucose disposal aside from incorporation into muscle and liver glycogen, with elevated lipogenesis initially playing a relatively minor role.

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JLF

Publications

Unless otherwise indicated by acknowledgements or references to published literature, the work contained herein is that of the author. The findings presented in this thesis have been reported, in part, in the following publications.

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*To Mum and Dad
with love and gratitude*

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Introduction

'There's an almost primitive belief that there's something, somewhere, that you can eat in order to perform better - that there's just got to be some secret.'

- Peter Jokl, MD (1976)

(Cited: Coleman, 1988)

Sports performers are constantly looking for that special something that will make them different from the rest, that will raise them above the rest. As the body is honed and finely tuned, careful preparation and attention to detail will maximise any training advantage. For at the highest level, where performers are on a physical and technical par, the difference between a winner and a loser is less than the blink of an eye.

Over the last twenty years, the importance of nutrition in this process of fine tuning has become increasingly evident.

'Diet significantly influences athletic performance. An adequate diet, in terms of quantity and quality, before, during and after training and competition will maximise performance.'

- Devlin and Williams (Eds) Proceedings of an
IOC International Scientific Consensus,
4-6 February 1991, Lausanne, Switzerland.

Despite a general acceptance of the importance of appropriate nutrition for health and well-being, as well as for performance, the concept of 'optimal nutrition' remains a confusing and contradictory issue (Wootton, 1988). In a quest for that 'winning edge', athletes are susceptible to commercially driven fadism on the one hand, or folk lore and myth perpetuated through the traditions of their sport on the other. Both serve to confound the principals underlying a well balanced and healthy diet.

The positive effects of appropriate nutrition is no more evident than during the recovery from prolonged, constant pace running. At a time when endogenous fuel reserves are relatively low, the benefits of providing exogenous fuel become rapidly apparent. Exercise represents a

severe challenge to metabolism. Fatigue ensues during repeated bouts of muscular activity when energy metabolism fails to rise to this challenge. However, the precise cause of a subsequent decline in performance still remains elusive (Green, 1991). The recovery process is concerned with re-instating a normal physiological balance, such that further activity is made possible. Redressing this balance following exercise takes place in a hierarchical order, with disturbances representing the greatest threat to normal physiological functioning being alleviated first. ←

The ability to sustain prolonged, constant pace running is limited by several factors. The main limitation is the availability of an appropriate energy supply (Ahlborg, Bergstrom, Brohult, Ekelund, Hultman and Maschio, 1967a). Adenosine triphosphate (ATP) is the only fuel for muscular contractile activity (Newsholme and Leech, 1983). The body regenerates ATP through various metabolic pathways. These pathways are fuelled during submaximal exercise by a combination of carbohydrates and fats. Protein may also contribute to the provision of energy (Felig and Wahren, 1975). However, it could be argued that the primary metabolic role of protein is the anaplerotic replacement of pathway intermediates during increased oxidative phosphorylation, rather than fuel provision per se (Green, 1991). ←

The re-introduction of the needle biopsy technique in the early 1960's (Bergstrom, 1962), allowed this inter-play between the different fuels for energy metabolism to be examined at a muscle tissue level. Endogenous carbohydrate reserves were found to be finite, and represented only a fraction of the potential energy store of fat. As a consequence, a reduction in the body's reserves compromised carbohydrate availability during exercise, and contributed to the onset of fatigue (Ahlborg, Bergstrom, Ekelund and Hultman, 1967b; Bergstrom and Hultman, 1966b; Hermansen, Hultman and Saltin, 1967; Hultman, 1967). Thus, maintaining adequate carbohydrate reserves is an important factor in ensuring successful endurance performances (Hultman, 1967; Karlsson and Saltin, 1971). Similarly, ← replenishing these reserves following exercise is essential if the capacity to perform endurance exercise is to be restored.

The early work of Christensen and Hansen (1939)¹ firmly established a link between a high carbohydrate diet and improvements in the capacity to perform endurance exercise. Various feeding strategies have subsequently been found to influence the physiological availability of carbohydrates during exercise (Coyle, 1991). Exogenous supplies of carbohydrate have been provided both before and during exercise, as well as combinations thereof. It is suggested that the onset of fatigue may be delayed through such feeding strategies by decreasing the utilisation rate of limited endogenous reserves (Coyle and Coggan, 1984). Moreover, there is evidence of muscle glycogen resynthesis taking place during low intensity exercise when an adequate carbohydrate availability is maintained (Constable, Young, Higuchi and Holloszy, 1984). In addition, it might be argued that decreasing disturbances in the body's energy balance during activity may hold some benefit for enhancing post-exercise recovery.

As mentioned previously, the recovery process is concerned with reinstating a normal physiological balance to allow further activity. There has been considerable recent interest in the replenishment of carbohydrate stores post-exercise through dietary manipulation. The return of liver and muscle glycogen reserves to normal levels following exercise is determined by a number of inter-related factors. Such factors include: the extent of prior depletion; the timing of carbohydrate ingestion; the amount of carbohydrate per feeding (ie. the rate of ingestion); the type of carbohydrate consumed; and, the form in which the carbohydrate is administered (Blom, 1989a). The recovery process is further influenced by the prevalence of muscle damage, which may be incurred during a prior bout of exercise (O'Reilly, Warhol, Fielding, Frontera, Meredith and Evans, 1987; Sherman, Costill, Fink, Hagerman, Armstrong and Murray, 1983). This can retard the recovery process as a consequence of the inherent energy demands of tissue repair (Costill, Pascoe, Fink, Robergs, Barr and Pearson, 1990), as well as through an associated reduction in the efficiency of carbohydrate storage (Kirwan, Hickner, Yarasheski, Kohrt and Wiethop, 1992; Lash, Sherman and Bloomfield, 1987). However, few studies investigating this repletion of endogenous carbohydrate reserves have progressed to examine whether exercise capacity is also restored. Furthermore, the majority of work has been based upon cycling as the mode of exercise, with a paucity of studies examining the responses to constant pace treadmill

¹ Cited Astrand (1967)

running. This has resulted in much of the current sports nutrition advice being based upon cycling research, as opposed to studies investigating the more fundamental whole body activity of running.

Thus, the principal aim of the present thesis was to investigate if increasing carbohydrate provision during the immediate post-exercise period returns an individual to an optimum state of fitness, and enables previous running performances to be reproduced. Implicit in this aim was a desire to bridge what remains a rather grey area between accepted theory and its practical application. An experimental procedure was used for quantitatively assessing recovery. This procedure involved a standardised endurance run (ie. 90 min constant pace run at 70% $\dot{V}O_2\text{max}$) followed by a prescribed recovery and a further run to exhaustion (ie. open-ended constant pace run at 70% $\dot{V}O_2\text{max}$). The return of endurance capacity, and hence the efficacy of a prescribed recovery, was reflected in terms of run time to fatigue during the second exercise bout. This procedure provided a reliable measure of recovery from prolonged, constant pace running.

1.1 Thesis overview

This thesis is sub-divided and presented in an order that progresses from a consideration of recovery on a day-to-day basis, to the more immediate concerns for optimising short-term recovery. The Review of Literature (Chapter 2) initially addresses the relationship between energy metabolism and constant pace running. Possible causes of fatigue are identified, where a failure of thermoregulatory processes may play a significant role. A link between diet and exercise performance is established, before the review concentrates on our current understanding of the main physiological adjustments with respect to recovery during the immediate post-exercise period. These adjustments, in association with appropriate dietary manipulation, result in the replenishment of endogenous carbohydrate reserves, and hence, a possible restoration of functional capacity.

The first study (Chapter 4) examined the influence of increasing dietary carbohydrate intake on functional capacity after a recovery period of 22.5-h. A need to recover from intense daily training

sessions is a very real problem for dedicated sports performers. Increasing carbohydrate intake from ~ 4.0 to ~ 8.0 $\text{g}\cdot\text{kg}^{-1}\text{body wt}\cdot 24\text{h}^{-1}$ is reported to restore muscle glycogen levels to normal resting values (Costill, Sherman, Fink, Maresh, Witten and Miller, 1981). Thus, the aim of this study was to assess whether functional capacity can similarly be restored over this period of recovery.

Dehydration represents a further limitation to prolonged exercise (Armstrong, Costill and Fink, 1985), influencing both cardiovascular activity and temperature regulation (Costill and Sparks, 1973). Peripheral circulation is restricted as the body attempts to maintain central blood pressure in the face of a declining blood volume (Fortney, Wenger, Bove and Nadel, 1983). A compensatory decrease in the rate of sweating further compromises heat dissipation and results in a rapid increase in core temperature (Gisolfi and Copping, 1974; Sawka, Young, Francesconi, Muza and Pandolf, 1985). Fluid ingestion during exercise can attenuate this rise in body temperature (Costill, Kammer and Fisher, 1970).

As well as adversely affecting exercise capacity, dehydration may also impinge upon the body's recovery capacity. This may not necessarily be evident in impaired replenishment of muscle fuel reserves (Neufer, Sawka, Young, Quigley, Latzka and Levine, 1991). Rather, this may be a more insidious effect with whole-body implications. As such, implementing strategies to maintain fluid balance during an exercise bout may offer additional benefits over the immediate post-exercise period.

The second study (Chapter 5) examined whether fluid provision during constant pace running can limit dehydration. Performance benefits of drinking water throughout constant pace running were assessed in terms of exercise time to fatigue. Post-exercise physiological responses were determined in order to establish the nature and extent of metabolic disturbances arising from a bout of prolonged running. These disturbances must then be promptly redressed if a rapid recovery is to be ensured.

Energy and fluid deficits may be alleviated during a short recovery period through ingestion of carbohydrate-electrolyte (CE) beverages (Carter and Gisolfi, 1989). Thus, the immediate needs for both fluid and carbohydrate may be accommodated.

The third study (Chapter 6) examined the influence of ingesting a carbohydrate-rich drink on functional capacity after a recovery period of 4-h. Ingestion of a CE beverage during exercise has been observed to enhance performance (Maughan, Fenn and Leiper, 1989; Tsintzas, Liu, Williams, Campbell and Gaitanos, 1993a), whilst reducing the effects of dehydration (Carter and Gisolfi, 1989). However, it was still to be determined whether ingestion of such beverages during short-term post-exercise recovery could similarly promote rehydration and carbohydrate availability, such that functional capacity would be restored during a later exercise bout. As such, this study represented the practical application of dietary recommendations for optimising short-term recovery (Blom, Hostmark, Vaage, Kardel and Mæhlum, 1987; Ivy, Lee, Brozinick and Reed, 1988b; Brouns, 1991a; Gisolfi and Duchman, 1992; Maughan, 1991). Such strategies would be of particular interest to sports performers involved in repeated bouts of intense activity on the same day.

An adequate carbohydrate intake is essential for maintaining an optimal rate of glycogen resynthesis throughout the recovery period (Ahlborg et al, 1967a; Bergstrom, Hermansen, Hultman and Saltin, 1967). Fasting or delaying carbohydrate ingestion following exercise restricts this process (Mæhlum and Hermansen, 1978; Ivy, Katz, Cutler, Sherman and Coyle, 1988a), whereas increasing post-exercise carbohydrate intake to $1.5 \text{ g}\cdot\text{kg}^{-1}\text{body wt}\cdot 2\text{h}^{-1}$ enhances muscle glycogen synthesis (Blom et al, 1987b; Ivy et al, 1988b). Further increases do not appear to yield any additional benefit at a muscle tissue level (Blom et al, 1987b; Ivy et al, 1988b). The third study examined the functional advantage of ingesting $1.0 \text{ g}\cdot\text{kg}^{-1}\text{body wt}\cdot 2\text{h}^{-1}$ of carbohydrate during 4-h recovery from prolonged running. However, the fate of carbohydrate ingested in excess of this recommended amount still remained unclear.

The fourth study (Chapter 7) was designed to expand on the findings of previous time-course studies (see Blom et al, 1987b; Ivy et al, 1988b; Piehl, 1974) by examining the influence of increasing carbohydrate intake during 4-h recovery on metabolism and subsequent constant pace running performance. Providing a high carbohydrate feeding following exercise will optimise both muscle and blood reserves. It was further speculated that excess carbohydrate may be stored in the liver, and as such could provide additional fuel during a later exercise bout. It was hypothesised that this might be reflected in a performance study by a more favourable energy balance during subsequent exercise, and a delaying of fatigue.

Finally, the general discussion (Chapter 8) draws together the findings of the studies reported in this thesis and those reported in the literature. The question is then addressed as to what are the limitations of the recovery process? For despite the provision of adequate dietary carbohydrate during the immediate post-exercise period, individuals are generally unable to reproduce their previous performance.

Review of Literature

2.1 Overview

The ability to sustain prolonged exercise is limited by several factors. One such limitation is exercise-induced dehydration (Armstrong et al, 1985), which adversely effects cardiovascular functioning and temperature regulation (Costill and Sparks, 1973). Another limitation is carbohydrate availability (Ahlborg et al, 1967a). Prolonged exercise at 60 to 80% of maximal oxygen uptake ($\dot{V}O_{2max}$) reduces the carbohydrate reserves of the body, and contributes to the onset of fatigue (Ahlborg et al, 1967b; Bergstrom and Hultman, 1966b; Hermansen et al, 1967; Hultman, 1967).

This thesis examines if the provision of adequate carbohydrate and fluid during the immediate post-exercise period facilitates the recovery process, such that an individual is returned to a normal state of fitness. This will be referred to as the 'functional capacity' of an individual, as reflected by an ability to reproduced their previous running performance. The reason for examining this question is the lack of previous research linking the replenishment of endogenous carbohydrate reserves and the restoration of endurance running capacity. Furthermore, a current understanding of recovery, whilst focussing upon redressing an energy imbalance, does not satisfactorily examine the possible limitations of this process.

The review initially considers energy metabolism during constant pace running. Thermoregulatory factors play an influential role where failure to effectively regulate temperature, primarily as a consequence of exercise-induced dehydration, may be associated with the onset of fatigue. Further causes of fatigue which may limit physical activity are examined. The links between diet and exercise performance are discussed, where optimum feeding strategies implemented before and during exercise may offer some benefit in alleviating the symptoms of fatigue.

The review then considers the main physiological adjustments during the immediate post-exercise period. These adjustments, in conjunction with appropriate dietary manipulation, will result in the replenishment of endogenous carbohydrate reserves. Current understanding would suggest that this is but one factor associated with the restoration of functional capacity.

2.2 Energy metabolism during constant pace running

Carbohydrates and fats are the primary fuels for prolonged exercise. Their degradation through anaerobic and aerobic metabolism yields adenosine triphosphate (ATP), which is the immediate energy source of muscular contractile activity. The carbohydrate stores of the body in muscle, liver and blood amount to less than 2000 kcal (8 MJ), whereas fat stores exceed 100 000 kcal (420 MJ) or 80-85% of body fuel. Structural protein theoretically represents 15-20% of body fuel (Felig and Wahren, 1975). Thus, carbohydrate stores limit endurance capacity, and need to be optimised for successful endurance performances (Sherman and Costill, 1984).

The relative utilisation of carbohydrate and fat is primarily influenced by the nature and intensity of activity (Costill, Sparks, Gregor and Turner, 1971b; Costill, Gollnick, Jansson, Saltin and Stein, 1973b; Romijn, Coyle, Sidossis, Gastaldelli, Horowitz, Endert and Wolfe, 1993a). In addition, diet, training status and health have parts to play (Costill and Miller, 1980; Essen, 1977; Gollnick, 1985). Christensen and Hansen (1939)² examined the contribution of carbohydrate and fat to energy metabolism during exercise of varying intensities. This was assessed from respiratory exchange ratio (R) values estimated from pulmonary ventilation. Subjects on a normal diet engaged in aerobic activity obtained 50 to 60% of their energy from fat. As exercise duration was extended to 3-h, R-values decreased as dependency upon fat increased. On the other hand, elevated R-values during heavy exercise were consistent with an increased dependency on carbohydrate. Hultman (1967) similarly observed a decrease in R-values during prolonged activity, where low-intensity exercise was associated with a greater reduction. This was true up to a critical load equivalent to

² Cited Astrand and Rodahl (1986)

~75% $\dot{V}O_2$ max, beyond which R values remained constant or increased. The provision of oxygen to active muscle at high relative exercise intensities becomes increasingly inadequate to satisfy the rate of energy demand (Hultman and Sjoholm, 1983). This necessitates enhanced anaerobic metabolism of high energy phosphates, muscle glycogen and cellular free glucose, whilst the aerobic contribution from fat metabolism declines (Saltin and Karlsson, 1971; Walker, Mickle, Tanner, Harding and Romaschin, 1984). This gives rise to an exponential relationship between exercise intensity and the rate of muscle glycogen utilisation (Ahlborg et al, 1967a).

2.2.1 Metabolism of carbohydrate

Glycogen depots in muscle and liver are the main carbohydrate stores in the body, with blood glucose providing a small transient reserve. As well as a high potassium content, glycogen has a high water content being deposited in a one part glycogen to three parts water ratio (Fenn, 1939; Olsson and Saltin, 1970). Liberation of this bound water under conditions of thermal distress may assist in the maintenance of blood volume (Plyley, Costill and Fink, 1980). Granules of glycogen also contain the enzymes phosphorylase and glycogen synthase, which are respectively involved in the degradation and synthesis of glycogen.

The three carbohydrate pools are in a continuous state of flux, operating in concert to meet the energy demands of active tissue. The relative importance of each pool with respect to limiting endurance capacity depends upon work load and previous diet (Pruet, 1970a). For simplicity, each pool will be discussed independently as well as considering their combined activities. This discussion will introduce 'secondary' carbohydrate sources, which also play important roles in energy metabolism during constant pace running. For example, incomplete glucose degradation produces lactate, which is either metabolised directly or is converted back to glucose via gluconeogenesis. Other gluconeogenic precursors which may also be regarded as secondary sources of glucose include pyru-

vate, glycerol, and the branched-chain amino acids (BCAA) leucine, isoleucine and valine (Wahren, 1977).

Muscle glycogen provides an immediate fuel for energy production via glycolysis and oxidative phosphorylation (Reichard, Issekutz, Kimbel, Putnam, Hochella and Weinhouse, 1961). Individuals on a mixed diet will normally have muscle glycogen concentrations of $\sim 80 \text{ mmol}\cdot\text{kg}^{-1}\text{wet wt}$ (Hultman and Sjöholm, 1983). Assuming an average total muscle mass of $\sim 25\text{-}30 \text{ kg}$, this would allow $\sim 350\text{g}$ of carbohydrate to be stored as muscle glycogen (Essen, 1977). The absolute content varies with diet and exercise, and an underlying diurnal variation may also exist (Conlee, Rennie and Winder, 1976), though this has not consistently been demonstrated in humans (Hultman, Bergstrom and Roch-Norlund, 1971). As will be discussed later, a further $\sim 90\text{g}$ of carbohydrate is stored in the liver (Hultman and Nilsson, 1971). Thus, Newsholme (1983) estimated that total body carbohydrate stores are of the order of 440g .

Mobilisation of muscle glycogen during prolonged, constant pace running is tri-phasic: an initial fast phase of rapid glycogenolysis (15-20 min) is accompanied by a relatively high level of lactate production; this is followed by a steady state period in which mobilisation remains stable; finally, low glycogen levels over the latter stages of exercise result in slower rates of mobilisation (Bergstrom and Hultman, 1967; Hultman, 1967). The high demand for energy at the onset of exercise coincides with a period of limited cellular glucose transport (Jansson and Kaijser, 1982a; Reichard et al, 1961). Blood borne glucose supply and cellular transport are enhanced as exercise continues (Felig and Wahren, 1975), whereas muscle glycogen utilisation invariably plateaus and starts to decrease. A point is reached during prolonged exercise when the total rate of carbohydrate supply is insufficient to meet the rate of energy demand. Muscle content is low and cellular glucose transport becomes limiting. At this point the rate of energy demand, and therefore the intensity of work, must be reduced if exercise is to continue (Davies and Thompson, 1979).

Local muscle glycogen stores influence the relative contribution of carbohydrate and fat to energy metabolism (Costill, Bowers, Branam and Sparks, 1971a; Gollnick, Pernow, Essen, Jansson and Saltin, 1981). Elevating pre-exercise muscle glycogen stores allows carbohydrate metabolism to be maintained at a relatively high level (Bosch, Dennis and Noakes, 1993). In contrast, Costill et al (1971a) observed a compensatory increase in lipid metabolism in response to reduced carbohydrate availability during three successive days of running 16.1 km at 80% $\dot{V}O_2$ max. Muscle low in glycogen extracts more lactate and glucose from blood, and oxidises fat to a greater extent than normally stocked muscle (Gollnick et al, 1981).

Plasma catecholamine levels influence glycogen availability (Banister and Griffiths, 1972). Adrenaline stimulates muscle glycogenolysis through interaction with 3',5' cyclic adenosine monophosphate (cAMP) (Jansson, Hjendahl and Kaijser, 1986; Richter, Galbo and Christensen, 1981). Adenyl cyclase activation is increased by cAMP, which in turn stimulates phosphorylase activity by converting the 'less active' b-form to the 'more active' a-form (Chasiotis, Sahlin and Hultman, 1983; Drummond, Harwood and Powell, 1969). Contractile activity also stimulates glycogenolysis (Kjær, Secher, Bach, Sheikh and Galbo, 1989; Richter et al, 1981), with calcium ions activating phosphorylase kinase to trigger the dephosphorylation of phosphorylase-b (Drummond et al, 1969; Entman, Keslensky, Chu and Van Winkle, 1980). Thus, muscle glycogen mobilisation is jointly regulated by adrenaline and muscular contraction (Richter, Ruderman, Gavras, Belur and Galbo, 1982b), though the latter represents a more transient stimulus having its greatest effect over the initial stages (Conlee, McLane, Rennie, Winder and Holloszy, 1979). The relative importance of adrenaline and contractile activity in stimulating muscle glycogenolysis depends upon exercise intensity and duration, muscle fibre recruitment, the adequacy of blood flow (Richter et al, 1982b), and local glycogen levels (Constable, Favier and Holloszy, 1986). The influence of both of these mechanisms is blunted following exhaustive exercise (Constable et al, 1986).

The initial 5-10 min of submaximal exercise is associated with a burst of glycogenolysis (Bergstrom, Guarnieri and Hultman, 1971). This is accompanied by a rapid increase in muscle lactate concentration (Rowell, Kraning, Evans, Kennedy, Blackmon and Kusumi, 1966), which reflects an imbalance between glycolysis and the subsequent oxidation of pyruvate (Wahren, 1977). There appears to be a maximal rate at which lactate is released from muscle equivalent to $\sim 5.0 \text{ mmol}\cdot\text{min}^{-1}$ (Jorfeldt, Juhlin-Dannfelt and Karlsson, 1978).

Blood lactate concentrations correlate with the rate of lactate disappearance, indicating that a 'mass action effect' is operating in this process (Issekutz, Shaw and Issekutz, 1976). Trace amounts of lactate are lost in sweat and urine, but the major routes of removal are via oxidation in cardiac and non-active muscle, gluconeogenesis in the liver and kidneys, and possibly glyconeogenesis in skeletal muscle (Bangsbo, Gollnick, Graham and Saltin, 1991; Issekutz et al, 1976; Rowell et al, 1966). An increasing proportion of lactate production during prolonged, constant pace running will result from glycogenolysis in non-active muscle, possibly in response to elevated plasma adrenaline concentrations (Ahlborg and Felig, 1982; Ahlborg, 1985).

Blood glucose remains relatively stable during prolonged running. Studies using the continuous isotope infusion technique have demonstrated that blood glucose is actively metabolised, and that a powerful homeostatic mechanism operates to maintain systemic concentrations (Issekutz, Issekutz and Nash, 1970; Issekutz and Vranic, 1980; Vranic, Kawamori, Pek, Kovacevic and Wrenshall, 1976). In addition, extracellular glucose temporarily buffers imbalances in demand and supply (Coggan, 1991). The brain is a major consumer of blood borne glucose under resting conditions (Reichard et al, 1961; Wahren, 1977). Cerebral glucose utilisation as a proportion of total glucose turnover decreases during exercise though absolute levels remain unchanged (Ahlborg and Wahren, 1972; Wahren, Felig, Ahlborg and Jorfeldt, 1971), whilst peripheral uptake increases (Keul, Doll and Keppler, 1967; Romijn et al, 1993a). Rates of

plasma glucose appearance (Ra) and disappearance (Rd) increase within 10 min of the onset of exercise, to accommodate an increasing metabolic role (Miles, Finegood, Lickley and Vranic, 1992).

Muscle glucose uptake may be enhanced twenty-fold (Galbo, Kjaer, Mikines, Christensen, Tronier, Sonne, Hilsted and Richter, 1983) through an insulin-independent transport process, which is influenced by muscle fibre glycogen content (Hespel and Richter, 1990; Ploug, Galbo and Richter, 1984; Wallberg-Henriksson and Holloszy, 1984). Once in the muscle cell, 5-10% of free glucose is converted to lactate. Aerobic oxidation of free glucose covers an increasing fraction (28-37%) of the total cellular energy requirement, and an increasing fraction (75-90%) of the carbohydrate component of this requirement (Wahren et al, 1971). Cellular transport peaks after 90 min of moderate leg exercise and remains stable, before declining as Ra falls (Wahren, 1977). This transport mechanism will be discussed in Section 2.8.1.

The primary role of the liver is in the homeostatic control of blood glucose. There is an underlying diurnal variation to liver glycogen content which is modulated by activity levels and diet (Fuller and Diller, 1970; Hultman and Nilsson, 1971; Sollberger, 1964). Starvation or a low-carbohydrate diet reduces this energy store even under resting conditions (Hultman and Nilsson, 1971; Nilsson and Hultman, 1973). At rest, the release of lipid from the splanchnic bed represents less than 5% of the body's total energy expenditure, triglyceride release may contribute 2-5%, and ketone body release 1-3% (Rowell, Masoro and Spencer, 1965). In contrast, glucose release may account for 20%, increasing six-fold during heavy exercise despite reduced splanchnic blood flow (Ahlborg and Felig, 1982; Hultman, 1967; Rowell, 1971; Wahren et al, 1971).

The rapid increase and decrease in hepatic glucose production at the onset and cessation of exercise, suggests that factors intimately associated with motor activity in the central nervous

system (CNS) are involved in the control pathway (Sonne and Galbo, 1985). The sympathetic nervous system may also play a part, with noradrenaline acting as a potent gluco-regulator (Hoelzer, Dalsky, Schwartz, Clutter, Shah, Holloszy and Cryer, 1986). Mobilisation of hepatic glucose appears to be a primary (ie. feed-forward) event, rather than a secondary (ie. feed-back) event in response to increased muscular demand (Kjær, Secher, Bach and Galbo, 1987; Vissing, Sonne and Galbo, 1988). Thus, blood glucose concentrations are not accurately balanced on a moment-to-moment basis (Kjær, Farrel, Christensen and Galbo, 1986), such that hyperglycaemia *per se* does not consistently inhibit further glucose production (Muller, Acheson, Burger and Jequier, 1990).

Initially, ~75% of augmented hepatic glucose production results from glycogenolysis, with gluconeogenesis accounting for ~25% (Wahren, 1977). As exercise continues, the contribution of gluconeogenesis may increase three-fold to cover ~40% of an elevated total glucose production. Both active (pyruvate, alanine, glycerol and BCAA), and non-active (lactate) muscle provide gluconeogenic substrates (Bonen, McDermott and Hutber, 1989). Fractional extraction of these precursors from blood is enhanced during prolonged exercise (Ahlborg, Felig, Hagenfeldt, Hendler and Wahren, 1974; Holm, Bjorntorp and Jagenburg, 1978; Rowell et al, 1966).

Elevated glycogenolysis and gluconeogenesis may be due to increased glucagon secretion in the face of decreasing circulatory insulin concentrations (Felig and Wahren, 1979; Issekutz and Vranic, 1980; Pruett, 1971; Wasserman, Spalding, Brooks Lacy, Colburn, Goldstein and Cherrington, 1989). A decrease in blood glucose concentration directly stimulates glucagon secretion (Richter et al, 1981). Nevertheless, an exercise-induced increase in plasma glucagon in association with increases in blood glucose and plasma alanine may also be evident (Felig, Wahren, Hendler and Ahlborg, 1972; Muller, Faloona and Unger, 1971). Hyperalaninaemia appears to play a direct role in the regulation of glucagon secretion (Felig et al, 1972), whereas

increases in adrenaline and noradrenaline play indirect roles via insulin suppression (Porte, 1967; Porte and Williams, 1966; Richter et al, 1981). When insulin concentrations are low, glucose-sensitive cells in the CNS and the pancreas respond to small changes in systemic glucose levels (Galbo, Holst, Christensen and Hilsted, 1976). Thus, increasing plasma glucagon concentrations parallel a decline in blood glucose levels. This results in a glucagon-to-insulin ratio favouring glucose production and lipid mobilisation (Galbo et al, 1976; Miles et al, 1992). Moates, Brooks Lacy, Goldstein, Cherrington and Wasserman (1988) suggest that glucagon and insulin are the primary regulators during the early stages, whereas adrenaline plays a critical role over the later stages of prolonged exercise.

Dohm and Newsholme (1983) suggest that glucagon, noradrenaline and adrenaline influence gluconeogenesis by increasing liver cAMP content. Increased gluconeogenic flux is mediated through enhanced activation of pyruvate carboxylase, fructose 1,6-bisphosphatase and glucose 6-phosphatase, and reduced activation of pyruvate kinase and phosphofructokinase (PFK). Weber, Hird-Convery, Lea and Stamm (1966) observed a rapid gluconeogenic effect of elevated FFA concentrations. This was partly explained by an inhibitory effect of FFA on the glycolytic enzymes glucokinase, hexokinase, PFK and pyruvate kinase. Thus, the rate limiting reactions of glycolysis are inhibited as the 'by-passing' reactions of gluconeogenesis are enhanced.

Gluconeogenesis cannot supply carbohydrate at a rate commensurate with peripheral demand, such that blood glucose progressively declines as endogenous carbohydrate stores are reduced (Ahlborg and Felig, 1982). Hypoglycaemia *per se* may cause exercise cessation in rats (Arogyasami, Sellers, Wilson, Jones, Duan and Winder, 1992), but is not thought to be a major limiting factor in humans (Felig, Cherif, Minagawa and Wahren, 1982), and is rarely observed (Wahren, 1977). A fall in blood glucose necessitates a reversion to a primary dependency upon muscle glycogen, which in turn accelerates the onset of fatigue (Costill, 1988).

2.2.2 Metabolism of fat

It has been mentioned previously that carbohydrates are not the only source of energy. Indeed, fat is an important fuel for prolonged, constant pace running. Fatty acids are the energy molecules of fat (Fritz, Davis, Holtrop and Dundee, 1958). They are stored as triglycerides, where three fatty acid carbon chains form uncharged esters with glycerol (Stryer, 1988). Intramuscular stores vary with fibre type; type-I fibres, with a high aerobic activity, contain 2-3 times the triglyceride stores of glycolytic type-II fibres (Essen, Jansson, Henriksson, Taylor and Saltin, 1975). Triglycerides are mainly stored in the cytoplasm of adipocytes, where cellular organelles are displaced in order to maximise storage capacity. These fat cells are congregated into adipose tissue, which is deposited at various functional sites around the body. A temporary store in blood in the form of triglycerides, lipoproteins and chylomicrons provides a third storage site of fat (Bjorntorp, 1991).

Mobilisation of fat is initiated by sympathetic nervous activity, and is mediated via the action of lipoprotein lipase (Oscai, Essig and Palmer, 1990). Transport of FFA into the muscle cell from plasma is thought to be regulated by 'mass action' (Armstrong, Steele, Altszuler, Dunn, Bishop and DeBodo, 1961). Thus, changes in FFA mobilisation determine both systemic FFA concentrations and cellular uptake (Havel, Pernow and Jones, 1967; Costill, Fink, Getchell, Ivy, Witzmann, 1979). Mobilisation is dependent upon relative exercise intensity (Romijn et al, 1993a), as increasing blood lactate concentrations associated with intense activity exert an inhibitory effect (Boyd, Giamber, Mager and Lebovitz, 1974; Issekutz, Shaw and Issekutz, 1975; Pruett, 1970b). Other factors such as plasma adrenaline and growth hormone concentrations are also implicated (Klein, Holland and Wolfe, 1990; Pruett, 1970b; Savard, Despres, Marcotte, Theriault, Tremblay and Bouchard, 1987).

Havel, Carlson, Ekelund and Holmgren (1964) examined FFA turnover during 2-h of moderate cycling. Oxidation of plasma

FFA accounted for ~50% of exercise lipid metabolism. Intramuscular triglyceride stores are believed to supply fatty acids directly to muscle fibres to account for the remaining ~50% (Havel et al, 1967). Increases in plasma glycerol concentration closely follow changes in plasma FFA but are greater in magnitude (Havel et al, 1964; Wolfe, Klein, Carraro and Weber, 1990). The liberation of glycerol from triglycerides provides gluconeogenic precursors for carbohydrate synthesis (Paul and Holmes, 1975). A similar pattern of FFA and glycerol mobilisation was observed following 60 min of treadmill running at 70% $\dot{V}O_2$ max (Costill et al, 1979). Moreover, Romijn, Klein, Coyle, Sidossis and Wolfe (1993b) concluded that strenuous endurance training has a potentiating effect on fatty acid oxidation through enhancing basal FFA and glycerol turnover rates during exercise. Thus, triglyceride-fatty acid cycling plays an instrumental role in the rapid response of fatty acid metabolism in meeting the increased energy demands of submaximal exercise (Wolfe et al, 1990).

Elevating plasma FFA concentrations by feeding corn oil and subcutaneously injecting heparin spared muscle and liver glycogen during treadmill running in rats (Hickson, Rennie, Conlee, Winder and Holloszy, 1977; Rennie, Winder and Holloszy, 1976). This effect was limited to type I and IIa fibres and was accompanied by elevated citrate levels and a blunted glucagon response. Citrate inhibits PFK activity and results in glucose-6-phosphate (G-6-P) accumulation (Garland, Randle and Newsholme, 1963; Garland and Randle, 1964; Randle, Newsholme and Garland, 1964), which in turn inhibits hexokinase to reduce glucose uptake (Garland, Newsholme and Randle, 1964).

Ferrannini, Barrett, Bevilacqua and DeFronzo (1983) demonstrated a similar effect in man. Elevated plasma FFA levels inhibited insulin-stimulated glucose metabolism through competition with blood glucose for cellular uptake. Costill, Coyle, Dalsky, Evans, Fink, and Hoopes (1977) increased plasma FFA concentrations in humans with a high-fat meal and

heparin infusion. Subjects then performed a 30 min treadmill run at 68% $\dot{V}O_2$ max. Increasing FFA availability reduced skeletal muscle carbohydrate utilisation in comparison with a carbohydrate-fed condition. Thus, there is evidence of a glucose-fatty acid cycle (Randle, Garland, Hales and News-holme, 1963) operating in rat and human skeletal muscle (Costill et al, 1977; Ferrannini et al, 1983; Hickson et al, 1977; Rennie et al, 1976; Rennie and Holloszy, 1977).

Hargreaves, Kiens and Richter (1991) suggest that a sparing of endogenous carbohydrate may be mediated through a direct action of lipid metabolites on the cellular glucose transporter mechanism, as opposed to the classical glucose-fatty acid cycle theory. One hour of dynamic knee extensions were performed at 80% of knee-extensor maximum work capacity. The energy requirement was equally shared between carbohydrate and fat metabolism. Increasing arterial FFA concentrations by Intra-lipid infusion did not spare muscle glycogen, but cellular glucose uptake at rest and during work was decreased. This reduced transport was not associated with G-6-P accumulation or increased leg citrate release, as would be consistent with the classical theory. The possibility that this divergence from earlier investigations may be due to differences in experimental methodology and design cannot be excluded.

2.2.3 Metabolism of amino acids

Amino acids are the building blocks of protein, and outside of their functional roles, they are not stored as a fuel for exercise. The actual contribution of protein is estimated to be ~4-10% of the body's energy expenditure during prolonged exercise (Dohm, Williams, Kasperek and Van Rij, 1982), this value being influenced by carbohydrate availability (Brouns, Beckers, Wagenmakers and Saris, 1990; Wagenmakers, Beckers, Brouns, Kuipers, Soeters, Van Der Vusse and Saris, 1991).

The majority of amino acids in the body are incorporated into muscle tissue (Brodan, Kuhn, Pechar and Tomkova, 1976).

Absolute muscle content is stable under resting conditions, though levels of individual amino acids are in a state of flux (Cahill, 1971; Dohm, Tapscott and Kasperek, 1987). Exercise increases amino acid metabolism, such that tissue demand will exceed the available supply provided by a circulatory 'free pool' (Dohm, Kasperek, Tapscott and Barakat, 1985). Thus, amino acid availability is reduced over the early stages of prolonged exercise (Haralambie and Berg, 1976), necessitating an increase in protein turnover (Lemon and Mullin, 1980; Lemon and Nagle, 1981).

Contractile activity accelerates amino acid cycling, possibly through a direct action of calcium ions on protein degradation (Lundholme, Edstrom, Ekman, Karlberg, Walker and Schersten, 1981). This exercise effect on protein turnover is well documented (Haralmbie and Berg, 1976; Refsum, Gjessing and Stromme, 1979; Refsum and Stromme, 1974; Rennie, Edwards, Krywawych, Davies, Halliday, Waterlow and Millward, 1981; White and Brooks, 1981). Amino acid availability is enhanced through increased protein degradation and decreased protein synthesis (Dohm, Beecher, Warren and Williams, 1981; Rennie et al, 1981).

Muscle amino acid cycling during exercise is characterised by a greater uptake of circulating amino acids, and a simultaneous efflux of notably alanine and glutamine (Ahlborg et al, 1974; Bergstrom, Furst and Hultman, 1985; Felig and Wahren, 1971; Felig, Pozefsky, Marliss and Cahill, 1970). Enhanced BCAA metabolism appears to be the major event in elevated protein turnover during exercise (Kasperek and Snider, 1987). The liver is the main source of BCAA, and muscle tissue is the principal site of oxidation (Ahlborg et al, 1974; Felig and Wahren, 1975). Decreased plasma insulin, (and possibly testosterone), and increased plasma glucagon (Ahlborg et al, 1974), catecholamine and cortisol (Galbo, Richter, Hilsted, Holst and Christensen, 1977b) levels enhance BCAA mobilisation. Elevated BCAA concentrations inhibit oxidation of pyruvate (Chang and Goldberg, 1978c), resulting in increased muscle

efflux of lactate and pyruvate (Ahlborg et al, 1974). This liberation of gluconeogenic precursors may indirectly contribute to a 'carbohydrate sparing effect'. Thus, hepatic BCAA provide an alternative source of carbon skeletons for respiration in muscle tissue (Odessey, Khairallah and Goldberg, 1974). This pathway between the liver and muscle forms one arm of the 'glucose-alanine cycle' (Felig et al, 1970; Felig and Wahren, 1971).

Alanine and glutamine account for less than 15% of muscle contractile protein, so their increased efflux during exercise is believed to result from *de novo* synthesis (Babij, Mathews, Wolman, Halliday, Millward, Mathews and Rennie, 1983b; Odessey et al, 1974). This process involves transamination of the amino acids aspartate, asparagine, arginine, proline, and the BCAA (Chang and Goldberg, 1978a; Odessey et al, 1974). Aminotransferase enzymes of the cytosol catalyse transfer of the amino group to α -ketoglutarate (Goldberg and Chang, 1978), to yield α -keto acids, glutamate, and ammonia.

The α -keto acids are either oxidised in the mitochondria or are released into the systemic circulation. The rate-limiting step in BCAA metabolism is the decarboxylation of α -keto acids by branched chain α -keto acid dehydrogenase (Stryer, 1988). This enzyme complex is largely inactive in resting muscle (Wagenmakers, Brookes, Coakley, Reilly and Edwards, 1989b), being activated by contractile activity (Kasperek and Snider, 1987; Wagenmakers et al, 1989b) and increases in α -keto acids and insulin (Odessey et al, 1974). This activation appears to be maximal during prolonged exercise when glycogen levels are low (Decombaz, Reinhardt, Anantharaman, Von Glutz and Poortmans, 1979). Whereas, low BCAA levels reduce α -keto acid availability and inhibit activation. Amino acid oxidation is speculated to have a negative feedback effect upon exercise capacity, limiting activity levels in order to prevent permanent tissue damage (Wagenmakers et al, 1989b).

The fates of glutamate are four-fold. First, it may donate amino groups to pyruvate under the action of alanine aminotransferase to yield alanine (Chang and Goldberg, 1978a; Felig and Wahren, 1975). Second, amidation of glutamate through glutamine synthase yields glutamine (Bergstrom et al, 1985; Chang and Goldberg, 1978b), whilst also facilitating the safe removal of ammonia from the muscle cell (Banister, Allen, Mekjavic, Singh, Legge and Mutch, 1983). Though notably, release of glutamine into the general circulation represents a substrate drain from the glucose-alanine cycle (Goldberg and Chang, 1978; Wagenmakers, Coakley and Edwards, 1990). A third fate of glutamate is transamination to aspartate, providing an important urea cycle precursor (Babij et al, 1983b). Aspartate may combine with citrulline to form argininosuccinate, to provide an avenue for substrate re-entry into the TCA cycle (Stryer, 1988). Finally, recycling of glutamate within the muscle cell through glutamate dehydrogenase activity maintains α -ketoglutarate availability for both glucose-alanine and TCA cycling.

Alanine is transported in the blood primarily to the liver (Felig et al, 1970; Haralambie and Berg, 1976). Subsequent deamination liberates carbon skeletons for gluconeogenesis (Favier, Koubi, Mayet, Sempore, Simi and Flandrois, 1987), whilst the amino groups enter the urea cycle (Stryer, 1988). Alanine production is linearly correlated with glucose utilisation (Felig and Wahren, 1971), though the two processes are functionally independent. The major fraction of circulatory glutamine is extracted by the kidneys (Babij et al, 1983b). The ammonia fraction is removed and excreted from the body in urine (Van Slyke, Phillips, Hamilton, Archibald, Fitcher and Hiller, 1943), whilst the remaining carbon skeleton is either oxidised or directed into gluconeogenesis (Goldberg and Chang, 1978).

Rates of both alanine and glutamine synthesis are governed by the availability of amino acid precursors (Chang and Golberg, 1978a), whilst their relative levels of production appear to depend upon ambient ammonia concentrations (Goldberg and

Chang, 1978). Plasma ammonia levels reflect exercise intensity and duration (Babij, Mathews and Rennie, 1983a), such that concentrations increase during the course of prolonged exercise (Broberg and Sahlin, 1988; Eriksson, Broberg, Bjorkman and Wahren, 1985; Graham, Pedersen and Saltin, 1987). Ammonia is produced through amino acid catabolism, as well as during AMP deamination via the purine nucleotide cycle (PNC) (Lowenstein, 1972). Usually associated with high intensity exercise and recruitment of type-II muscle fibres (Dudley, Staron, Murray, Hagerman and Luginbuhl, 1983; Meyer, Dudley and Terjung, 1980; Meyer and Terjung, 1979), PNC activity is also enhanced over the later stages of prolonged exercise (Broberg and Sahlin, 1989; MacLean, Spriet, Hultman and Graham, 1991). As glycogen becomes low in type-I fibres, type-II fibres play an increasing role in force generation (Gollnick, Armstrong, Saubert, Sembrowich, Shepherd and Saltin, 1973). Operation of the PNC plays an indirect regulatory role in glycolysis, as changes in AMP and ammonia levels influence PFK activity (Buono, Clancy and Cook, 1984; Goodman and Lowenstein, 1977; Tornheim and Lowenstein, 1975).

Increased BCAA metabolism provides substrates for anaplerotic reactions, whereby reserves of pathway intermediates are replenished (Lee and Davis, 1979; Wagenmakers et al, 1990). In addition to aspartate and glutamate recycling, enhanced oxidation of valine and isoleucine supplies succinyl Co-A to the TCA cycle (Kasperek and Snider, 1987). However, acceleration of BCAA oxidation reduces TCA cycle flux and impedes aerobic oxidation of glucose and FFA (Wagenmakers et al, 1990). Thus, complex physiological inter-relationships exist between the metabolic degradation of BCAA, FFA and carbohydrates.

2.3 Fluid balance and thermoregulation

A prerequisite of energy metabolism during constant pace running is an optimal milieu in which physiological processes may take place. This is not only dependent upon energy availability, but is also influenced by pH, fluid balance and temperature regulation. The human

body is ~20% efficient, such that a relatively high proportion of energy generated during exercise is in the form of heat (Harrison, 1986). Failure to dissipate heat will result in a rapid increase in body temperature (Nadel, 1977). However, core temperature rarely rises above 40°C during prolonged running (Pugh, Corbett and Johnson, 1967). This is mainly due to a transfer of heat from the principle sites of production (ie. working muscle) to the site of elimination (ie. skin) via the vascular system (Gisolfi and Wenger, 1984).

On reaching the cutaneous circulation, metabolic heat is removed through conduction, convection and radiation, as well as evaporation of sweat from the skin surface and water from respiratory surfaces (Gisolfi and Wenger, 1984). Changes in sweating rate are linearly related to increases in deep body temperature (Nielsen and Nielsen, 1962), such that evaporative heat loss represents the dominant cooling mechanism (Gisolfi and Wenger, 1984). As a consequence, temperature regulation during constant pace running will be influenced by body fluid balance, which in turn is jointly determined by fluid and electrolyte levels (Nielsen, 1974; Senay, 1979).

The sweating response represents an avenue of high fluid loss during submaximal steady state exercise. In comparison, respiratory fluid losses amount to ~2.0-5.0 ml·min⁻¹ (Mitchell, Nadel and Stolwijk, 1972), which is equivalent to ~0.12-0.30 l·h⁻¹. Whereas, in excess of 1.0 l·h⁻¹ is lost through sweating (Carter and Gisolfi, 1989; Costill et al, 1970). A movement of vascular fluid into sweat glands and subsequently out of the body reduces blood volume and elevates plasma osmolality (Sawka, 1992). This movement ultimately compromises both temperature regulation and cardiovascular functioning (Fortney, Nadel, Wenger and Bove, 1981a). Thus, competition for a limited fluid supply between the metabolic demands of active tissue and a whole body need to dissipate heat accompanies prolonged exercise (Costill, 1972).

This competition is inherently debilitating, such that dehydration limits aerobic work capacity (Astrand and Saltin, 1964; Kozlowski and Saltin, 1964). Fluid loss equivalent to a ~2.0% decrease in body weight results in impaired submaximal exercise performance (Armstrong et

al, 1985; Craig and Cummings, 1966). For each 1.0% decrease in body weight there is an increase in core temperature of between 0.1 to 0.4°C (Ekblom, Greenleaf, Greenleaf and Hermansen, 1970; Greenleaf and Castle, 1971; Sawka et al, 1985). A reduction in body weight of 1.0% is also associated with a ~2.4% decrease in plasma water and a ~1.2% decrease in muscle water (Costill, Cote and Fink, 1976). Resultant increases in serum osmolality, specifically through changes in magnesium, sodium and potassium levels, may be involved in a reduced sweating response (Costill et al, 1976; Greenleaf and Castle, 1971; Nielsen, 1974). These changes may operate locally by decreasing the sensitivity of sweat glands to neural innervation or by interfering with the glandular secretory mechanism (Nielsen, 1974). Alternatively, increased osmolality may operate centrally through an effect upon hypothalamic thermoregulatory centres (Ekblom et al, 1970; Greenleaf and Castle, 1971). As such, deep body temperature will continue to increase in the face of reduced SBF (Horstman and Horvarth, 1972), and declining sweat gland stimulation (Ekblom et al, 1970).

Saltin and Stenberg (1964) observed that stroke volume (V_s) decreased ~15% during 3-h cycling or running (75% $\dot{V}O_2$ max), in which body weight was reduced by ~4%. With only a small compensatory increase in cardiac output (Q), mean arterial blood pressure decreased by ~10%. Thus, exercise-induced dehydration resulted in a decreased V_s and increased heart rate (HR), such that Q remained relatively constant. The magnitude of cardiovascular adjustments correlate with the degree of hydratory distress, which in turn is reflected in body weight and blood volume reductions (Saltin, 1964b).

A negative fluid balance depresses Q due to a reduction in V_s and an inadequate increase in HR (Fortney et al, 1983; Nadel, Fortney and Wenger, 1980). Decreasing arterial blood pressure stimulates peripheral vasoconstriction (ie. baroreceptor reflex) such that SBF is reduced in order to maintain cardiac filling pressure and deep muscle blood flow (Fortney et al, 1983; Nadel, Fortney and Wenger, 1980). Decreases in Q and SBF are proportional to absolute changes in blood volume (Fortney et al, 1981a; Montain and Coyle, 1992b). Blood volume in turn influences the sweating response, independent of plasma osmolality modulation (Fortney, Nadel, Wenger and Bove,

1981b). Both hypovolaemia and hyperosmolarity delay the onset of sweating by elevating the internal threshold temperature for cutaneous vasodilation (Fortney, Wenger, Bove and Nadel, 1984; Nadel et al, 1980). This in turn decreases core-to-skin heat transfer and increases core temperature. Fluid replacement during exercise attenuates hyperthermia largely by maintaining SBF (Montain and Coyle, 1992a). This is achieved through a lowering of serum osmolality, especially serum sodium concentration, rather than through increases in blood volume (Fortney et al, 1981b; Montain and Coyle, 1992a; Sawka, Hubbard, Francesconi and Horstman, 1983). Nevertheless, small adjustments to blood volume may improve cardiovascular functioning (Fortney et al, 1981a; Montain and Coyle, 1992a).

Fortney, Vroman, Beckett, Permutt and LaFrance (1988) suggest that the greatest change in plasma volume occurs during the initial 6-10 min of exercise. This early change contributing to an elevated HR primarily reflects postural adjustments and transient vasodilatory responses associated with the rest-to-exercise transition (Gore, Scroop, Marker and Catcheside, 1992; Harrison, 1985). Gore et al (1992) suggest that there is a lower limit to plasma volume reductions, with further decreases in total body water being drawn from the extra-vascular space. A later 'secondary rise' in HR, or cardiovascular drift, may be due to a progressive decrease in cardiac filling (Nielsen, Sjogaard and Bond-Petersen, 1984). This is partly due to thermoregulatory factors, and partly due to enhanced sympathetic nervous activity (Hamilton, Gonzalez-Alonso, Montain and Coyle, 1991; Kalis, Freund, Joyner, Jilka, Nittolo and Wilmore, 1988; Nose, Mack, Shi, Morimoto and Nadel, 1990).

Mobilisation of endogenous energy reserves elevates muscle solute content. A resultant change in osmotic forces draws in extracellular fluid to increase muscle water content (Nielsen, Sjogaard, Ugelvig, Knudsen and Dohlmain, 1986). This in turn will elevate osmolality in the extracellular and vascular compartments. Increases in core temperature during exercise are observed to correlate with changes in plasma osmolality (Greenleaf, Kozlowski, Nazar, Kaciuba-Uscilko, Brzezinska and Ziemba, 1976; Harrison, Edwards and Fennessy, 1978). However, elevated plasma osmolality is not always associated with

decreases in vascular volume (Montain and Coyle, 1992b), such that increasing initial plasma volume does not consistently prevent an upward drift in exercise HR (Nielsen et al, 1986). Changes specifically in sodium ion concentrations appears to represent an important non-thermal input in temperature regulation (Greenleaf et al, 1976), providing an index of osmotic pressure which reflects total body water volume rather than transient fluctuations in compartmental water volumes. Osmotic pressure in turn provides an index of fluid balance, which may act synergistically with thermoreceptor inputs in the regulation of deep body temperature.

Nose, Morita, Yawata and Morimoto (1986) examined fluid intake and changes in blood volume during recovery from thermal dehydration. Water and 0.45% NaCl. solution were provided ad libitum during a 6-h rehydration period. Ingestion of water inhibited the stimulus to drink, (ie. reduced dipsogenic drive), whereby a rapid decrease in circulatory osmolality superceded blood volume regulation. Plasma hyposmolality blunts the thirst stimulus in order to prevent further reductions in osmolality and ensuing cellular hyperhydration. Recovery of ~80% blood volume appears to inactivate the volume-dependent thirst mechanism. A continuing involuntary dehydration, or a delayed rehydration, results from a rapid decrease in dipsogenic drive (Nose, Mack, Shi and Nadel, 1988b). A transient rise in plasma volume following exercise is due to a rapid efflux of water from muscle (Nielsen et al, 1986). This is in response to electrolyte deficits from intra- and extracellular spaces (Nose, Mack, Shi and Nadel, 1988a), which artificially suppress the thirst drive. However, subsequent fluid and osmotic equilibration between body compartments unmasks a fluid deficit and evokes a desire to drink. Thus, total body water balance is determined by the proportional distribution of fluid and electrolytes between fluid compartments (Nose et al, 1988a; Nose et al, 1988b).

As discussed previously, peripheral displacement of central blood volume during submaximal exercise reduces V_s (MacDougall, Reddan, Layton and Dempsey, 1974). The total oxygen cost of exercise gradually increases due to the greater metabolic cost of, amongst other concerns, maintaining a stable core temperature (Hamilton et al,

1991). Hamilton et al (1991) speculated that impaired cardiovascular functioning may reduce muscle cell phosphorylative efficiency. Fink, Costill and Van Handel (1975) observed that muscle blood flow was compromised during exercise in the heat, resulting in a decrease in oxygen supply and an increase in muscle glycogenolysis. Nadel, Fortney and Wenger, (1980) suggest that decreasing cardiovascular efficiency is compensated for by either an increase in tissue oxygen extraction or enhanced anaerobic metabolism, or both, in an attempt to maintain the required energy flux. Elevated lactic acid production during a 30 min dehydrating exercise bout was regarded as symptomatic of accelerated anaerobic glycolysis (Nadel et al, 1980).

Exercise hyperthermia has been associated with lactate accumulation and a more rapid rate of glycogen depletion, which might ultimately contribute to localised muscle fatigue (Kozlowski, Brzezinska, Kruk, Kaciuba-Uscilko, Greenleaf and Nazar, 1985). However, Nielsen, Savard, Richter, Hargreaves and Saltin (1990) observed that compromised muscle blood flow, and altered cellular metabolism, did not limit performance during uphill walking in the heat. Leg blood flow, femoral arteriovenous oxygen difference, (and hence oxygen extraction), glycogen utilization, muscle uptake of glucose and FFA, and production of lactic acid, did not differ between exercise in a cool environment and exercise in a hot environment. In contrast with previous arguments, Nielsen et al (1990) suggest that rather than a peripheral effect limiting exercise performance, elevated core temperature may operate centrally to reduce motor drive. Though notably, the 'cool' condition was experienced over the initial 30 min of exercise and the 'hot' condition over the latter 60 min of exercise. Section 2.2 discussed the processes underlying a shift in energy metabolism during prolonged exercise, where the early phase is associated with a greater dependency upon carbohydrate metabolism, whilst the later phase is associated with a greater dependency upon fat metabolism. A high environmental temperature may have inhibited this normal exercise-dependent shift in energy metabolism. Thus, the evidence provided by Nielsen et al (1990) does not conclusively negate the possibility of a temperature effect on metabolism in skeletal muscle.

Cardiovascular integrity takes precedence over temperature regulation when thermo-hydratory conditions exceed the control of normal homeostatic mechanisms (Nadel, Cafarelli, Roberts and Wenger, 1979). During exercise of increasing intensity in the heat, Q and V_s are maintained at the expense of a regulated deep body temperature. The threat to venous return is three-fold: hydrostatic and osmotic pressures result in a filtration of fluid out of the vascular volume; thermoregulatory increases in SBF increase cutaneous venous volume; and, evaporative water losses through sweating and respiration progressively decrease total body fluid. The most important factor contributing to reduced cardiac filling pressure is the pooling of blood in peripheral veins, which becomes more significant than plasma volume losses (Fortney et al, 1983).

In contrast with submaximal exercise performance, dehydration does not appear to reduce the ability of muscle to maximally contract (Craig and Cummings, 1966; Saltin, 1964a). Nerve impulse propagation and the responsiveness of muscle fibres is not impaired by dehydration (Saltin, 1964a). Costill et al (1976) observed that water and electrolyte losses following exercise-induced dehydration did not alter muscle cell membrane excitability, even though total maximal work time and cumulative blood lactate concentrations were reduced (Saltin, 1964a; Saltin, 1964b). This suggests that a dehydratory limitation to performance lies within the muscle cell (Kozlowski and Saltin, 1964; Saltin, 1964a; Saltin, 1964b).

2.4 Causes of fatigue during constant pace running

Prolonged muscular activity is inevitably accompanied by fatigue and a progressive decline in performance (Green, 1991). There does not appear to be a simple definitive cause underpinning this decline, rather a whole spectrum of events occur which differentially combine to limit exercise capacity (Roberts and Smith, 1989). The interaction of these events is determined by characteristics defining the nature of activity. However, a point is reached within this matrix of metabolic and non-metabolic processes where one or more factors become incapacitating (Edwards, 1983).

As mentioned previously, the ability to sustain prolonged exercise is principally limited by fluid balance (Armstrong et al, 1985), and carbohydrate availability (Ahlborg et al, 1967a). The former, which was addressed in Section 2.3, exerts a relatively global influence over physiological processes. In contrast, the latter may arise more discretely, such that exercise cessation is associated with local decreases in muscle glycogen. Though notably, absolute glycogen concentrations never reach zero (Conlee, 1987; Tsintzas, 1993). Low muscle glycogen levels ultimately result in an 'energy deficit' at the adenine nucleotide level, with an inability to regenerate ATP at the required rate (Sahlin, 1992). Reduced carbohydrate supplies limit energy production via glycolysis, whilst a subsequent reduction in the supply of TCA cycle precursors will impair oxidative phosphorylation.

Muscle fibre glycogen depletion is selective, reflecting the nature and intensity of exercise (Costill et al, 1973b; Costill, Jansson, Gollnick and Saltin, 1974; Gollnick et al, 1973; Gollnick, Piehl and Saltin, 1974; Tsintzas, 1993; Vollestad and Blom, 1985). This is partially due to the higher oxidative potential of type-I fibres, and the higher glycolytic potential of type-II fibres (Essen et al, 1975; Gollnick, Armstrong, Saubert, Piehl and Saltin, 1972a; Greenhaff, Ren, Soderlund and Hultman, 1991). Baldwin, Reitman, Terjung, Winder and Holloszy (1973) examined substrate utilisation of different fibres in running rats. Glycogen depletion patterns suggested a minimal recruitment of type-IIb (fast twitch, glycolytic) fibres, with type-I (slow twitch, oxidative) and intermediary type-IIa (fast twitch, oxidative-glycolytic) fibres performing most of the work. A substantial recruitment of type-IIb fibres was only apparent over the later stages of exercise as type-I and IIa fibres became fatigued.

A similar pattern occurs in humans during 60 min of cycling at 50-60% $\dot{V}O_2$ max (Essen, 1978). Resting muscle glycogen concentrations were greater in type-IIa and IIb fibres, than type-I fibres. Continuous exercise resulted in a greater glycogen reduction in type-I fibres, whilst intense intermittent exercise resulted in similar reductions in all fibre types. Vollestad, Vaage and Hermansen (1984) undertook histochemical examinations of muscle glycogen depletion patterns during both exhaustive cycling and running at 75% $\dot{V}O_2$ max. Type I and IIa

fibres were recruited at the start of exercise. Type IIb and intermediate IIab fibres were not recruited until 20 min of exercise, or later. This resulted in sequential reductions of carbohydrate reserves in different muscle fibre groups. Costill et al (1971b) report that absolute decreases in muscle glycogen concentration during running at $\sim 80\%$ $\dot{V}O_2\text{max}$ were less than during cycling of a similar intensity, with substantial glycogen reserves remaining in muscle following an exhaustive run. Costill et al (1971b) concluded that running permits longer periods of intense, submaximal work in comparison with cycling, and that fatigue is less localised. This may be a contributory factor in the less exact perception of exhaustion during prolonged running (Sherman and Costill, 1984). However, the findings of Costill et al (1971b) may have reflected inherent differences in sampling site and muscle fibre recruitment with respect to the activities undertaken. Thus, there is a need for caution when generalising from studies involving different species and different exercise modes.

More recently, single fibres from the m.vastus lateralis were analysed before and after exhaustive running at 70% $\dot{V}O_2\text{max}$ (Tsintzas, 1993). Glycogen concentrations in type-I fibres decreased from $317.0 (\pm 34.2)$ to $31.6 (\pm 10.3)$ $\text{mmol}\cdot\text{kg}^{-1}\text{dry wt}$, and in type-II fibres from $443.4 (\pm 44.9)$ to $103.9 (\pm 29.2)$ $\text{mmol}\cdot\text{kg}^{-1}\text{dry wt}$. Tsintzas (1993) concluded that compromised carbohydrate availability specifically in type-I fibres is associated with fatigue during prolonged, constant pace running.

Despite a reduction in muscle glycogen levels during constant pace running, blood glucose and cellular ATP levels remain approximately constant (Broberg and Sahlin, 1989; Norman, Sollevi, Kaijser and Jansson, 1987). Notwithstanding the possibility that changes in ATP concentrations too subtle to detect may influence functional capacity, this would suggest that further physiological mechanisms are operating to precipitate fatigue which results in exercise cessation.

Low blood glucose levels may not necessarily exert their greatest effect upon peripheral processes. Reduced systemic carbohydrate availability may result in a decline in central drive from higher motor centres, or indirectly influences circulatory concentrations of neurotransmitters (Green, 1991). A decrease in muscle contractile activity

would accompany any reduction in α -motoneuron output. However, changes in catecholaminergic and serotonergic transmitter levels have not consistently been associated with low carbohydrate availability (Green 1991). Furthermore, restoration of normal blood glucose levels neither alleviates central symptoms of fatigue, nor results in a return of exercise capacity (Bergstrom and Hultman, 1967).

Elevated plasma FFA levels are associated with impaired glucose tolerance by impinging upon the sensitivity of tissue to insulin (Paul and Holmes, 1975; Schalch and Kipnis, 1965). Furthermore, plasma FFA may directly reduce cellular glucose uptake, at a time when muscle glycogen concentrations are increasingly limited (Rennie and Holloszy, 1977). Oxidation of FFA gives rise to citrate, which inhibits PFK activity and results in G-6-P accumulation (Randle et al, 1963). Hexokinase is in turn inhibited by G-6-P such that cellular glucose transport is compromised, further limiting carbohydrate availability in the muscle fibre.

Exercise is accompanied by an increase in BCAA metabolism (Kasperek and Snider, 1987). Elevated muscle BCAA concentrations may impair pyruvate oxidation (Chang and Goldberg, 1978c), such that pyruvate and lactate efflux from muscle is increased (Ahlborg et al, 1974). Thus, high BCAA cycling may reduce TCA cycle flux and limit oxidation of glucose and FFA (Wagenmakers et al, 1990). As discussed previously, this negative feedback effect of amino acid metabolism on exercise capacity may provide a safety mechanism to limit physical activity in order to prevent permanent tissue damage (Wagenmakers et al, 1989b).

Changes in muscle BCAA metabolism may also influence tryptophan metabolism in the brain (Newsholme, Blomstrand, Hassmen and Ekblom, 1991). Tryptophan is a precursor of the brain neurotransmitter 5-hydroxytryptamine (5-HT), (also known as serotonin), which is involved in the control of sleep, food intake, mood, pain sensitivity and pituitary hormone release (Newsholme and Leech, 1983). Tryptophan is transported bound to albumin, but is displaced by increasing plasma concentrations of FFA and large neutral amino acids such as glutamine (Curzon, Friedel and Knott, 1973; Newsholme and

Leech, 1983). Enhanced competition for albumin binding sites during prolonged exercise results in elevated plasma free tryptophan concentrations, and it is in this 'free' form that tryptophan is carried across the blood brain barrier. The same carrier molecule is involved in the transport of BCAA (Newsholme and Leech, 1983). Increased muscle BCAA utilisation reduces plasma BCAA concentrations (Decombaz et al, 1979). Thus, decreased competition from BCAA will enhance the transport of tryptophan into the brain. Subsequent hydroxylation of tryptophan results in 5-HT synthesis (Newsholme and Leech, 1983). Elevated 5-HT levels in specific areas of the brain are speculated as playing a role in the onset of fatigue (Newsholme et al, 1991; Parry-Billings, Blomstrand, McAndrew and Newsholme, 1990).

Mobilisation of muscle and liver glycogen is associated with a cellular efflux of potassium ions (Hultman, 1967; Sjogaard, 1986; Sjogaard, 1989), resulting in increased concentrations in plasma (Bergstrom and Hultman, 1966b; Laurell and Pernow, 1966). A decrease in muscle cell potassium has been implicated in reduced contractility, as processes involved in normal excitation-contraction coupling are disturbed (Sjogaard, 1990). Whether this disturbance is of sufficient magnitude to compromise muscle function during voluntary activity remains a matter for debate (Ahlborg et al, 1967b; Vollestad and Sejersted, 1988). Changes in intracellular potassium levels may disrupt membrane integrity of the sarcoplasmic-reticulum, which would effect calcium ion transport (Vollestad and Sejersted, 1988). Low muscle glycogen concentrations have been linked with decreases in calcium re-uptake and diminished calcium ATPase activity (Byrd, Bode and Klug, 1989). Thus, muscle relaxation would become retarded, resulting in a persistent state of semi-rigor. Carbohydrate ingestion may indirectly buffer potassium ions through potassium incorporation into glycogen granules (Hultman, 1967).

2.5 Diet and exercise performance

A close association between diet and performance was evident in the early work of Christensen and Hansen (1939)³. Subjects on high-carbohydrate diets had an improved capacity for prolonged work,

³ Cited Astrand (1967)

whereas consuming fat-protein diets low in carbohydrate reduced exercise tolerance. Fat-rich, carbohydrate-deficient diets are associated with increased lipid oxidation and decreased carbohydrate oxidation during submaximal cycling and constant pace running (Gollnick, Piehl, Saubert, Armstrong and Saltin, 1972b; Galbo, Holst and Christensen, 1979; Jansson and Kaijser, 1982a; Jansson and Kaijser, 1982b). This is reflected in elevated plasma concentrations of FFA, glycerol and β -hydroxybutyrate, whilst glucose and lactate concentrations in plasma and α -glycerophosphate in muscle are reduced (Jansson and Kaijser, 1982a). This shift in energy metabolism is partly mediated through substrate inhibition of carbohydrate metabolism (Costill et al, 1977; Ferrannini et al, 1983; Rennie and Holloszy, 1977), and partly through changes in hormonal secretions (Galbo, Christensen and Holst, 1977a; Galbo et al, 1979; Jansson, Hjendahl and Kaijser, 1982). The net result is a reduction in both muscle glycogenolysis and hepatic glucose output (Jansson and Kaijser, 1982b). In contrast, a high-carbohydrate diet elicits a contra-shift in energy metabolism, whereby plasma FFA and glycerol concentrations are suppressed whilst blood lactate is elevated (Kelman, Maughan and Williams, 1975).

The re-introduction of the needle biopsy technique in the 1960's allowed closer examination of the mechanisms underlying shifts in substrate utilisation (Bergstrom, 1962). Consuming a fat-protein diet following exhaustive exercise resulted in slower rates of muscle glycogen resynthesis, whereas a high carbohydrate diet was associated with a rapid rate of resynthesis (Ahlborg et al, 1967a). Bergstrom et al (1967) varied muscle glycogen concentrations through a combination of exercise and dietary manipulation. Prescribed diets were ingested for 72-h prior to exhaustive cycling at 75% $\dot{V}O_2$ max. A normal mixed-diet resulted in pre-exercise glycogen concentrations of 97 mmol \cdot kg⁻¹wet wt in the m.vastus lateralis and an exercise time to exhaustion of 114 min. A fat-protein diet resulted in muscle glycogen concentrations of 35 mmol \cdot kg⁻¹wet wt and an exercise time of 57 min. Whilst a high-carbohydrate diet resulted in 184 mmol \cdot kg⁻¹wet wt of glycogen and an exercise time of 167 min. Thus, local muscle glycogen stores appear to be a primary determinant of endurance capacity (Ahlborg et al, 1967b), and these stores may be increased through

dietary manipulation (Bergstrom et al, 1967; Hultman and Bergstrom, 1967).

Karlsson and Saltin (1971) demonstrated the practical implications of this relationship between diet and exercise performance during a 30 km running race. Elevating pre-exercise muscle glycogen concentrations through diet and exercise (Astrand, 1967), improved the ability of runners to maintain an optimal running speed. Williams, Brewer and Walker (1992) supplemented the normal diet of runners with additional carbohydrate during 7-d recovery from a 30 km treadmill time trial. When subjects repeated the time trial, a faster pace was maintained over the last 5 km compared to their first trial performance. A similar improvement was not evident in a control group prescribed an isocaloric diet in which additional energy was provided in the form of fat and protein.

Sherman, Costill, Fink and Miller (1981) elevated muscle glycogen levels through a regimen of increased dietary carbohydrate intake and training taper. A strenuous bout of depletory exercise as prescribed in Astrand's (1967) classical 7-d 'super-compensating' regimen was not performed. This has been shown to be unnecessary for eliciting a carbohydrate loading effect (Blom, Costill and Vollestad, 1987a). However, there is some evidence that muscle glycogen *per se* is not the only factor determining exercise capacity. Despite elevating muscle glycogen concentrations, endurance capacity in trained runners was not improved (Madsen, Pedersen, Rose and Richter, 1990). Madsen et al (1990) were unable to offer a satisfactory explanation for exercise cessation in carbohydrate 'super-compensated' highly trained endurance runners after 77 (\pm 13) min at 75-80% $\dot{V}O_2$ max. Blood glucose concentrations were maintained during exercise and blood lactate concentrations were not exceptionally high, whilst substantial stores of glycogen remained in muscle at the end of exercise. A high estimated rate of liver glycogen utilisation may have compromised carbohydrate availability late in exercise, though this was not evident from the maintenance of blood glucose homeostasis. Madsen et al (1990) speculated that an imbalance between intra- and extra-cellular potassium concentrations may have been associated with the onset of fatigue (Sjogaard, 1986), whilst interference of excitation-contraction

coupling might also have played a part (Belcastro, Maclean and Gilchrist, 1985), though data in support of such contentions was not provided.

2.5.1 Gastric emptying and intestinal absorption of carbohydrates

The efficacy of dietary manipulation primarily depends upon the rate at which food is made available to active tissue. This is determined in the first instance by the transit time of ingesta along the gastro-intestinal (G-I) tract. Whilst the rate of intestinal absorption represents a second determinant factor. A single 300 ml feeding of a 10.6% carbohydrate solution may appear as serum glucose ~5-7 min following ingestion (Costill, Bennet, Branam and Eddy, 1973a). This serves to illustrate the relative rapidity of gastric emptying and intestinal absorption, which is still apparent even under the duress of exercise.

Gastric motor activity is controlled by a combination of neural and humoral feed-back mechanisms (Murray, 1987). Receptors located in the walls of the duodenum and jejunum are sensitive to changes in volume, energy density, osmolality, pH, temperature, fat and amino acid levels. The effects of physical activity overlie these stimuli, where the nature and intensity of activity are speculated to differentially influence G-I transit. Exercise of moderate intensity (ie. <70% $\dot{V}O_2\text{max}$) did not effect gastric emptying and intestinal absorption of a 13.3% glucose solution (Fordtran and Saltin, 1967), but more intense activity (ie. >70% $\dot{V}O_2\text{max}$) elicited an inhibitory effect (Costill and Saltin, 1974). This may be in response to elevated plasma catecholamines or endogenous opioids which inhibit both splanchnic blood flow and gastric motility. Gastric emptying of water and carbohydrate solutions (ie. 5.0-7.1% maltodextrin-glucose) was enhanced during moderate running (ie. 50-70% $\dot{V}O_2\text{max}$) in comparison with a resting condition (Neufer, Costill, Fink, Kirwan, Fielding and Flynn, 1986). Contractile activity of the abdominal muscles as part of the upper-body component to the running action may increase intragastric pressure (Neufer, Young and Sawka, 1989b). At rest, intra-

gastric pressure is generated by rhythmical contractions of the proximal stomach wall whilst the pyloric sphincter muscle occludes the distal exit. Upper body movement during cycling is minimal in comparison to running. However, recent studies of cycling and running have found gastric emptying and intestinal absorption of isotonic and hypertonic carbohydrate solutions to be independent of exercise mode (Houmard, Egan, Johns, Neuffer, Chenier and Israel, 1991; Rehrer, Brouns, Beckers, Ten Hoor and Saris, 1990b).

In contrast to the equivocal indirect effects of exercise, osmolality, energy density and volume exert more direct influences over G-I events (Murray, 1987; Rehrer, Beckers, Brouns, Ten Hoor and Saris, 1989). This is via activation of duodenal and jejunal receptors (Hunt and Pathak, 1960). The majority of solutes entering the intestine do not penetrate the receptor membrane, such that an osmotic gradient develops between the intestinal lumen and receptor vesicle. This draws fluid out of the receptor vesicles and surrounding tissue, and into the lumen (Barker, Cochrane, Corbett, Hunt and Kemp Roberts, 1974; Leiper and Maughan, 1986; Maughan, Fenn, Gleeson and Leiper, 1987; Rehrer, Beckers, Brouns, Saris and Ten Hoor, 1993). Dehydration of the receptors triggers inhibition of gastric emptying. Thus, hypertonic solutions maintain the vesicles in a shrunken state and impair G-I transit (Barker et al, 1974). Such solutions may gain in volume during their passage along the G-I tract through increases in gastric secretion (Barker et al, 1974; Coyle, Costill, Fink and Hoopes, 1978).

Solutes which penetrate the receptors, (ie. sodium, urea and glycerol), reverse the osmotic gradient to favour a fluid movement into the vesicles (Hunt and Pathak, 1960). The reduced neural drive of enlarged vesicles allows gastric emptying to be accelerated. Isotonic glucose solutions enhance receptor transport of sodium and indirectly promote G-I transit (Hunt and Pathak, 1960; Leiper and Maughan, 1986). The access of penetrating solutes into the receptor depends upon their intestinal concentration (Hunt and Pathak, 1960). Low concentrations are

actively transported into the vesicle and provide for facilitated receptor rehydration. Whereas, high concentrations must rely upon membrane diffusion, making reversal of receptor-inhibition a slower process.

Increasing the glucose content, and consequently the osmolality, of a solution slows G-I transit (Coyle et al, 1978). It was speculated that glucose polymers (GP) may reduce this inhibitory effect, as polymerised glucose has a higher molecular weight and lower osmolality (Murray 1987). Foster, Costill and Fink (1980) measured gastric emptying rates of glucose and GP solutions. A 5.0% glucose solution emptied at $\sim 10 \text{ ml}\cdot\text{min}^{-1}$, whereas a 5.0% GP solution emptied at $\sim 17 \text{ ml}\cdot\text{min}^{-1}$. Thus, Foster et al (1980) suggested that reducing osmolality through polymerisation may be advantageous for G-I transit. However, the advantage is probably not as great as originally anticipated, as calculations of emptying rates did not account for gastric secretions.

Hunt and Stubbs (1975) identified energy density as a primary determinant of gastric emptying, such that isocaloric portions of carbohydrate, fat and protein elicited similar G-I inhibitory effects. Energy-deficient saline meals empty from the stomach more rapidly than energy-rich carbohydrate meals (McHugh and Moran, 1979). The former empty exponentially, whereas the latter empty linearly over most of their time-course (Hunt and Spurrell, 1951; McHugh and Moran, 1979). However, concentrated feedings maintain a faster rate of energy delivery to the duodenum in comparison with less concentrated feedings, despite retarding gastric emptying (Hunt, Smith and Jiang, 1985; Hunt and Stubbs, 1975; Mitchell, Costill, Houmard, Fink, Robergs and Davis, 1989b).

Brener, Hendrix and McHugh (1983) proposed a two-phase model of gastric emptying. An initial exponentially rapid phase is controlled by an 'open-loop' mechanism. Feeding volume and intragastric pressure interact to deliver $\sim 2.13 \text{ kcal}\cdot\text{min}^{-1}$ to the duodenum. This is followed by a slow phase,

characterised by a relatively constant emptying rate under feedback regulation. An energy dependent 'closed-loop' system inhibits gastric emptying at a rate of $\sim 0.46 \text{ min}\cdot\text{kcal}^{-1}$. Thus, a reciprocal control mechanism is proposed which assumes a steady balance between duodenal delivery and gastric emptying. Hunt et al (1985) similarly report a two-phase mechanism where duodenal energy delivery may be enhanced by increasing initial volume and energy density. Gastric emptying was equivalent to $\sim 5.3 \text{ kcal}\cdot\text{min}^{-1}$ over 30 min proceeding ingestion of 600ml of a GP solution, although this declined to $\sim 2.6 \text{ kcal}\cdot\text{min}^{-1}$ by 120 min post-absorption.

Mitchell, Costill, Houmard, Flynn, Fink and Beltz (1988) examined the influence of serial carbohydrate feedings on gastric emptying and intermittent exercise performance. Carbohydrate drinks, (ie. 5.0 to 7.5 % solutions), were ingested at a rate of $8.5 \text{ ml}\cdot\text{kg}^{-1}\text{body wt}\cdot\text{h}^{-1}$ during each rest period of an interval session. The intestinal fluid delivery of low concentration carbohydrate solutions did not differ from water. Exercise capacity during a final 12 min performance ride was improved, possibly due to enhanced blood glucose availability. In a subsequent study, Mitchell et al (1989b) employed a similar intermittent exercise model but failed to observe the regulated G-I energy flow proposed by Brener et al (1983). Mitchell et al (1989b) reported a greater energy delivery with solutions of equal volume but increasing concentration.

Rehrer et al (1989), and Sole and Noakes (1989), report findings which are further anomalous with an 'energy control' model. A stable relationship between energy density and a delay in gastric emptying was not observed during single bolus or serial feedings. Energy delivery was increased through manipulation of feeding size and solution content. An initial fast emptying phase was evident, the characteristics of which reflected initial bolus size (Rehrer et al, 1989).

Increasing the volume of a feeding improves G-I transit (Costill and Saltin, 1974; Mitchell and Voss, 1991). Costill and

Saltin (1974) suggested a threshold of ~600 ml for a single feeding, whereas serial feedings may maintain high gastric emptying rates of ~10 to 15 ml·min⁻¹ (ie. 0.6 to 0.9 l·h⁻¹) throughout their course (Mitchell et al, 1989b; Ryan, Bleiler, Carter and Gisolfi, 1989). Distension of the stomach during filling increases intragastric pressure, whilst stimulation of mechanoreceptors sited in the stomach wall accelerates gastric emptying (Murray, 1987). This 'volume effect' appears to be the most important regulatory determinant immediately following ingestion. The G-I flow is maintained in order to minimise possible mechanical damage of the tract. Flow rates rapidly reach maximal following ingestion of a single bolus and then decline exponentially. Serial feedings prevent this exponential decline by maintaining a constant G-I flow (Noakes, Rehrer and Maughan, 1991). Under such circumstances, neither gastric emptying nor intestinal absorption are limiting (Rehrer, Wagenmakers, Beckers, Halliday, Leiper, Brouns, Maughan, Westerterp and Saris, 1992).

A further point to consider is the influence of ambient temperature, where high temperatures inhibit gastric emptying. This effect was observed during constant pace running (65% $\dot{V}O_2$ max) in a warm (35°C) environment, in comparison with a neutral (25°C) environment (Owen, Kregel, Wall and Gisolfi, 1986). The magnitude of this inhibition is dependent upon the severity of the exercise or heat-induced thermal stress, as well as physiological fluid status (Neufer, Young and Sawka, 1989a). Elevated core temperature may contribute to a reduced splanchnic blood flow or elevated plasma β -endorphin levels, or both, which in turn inhibit G-I motility (Owen et al, 1986). This phenomenon is implicated in the greater incidence of G-I disturbances in warmer environments (Rehrer, Beckers, Brouns, Ten Hoor and Saris, 1990a).

The composition of a solution on reaching the intestine influences subsequent absorption. Movement of fluid and solutes across the luminal mucosa and into splanchnic capillaries is either via simple diffusion, or via an active transport or carrier

system. Glucose and sodium are actively co-transported, and this movement facilitates the passive absorption of water by osmosis (Schedl and Clifton, 1963; Sladen and Dawson, 1969). The active transport of glucose and sodium increases markedly with intestinal content, despite a prevailing concentration gradient favouring gastric secretion (Schedl and Clifton, 1963). Cycling at moderate intensities has been observed to reduce intestinal electrolyte (ie. sodium, chloride and potassium ions) and water absorption (Barclay and Turnberg, 1988). This may be associated with exercise-induced increases in autonomic nervous activity (Barclay and Turnberg, 1988). However, any inhibitory effect rapidly diminishes after exercise cessation, except for a small residual effect restricting the uptake of potassium (Barclay and Turnberg, 1988). Thus, of importance with regards to the present thesis, post-exercise intestinal absorption is largely limited by solute and fluid supply.

2.5.2 Pre-exercise carbohydrate feeding

The influence of pre-exercise carbohydrate feeding within the context of the present thesis will assume increasing importance as the duration of the recovery period is reduced. Pre-exercise feeding will be considered over the 4-h preceding exercise, where ingestion during the final 60 min has received the greatest attention. This is one area of sports nutrition that is still not clearly defined with respect to the performer.

Pre-exercise carbohydrate ingestion increases blood glucose concentrations, which in turn stimulates insulin release and inhibits glucagon secretion (Ahlborg and Felig, 1977). There is a shift in energy metabolism to favour carbohydrate oxidation, whilst lipolysis and gluconeogenesis are suppressed (Ahlborg and Bjorkman, 1987). Despite changes in hormonal balance, muscle glycogen utilization during light exercise is not increased (Hughes, Edwards, Meredith, Evans, Martin and Young, 1984). Indeed, glucose ingested prior to low intensity exercise may elicit a glycogen sparing effect (Ahlborg and Bjorkman, 1987). 'Glycogen sparing' refers to a reduction in the degrada-

tion rate of endogenous carbohydrate stores. As such, exercise may be maintained for a longer period as utilisation of finite endogenous reserves is reduced. Thus, any intervention eliciting a 'glycogen sparing effect' may: shift the nature of energy metabolism towards a greater dependency upon fuels other than glycogen, (eg. FFA); or alternatively, provide exogenous carbohydrate of an appropriate form and at an appropriate rate to supplement endogenous reserves.

Costill et al (1977) observed a reduction in blood glucose early in exercise when glucose was consumed 45 min before treadmill running (~68% $\dot{V}O_2$ max). This was associated with a decrease in FFA availability and a compensatory increase in muscle glycogen utilisation. Hargreaves, Costill, Katz and Fink (1985) similarly report a decline in blood glucose and a slight increase in muscle glycogen utilisation following pre-exercise glucose ingestion. Foster, Costill and Fink (1979) provided 75 g of glucose 30 min prior to intensive cycling (84% $\dot{V}O_2$ max). Elevated plasma insulin levels early in exercise precipitated hypoglycaemia. However, this was a transient response and of negligible functional significance, though an anti-lipolytic effect of insulin was implicated in a reduced exercise time. ←

Fielding, Costill, Fink, King, Kovaleski and Kirwan (1987) report that carbohydrate supplements ingested 30-45 min before exercise do not accelerate muscle glycogen utilisation, despite eliciting a rapid decline in blood glucose. Similarly, endurance cycling capacity was not found to be impaired by pre-exercise carbohydrate provision (Hargreaves, Costill, Fink, King and Fielding, 1987; Koivisto, Karonen and Nikkla, 1981). Gleeson, Maughan and Greenhaff (1986) found that glucose feeding 45 min prior to exhaustive cycling (~73% $\dot{V}O_2$ max) did not adversely effect performance. Exercise in a glucose pre-fed group relative to a placebo group was accompanied by elevated R values and enhanced carbohydrate oxidation, whilst plasma FFA and β -hydroxybutyrate levels were reduced. In contrast to previous reports, hypoglycaemia was not evident either before or during exercise. Moreover, Chryssanthopoulos, Hennessy ←

and Williams (1994) observed that providing 75g of glucose in 300 ml of water 30 min before an exhaustive bout of constant pace running at 70% $\dot{V}O_2$ max elevated pre-exercise blood glucose concentrations but did not impair endurance capacity. Thus, pre-exercise carbohydrate feedings possibly enhances oxidation of endogenous reserves, though this may in turn be compensated for through exogenous provision (Jandrain, Krzentowski, Pirnay, Mosora, Lacroix and Luyckx, 1984).

Physiological responses to carbohydrate ingestion are influenced by the nature of the supplement with respect to the glycaemic index (GI) (Coyle, 1991; Guezennec, Satabin, Duforez, Koziat and Antoine, 1993). This is a measure of the rate at which carbohydrates become metabolically available as blood borne glucose (Jenkins, Thomas, Wolever, Taylor, Barker, Fielden, Baldwin, Bowling, Newman, Jenkins and Goff, 1981). Glucose and potatoes elicit higher blood glucose and insulin responses than rice or pasta; whereas fructose elicits a higher blood lactate response (Calles-Escandon, Devlin, Whitcomb and Horton, 1991; Guezennec et al, 1993; Hargreaves et al, 1985; Koivisto et al, 1981; McMurray, Wilson and Kitchell, 1983), though fluctuations in blood glucose and insulin are blunted (Koivisto et al, 1981; Levine, Evans, Cadarette, Fisher and Bullen, 1983). Despite smaller disturbances in plasma glucose and insulin concentrations, fructose is no more effective than other sugars in sparing muscle and liver glycogen (Koivisto, Harkonen, Karonen, Groop, Elovainio, Ferrannini, Sacca and DeFronzo, 1985). Such differences in response are partly due to variations in G-I transit, digestion and absorption, which ultimately determine physiological availability (Levine et al, 1983). A longer transit time between ingestion and absorption of fructose and fibre-rich carbohydrates delays physiological availability, but is also implicated in greater G-I disturbances (Levine et al, 1983; Rehrer, Van Kemenade, Meester, Saris and Brouns, 1990c).

Exercise performance and substrate utilisation are similar regardless of whether a pre-exercise meal is consumed 4 or 8-h

prior to an event (Flynn, Michaud, Rodriguez-Zayas, Lambert, Boone and Moleski, 1989). Transient increases in blood glucose and plasma insulin preceded ingestion of 100 to 312 g of carbohydrate (Coyle, Coggan, Hemmert, Lowe and Walters, 1985; Jandrain et al, 1984; Sherman, Brodowicz, Wright, Allen, Simonsen and Derbach, 1989). Coyle et al (1985) observed that these responses were associated with a 42% increase in the glycogen content of m.vastus lateralis. Normal blood glucose concentrations were restored prior to exercise, and were then maintained. Exercise performed 3 to 4-h later was associated with enhanced carbohydrate metabolism, whilst fat oxidation was suppressed (Coyle et al, 1985; Sherman et al, 1989). It is possible that this shift in energy metabolism was accommodated by the rapid availability of exogenous substrate (Jandrain et al, 1984), such that post-exercise muscle glycogen levels were similar in the fasted and fed state (Coyle et al, 1985).

Pre-exercise carbohydrate feeding following a 3-d diet and training regimen which elevated muscle glycogen content has also been examined (Levine et al, 1983). A light breakfast, (~80 g of carbohydrate), was provided 4-h before each trial to replenish hepatic glucose reserves. Fructose, glucose or water was subsequently ingested 45 min prior to a treadmill run (~75% $\dot{V}O_2$ max). Carbohydrate oxidation was elevated but was not associated with hypoglycaemia. In contrast, Neuffer, Costill, Flynn, Kirwan, Mitchell and Houmard (1987) provided carbohydrate supplements 5 min before exercise in the 4-h post-absorptive state. This dietary combination improved sub-maximal cycling performance, apparently due to maintaining high carbohydrate oxidation rates. Brouns, Rehrer, Saris, Beckers and Ten Hoor (1989) provided carbohydrate during a 7 min rest separating a 20 min standardised warm-up from 45 min of moderate cycling. Both exercise sessions stimulated catecholamine release and suppressed insulin secretion, whereas carbohydrate ingestion reversed this response and improved blood glucose availability.

Wright and Sherman (1989) compared the efficacy of pre-exercise carbohydrate ingestion, with further supplementation during exercise. Intermittent cycling performance was enhanced following carbohydrate ingestion either before or during exercise, with further improvements being achieved by combining the two treatments. Similarly, endurance capacity was improved during steady state cycling (70% $\dot{V}O_2$ max) with a combination of pre-exercise and during exercise feedings (Wright, Sherman and Dernbach, 1991). It is speculated that the benefits of such dietary strategies arise from enhanced carbohydrate availability, especially over the later stages of exercise, such that carbohydrate oxidation is maintained.

Thus, pre-exercise carbohydrate ingestion shifts energy metabolism to favour its enhanced oxidation. Hepatic glycogen is of increasing importance for maintaining blood glucose stability during exercise, ultimately determining net systemic concentrations. If liver glycogen reserves are low, hypoglycaemia and hyperinsulinaemia may accompany the onset of exercise after pre-exercise carbohydrate ingestion, though normal concentrations are quickly restored (Sherman et al, 1989). The available evidence suggests that pre-exercise carbohydrate ingestion improves endurance exercise performance, whilst further feedings during exercise will maximise the effect (Coyle, 1991).

2.5.3 Carbohydrate feeding during exercise

Carbohydrate ingestion during exercise has been reported to have no effect on performance (Burgess, Davis, Bartoli and Woods, 1991). However, the weight of available evidence supports a performance enhancing effect (Coyle, Hagberg, Hurley, Martin, Ehsani and Holloszy, 1983; Fenn, Leiper, Light and Maughan, 1983; Murray, Eddy, Murray, Seifert, Paul and Halaby, 1987; Tsintzas et al, 1993a; Tsintzas, Williams, Boobis and Wilson, 1993b; Wilber and Moffat, 1992).

The efficacy of supplementation is influenced by the type of carbohydrate provided (Massicotte, Peronnet, Allah, Hillaire-

Marcel, Ledoux and Brisson, 1986; Murray, Paul, Seifert, Eddy and Halaby, 1989). The rate of ingestion is also of importance, reflecting the pattern of feeding and individual feeding volumes. Ingestion of a 1.8% carbohydrate-electrolyte beverage providing 13 g CHO·h⁻¹, was insufficient to maintain plasma glucose or influence performance during 165 min of moderate cycling (Burgess et al, 1991). Similarly, Mitchell, Costill, Houmard, Fink, Pascoe and Pearson (1989a) report that 37 g CHO·h⁻¹ does not influence isokinetic work output, whilst a positive effect resulted from increasing intake to 74 g·CHO·h⁻¹. Thus, a rate of ingestion between ~43 and 84 g·CHO·h⁻¹ during exercise appears to elicit a performance enhancing effect (Coyle et al, 1983; Coyle, Coggan, Hemmert and Ivy, 1986; Hargreaves, Costill, Coggan, Fink and Nishibata, 1984; Pallikarakis, Jandrain, Pirnay, Mosora, Lacroix, Luyckx and Lefevre, 1986).

The principal theses underlying the case for supplementation include: relieving CNS dysfunctioning by alleviating hypoglycaemia; providing an alternate carbohydrate source, and hence spare endogenous reserves; maintaining blood glucose availability when liver and muscle glycogen levels are low and blood glucose uptake is increased. Ultimately, tolerance to prolonged exercise is enhanced and the onset of fatigue is delayed. Christensen and Hansen (1939)⁴ suggested that 'frank hypoglycaemia' (ie. blood glucose <2.5 mmol·l⁻¹), was associated with CNS dysfunctioning, the symptoms of which included lightheadedness, general weakness and nausea. Ingesting 200 g of glucose at the point of fatigue from endurance exercise alleviated these hypoglycaemic symptoms and allowed activity to continue for a further 60 min. As R values had not decreased before fatigue, nor increased following glucose ingestion, Christensen and Hansen (1939) speculated that low blood glucose concentrations led to fatigue through an effect on 'higher processing centres'. In the studies reported in the present thesis, fatigue during constant pace running was not accompanied by hypoglycaemia, though subjective responses of the runners did include lightheadedness, general weakness

⁴ Cited Astrand and Rodahl (1986)

and nausea. This indicates that a causal link between these subjective responses and blood glucose availability may not be as simple as Christensen and Hansen (1939) would suggest.

More recently Felig et al (1982) observed that blood glucose concentrations of less than $2.5 \text{ mmol}\cdot\text{l}^{-1}$ are not always associated with exercise cessation. Hypoglycaemia was measured in 7 out of 10 subjects during exhaustive submaximal cycling, nevertheless performance was maintained. Glucose ingestion at rates of 40 or $81 \text{ g}\cdot\text{h}^{-1}$ stabilised blood levels whilst suppressing plasma adrenaline concentrations. Similarly, endurance capacity was improved when GP was ingested at 30 min intervals during exhaustive walking (Ivy, Miller, Dover, Goodyear, Sherman, Farrell and Williams, 1983). Blood glucose availability was maintained and exercise cessation was not associated with hypoglycaemia nor CNS dysfunctioning. Thus, hypoglycaemia *per se* does not appear to limit endurance performance, such that its prevention by supplementation does not consistently delay fatigue.

Pirnay, Lacroix, Mosora, Luyckx and Lefebvre (1977a) provided a 400 ml (25%) glucose feeding after 15 min of walking ($\sim 50\% \dot{V}\text{O}_2\text{max}$). The oral glucose feeding increasingly contributed to energy metabolism, progressively replacing the contributions of fat and protein. Total carbohydrate and fat oxidation, the contribution of exogenous glucose, and work intensity were linearly related during low-intensity exercise (ie. 22-51% $\dot{V}\text{O}_2\text{max}$). At higher exercise intensities (ie. 51-64% $\dot{V}\text{O}_2\text{max}$) fat and exogenous glucose oxidation tended to plateau as endogenous carbohydrate oxidation continued to increase (Pirnay, Crielaard, Pallikarakis, Lacroix, Mosora, Krezentowski, Luyckx and Lefebvre, 1982). A maximum contribution of exogenous glucose to energy metabolism was reached within ~ 60 min of ingestion and declined after ~ 90 min (Pirnay et al, 1977a). Notably, this oxidation pattern of a single bolus appears to be independent of the timing of ingestion during exercise (Krezentowski, Jandrain, Pirnay, Mosora, Lacroix, Luyckx and Lefebvre, 1984). Glucose feeding increased total carbohydrate

utilisation, but this increase was more than accommodated by exogenous provision. Thus, supplementation maintained blood glucose levels, whilst endogenous carbohydrate reserves appeared to be spared (Pirnay et al, 1977a; Pirnay, Lacroix, Mosora, Luyckx and Lefebvre, 1977b).

Providing carbohydrate during prolonged exercise elevates plasma insulin and blood glucose (Coyle et al, 1983; Hargreaves et al, 1984), and is associated with increased peripheral carbohydrate oxidation (Coyle et al, 1983; Pallikarakis et al, 1986). Whilst plasma FFA and glycerol concentrations are suppressed (Ahlborg and Felig, 1976; Hargreaves et al, 1984). Splanchnic glucose escape increases in the face of reduced hepatic glycogenolysis and gluconeogenesis, suggesting an increased contribution of exogenous carbohydrate to systemic glucose concentrations (Ahlborg and Felig, 1976). This may be associated with a sparing of muscle glycogen (Coyle et al, 1983; Hargreaves et al, 1984; Tsintzas, Williams and Wilson, 1992; Tsintzas et al, 1993b), though this is not a consistent observation (Coyle et al, 1986; Flynn, Costill, Hawley, Fink, Neuffer, Fielding and Sleeper, 1987). Tsintzas (1993) observed glycogen sparing in type-I muscle fibres when a 6.9% carbohydrate-electrolyte solution was ingested during constant pace running (70% $\dot{V}O_2$ max). This resulted in a ~28% reduction in muscle glycogen utilisation over the initial 60 min of exercise.

The provision of carbohydrate during exercise may delay fatigue by maintaining blood glucose availability (Costill et al, 1970), such that total carbohydrate oxidation is not compromised (Coyle et al, 1986; Hargreaves and Briggs, 1988; Mitchell et al, 1989a), especially when muscle glycogen reserves are low (Coggan and Coyle, 1987). Coggan and Coyle (1987, 1989) provided a single feeding late in exercise, which reversed a decline in carbohydrate oxidation and restored euglycaemia (ie. normal blood glucose concentrations), such that fatigue from strenuous cycling was delayed. Similarly, the capacity to sustain a higher running speed is associated with improved blood glucose availability (Williams, Nute, Broadbank and Vinall,

1990). Widrick, Costill, Fink, Hickey, McConnell and Tanaka (1993) found that optimising carbohydrate availability through supplementation, as well as through prior elevation of endogenous reserves, enabled self-paced submaximal cycling performance to be maintained. In contrast, reduced exercise capacity in the face of falling plasma glucose levels and carbohydrate oxidation rates is consistent with a thesis whereby fatigue results from inadequate substrate supply to working muscle.

Ingestion of carbohydrate during prolonged cycling or running inhibits the normal gluco-regulatory hormonal response (Deuster, Singh, Hofman, Moses and Chrousos, 1992; Mitchell, Costill, Houmard, Flynn, Fink and Beltz, 1990). Lower plasma cortisol concentrations and a reduced glucagon-to-insulin ratio may suppress hepatic glucose release, possibly eliciting a liver glycogen sparing effect. Thus, carbohydrate ingestion may delay fatigue by reducing mobilisation of glycogen in the liver (Bosch et al, 1991; Van Handel, Fink, Branam and Costill, 1980). In agreement, Coyle, Hamilton, Gonzalez Alonso, Montain and Ivy (1991) observed that hyperglycaemia does not influence the rate of muscle glycogen degradation, but appeared to elicit a sparing of liver glycogen.

2.6 Replenishment of carbohydrate reserves during exercise

Glycogenolysis and glycogenesis occur concomitantly in muscle (Hutber and Bonen, 1989), although the latter is a much slower process with a maximal rate in man of ~1% of the former (Hultman et al, 1971). A subtle physiological balance determines whether net glycogen breakdown or net glycogen accumulation prevails. This balance is influenced by the rate of energy demand, the prevailing hormonal milieu, as well as current nutritional status (Constable et al, 1984).

Constable et al (1984) hypothesised that supplying exogenous carbohydrate during exercise would maintain systemic levels, and provide sufficient glucose for working muscle to fuel metabolism and synthesise glycogen. It is apparent that reduced glycogen levels rather than glucose feeding per se stimulate resynthesis, even under conditions

of continued degradation in rat and human skeletal muscle (Kuipers, Costill, Porter, Fink and Morse, 1986; Kuipers, Keizer, Brouns and Saris, 1987). Muscle glycogen synthesis was maintained during light exercise which preceded multi-sprint exercise (Peters Futre, Noakes, Raine and Terblanche, 1987). It was suggested that glycogenic substrates were provided in the form of glucose and lactate. In contrast, Bonen, Ness, Belcastro and Kirby (1985) observed that mild exercise delayed glycogen replenishment in humans in both active and non-active tissue. It was speculated that this may be due to the inhibitory effect of elevated plasma adrenaline.

A number of studies have demonstrated significant glycogen synthesis during exercise (Hutber and Bonen, 1989; Kuipers et al, 1986; Kuipers et al, 1987; Peters Futre et al, 1987), but this is not a consistent finding (Bonen et al, 1985; Kuipers, Saris, Brouns, Keizer and Ten Bosch, 1989). Equivocal observations may arise from differences in exercise protocol and individual training status (Kuipers et al, 1987; Kuipers et al, 1989). The present consensus suggests a net glycogen gain with carbohydrate feeding in trained athletes during mild exercise in relatively non-active muscle fibres (ie. type IIa and IIb). Thus, glycogenesis should be thought of as an extremely dynamic process that is usually increased during as-well as following exercise, without the necessity for extensive prior depletion (Bonen et al, 1989; Hutber and Bonen, 1989).

2.7 Excess post-exercise oxygen consumption (EPOC)

Cycling or running at 70% $\dot{V}O_2$ max for longer than 80 min results in elevated post-exercise resting oxygen consumption (Bahr, 1992; Bahr, Inges, Vaage, Sejersted, Newsholme, 1987; Gore and Withers, 1990). This excess post-exercise oxygen consumption (EPOC) corresponds to as much as ~14% of the energy expended during previous activity (Bahr and Mæhlum, 1986), and may persist for more than 12-h (Bielinski, Schutz and Jequier, 1985; Mæhlum, Grandmontagne, Newsholme and Sejersted, 1986). The magnitude of EPOC depends upon both exercise intensity and exercise duration, there being a minimum threshold level of activity below which a protracted effect is not apparent (Bahr et al, 1987; Bahr and Sejersted, 1991b).

The removal of lactate and repletion of haemoglobin and myoglobin bound oxygen stores are all speculated as contributing to EPOC (Bahr and Mæhlum, 1986). However, the former is unlikely, especially following constant pace running, whereas the latter requires a combined total of only ~0.5-2.0 litres of oxygen. A further ~10% may be accounted for as a consequence of a post-exercise tachycardia (Gore and Withers, 1990).

The major fraction of EPOC is believed to arise from the resynthesis of muscle and liver glycogen, and enhanced rates of 'futile' energy cycling (Bahr and Mæhlum, 1986). With regards to the latter, elevated post-exercise plasma catecholamine levels may stimulate substrate cycles, thereby increasing energy metabolism (Bahr et al, 1987). Such futile cycling of fats and carbohydrates is evident in both non-active and active muscle (Bahr, Hansson and Sejersted, 1990; Ivy et al, 1988b), where the rate of post-exercise FFA utilisation and triglyceride-fatty acid cycling reflects the intensity and duration of prior exercise (Bahr, Hostmark, Newsholme, Gronnerod and Sejersted, 1991).

The consumption of food during the immediate post-exercise period is observed to further increase oxygen consumption (Bielinski et al, 1985; Gore and Withers, 1990). A 'thermic effect of food' (TEF) results from the energy requirements of digesting, absorbing and disposing of a meal. This effect is additive with the recovery energy requirements following exercise (Young, Treadway and Ruderman, 1985). A stimulatory effect of diet on metabolism may be mediated through increased insulin secretion in response to elevated circulatory macronutrients (Balon, Zorzano, Goodman and Ruderman, 1984). The thermic effect of physiological insulin concentrations is negligible under resting conditions (Christin, Nacht, Vernet, Ravussin, Jequier and Acheson, 1986). However, prior exercise appears to potentiate the activity of enhanced systemic insulin concentrations (Young et al, 1985). Thus, substrate cycles are stimulated, as are ion transporter systems, and possibly further unidentified energy requiring processes (Balon, Treadway, Hughes, Young and Ruderman, 1992). However, the contribution of TEF in response to post-exercise dietary intake to EPOC is relatively small in comparison with the residual effects of prior exercise (Bahr and Sejersted, 1991a). Nevertheless, such consid-

erations need to be taken into account when examining the influence of nutrition on recovery from prolonged, constant pace running.

2.8 Post-exercise carbohydrate replenishment

The essentiality of muscle glycogen for physical activity necessitates its rapid resynthesis following exercise (Costill and Hargreaves, 1992). If the level of glycogen depletion is less than ~60%, then repletion with a carbohydrate-rich diet may be complete within 24-h (Costill et al, 1981; Kochan, Lamb, Lutz, Perril, Reimann and Schlender, 1979). However, restoration to pre-exercise levels may take longer if this prior depletion is greater than ~60% (Piehl, 1974; Sherman et al, 1983). The influence of muscle glycogen concentration on the resynthesis rate is greatest immediately post-exercise (Zachwieja, Costill, Pascoe, Robergs and Fink, 1991). This rate is also influenced by: the nature of prior exercise; the amount and timing of post-exercise carbohydrate feeding; the type of carbohydrate; and, the method of administration (Blom, 1989a).

2.8.1 The pattern of post-exercise glycogen resynthesis

Post-exercise muscle glycogen resynthesis follows a curvilinear pattern. This response is characterised by an initial and relatively short fast phase, which is followed by a longer slow phase (Adolfson and Ahren, 1971; Garetto, Richter, Goodman and Ruderman, 1984). The duration of the fast phase is ~4 to 6-h, where the first 2-h is most rapid. The slow phase, which is characterised by less transient metabolic adjustments, may last for 2 to 3 days after severe, prolonged exercise (Bergstrom and Hultman, 1966a).

The pattern of glycogen resynthesis reflects previous muscle fibre recruitment, and differs between fibre types. Type II fibres resynthesise glycogen 65% faster than type I fibres during the early phase (McLane and Holloszy, 1979; Terjung, Baldwin, Winder and Holloszy, 1974; Vollestad, Blom and Gronnerod, 1989), but this difference was no longer evident after 60 min. This suggests a slower initial acceleration of the synthetic

pathway in type-I fibres. However, the highest rate of glycogen resynthesis takes place in the liver, and is 75% faster than type-II fibres (Terjung et al, 1974). Differences in synthetic rates between tissues can be explained partly by variant activity levels of the enzyme glycogen synthase (Conlee, Hickson, Winder, Hagberg and Holloszy, 1978).

Glycogen synthase (GS) is the rate limiting step in the synthetic pathway (Young, Bogardus, Stone and Mott, 1988). It catalyses the transfer of free glucosyl units to the glycogen skeleton (Stryer, 1988). Being a very potent enzyme, small changes in activity will yield relatively large changes in the synthetic rate. Whilst factors associated with its activation may simultaneously inhibit phosphorylase activity, and impede further glycogen degradation (Constable et al, 1986).

There are at least two inter-convertible forms of GS: GS-D, a phosphorylated D-form which requires the presence of G-6-P; and GS-I, a dephosphorylated I-form which operates independent of G-6-P (Larner and Villar-Palasi, 1971). The activation of GS (ie. a D-to-I conversion) involves the hydrolysis of a phosphate moiety. This process is catalysed by the enzyme D-phosphatase (Larner and Villar-Palasi, 1971). Both GS-D and D-phosphatase are bound into glycogen in the basal fed state, thus protecting GS-D. Mobilisation of glycogen during exercise liberates the two enzymes and allows interaction in the cellular cytosol. Deactivation of GS (ie. an I-to-D conversion) involves the addition of a phosphate moiety. This process is catalysed by the enzyme I-kinase (Larner and Villar-Palasi, 1971), whilst D-phosphatase is inhibited through incorporation into glycogen (Villar-Palasi, 1969).

Insulin allosterically shifts the emphasis of control to favour GS activation (Roch-Norlund, Bergstrom and Hultman, 1972), whereas adrenaline and food deprivation shifts the emphasis towards deactivation (Danforth, 1965). High GS levels in the glycogen-depleted state modulate the influence of insulin on skeletal muscle (Bogardus, Thuillez, Ravussin, Vasquez,

Narimiga and Amhar, 1983). Further control of GS is exerted by cellular metabolites: UDP-glucose, magnesium and G-6-P are positive effectors; whereas UDP, inorganic phosphate and the adenine nucleotides (ie. ATP, ADP and AMP) are negative effectors (Roach and Larner, 1976).

Decreasing muscle glycogen levels elicits a strong glycogenic drive (Fell, Terblanche, Ivy, Young and Holloszy, 1982). There is an inverse relationship between %GS-I activity (ie. GS-I/GS (I+D) %) and muscle glycogen content (Bergstrom, Hultman and Roch-Norlund, 1972; Roch-Norlund et al, 1972). Exercise increases %GS-I activity, though total enzyme activity (ie. GS [D +I]) remains unchanged (Adolfsson, 1973; Jeffress, Peter and Lamb, 1968; Piehl, Adolfsson and Nazar, 1974). Following exercise %GS-I activity quickly returns to basal levels (Kochan et al, 1979), suggesting that other mechanisms must operate to sustain an elevated rate of glycogen synthesis (Piehl et al, 1974). One mechanism is thought to be an increased sensitivity of GS-D to G-6-P, allowing stimulation of GS-D by lower G-6-P concentrations (Bak and Pedersen, 1990; Kochan et al, 1979). Thus, G-6-P formation is likely to be an important regulator of glycogen synthesis. Exercise may enhance glucose phosphorylation through a positive effect on hexokinase activity (Lamb, Peters, Jeffress and Wallace, 1969; Van Houten, Davis, Meyers and Durstine, 1992), such that cellular glucose transport may limit the recovery process (Ziel, Venkatesan and Davidson, 1988).

Insulin enhances post-exercise membrane permeability by interaction with a specific receptor mechanism (Narahara and Ozand, 1963; Park, Reinwein, Henderson, Cadenas and Morgan, 1959). Total glucose disposal is accelerated, with storage being the preferred pathway over oxidation (DeFronzo, Jacot, Jequier, Maeder, Wahren and Felber, 1981). Elevated plasma catecholamines inhibit insulin secretion (Galbo et al, 1977b), though this is countered by a local contraction-induced increase in muscle sensitivity to insulin (Heath, Gavin, Hinderliter, Hagberg, Bloomfield and Holloszy, 1983; Richter, Garetto, Goodman and Ruderman, 1982a; Richter, Garetto,

Goodman and Ruderman, 1984; Richter, Ploug and Galbo, 1985). The sensitivity and responsiveness of muscle to insulin varies with fibre type (James, Kraegen and Chisholm, 1985b). Type I and IIa fibres are more sensitive than type-IIb fibres (James, Jenkins and Kraegen, 1985a). Increased sensitivity is not due to changes in receptor-binding characteristics (Zorzano, Balon, Garetto, Goodman and Ruderman, 1985), but is associated with reductions in muscle glycogen (Ivy, Frishberg, Farrell, Miller, and Sherman, 1985). This suggests that regulatory alterations are taking place distal to the receptor site (James et al, 1985b; Treadway, James, Burcel and Ruderman, 1989).

Contractile activity also enhances membrane permeability and promotes glucose transport (Goldstein, Mullick, Huddlestun and Levine, 1953; Ivy, 1987; Richter, Mikines, Galbo and Kiens, 1989; Wallberg-Henriksson and Holloszy, 1985). This effect is localised to previously active muscle (Bergstrom and Hultman, 1966a). Berger, Hagg and Ruderman (1975) speculated that insulin played a permissive role, being an essential prerequisite for glucose transport. However, the exercise effect appears to be independent of insulin (Ivy and Holloszy, 1981; Nesher, Karl and Kipinis, 1985; Ploug et al, 1984). Indeed, Ivy (1977) suggests that insulin is not essential for glucose transport and glycogen resynthesis during the early phase of recovery, but plays a more prominent role in the later phase.

The combined effects of insulin and muscular contraction did not exceed their maximal independent effects in frog muscle (Holloszy and Narahara, 1965). Though their activities were approximately additive following submaximal contractions. In contrast, maximal additive effects were observed in rat skeletal muscle, suggesting that these stimuli operated via independent mechanisms (Nesher et al, 1985; Zorzano, Balon, Goodman and Ruderman, 1986). Ploug, Galbo, Vinten, Jorgensen and Richter (1987) reported that an additive effect was limited to type I and IIa fibres, being absent in type-IIb fibres. Wallberg-Henriksson, Constable, Young and Holloszy (1988) suggest that a combined maximal effect only persists during the early fast

phase of recovery. Thus, high insulin concentrations and prior exercise may act synergistically to enhance the disposal of blood glucose in muscle tissue (DeFronzo, Ferrannini, Sato, Felig and Wahren, 1981; Ploug et al, 1987; Wasserman, Geer, Rice, Flakoll, Brown, Hill and Abumrad, 1991).

As contraction-induced transport declines, the enhanced sensitivity and responsiveness of skeletal muscle to insulin increases (Richter et al, 1989; Wallberg-Henriksson et al, 1988). The development of insulin sensitivity is a delayed response (Gulve, Cartee, Zierath, Corpus and Holloszy, 1990), and is confined to de-glycogenated muscle fibres (Mikines, Sonne, Farrell, Tronier and Galbo, 1988; Richter et al, 1989). As such, a rapid resynthesis seems to be facilitated through prioritising previously active tissue.

Glucose enters the muscle cell via transporter proteins which span the sarcolemma (ie. facilitated diffusion). A family of such proteins has been identified (Mueckler, Caruso, Baldwin, Panico, Blench, Morris, Allard, Lienhard and Lodish, 1985; Klip and Paquet, 1990), of which GLUT-4 is the major transporter species in human muscle (Friedman, Neuffer and Dohm, 1991). The greater distribution of GLUT-4 in type I and IIa muscle fibres appears to be a major determinant in their greater responsiveness to insulin (Kern, Wells, Stephens, Elton, Friedman, Tapscott, Pekala and Dohm, 1990). In addition, GLUT-4 distribution is influenced by training, which has been observed to elicit increases in membrane transporter protein (Sherman, Friedman, Gao, Reed, Elton and Dohm, 1993).

Enhanced post-exercise glucose transport involves a translocation of transporters from an inner cellular pool to the outer plasma membrane (Fushiki, Wells, Tapscott and Dohm, 1989; King, Hirshman, Horton and Horton, 1989). Both insulin and muscle contraction stimulate a movement of hexose-complexes across the sarcolemma (Goodyear, King, Hirsham, Thompson, Horton and Horton, 1990b; Klip, Ramlal, Young and Holloszy, 1987; Wallberg-Henriksson and Holloszy, 1985),

possibly through the recruitment of transporters from different intracellular pools (Douen, Ramlal, Klip, Young, Cartee and Holloszy, 1989). The total transport rate is accelerated by recruitment of more transporter molecules and by increasing their intrinsic level of activity (DeFronzo et al, 1981; King et al, 1989; Neuffer et al, 1985; Richter et al, 1984; Young, Uhl, Cartee and Holloszy, 1986). Reversal of enhanced glucose transport correlates with a reversal in transporter turnover (Goodyear, Hirsham, King, Thompson and Horton, 1990a). The elevated level of intrinsic activity is lost in advance of a reduction in transporter number (Goodyear et al, 1990a).

The onset of the late phase in muscle glycogen replenishment is marked by a decrease in both the rate of glucose uptake and the rate of glycogenesis. As muscle glycogen increases towards base-line levels the stimulatory effect of prior exercise quickly recedes (Garetto et al, 1984). An underlying component of this reversal is evident in all fibre types in the absence of glycogen repletion, but is more rapid in type I and IIa fibres than in type IIb (Ploug et al, 1987; Young, Wallberg-Henriksson, Sleeper and Holloszy, 1987). However, an 'exercise effect' persists for longer when exogenous carbohydrate is not available (Cartee, Young, Sleeper, Zierath, Wallberg-Henriksson and Holloszy, 1989; Young, Garthwaite, Bryan, Cartier and Holloszy, 1983). A decline in the stimulatory effect of prior activity may be due to a gradual intra-cellular sequestration of previously mobilised glucose transporters (Young et al, 1983). Thus, elevated glucose transport is sustained over the late phase through enhanced cellular sensitivity to, and hormonal responsiveness per se, of increasing plasma insulin concentrations (Zorzano et al, 1986).

Splanchnic glucose output rapidly returns to resting levels during the immediate post-exercise period (Wahren, Felig, Hendler and Ahlborg, 1973). This is despite enhanced gluconeogenic precursor uptake in response to greater availability and altered hepatic metabolism. Thus, glucagon stimulated gluconeogenesis covers an increasing proportion of the total glucose output (Felig et al, 1972; Wahren et al, 1973). Alanine is

the major gluconeogenic substrate following exhaustive exercise, with lactate and glycerol playing relatively minor roles (Favier et al, 1987).

To summarise, the fast phase of muscle glycogen resynthesis results from increased membrane permeability in response to previous contractile activity and insulin. Cellular glucose transporters are mobilised and their intrinsic level of activity enhanced. Substrate availability is maintained through increased blood flow, which is a persistent cardiovascular adjustment to prior exercise (Schultz, Lewis, Westbie, Gerich, Rushakoff and Wallin, 1977). Activation of GS sustains glucose flux through the synthetic pathway. Glycogenic drive decreases over time as the magnitude of provocative stimuli diminish. There is a reversal of the direct exercise-induced effect as well as GS-I activation, though increased tissue sensitivity to insulin maintains elevated glycogen synthesis over the late phase of recovery (Adolfsson and Ahren, 1971).

2.8.2 The importance of timing post-exercise carbohydrate ingestion

Ivy et al (1988a) demonstrated the practical implications of this bi-phasic pattern of muscle glycogen resynthesis. Carbohydrate feedings equivalent to $1.0 \text{ g}\cdot\text{kg}^{-1}\text{body wt}\cdot\text{h}^{-1}$ were ingested immediately post-exercise, or 2-h post-exercise. This delay in carbohydrate ingestion resulted in glycogen synthesis being reduced to ~50% of that measured during the first 2-h, when carbohydrate was fed immediately on cessation of exercise.

If a fasting state is maintained over the initial 4-h of recovery from exhaustive exercise ($70\% \dot{V}O_2\text{max}$), resynthesis of muscle glycogen takes place at $\sim 1.8 \text{ mmol}\cdot\text{kg}^{-1}\text{wet wt}\cdot\text{h}^{-1}$ (Mæhlum and Hermansen, 1978). Similarly, Ivy et al (1988a) measured a fasting rate of $\sim 2.5 \text{ mmol}\cdot\text{kg}^{-1}\text{wet wt}\cdot\text{h}^{-1}$ over the first 2-h following prolonged, interval cycling. In contrast, a resynthesis rate equivalent to ~ 5.0 to $8.0 \text{ mmol}\cdot\text{kg}^{-1}\text{wet wt}\cdot\text{h}^{-1}$ is maintained with carbohydrate ingestion (Blom et al, 1987b; Ivy et al, 1988b; Keizer, Kuipers, van Kranenburg and Geurten, 1987; Mæhlum,

Felig and Wahren, 1978; Mæhlum, Hostmark and Hermansen, 1977; Reed, Brozinick, Lee and Ivy, 1989). Thus, the timing of post-exercise carbohydrate ingestion is critical in optimising the recovery process. Feedings should be provided immediately after exercise in order to capitalise upon favourable conditions for muscle glycogen replenishment.

2.8.3 Short-term recovery from submaximal exercise - Optimising the early phase

Increasing the amount of carbohydrate consumed over this critical period from 0.35 to 0.70 g·kg⁻¹body wt·2h⁻¹ increases muscle glycogen resynthesis (Blom et al, 1987b). Further increases in excess of 1.50 g·kg⁻¹body wt·2h⁻¹ do not appear to yield additional benefits at a muscle tissue level (Blom et al, 1987b; Ivy et al, 1988b). Thus, post-exercise muscle glycogen resynthesis can be maintained at an optimal rate of ~6.0 mmol kg⁻¹ wet wt·h⁻¹, provided that carbohydrate intake exceeds 1.0 g·kg⁻¹body wt·2h⁻¹ (Ivy, 1991).

Under basal conditions, ~15% of an oral glucose load escapes the splanchnic bed and is available for peripheral metabolism (Felig, Wahren and Hendler, 1975). The primary fate of liver glucose uptake during exercise is storage, whereas in muscle the primary fate is oxidation (Kelley, Mitrakou, Marsh, Schwenk, Benn, Sonnenberg, Arcangeli, Aoki, Sorensen, Berger, Sonksen and Gerich, 1988). Following exhaustive cycling (70% $\dot{V}O_2$ max), hepatic glucose output increased up to ~200% (Wahren, Felig and Mæhlum, 1977). Krzentowski, Pirnay, Luyckx, Pallikarakis, Lacroix, Mosora and Lefebvre (1982) suggest that high plasma glucagon concentrations and a delayed insulin response are partly responsible for a decrease in hepatic glucose retention. This gluco-regulatory hormonal balance is also involved in prioritising the replenishment of muscle glycogen over liver glycogen (Fell, McLane, Winder, and Holloszy, 1980; Mæhlum, 1978; Mæhlum et al, 1978). Thus, the majority of glucose ingested immediately post-exercise escapes the liver and is transported directly to skeletal muscle.

Meanwhile, liver glycogen resynthesis is ensured through increased fractional extraction of gluconeogenic precursors (notably lactate and alanine) from blood (Ahlborg et al, 1974; Holm et al, 1978; Rowell et al, 1965).

The upper limit of 'useful' carbohydrate ingestion largely depends upon a maximal rate of incorporation into muscle as glycogen. A normal dietary carbohydrate intake is of the order of $\sim 350 \text{ g}\cdot 24\text{h}^{-1}$ (Office of Population Censuses And Surveys, 1990). The recommended intake for active individuals is ~ 600 to $700 \text{ g}\cdot 24\text{h}^{-1}$ (Coyle, 1991). Carbohydrate over-feeding is disposed of through elevated oxidation and 'de novo' lipid synthesis (Acheson, Schutz, Bessard, Anantharama, Flatt and Jecquier, 1988; Bjorntorp and Sjostrom, 1978). Lipogenesis has the capacity to convert a dietary carbohydrate excess of $\sim 475 \text{ g}\cdot 24\text{h}^{-1}$ into $\sim 150 \text{ g}$ of fat (Acheson et al, 1988). Thus, there is an optimal level of daily carbohydrate ingestion which is consonant with both utilisation rates and a maximal rate of glycogen repletion.

2.8.4 Long-term recovery from submaximal exercise - Optimising the late phase

Daily training at a moderate intensity for longer than 60 min will reduce endogenous carbohydrate stores. Thus, if training is to be effective there is a need to consider replenishing these fuel reserves on a 24-h basis. Extrapolating from the requirements of 'short-term' recovery following a strenuous exercise bout, $\sim 600 \text{ g}\cdot \text{CHO}$ represents a minimum daily intake for active individuals (Costill and Hargreaves, 1992; Coyle, 1991).

Resting muscle glycogen content was restored in a day when a dietary intake of $\sim 600 \text{ g}\cdot 24\text{h}^{-1}$ of carbohydrate was consumed following exhaustive single-legged cycling (Bergstrom and Hultman, 1966a). Similarly, a high-carbohydrate diet providing $809 \text{ g}\cdot 24\text{h}^{-1}$ (ie. $11.0 \text{ g}\cdot \text{kg}^{-1}\text{body wt}$) replenished glycogen stores after 60 min of single-legged cycling ($75\% \dot{V}\text{O}_2\text{max}$) within 24-h (Kochan et al, 1979). MacDougall, Ward, Sale and Sutton (1977) examined glycogen resynthesis after supramaximal cycling

which reduced muscle stores by ~70%. A 'normal' diet, (ie. ~4.0 to 5.0 g·kg⁻¹body wt), provided adequate carbohydrate to replenish muscle within 24-h. However, blood lactate was elevated following the intense exercise bout, and this would provide substrate in the absence of carbohydrate feeding for a considerable degree of glycogen resynthesis. This would largely have been in type IIa and IIb fibres, as fructose 1,6 bisphosphatase activity is low in type-I fibres relative to type-II fibres (McLane and Holloszy, 1979). Hence, the capacity of type-I fibres for converting lactate to glycogen is limited (McLane and Holloszy, 1979).

A programme of daily endurance training will elicit a high energy demand. Pascoe, Costill, Robergs, Davis, Fink and Pearson (1990) reported that a carbohydrate intake equivalent to 5.0 g·kg⁻¹body wt·24h⁻¹ was insufficient to maintain optimal muscle glycogen levels over 3 successive days of hard exercise (ie. 60 min at 75% $\dot{V}O_2$ max). This study did not include an objective assessment of post-recovery performance, such that ratings of perceived exertion provided the only indication of exercise tolerance.

Costill, Flynn, Kirwan, Houmard, Mitchell, Thomas and Park (1988) observed that swimmers ingesting 5.3 g·CHO kg⁻¹body wt 24h⁻¹ experienced difficulty in completing daily training sessions during an intensive 10-d programme, in comparison with swimmers consuming 8.2 g·kg⁻¹body wt·24h⁻¹. However, Kirwan, Costill, Mitchell, Houmard, Flynn, Fink and Beltz (1988) found that an intake as high as 8.0 g·kg⁻¹body wt·24h⁻¹ in runners was still inadequate to prevent a cumulative glycogen depletion over 5-d of hard training (ie. 20 km run at 80% $\dot{V}O_2$ max). As such, an even greater carbohydrate intake may be required to sustain this level of running. Alternatively, there may be a physiological limitation that acts as a metabolic safety mechanism (Kirwan et al, 1988). This limitation is probably operating at a cellular level, and is possibly associated with the capacity to store carbohydrate (Richter, Hansen and Hansen, 1988). Simonsen, Sherman, Lamb, Dernbach, Doyle and

Strauss (1991) examined muscle glycogen content and rowing performance during 4-wk of intense training whilst consuming either a moderate (ie. $5.0 \text{ g}\cdot\text{kg}^{-1}\text{body wt}\cdot 24\text{h}^{-1}$) or a high (ie. $10 \text{ g}\cdot\text{kg}^{-1}\text{body wt}\cdot 24\text{h}^{-1}$) carbohydrate diet. Both diets maintained muscle glycogen and power output, though the high carbohydrate diet was associated with enhanced glycogen resynthesis and a greater mean power output. In a more recent, study Sherman, Doyle, Lamb and Strauss (1993) observed that muscle glycogen was reduced by 30-36% in runners and cyclists consuming a moderate carbohydrate diet (ie. $5 \text{ g}\cdot\text{kg}^{-1}\text{body wt } 24\text{h}^{-1}$) during 7-d of intense training. Whereas, a high carbohydrate diet (ie. $10 \text{ g}\cdot\text{kg}^{-1}\text{body wt}\cdot 24\text{h}^{-1}$) maintained muscle glycogen concentrations, though this did not appear to offer additional training benefits or improve performance in a subsequent bout of high intensity exercise.

A daily carbohydrate intake equivalent to $\sim 9.0 \text{ g}\cdot\text{kg}^{-1}\text{body wt}$ is recommended for endurance sports participants (Costill, 1988). Muscle glycogen stores were replenished within 24-h after a 16.1 km run at $\sim 80\% \dot{V}\text{O}_2\text{max}$ when diets providing 525 to 648 g of carbohydrate (ie. 7.3 to $8.2 \text{ g}\cdot\text{kg}^{-1}\text{body wt}$) were consumed (Costill et al, 1981). Ingesting carbohydrate in frequent feedings did not further improve total muscle glycogen resynthesis over 24-h, in comparison with ingesting the same amount of carbohydrate in fewer but larger feedings (Burke, Collier and Hargreaves, 1993b; Costill et al, 1981).

The studies cited previously investigating glycogen resynthesis have not examined the return in functional capacity along with repletion of the body's fuel reserves. Keizer et al (1987) presents one of the few studies to examine post-recovery exercise performance. The relationship between glycogen restoration and the ability to perform maximal work (ie. maximal physical work capacity, MPWC) was examined. The MPWC of subjects was initially determined during a graded exercise test on a cycle ergometer. This was followed by a bout of exhaustive interval work, after which prescribed carbohydrate-rich diets (ie. $\sim 590 \text{ g}$ or $8.0 \text{ g}\cdot\text{kg}^{-1}\text{body wt}\cdot 24\text{h}^{-1}$) were consumed. Self-

selection of food intake resulted in less effective muscle glycogen repletion in subjects, than was achieved through dietary prescription. This may reflect an appetite suppressive effect of previous exercise, possibly due to increased body temperature and elevated systemic metabolite concentrations. The MPWC of subjects were again determined after 22-h recovery. Despite restoration of muscle glycogen to pre-exercise values, MPWC was impaired by 7.3%. Thus, replenishing muscle glycogen alone was not sufficient to restore MPWC. Changes in glycogen content per se do not appear to result in a return in the force generating capacity of skeletal muscle (Housh, deVries, Johnson, Evans, Tharp, Housh and Hughes, 1990; Young and Davies, 1984), suggesting that other factors may be important for recovery from maximal exercise bouts.

Nevill, Williams, Roper, Slater and Nevill (1993) examined the influence of dietary manipulation on recovery from intense, intermittent exercise. Participants in multi-sprint sports such as field hockey, rugby and soccer, rarely sprint for more than 5 or 6 s⁵. Thus, a group of games players completed 30 maximal 6 s sprints on a non-motorised treadmill. Each sprint was separated by 54 s of walking, and 60 s of jogging at 40% of pre-determined maximal speed. During a ~22-h recovery period, subjects were assigned to one of three groups: a high carbohydrate group (ie. 644 g or 8.7 g·kg⁻¹body wt); a low carbohydrate group (ie. 80 g or 1.1 g·kg⁻¹body wt); or a normal carbohydrate group (ie. 322 g or 4.6 g·kg⁻¹body wt). Subjects repeated the 60 min bout of intermittent exercise following the recovery. Power output did not differ between the groups during the first exercise bout, nor 22-h later. All maximal sprint performances were reduced during the repeated exercise test. Thus, a carbohydrate intake as high as 8.7 g·kg⁻¹ body wt was inadequate for restoring maximal intermittent sprint exercise capacity after 22-h of recovery. Nevill et al (1993) suggested that games players may require a daily carbohydrate intake in excess of ~9.0 g·kg⁻¹ body wt in order to maintain performance.

In an adaptation of Nevill et al's (1993) study, Nicholas,

⁵ Cited Williams (1992) Proceedings of the Second IOC Congress on Sports Science. pp. 99-103

Nuttall, Green, Hawkins and Williams (1993) designed a field-based intermittent exercise model, incorporating the Multi-Stage Shuttle Run Test (Ramsbottom, Brewer and Williams, 1988). Two bouts of intermittent exercise were performed on consecutive days, separated by 22-h recovery. Each bout comprised of two parts: an initial 70 min at varying predicted relative exercise intensities (ie. walk, jog, steady running, and maximal sprint); the second part following a 5 min rest, consisted of exhaustive intermittent running at speeds equivalent to 55% and 95% of predicted⁶ $\dot{V}O_2$ max. During the recovery, one group supplemented their normal diet with additional carbohydrate, increasing their daily intake to 10.0 g·kg⁻¹body wt (CHO group). A control group consumed their normal diet, which was made isocaloric to the CHO diet with additional energy from dietary fat and protein (CON group). Intermittent running capacity was restored in both groups after 22-h, but the CHO group exceeded their performance of the previous day in the second part of the exercise bout, the open-ended run. The difference in open-ended run times under the two dietary conditions was 345 (±100) s. This finding once again demonstrates the performance benefits of a high carbohydrate diet during post-exercise recovery.

Brewer, Williams and Patton (1988) investigated the influence of a high-carbohydrate diet on recovery of endurance running capacity. Subjects were required to run to exhaustion at a speed equivalent to 70% $\dot{V}O_2$ max on two occasions which were separated by an interval of 72-h. The normal diet was supplemented during the recovery with either 'simple' or 'complex' carbohydrates, increasing the daily intake from 4.4 (±1.1) to 7.3 (± 1.5) g·CHO·kg⁻¹ body wt. The high-carbohydrate diets were associated with suppressed plasma FFA concentrations and elevated R-values in comparison with a control-group. Nevertheless, endurance capacity was improved by 25% during the second exercise bout performed 72-h later.

2.8.5 Influence of carbohydrate type

⁶ Predicted from the Multi-Stage Shuttle Run Test

The nature of carbohydrate administered following prolonged exercise also influences the glycogen resynthesis rate (Blom et al, 1987b; Burke, Collier and Hargreaves, 1993a; Costill et al, 1981). Costill et al (1981) did not observe differences in glycogen resynthesis during the initial 24-h of recovery, when diets high in what are classically referred to as either 'simple' or 'complex' carbohydrates were provided. Notably, the complex carbohydrate diet resulted in higher muscle glycogen levels after 48-h of recovery.

As discussed previously, it is of more physiological significance to categorise carbohydrates in terms of the glycaemic index (GI) (Coyle, 1991). Kiens, Raben, Valeur and Richter (1990) examined post-exercise muscle glycogen repletion following ingestion of a diet high in low-GI, fibre rich carbohydrates, in comparison with a diet providing high-GI, low-fibre carbohydrates. A more rapid resynthesis was achieved with the high-GI diet during the first 6-h. This was probably due to faster rates of digestion and absorption (Swan, Davidson and Albrink, 1966), with the glucose load being rendered more readily available during the fast phase of recovery (Coyle, 1991). However, glycogen repletion was independent of carbohydrate type after 20-h recovery, which contrasts the findings of Costill et al (1981).

Thus, supplements providing high-GI carbohydrates are most effective for replenishing the body's glycogen reserves during the immediate post-exercise period (Burke et al, 1993a; Ivy, 1991). However, there are subtle differences in physiological responses to the various forms of simple sugars (Conlee, Lawler and Ross, 1982). For example, glucose and sucrose are twice as effective as fructose for muscle glycogen resynthesis (Blom et al, 1987b). This reflects the different ways in which the body disposes of these sugars (Costill, Craig, Fink and Katz, 1983). Glucose is preferentially metabolised in muscle tissue (Maehlum, 1978; Maehlum et al, 1978), whilst fructose is preferred by the liver (Zakim, Herman and Gordon, 1969). This has been demonstrated during intravenous infusion studies in

which blood glucose concentrations are maximised (Blom, 1989b). Fructose infusion increased liver glycogen content to levels four times higher than those achieved following glucose infusion (Nilsson and Hultman, 1974). Whereas, glucose infusion achieved higher muscle glycogen concentrations (Bergstrom and Hultman, 1967).

Sucrose is a disaccharide made up of equimolar fractions of glucose and fructose (Ivy, 1991). The physiological response to sucrose ingestion may reflect an hepatic inhibition of glucose uptake by the presence of fructose. In this way, a larger fraction of the total glucose absorbed is rendered available for muscle metabolism (Blom et al, 1987b). Thus, substrate is provided for both liver and muscle glycogen resynthesis (Blom et al, 1987b). As such, sucrose appears to be an ideal carbohydrate for optimising short-term recovery from glycogen depleting exercise, simultaneously repleting two major carbohydrate depots in the body.

Bovens, Keizer and Kuipers (1985) demonstrated that isocaloric liquid and solid carbohydrate feedings do not differentially influence muscle glycogen resynthesis over the initial 5-h of recovery from exhaustive cycling. Keizer et al (1987) further examined the influence of carbohydrate form on post-exercise recovery. A liquid carbohydrate diet providing 577 g (ie. 7.8 g·kg⁻¹body wt), and a solid carbohydrate diet providing 602 g (ie. 8.2 g·kg⁻¹body wt), were prescribed during 22-h recovery. There were no differences in muscle glycogen repletion between the two dietary treatments. Similarly, Reed et al (1989) observed that despite differences in blood glucose and insulin response, post-exercise glycogen resynthesis was similar when 3.0 g·CHO kg⁻¹body wt were administered in either liquid or solid forms.

2.9 Exercise-induced tissue damage and recovery

It is possible that muscle functional capacity is impaired to a greater extent following eccentric exercise (ie. tension development during muscle fibre lengthening) in comparison with concentric exercise (ie.

tension development during muscle fibre shortening) (Armstrong, Ogilvie and Schwan, 1983; Clarkson, Byrnes, McCormick, Turcotte and White, 1986; Newham, Mills, Quigley and Edwards, 1983b). A difference has also been observed in post-exercise muscle glycogen replenishment, where prior eccentric exercise exerts a more persistent inhibitory effect upon the recovery process (Doyle, Sherman and Strauss, 1993). The running action is made up of both eccentric and concentric components, and is associated with muscle fibre ultrastructural disturbances (Hikida, Staron, Hagerman, Sherman and Costill, 1983; Sherman et al, 1983).

Hikida et al (1983) examined muscle fibre integrity immediately prior and for 7-d following a marathon race. Signs of tissue damage were apparent even in resting samples, which reflected the rigorous nature of pre-race training. However, muscle fibre necrosis and inflammation were much greater in post-race samples, and resulted in the spilling of cellular contents into extracellular and extravascular spaces. This may have resulted from ionic imbalances within the cell. Changes in intracellular calcium ion concentrations have been implicated in reduced tension development and a delaying of muscle fibre relaxation (Allen, Lee and Westerblad, 1989). This partially results from elevated calcium ion concentrations activating neutral proteases, which in turn may be responsible for Z-line breakdown (Busch, Strommer, Goll and Suzuki, 1962; Hikida et al, 1983). Such disturbances become manifest in the clinical symptoms of rhabdomyolysis and myoglobinuria, which were most pronounced during the first 3-d of recovery (Hikida et al, 1983).

The development of post-exercise tissue damage follows a biphasic pattern (Newham, McPhail, Mills and Edwards, 1983a). Initially, the mechanical trauma of prior exercise is paramount, and is associated with an absence of pain but a reduced ability to generate force. The later phase is characterised by increasing muscle soreness and chemically-mediated tissue damage. This pattern reflects the time course of delayed creatine kinase (CK) release (Armstrong et al, 1983; Jones, Newham, Round and Tolfree, 1986).

Pascoe et al (1990) failed to demonstrate evidence of fibre injuries after 60 min of running (75% $\dot{V}O_2$ max). Leukocyte infiltration was negligible and blood CK concentrations were low, providing no evidence of muscle damage. It is possible that training status may influence the susceptibility of individuals to mechanical trauma, which would explain Pascoe et al's (1990) observations in highly trained athletes (Hikida et al, 1983; Newham, Jones and Edwards, 1986). O'Reilly et al (1987) examined the effects of eccentric cycling in untrained subjects. Exercise reduced muscle glycogen by ~61% and was associated with myofibrillar tearing and oedema. After 10-d on a normal diet (ie. a carbohydrate intake equivalent to ~54% of total energy) muscle fibre necrosis and inflammatory cell infiltration was still evident. Thus, the recovery process in terms of tissue repair is relatively slow (Hikida et al, 1983; Jones et al, 1986). Additionally, the heterogeneity of human muscle also appears to be influential, with type-II fibres being more susceptible to damage (Jones et al, 1986).

Muscle damage incurred during eccentric exercise impairs glycogen resynthesis (O'Reilly et al, 1987; Costill et al, 1990; Sherman et al, 1983). This is a delayed response and reflects the biphasic nature of events (Widrick, Costill, Fink, McConell, Anderson, Pearson and Zachwieja, 1992). Costill et al (1990) suggest that impaired glycogen resynthesis is partly attributable to an infiltration of damaged muscle by inflammatory cells. These have been shown to increase glucose metabolism by the release of as yet unknown soluble factors (Shearer, Amaral and Caldwell, 1988). Reduced glycogen storage may reflect substrate competition between inflammatory cells and muscle fibres. Improving substrate availability by increasing dietary carbohydrate intake may alleviate this situation (Costill et al, 1990; Doyle et al, 1993). However, the time course of impaired glycogen accumulation does not closely parallel that of inflammatory cell infiltration (Widrick et al, 1992).

Alternatively, reduced muscle glycogen resynthesis may result from a failure of the cellular glucose transport mechanism (Kirwan et al, 1992; Lash et al, 1987). Tissue damage associated with eccentric exercise appears to impair the responsiveness of muscle cells to insulin, whilst disturbances of the sarcolemma may directly interfere with

glucose transport (O'Reilly et al, 1987). A resultant decrease in cellular glucose availability would reduce muscle glycogen replenishment, and hence delay recovery.

2.10 Towards an understanding of the limitations to recovery

Fatigue during prolonged, constant pace running ultimately results from a mismatch between ATP utilization and ATP resynthesis. This imbalance in energy metabolism is not necessarily accompanied by an observable disturbance in cellular ATP concentrations, nor in the concentrations of products resulting from ATP hydrolysis (Green, 1991). The physiological availability of an appropriate fuel for maintaining cellular ATP flux is of paramount importance if exercise is to continue. Whereas, replenishment of these fuel reserves following exercise is essential if functional capacity is to be restored.

The following studies have attempted to further clarify our understanding of recovery from prolonged, constant pace running, and the inherent limitations of what is an extremely complex process.

General Methods

The specific procedures followed in each study are briefly described in the methods section of each experimental chapter. Common methods are reported below.

All procedures were approved by the Ethical Advisory Committee of Loughborough University and were carried out in accordance with the 'Code of Practice for Workers having Contact with Body Fluids'. Before receiving written consent to participate in the studies, subjects were informed of the demands that would be placed upon them and possible risks and discomforts. Subjects were given every opportunity to ask for further information and for clarification of the tests to be performed. Subjects were also required to complete a medical history questionnaire and provide general details of their running ability (Appendix A). Subjects with diabetes mellitus or any other medical condition potentiating an undue personal risk or introducing bias were excluded.

3.1 Apparatus and instrumentation

Studies were performed on a motorised treadmill (Quinton, Model 24-72), which had a dual speed range of either 2.4 to 24.2 km·h⁻¹, or 4.0 to 40.2 km·h⁻¹. The lower range was selected for consistency with previous work carried out in this laboratory. The treadmill elevation ranged from 0 to 40%, which fulfilled the requirements of all experimental protocols.

Before commencing each study the treadmill speed calibration was validated and the reliability of the analogue speedometer confirmed. The treadmill belt length was measured and time recorded for the belt to complete fifty revolutions at various speeds spanning the experimental range. The total distance covered by the belt during each set of fifty revolutions was determined and the actual treadmill speed calculated.

The treadmill was linked to a microcomputer (BBC Master series), which was in turn interfaced with a switchable 40/80 track single disc

drive (Akhter Instruments Ltd, Type DS80TK) and a printer (Canon PW 1080-A). Using software developed in the department (© DG Kerwin 1988, Department of Physical Education, Sports Science and Recreation Management, Loughborough University) performance data from the treadmill was continuously monitored and recorded.

Heart rate (HR) and ECG profiles were also continuously monitored throughout the preliminary tests and experimental trials. These were displayed on a cardiometer (Rigel, Model 302), which was at all times visible to the experimenter. The cardiometer was interfaced with the computer to provide a permanent record of HR.

Electrical signals proportional to the treadmill speed and the cardiac responses of the subject enter two separate computer channels. Sampling took place at two levels. The first level, or 'inner loop', sampled data over intervals of 0.4s at a frequency of 50 Hz. Mean values for each sampling interval were calculated for the two channels. These averaged signals were processed by an analogue to digital converter (ADC) such that data is presented in a numerical form. The second level of sampling, or 'outer loop', overlies this continuous cycle of data retrieval and processing. The sampling interval in this case is pre-set by the experimenter (eg. 10s, 15s, or 30s). On completion of the outer loop the data displayed on the monitor was printed to provide a hard-copy. These two levels of data collection act to smooth data sampling.

Speed data was collected relative to time. Thus, distance completed by the subject was calculated and displayed on the monitor. The monitor display (providing information on run time, distance, speed, running pace, and heart rate) was updated every second.

3.2 Subjects and laboratory procedures

Prolonged running is extremely demanding and the exhaustive runs reported in this thesis warranted tremendous commitment and motivation on the part of the subjects. As such, men and women familiar with endurance exercise were accepted as volunteers. All regularly participated in submaximal running with weekly training

distances ranging from 35 to 100 km.

3.2.1 Height and mass

Height was measured using a wall mounted stadiometer with a maximum range of 200.00 cm and accurate to ± 0.01 cm (Holtain Ltd). Subjects were weighed on each visit to the laboratory using balance scales (Avery Ltd, Model 3306BV) with a capacity of 120 kg and accurate to ± 0.05 kg. Before each preliminary test subjects were weighed in light-weight running kit and bare feet. Nude body mass was measured before and after each long run, subjects having previously towelled down to remove all surface moisture.

3.2.2 Monitoring of heart rate

Four self-adhesive Ag/AgCl disposable chest electrodes (Red Dot 3M UK Ltd, type 2255) were positioned prior to exercise for monitoring HR. The first was located at the top of the sternum. Two more were located at either side of the rib cage, 15 cm below the level of the first. Finally, the fourth electrode was located in close proximity to the acromion process of the shoulder and functioned to earth the subject whilst running on the treadmill. Each site was abraded, degreased and an electrolyte gel applied to ensure good contact prior to adhering the electrodes. Additional tape was required to maintain electrode contact during the long runs as profuse sweating was found to lift the plates and disrupt the signal.

3.2.3 Expired air collection and analysis

Expired air was collected using Douglas bag techniques. Subjects were presented with a noseclip (Harvard Equipment) and mouthpiece (Harvard Equipment) 45s before each collection was due to be taken. This ensured evacuation of 'dead-space' with expiratory air. The mouthpiece communicated with a 150 l capacity Douglas bag (Harvard Equipment) via a lightweight two-way valve (Jakeman and Davies, 1979), a 1.5m

length of wide-bore (30mm) lightweight tubing (Fulconia; Baxter, Woodhouse and Taylor) and a two-way tap (Harvard Equipment). Thus, a closed-circuit was formed when the nose-clip and mouthpiece were correctly worn, allowing expired air to be collected over a measured time interval.

The percentage oxygen content of expired air was measured by a paramagnetic oxygen analyser (Taylor Servomex, Model 570A). This operates on the basis of the susceptibility of oxygen to a paramagnetic gas. Oxygen concentration accurate to $\pm 0.1\%$ was given as a digital readout. The percentage carbon dioxide of expired air was measured by an infra-red carbon dioxide analyser (Lira, Model 303; Mines Safety Appliances Ltd). This has an analogue readout from which carbon dioxide concentrations were calculated with reference to a calibration curve. The final value obtained was accurate to $\pm 0.01\%$. Both analysers were calibrated against nitrogen, a calibration gas, and room air immediately prior to each series of gas analyses. The analysers were found to be stable over 8-h provided barometric pressure remained stable. Nevertheless, analysers were checked each hour and adjusted if necessary.

A Harvard digital dry gas meter was used to determine gas volumes. This had previously been calibrated using a 6001 Tissot Spirometer (Collins Ltd, USA). The temperature of expired air was monitored as each bag was evacuated by a thermistor probe (Edale type 2984, Model C). This was fitted on the inner surface of the air inlet pipe connecting the Douglas bag to the gas meter. The analogue readout of the thermistor was calibrated prior to each set of analyses against two standard settings of 25°C (position A) and 50°C (position B).

Respiratory values were standardised for temperature, atmospheric pressure and water vapour content using software developed in the department (© Dr HKA Lakomy, Department of Physical Education, Sports Science and Recreation Management, Loughborough University).

3.2.4 Estimation of energy expenditure

Energy expenditure was estimated under steady state conditions by indirect calorimetry, using open circuit spirometry. The proportions of energy derived from carbohydrate and fat were estimated from the non-protein respiratory exchange ratio (R) value. This assumes that the contribution of protein to energy metabolism is relatively small (Consolazio, Johnson and Pecora, 1963).

The following method for calculating energy expenditure by indirect calorimetry is adapted from McArdle, Katch and Katch (1981):

The oxidation of 1.0 g of carbohydrate uses 0.828 l of oxygen, and produces 0.828 l of carbon dioxide and 17 kJ of energy. The oxidation of 1.0 g of fat uses 1.989 l of oxygen, and produces 1.419 l of carbon dioxide and 39 kJ of energy. Whole body oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) is calculated from expired air analyses. Rates of carbohydrate (x g·min⁻¹) and fat (y g·min⁻¹) oxidation can be determined using simultaneous equations:

$$\dot{V}O_2 = 0.828x + 1.989y \quad \dots \quad (i)$$

$$\dot{V}CO_2 = 0.828x + 1.419y \quad \dots \quad (ii)$$

Solving for 'x' and 'y' by subtracting (ii) from (i), gives rise to the following equations of energy metabolism:

$$x = \frac{\dot{V}O_2 - (y * 1.989)}{0.828} \quad \text{where, } y = \frac{\dot{V}O_2 - \dot{V}CO_2}{0.570}$$

Thus, total energy expenditure is given by:

$$\text{Energy expenditure} = [(x * 17) + (y * 39)] \text{ kJ}\cdot\text{min}^{-1}$$

3.2.5 Measurement of body temperature

Four interchangeable thermistor probes (Edale, type EU) were used to measure skin temperature. These were taped to the calf, thigh, upper arm and chest as described by Mitchell and Wyndham (1969). Appropriate siting and methods of adhesion were established through pilot-testing such that reliable temperature profiles were recorded during the exercise bouts. Weighted mean skin temperatures (T_{sk}) were calculated from values recorded at the four sites (Ramanathan, 1964), and calculated as follows:

$$T_{sk} = 0.3 (T_{chest} + T_{arm}) + 0.2 (T_{calf} + T_{thigh})$$

In Chapter 4 an aural probe (Edale) monitored tympanic temperature (T_{ty}), whereas in Chapters 5, 6, and 7, a rectal probe (Edale) was preferred to monitor body temperature (T_{rec}), providing a more representative indicator of changes in deep body temperature (Nadel and Horvarth, 1970; Nielsen and Nielsen, 1965). The aural probe, which was inserted through a small plastic ear piece, was placed directly onto the tympanum by the subject (Nadel and Horvarth, 1970). The ear was then insulated by a small gauze pad and secured with strips of tape over the outer ear. The rectal thermistor probe was inserted to a depth of 10 cm beyond the external anal sphincter muscle (Nielsen and Nielsen, 1962).

Core temperature can be estimated from hypothalamic, tympanic membrane, ear canal, oral, esophageal, rectal, or central blood temperatures (Gisolfi and Wenger, 1984). Tympanum (T_{ty}), oesophageal (T_{es}), and rectal (T_{rec}) temperature measurements are the most commonly used. Nadel and Horvarth (1970) compared T_{ty} and T_{rec} over a range of ambient temperatures. The T_{ty} estimate responded more rapidly than T_{rec} to transient changes in deep body temperature, providing a suitable measure during intensive dynamic activity. However, T_{ty} was influenced by environmental factors during steady state activity and was consistently lower than T_{rec} . In contrast,

T_{rec} was largely independent of environmental influences, being relatively stable during the early stages of steady state activity.

The response of each probe was validated over the physiological range using a water bath prior to each study. Temperatures were monitored during each study by two electronic thermometers (Edale Instruments, Model C) with ranges of 25°C to 45°C (increments of 0.2°C) and 0°C to 50°C (increments of 0.5°C) both accurate to $\pm 1.0\%$ of the full scale.

3.2.6 Estimation of whole body rehydration

Whole body rehydration was estimated according to the method of Gonzalez-Alonso, Heaps and Coyle (1992). The percent gain in body weight over the recovery period relative to weight loss during the previous exercise bout provided an index of rehydration. Percentage rehydration represented the amount of ingested fluid that was retained within the body after the specified recovery period, and was calculated as follows:

$$\% \text{ Rehydration} = \frac{[(BW_{pre} - BW_{post}) - (BW_{pre} - BW_{rec})]}{\text{Fluid intake (kg)}} * 100$$

Where, BW_{pre} = pre-exercise body weight (kg)

BW_{post} = post-exercise body weight (kg)

BW_{rec} = body weight following recovery (kg)

3.2.7 Subjective ratings of exertion

Perceptions of fatigue during the preliminary tests and the experimental trials were measured using the fourteen point Borg scale (Borg, 1973). In addition, the ten point category-ratio scale was applied during Chapters 5, 6, and 7 (Noble, Borg, Jacobs, Ceci and Kaiser, 1983). Both were shown to subjects during expired air collections, and their responses recorded.

The Borg scale is a linear rating scale graded from 6 to 20, where 7 is anchored by the expression 'Very, very light' and 19 is anchored by the expression 'Very, very hard'. Responses to the Borg scale have been shown to correlate with relative exercise intensity and heart rate (Borg, 1973).

The category-ratio scale is a curvi-linear scale graded from 0 to 10, which is anchored by the expressions 'nothing at all' and 'very, very strong' respectively. It was developed to assess perceptions of fatigue associated with non-linear physiological phenomena such as breathing difficulties, muscular aches, and pain (Borg, 1982). Responses in excess of 10 are recorded as 'maximal'. Ratings from the category-ratio scale correlate with the time course of exercise induced glycogenolysis, values increasing with lactate accumulation in blood and muscle tissue (Noble et al, 1983).

3.2.8 Environmental conditions

Ambient conditions in the laboratory were carefully monitored during all experimental trials, and controlled where possible. Wet and dry bulb temperatures were measured using a whirling hygrometer (Brannan Thermometers Ltd), which was operated in close proximity to the treadmill. Relative humidity was calculated from these values using a sliding scale. Barometric pressure was measured using a wall mounted barometer (Griffen and George).

3.3 Dietary analyses

Before commencing each study, subjects weighed and recorded their food intake over a continuous seven-day period (Anonymous, 1991). Analyses of the nutritional content of their normal diets were made from these food diaries (Paul and Southgate, 1978). This provided guidance for the preparation of individual dietary prescriptions and dietary manipulations where necessary.

Dietary analysis was based upon the weighed-food intake method (Marr, 1971). Subjects recorded the time of each meal and where the meal was consumed. In describing the food subjects were requested to provide as much detail as possible. Pertinent information to be included in the description would be: the brand name of food where possible; whether the food was fresh, frozen, dried or canned (ie. how the food is preserved and stored); details of the preparation (eg. the type of fat used in frying). The weight of each serving was determined using precalibrated digital scales, as was the weight of left-overs. Hanson electronic scales were used in Chapters 4 and 6, with a capacity of 2.0 kg and accurate to ± 2.0 g. In Chapter 7, EKS (Model 1002) electronic scales were used, which similarly had a maximum capacity of 2.0 kg and accurate to ± 2.0 g.

Each food item was coded in accordance with the index of foods in the MAFF/MRC Food Composition Tables (Paul and Southgate, 1978). The weight of food consumed and its item code was entered into a dietary analysis programme. This was originally devised by Dr John Challis (© Department of Physical Education, Sports Science, and Recreation Management, Loughborough University). An updated version was later developed by Dr Juliet Wiseman also formerly of this department. This was run using an Apple Macintosh-Plus computer interfaced with a Rodime 45-Plus hard drive.

Dietary prescriptions were made in accordance with each subject's normal intake of carbohydrate, fat and protein.

Chapter 5 was the exception to the above. Dietary control was achieved by recording the items consumed and approximate portion sizes in a food diary during the 48-h prior to the first experimental trial. Subjects then followed the same diet during the 48-h prior to the second trial. This system of dietary recall developed by Dr Juliet Wiseman was found to be reliable providing that subjects received careful instruction in describing portion sizes.

3.4 Preliminary Testing

Subjects were initially familiarised in the laboratory for 2-3 weeks. During this time they were introduced to running on a motorised treadmill, the laboratory setting, and the experimental protocols. In addition, three preliminary tests were completed determining the oxygen cost of submaximal running, maximal oxygen capacity ($\dot{V}O_2\text{max}$), and the relationship between oxygen consumption and blood lactate concentration during submaximal running.

3.4.1 Familiarisation

During the first visit to the laboratory subjects were thoroughly familiarised with running on a motorised treadmill. This was in the form of a progression, commencing with a walk, which was extended into a jog, and eventually into a run. Stopping procedures were explained and practised such that subjects felt confident and safe during all exercise bouts.

Subjects were instructed in the standard laboratory methods for sampling expired air and monitoring heart rate. In addition, they were introduced to the Borg Scale of perceived exertion, the Category Ratio Scale of muscular effort (Chapters 5-7), and the Gut Fullness Scale⁷ (Chapter 7).

3.4.2 Establishing a speed- $\dot{V}O_2$ relationship

The first performance test determined the oxygen cost of running over a range of submaximal speeds (speed- $\dot{V}O_2$ test). The speeds were selected with reference to each subject's running ability and lay between 60 and 90% $\dot{V}O_2\text{max}$. Actual speeds ranged from 2.79 to 5.04 m·s⁻¹ with increments of 0.45 m·s⁻¹. This was a continuous test with subjects running for 4 min at four different speeds. Expired air collections were made over the last minute of each stage (ie. 3-4, 7-8, 11-12, and 15-16 min) and analysed as described previously. Percentage oxygen and carbon dioxide concentrations were measured in each

⁷Stensel DJ (1988) Unpublished MSc thesis, Loughborough University

sample as was the total volume of expired air collected. Thus, rates of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were determined using the Haldane transformation, and the minute ventilation volume ($\dot{V}E$) was calculated. Responses to the Borg scale were recorded during each collection. By applying linear regression to the four co-ordinate values of $\dot{V}O_2$ and running speed, individual relationships were established for each subject.

3.4.3 Determination of maximal oxygen uptake ($\dot{V}O_{2max}$)

Maximal oxygen uptake was determined during continuous, incremental-grade uphill running. The method used was a modification of the Taylor treadmill test (Taylor, Buskirk and Henschel, 1955). The treadmill was initially set at a grade of 3.5%, and was subsequently increased by 2.5% every 3 min. Subjects ran at a constant submaximal speed throughout the test, and 1 min expired air collections were taken over the third min of each stage. Subjects aimed to run for as long as possible. A final expired air collection was taken when subjects felt that they could only maintain the required exercise intensity for one more minute. From this collection $\dot{V}O_{2max}$ was calculated. Criterion establishing a valid $\dot{V}O_{2max}$ test include: an R value greater than 1.15; plateauing of $\dot{V}O_2$ and $\dot{V}E$ values; and a heart rate which approximated a predicted maximal value (Astrand and Rodahl, 1986).

3.4.4 Establishing the speed-lactate relationship

The speed-lactate test established a relationship between exercise intensity and capillary blood lactate concentration. Subjects arrived at the laboratory having fasted overnight or in a 4-h post-absorptive state. After sitting quietly for 15 min, a pre-exercise capillary blood sample was taken for determining a base-line blood lactate concentration. The subject was then made ready for the test. Four speeds were calculated from data obtained during the previous tests (Ramsbottom, Nute and Williams, 1987), such that the respective $\dot{V}O_2$ demands of the

exercise bouts were equivalent to 60% to 90% of $\dot{V}O_2$ max. Capillary blood samples were taken at the end of each 4 min stage. Samples were analysed for blood lactate concentration as described in Section 3.7.1.

3.4.5 Assessing training status

Training status was assessed from the speed- $\dot{V}O_2$ and speed-lactate tests. Blood lactate concentrations during submaximal exercise are closely related to running performance (Jacobs, 1986). The running speed (ms^{-1}) and % $\dot{V}O_2$ max of subjects at blood lactate concentrations of $2 \text{ mmol}\cdot\text{l}^{-1}$ and $4 \text{ mmol}\cdot\text{l}^{-1}$ were determined. The former reference value is equivalent to the blood lactate concentration of endurance athletes at a self-selected running speed (Williams, Brewer and Patton, 1984), whilst the latter is equivalent to the 'onset of blood lactate accumulation' (OBLA) (Astrand and Rodahl, 1986). Thus, assessing exercise capacity at these two reference blood lactate concentrations provides a method for evaluating aerobic fitness (Williams, 1990).

3.5 Standardised test procedure

Run 1 in Chapters 4, 6 and 7 (Figure 3.1), and the long run of Chapter 5 (Figure 3.2), were performed early in the morning. Subjects arrived at the laboratory after an overnight fast of 10-h. They emptied their bladders before any pre-test measurements were made. Subjects were weighed nude before and after each run. Four ECG electrodes were positioned immediately before exercise for monitoring heart rate, and skin temperature probes were taped to the calf, thigh, upper arm and chest. An index of changes in body temperature was provided by either an aural (Chapter 4) or a rectal (Chapters 5, 6 and 7) thermistor probe.

Pre-exercise venous and capillary blood samples were taken as described in Section 3.7.1 and 3.7.2. Further capillary blood samples were taken during each exercise bout, and venous and capillary blood samples were taken at the end of the exercise bout.

Resting expired air collections were taken in Chapters 6 and 7 which allowed pre-exercise metabolic rate to be determined. A standardized 5 min warm-up at 60% $\dot{V}O_2$ max was then performed. The treadmill speed was increased to the test pace equivalent to 70% $\dot{V}O_2$ max immediately following the warm-up. In Chapters 4, 6 and 7 the first run was a standardized endurance task in which subjects were required to run for 90 min, or until volitional fatigue, whichever was reached first (R_1). Volitional fatigue was defined as the point at which the required running pace could not be maintained. During the second run subjects were required to run for as long as possible to assess endurance capacity (R_2). All subjects were highly motivated and were instructed to give a maximal effort. In order that subjects might more accurately assess their level of fatigue during the latter stages of each run they had the option of reducing their running pace for a period of 2 min on one occasion, after which the prescribed speed was resumed. When subjects needed to reduce the treadmill speed a second time the test was ended.

Expired air collections were taken during each exercise bout from which $\dot{V}E$, $\dot{V}O_2$ and $\dot{V}CO_2$ were determined and the respiratory exchange ratio (R) value calculated. Simultaneously, subjective ratings of perceived exertion (Chapters 4, 5, 6 and 7) and muscular effort (Chapters 5, 6 and 7) were obtained.

The laboratory was maintained at 20°C and subjects were cooled whilst running on the treadmill by electric fans. Wet sponges and drinking water were available for use *ad libitum* (Chapters 4, 6 and 7 only). The total fluid ingested during exercise was recorded at the end of each run and accounted for in post-exercise changes in body weight. Subjects maintained a constant level of training from commencing the preliminary tests until the completion of the experimental trials. In addition, subjects refrained from strenuous activity over the two days prior to the experimental day.

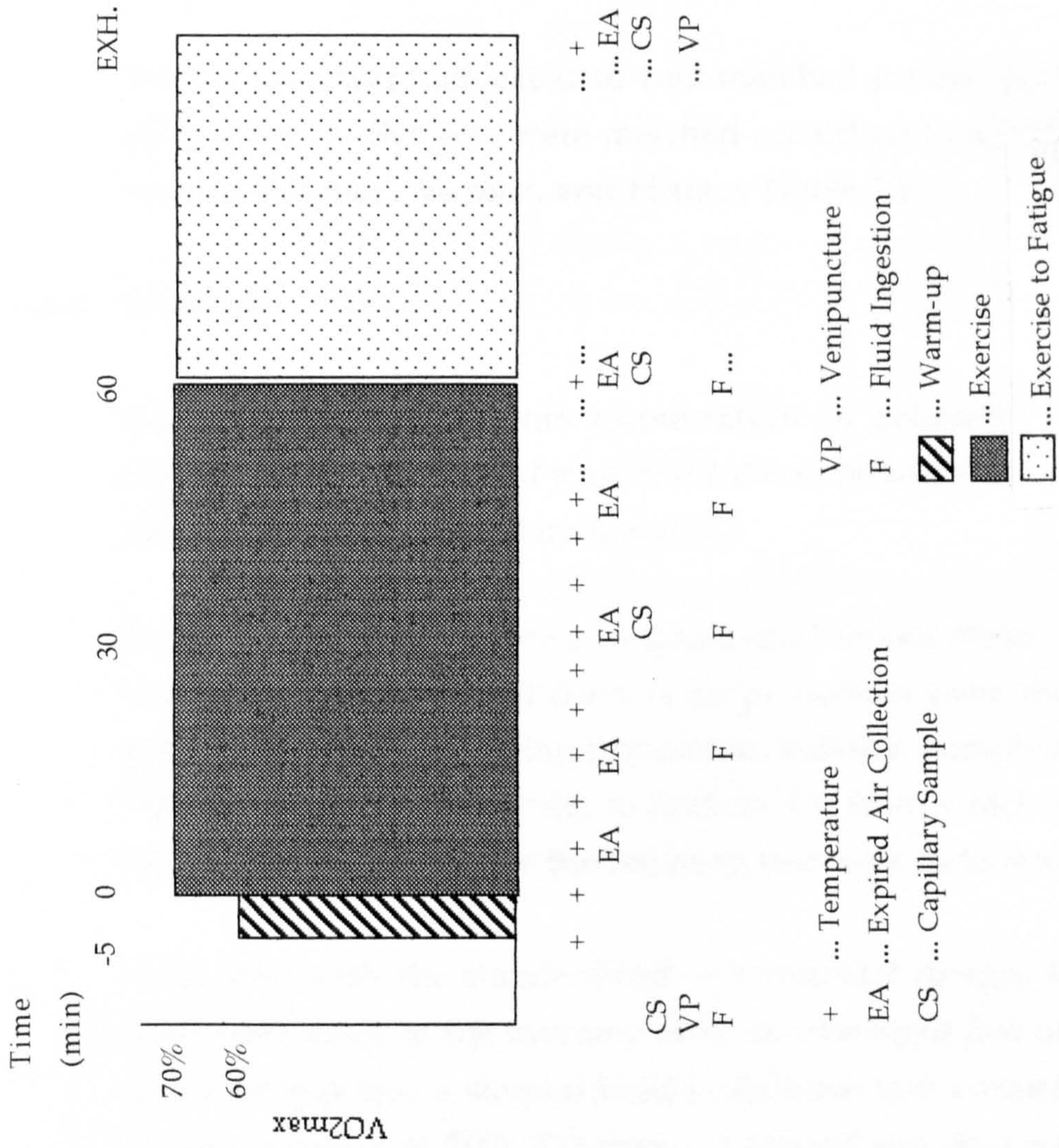


Fig 3.2 Schematic representation of the experimental procedures (NF vs FR trials)

3.6 An examination of the reliability of the experimental procedure

To examine the reliability of the recovery experimental procedure, the following data were retrospectively collated from Chapters 6 and 7. Male subjects who completed R_1 (ie. 90 min run at 70% $\dot{V}O_{2\max}$) were matched according to preliminary test measurements and physiological data relating to R_1 performance. Run times for R_2 were examined as a means of assessing reliability.

3.6.1 Subjects

Twelve men were allocated to two matched groups (group A and group B). Subjects were matched according to age, height, weight, $\dot{V}O_{2\max}$, $V_E \max$, and HR_{\max} (Table 3.1).

3.6.2 Protocol

The data reported in this examination of reliability were collected after subjects had completed the standardised preparations as reported in Chapters 4, 6 and 7.

In brief, subjects completed weighed-food intake dietary analyses from which normal diets were prescribed over the 48-h prior to the test. After familiarisation, subjects completed the three preliminary tests (refer to Section 3.4) from which appropriate running speeds for the recovery test were determined.

Consistent with the standardised experimental design, R_1 was performed early in the morning after an overnight fast of 10-h. This first run was a standardised endurance task consisting of 90 min running at 70% $\dot{V}O_{2\max}$. A second run (R_2) was performed 4-h later. During the recovery between R_1 and R_2 subjects in both groups ingested a 6.9% glucose-polymer solution immediately following R_1 and 2-h later. Each feeding provided the equivalent of 1.0 g·CHO·kg⁻¹body wt. During R_2 , subjects were required to run for as long as possible to assess the return of endurance capacity for each group. Expired air samples were collected during R_1 and R_2 , and subjective

ratings of perceived exertion and muscular effort were obtained.

Wet sponges and drinking water were available during exercise. The total fluid ingested was recorded at the end of each run and accounted for in post-exercise changes in body weight. Only the prescribed fluid was ingested during the recovery period.

Recovery performance times (R_2) were compared by a Mann-Whitney U test for small samples.

3.6.3 Results

Both groups completed the 90 min endurance task (R_1). The R_2 run time of group A was 56.1 (± 9.8) min, and the R_2 run time of group B was 56.8 (± 5.8) min (NS).

3.6.4 Conclusion

The submaximal exercise procedure adopted in the studies and reported in this thesis (ie. a standardised endurance task, a controlled recovery period, and an open-ended steady state performance test) provides a reliable measure for assessing recovery from an endurance activity.

Table 3.1 Physiological characteristics of subjects in group A and group B (mean \pm SE)

	Age (yrs)	Height (cm)	Weight (kg)	$\dot{V}_{E\max}$ (l \cdot min $^{-1}$)	HR max (b \cdot min $^{-1}$)	$\dot{V}O_{2\max}$ (ml \cdot kg $^{-1}$ min $^{-1}$)
A	29.4	174.6	67.5	116.5	192	57.3
\pm SE	3.8	1.9	2.2	5.1	4	2.5
B	31.6	178.8	73.4	126.5	185	60.6
\pm SE	3.0	3.6	2.4	7.5	6	1.6

3.7 The collection and analysis of blood samples

The characteristics of all commercial kits used in the biochemical analysis of blood samples were initially examined in the laboratory over a physiological range of standard concentrations. From these procedures the reliability and validity of micro-methods were ascertained. Assays were then performed with greater precision on smaller sample volumes.

3.7.1 Capillary blood samples

Capillary blood samples (20 μ l) were taken in duplicate from the thumb using micro-pipettes, the hand being pre-warmed for resting collections. Samples were immediately deproteinised in 200 μ l of cold 0.38 mM perchloric acid prior to centrifugation (Eppendorf, Model 5414) and storage at -20°C . These were later analysed for blood glucose and blood lactate.

Blood glucose concentration was determined by photometric analyses on 20 μ l aliquots of perchloric acid extract using the G.Od period method (Boehringer Mannheim GmbH Diagnostica, Appendix B). Blood lactate concentration was determined by fluorimetric analyses (Locarte, Model 8-9) on 20 μ l aliquots of perchloric acid extract using a method adapted from Maughan (1982) (Appendix C). A coefficient of variability of less than 3.0% (or a regression equation of correlation 0.999) on the standards was achieved before commencing sample analyses. The lactate standards ranged between 0.5 and 15.0 $\text{mmol}\cdot\text{l}^{-1}$, which accommodated the sample values measured in the studies reported in this thesis. The accepted coefficient of variation for these two assays was 2.0%.

3.7.2 Venous blood samples

Venous samples were drawn from an antecubital vein in the forearm.

From the venous blood samples, 1.5 ml aliquots were dispensed into heparinised Eppendorf tubes and immediately centrifuged for 5 min (Eppendorf, Model 5414). The plasma was removed and stored at -70°C , and analysed for ammonia (Boehringer Mannheim GmbH Diagnostica, Appendix D) within 48-h (Chapter 4) or within 24-h (Chapters 5, 6 and 7) (Tsintzas and Wilson, unpublished observations). This same plasma sample was also analysed for sodium and potassium by flame photometry (Ciba Corning, Model M435) (Appendix E).

The major fraction of each venous blood sample was dispensed into lithium heparin tubes to prevent coagulation. Two 20 μl aliquots of blood were drawn from each sample using calibrated micro pipettes (Acupette Pipettes, Scientific Industries Ltd) and mixed with 5.0 ml of Drabkins Reagent (Boehringer Mannheim GmbH Diagnostica). Thus, haemoglobin concentration was photometrically determined by the cyanmethaemoglobin method (Appendix F). Triplicate 50 μl samples of whole blood were drawn from each sample using heparinised pipettes. Following micro-centrifugation for 15 min at 11 000 $\text{rev}\cdot\text{min}^{-1}$ (Hawksely Ltd), packed cell volume was measured using a sliding haematocrit reader (Hawksley Ltd). Percent changes in plasma volume from rest were estimated by the method of Dill and Costill (1974), which is based upon changes in haemoglobin concentration and packed cell volume.

Serum was obtained in Chapters 5, 6 and 7. A 3 ml aliquot of whole blood was left to clot for 1-h and then centrifuged at 3°C for 15 min. Insulin and cortisol are reported to be stable in serum stored at -20°C for 6 months (Dr. J. Burrin, The London Hospital Medical College, personal communication) .

Plasma was obtained by centrifugation of the remaining whole blood at 6000 rpm for 15 min at 3°C (Burkard μP Koolspin). The operational range of the Koolspin was 100 to 99 000 $\text{rev}\cdot\text{min}^{-1}$. Aliquots were stored at -20°C and later analysed for FFA (method-i Chromy, Gergel, Voznicek, Krombholzova and

Musil, 1977; Noma, Okabe and Kita, 1973; method-ii Wako Chemicals GmbH), glycerol (Laurell and Tibling, 1966), and urea (Boehringer Mannheim GmbH Diagnostica). These assays are detailed in Appendices G - J). Serum insulin concentrations were determined by radioimmunoassay (Soeldner and Slone, 1965), and cortisol concentrations were determined using a kinetic enzyme immunoassay system (Euro/DPC Ltd, Llanberis, Gwynedd, UK). All hormone analyses were performed by Dr. J. Burrin of The London Hospital Medical College, where the coefficients of variation were reported to be 5.6% for serum insulin and 3.6% for serum cortisol.

3.8 The collection and analysis of sweat samples

Sweat was sampled in the male subjects from the scapula region at the end of R₁ and R₂ in Chapter 6. A pre-measured sampling area (5cm x 5cm) was wiped dry during the final minute of exercise. Five collections were made, each of 1 min duration, over the immediate post-exercise period. Sweat was absorbed using 25cm² filter paper swabs pressed against the sampling area with metal forceps. The swabs were placed into individual test tubes which were quickly sealed after each collection. Scapula skin temperature readings were recorded as each collection was completed. Each test tube and swab was then weighed to determine total sweat content, from which rates of secretion and electrolyte fluxes were calculated. The filter paper swabs were soaked in 15.0mM lithium solution prior to centrifugation. The upper phase was removed and analysed for sodium and potassium (Ciba Corning flame photometer, Model M435).

3.9 Statistical Analyses

Data are presented as means (\pm SE). The performance and physiological response data were analysed by parametric statistical methods, where values at the 0.05 level were accepted as being statistically significant. Details of the statistical tests are given in the respective chapters.

Carbohydrate intake and recovery from prolonged, constant pace running

4.1 Introduction

Optimal muscle glycogen stores are an important factor in successful endurance performances (Hultman, 1967; Karlsson and Saltin, 1971). Prolonged exercise at 60 to 80% $\dot{V}O_2$ max will reduce muscle glycogen, and this is believed to be a major contributor to the onset of fatigue (Bergstrom and Hultman, 1967; Hermansen et al, 1967). This carbohydrate store must be replenished if endurance capacity is to be restored, where endurance capacity refers to the exercise time to fatigue at a constant running speed. The restoration of normal resting muscle glycogen values is dependent upon post-exercise carbohydrate intake (Ahlborg et al, 1967a; Bergstrom et al, 1967).

Costill et al (1981) reported restoration of pre-exercise muscle glycogen 24-h after prolonged running, when a diet providing 525 to 648 g (ie. 7.3-8.2 g·kg⁻¹body wt) of carbohydrate was consumed. This high intake may be satisfactorily incorporated into a nutritionally well-balanced diet (Fogelholm, Tikkanen, Naveri and Harkonen, 1989).

Few studies have examined the return of exercise capacity along with replenishment of endogenous carbohydrate stores. Keizer et al (1987) examined muscle glycogen content and maximal physical work capacity (MPWC) before and after exhaustive intermittent cycling and a prescribed diet. A recovery diet providing ~590 g of carbohydrate (ie. 8.0 g·kg⁻¹body wt) replenished muscle glycogen stores within 22-h. Despite restoring the body's carbohydrate stores, MPWC was reduced by 7.3%. Nevill et al (1993) examined the effects of high (ie. 8.7 g·kg⁻¹ body wt), normal (ie. 4.6 g·kg⁻¹body wt) and low (ie. 1.1 g·kg⁻¹body wt) carbohydrate diets on intermittent sprint performance. Power output was reduced in all groups after ~22-h of recovery, there being no differences between dietary treatments with respect to maximal exercise performance.

However, the question of a return in endurance capacity along with replenishment of endogenous carbohydrate stores has still to be addressed. Thus, the aim of this study was to examine the influence of providing additional carbohydrate during 22.5-h recovery from prolonged, constant pace running on subsequent endurance capacity.

4.2 Methods

4.2.1 Subjects

Sixteen men volunteered to take part in the study. This sample included a wide range of performers, from recreational runners to county games players and international rowers. The subjects were divided into two matched groups (Table 4.1). The groups were matched in terms of physiological characteristics (age, height and weight), respiratory and cardiovascular responses ($\dot{V}_{E\max}$, HRmax and $\dot{V}O_{2\max}$), running economy and blood lactate responses to submaximal exercise. It was evident from the preliminary performance tests that the groups were of equal training status (Table 4.2). Subjects from each group were randomly assigned to either a control (CON) or a carbohydrate (CHO) trial.

4.2.2 Protocol

Subjects completed weighed-food intake dietary analyses before the start of the study (Section 3.3). From these analyses, prescribed diets were prepared for the 48-h period prior to R_1 .

After familiarisation, the three preliminary tests were completed to determine the oxygen cost of submaximal running, $\dot{V}O_{2\max}$ and the relationship between oxygen consumption and blood lactate concentrations during submaximal running (Section 3.4).

The treadmill runs R_1 and R_2 were performed at 08.30h on two consecutive days (Figure 3.1), subjects arriving at the laboratory after an overnight fast of 10-h on both occasions. After the pre-

viously described preparations and standardised warm-up, subjects completed R_1 . The interval between R_1 and R_2 was 22.5-h. During this controlled recovery period, food intake was prescribed to include additional energy. This was calculated on the basis of an increase in the carbohydrate component of each subject's habitual diet to $8.8 \text{ g}\cdot\text{CHO}\cdot\text{kg}^{-1}\cdot\text{body wt}$. The supplementary energy for the CHO group was provided in the form of a 16.5% glucose-polymer drink (Appendix K). The isocaloric equivalent was prescribed for the CON group in the form of dietary fat and protein, which was incorporated into the main meals. The proportions of additional fat and protein were determined in relation to each subject's normal levels of ingestion. Both groups were provided with their first meal in the laboratory immediately on completion of R_1 . Dietary prescriptions were prepared by a trained dietitian with reference to each subject's habitual food intake. As such, the prescribed foods were familiar and acceptable.

After the subject had been seated in a relaxed position for ~10 min, a 10 ml venous blood sample was drawn from the ante-cubital vein. Duplicate 20 μl capillary blood samples were also obtained. Further capillary samples were taken after 30 and 60 min during each run, with venous and capillary samples being obtained at the end of R_1 and R_2 . Packed cell volume and haemoglobin concentration were measured in whole blood, whilst plasma was analysed for FFA (method-i), glycerol, sodium, potassium, ammonia and urea. Lactate and glucose were determined in capillary samples. All blood samples were collected, treated, stored and analysed as previously described (Section 3.7).

Expired air samples were collected at 15 min intervals during both runs using the Douglas bag technique, and subjective ratings of perceived exertion were obtained using the Borg scale.

Performance times of the two groups were compared by analysis of covariance, where R_1 represented the covariate.

Differences in blood biochemical responses were examined using Student's independent T-test. Whereas, differences within each group were examined using a paired T-test. All other responses were analysed using two-way analysis of variance (ANOVA) with repeated measures (treatment-by-time). When differences were revealed using ANOVA, a Tukey post-hoc test was applied to identify the nature of these differences.

4.3 Results

4.3.1 Performance

Run times for R_1 were not different between the groups (CON group: 86.3 (± 3.8) min; CHO group: 82.7 (± 3.8) min). After 22.5-h recovery, run time of the CON group (R_2) was reduced by 15.6 min ($p < 0.05$) (mean 70.7 \pm 7.2 min; range 38.8 to 93.0 min), whilst run time of the CHO group was increased by 9.2 min (NS) (mean 91.9 \pm 9.0 min; range 60.0 to 131.0 min).

4.3.2 Pre-exercise energy intake and fuel utilisation during exercise

The energy intake of subjects during the 48-h prior to R_1 are given in Table 4.3. Carbohydrate provided 51 (± 3)% and 52 (± 3)% of total dietary energy of the CON and CHO groups respectively. The total carbohydrate in the CON recovery diet was the same in absolute terms as for the previous 48-h, but represented a reduction in relative terms to 36 (± 6)% of energy intake. Whilst, the carbohydrate content of the CHO recovery diet was increased, representing 63 (± 3)% of energy intake.

The eating patterns of the two groups during the recovery phase were analysed by examining the dietary content of breakfast, lunch, dinner and snacks. There were no differences in eating patterns between the two groups in terms of energy intake. Furthermore, the carbohydrate content of the first meal ingested immediately after R_1 was the same for both groups.

Respiratory exchange ratio (R) values were not different during the two runs (Figure 4.1). Estimated energy expenditure of the CON group during R₁ was 5.7 MJ, of which carbohydrate oxidation contributed 52%. This was equivalent to 173 (±8) g of carbohydrate during the first exercise bout. Whilst the energy expenditure of the CHO group during R₁ was 5.5 MJ, of which carbohydrate contributed 43% or 140 (±8) g. During R₂, 4.8 MJ of energy were expended by the CON group, of which 58% was in the form of carbohydrate. This compares with 6.2 MJ expended by the CHO group, of which 49% was provided by carbohydrate.

The oxygen cost of constant pace running for subjects in the CON and CHO groups during R₁ and R₂ are given in Table 4.4. There was evidence of $\dot{V}O_2$ -drift in both groups during R₁ ($p < 0.01$) and R₂ ($p < 0.05$), though there were no differences between the groups. This reflected an increase in the estimated energy cost of running during R₁ equivalent to ~3.8 KJ·min⁻¹, where more than 90% of the additional fuel requirement was provided from elevated fat metabolism.

4.3.3 Blood glucose and blood lactate responses

Both groups maintained normal blood glucose concentrations during R₁ and R₂ (Figure 4.2). The running speeds initially eliciting 70% $\dot{V}O_2$ max represented 106% of the 2.0 mmol·l⁻¹ blood lactate reference speed in the CON group, and 110% in the CHO group. Following the onset of R₁ exercise, blood lactate concentrations increased from 0.78 (±0.08) to 5.12 (±0.72) mmol·l⁻¹ in the CON group, and from 0.96 (±0.11) to 4.51 (±0.60) mmol·l⁻¹ in the CHO group (NS). Whereas, during R₂ blood lactate increased from 0.79 (±0.09) to 4.60 (±0.59) mmol·l⁻¹ in the CON group, and from 1.04 (±0.10) to 4.75 (±0.73) mmol·l⁻¹ in the CHO group. There were no differences between the two groups in blood glucose and blood lactate responses.

4.3.4 Plasma glycerol, FFA, ammonia and urea responses

Plasma glycerol increased seven-fold during R₁ in the CON group and eight-fold in the CHO group ($p < 0.01$) (Figure 4.3). Whereas, plasma FFA increased two-fold in both groups ($p < 0.01$). Pre-R₁ plasma glycerol and FFA concentrations were restored in both groups over the recovery period. During the second exercise bout plasma glycerol increased four-fold in the CON group and seven-fold in the CHO group ($p < 0.01$), whilst plasma FFA approximately doubled in both groups. However, the change in FFA concentration in the CHO group ($p < 0.01$) was greater than that in the CON group ($p < 0.05$).

Plasma ammonia increased in both groups during R₁ and R₂ ($p < 0.01$). Resting concentrations ranged from 39.02 to 54.12 $\mu\text{mol}\cdot\text{l}^{-1}$, increasing to 101.60 (± 7.97) $\mu\text{mol}\cdot\text{l}^{-1}$ following exercise. There were no differences in response between the two groups, with resting concentrations being restored during the recovery.

Plasma urea increased in both the CON ($p < 0.05$) and CHO ($p < 0.01$) groups during R₁ (Figure 4.4), though there were no differences between the groups. Pre-R₁ values were restored prior to R₂ in the CHO group, but not in the CON group ($p < 0.05$). Plasma urea remained stable in the CHO group during R₂ but increased in the CON group ($p < 0.01$). As such, plasma concentrations were persistently higher in the CON group ($p < 0.05$).

Table 4.1 Physiological characteristics of subjects in the control (CON) and carbohydrate (CHO) groups (mean \pm SE)

	Age (yrs)	Height (cm)	Weight (kg)	$\dot{V}_{E\max}$ (l \cdot min $^{-1}$)	HR max (b \cdot min $^{-1}$)	$\dot{V}O_{2\max}$ (ml \cdot kg $^{-1}$ min $^{-1}$)
CON	25.2	175.8	70.3	130.9	186	65.0
\pmSE	2.1	3.0	2.4	7.1	3	2.3
CHO	25.6	176.7	72.7	136.3	188	65.0
\pmSE	1.8	2.8	2.5	2.5	3	2.2

Table 4.2 Running speeds (ms^{-1}) and relative exercise intensities ($\% \dot{V}\text{O}_2\text{max}$) at blood lactate concentrations of $2 \text{ mmol}\cdot\text{l}^{-1}$ and $4 \text{ mmol}\cdot\text{l}^{-1}$, and running economy of subjects in the control (CON) and carbohydrate (CHO) groups (mean \pm SE)

	Speed		$\% \dot{V}\text{O}_2\text{max}$		Running Economy $\dot{V}\text{O}_2$ at 3.8 ms^{-1} ($\text{ml}\cdot\text{kg}^{-1}\text{min}^{-1}$)
	$2 \text{ mmol}\cdot\text{l}^{-1}$	$4 \text{ mmol}\cdot\text{l}^{-1}$	$2 \text{ mmol}\cdot\text{l}^{-1}$	$4 \text{ mmol}\cdot\text{l}^{-1}$	
CON	3.44	4.51	66.1	86.0	44.8
\pm SE	0.53	0.40	4.3	6.2	1.0
CHO	3.34	4.51	63.7	86.3	43.6
\pm SE	0.42	0.21	1.2	2.8	0.4

Table 4.3 The energy content and main dietary nutrients for the normal (ND) and recovery (RD) diets, of subjects in the control (CON) and carbohydrate (CHO) groups (mean \pm SE)

		Energy	Protein		Carbohydrate		Fat
		MJ	g	gkg ⁻¹ body wt	g	gkg ⁻¹ body wt	g
ND	CON	13.4	119.0	1.7	431.0	6.1	120.9
	\pm SE	1.3	14.1	0.2	45.5	0.6	12.1
	CHO	13.0	113.0	1.6	422.0	5.8	116.8
	\pm SE	0.9	8.3	0.1	32.5	0.5	16.2
RD	CON	17.4	171.8	2.4	446.5	6.3	197.2
	\pm SE	0.9	14.9	0.2	50.0	0.7	15.1
	CHO	16.3	a**118.5	a**1.6	b**631.8	b**8.8	a**113.3
	\pm SE	0.9	12.1	0.2	25.8	0.1	15.7

a** Denotes CHO group values significantly lower than CON group values (p<0.01)

b** Denotes CHO group values significantly higher than CON group values (p<0.01)

Table 4.4 Oxygen consumption ($l \cdot \text{min}^{-1}$) of the control (CON) and carbohydrate (CHO) groups during R₁ and R₂ (mean \pm SE)

	R ₁							R ₂						
	15	30	45	60	75	End	15	30	45	60	End			
CON	3.02	3.27	3.26	3.32	3.38	3.31	3.22	3.25	3.32	3.42	3.27			
\pm SE	0.14	0.13	0.14	0.14	0.16	0.13	0.13	0.14	0.12	0.19	0.14			
CHO	3.25	3.26	3.32	3.33	3.39	3.34	3.28	3.34	3.36	3.33	3.46			
\pm SE	0.10	0.10	0.12	0.11	0.14	0.12	0.15	0.13	0.12	0.12	0.14			

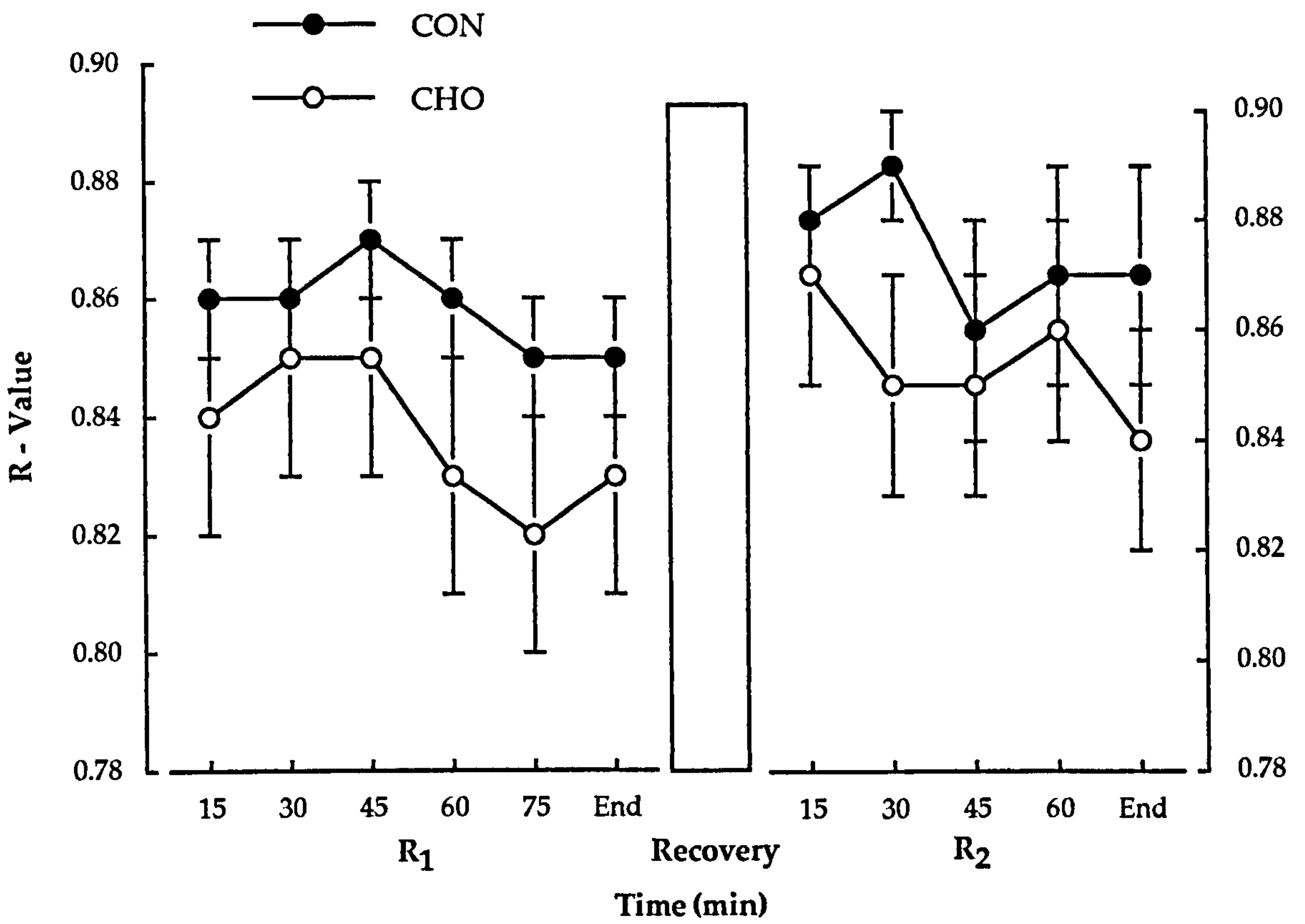


Fig 4.1 R values of the control (CON) and carbohydrate (CHO) groups during R₁ and R₂ (mean±SE)

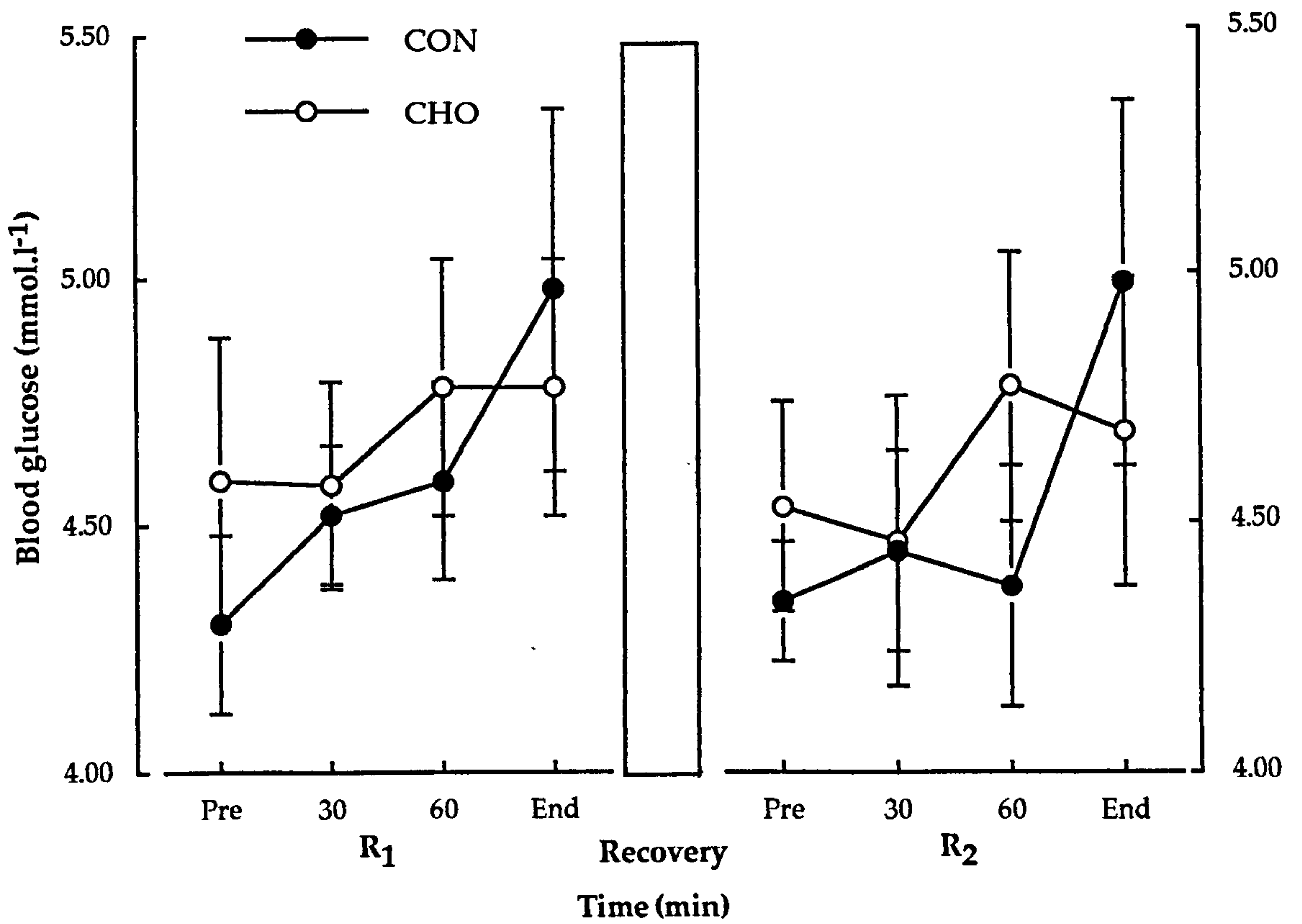


Fig 4.2 Blood glucose concentrations of the control (CON) and carbohydrate (CHO) groups during R₁ and R₂ (mean±SE)

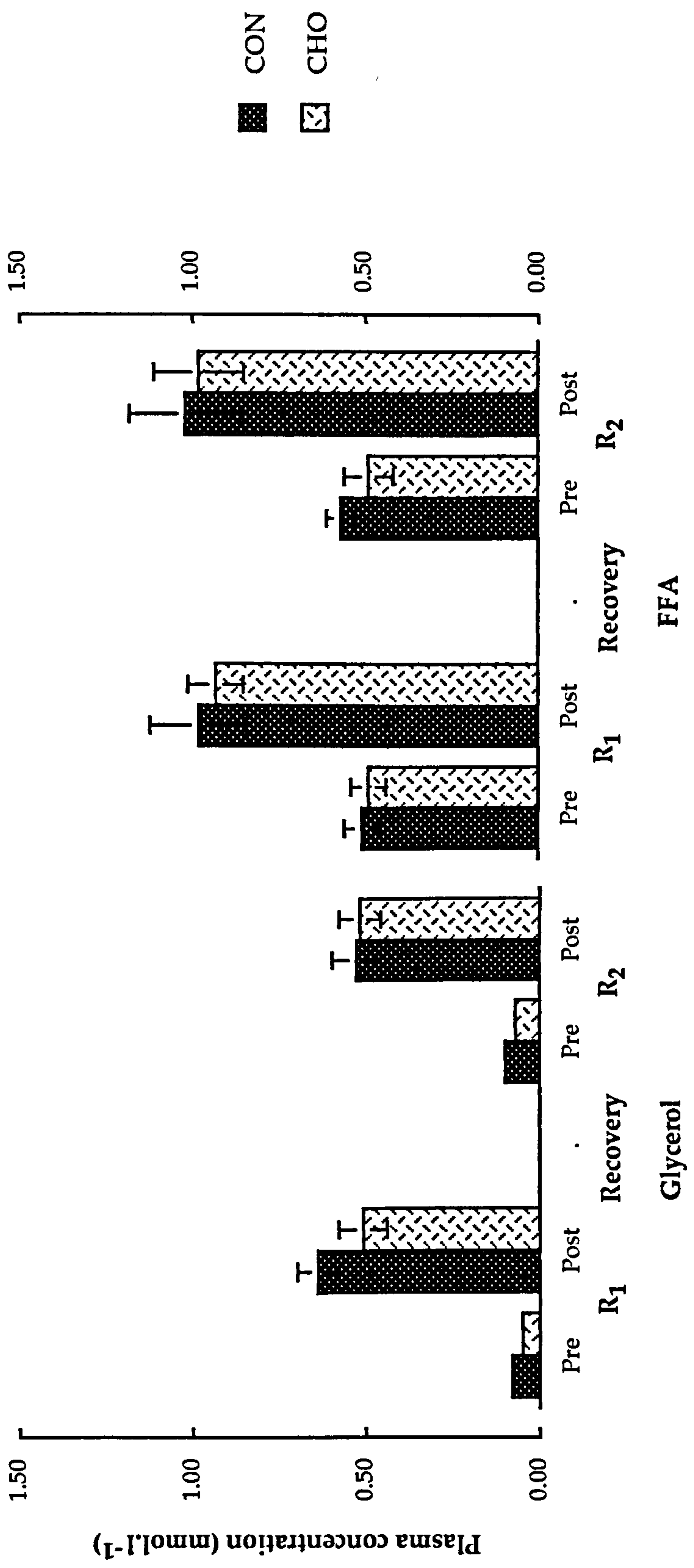


Fig 4.3 Plasma glycerol and FFA concentrations of the control (CON) and carbohydrate (CHO) groups for R₁ and R₂ (mean±SE)

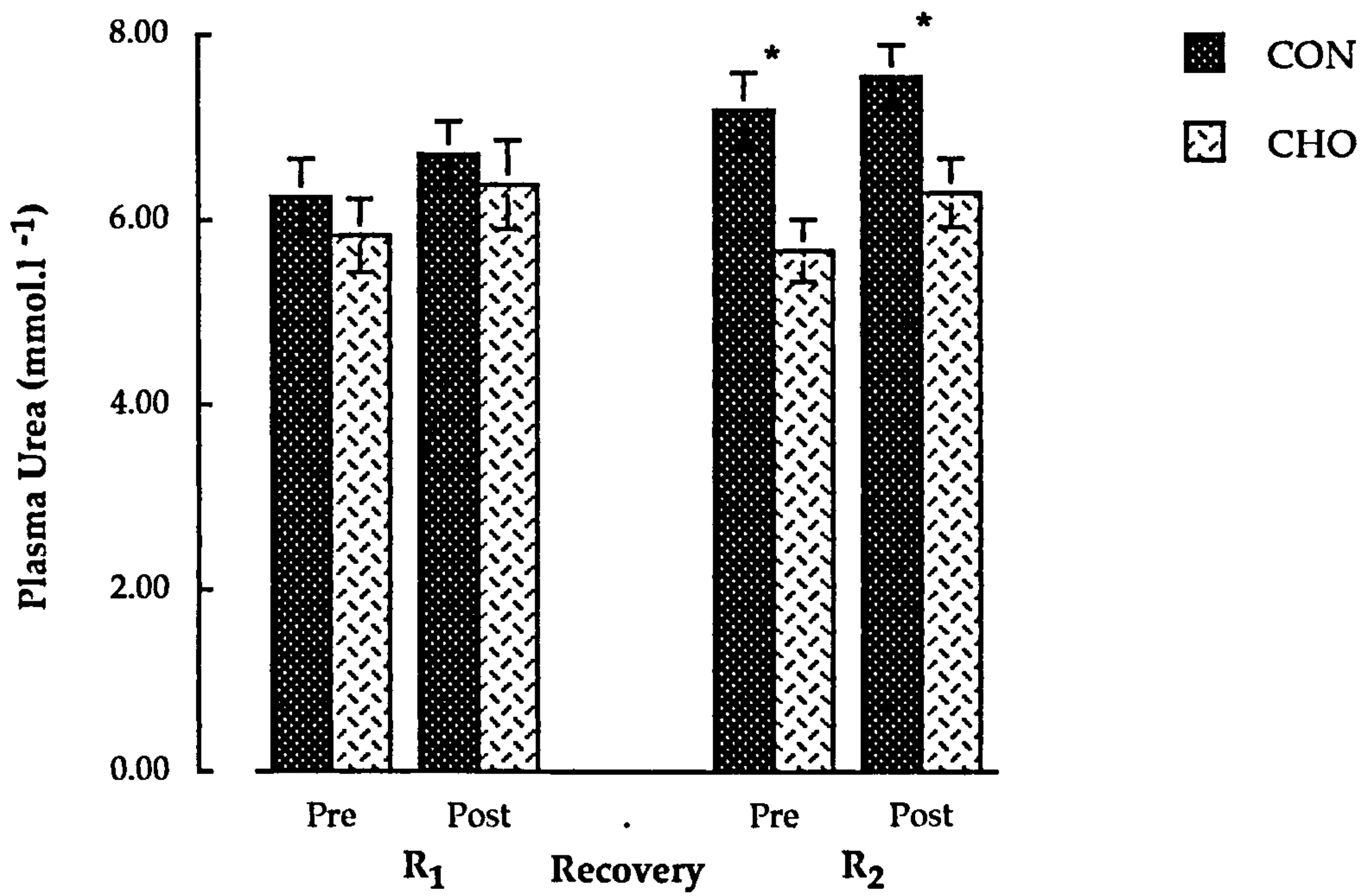


Fig 4.4 Plasma urea concentrations of the control (CON) and carbohydrate (CHO) groups during R₁ and R₂ (mean±SE)

* Denotes concentrations in the CON group greater than in the CHO group (p<0.05)

4.3.5 Plasma electrolyte responses

Plasma sodium concentration increased during R_1 by 2.7% ($p < 0.01$) in the CON group and 1.7% (NS) in the CHO group (Table 4.5). Resting values were restored in both groups after 22.5-h recovery. Plasma sodium remained unchanged in the two groups during R_2 .

Plasma potassium increased in the CON group by 11.6% over R_1 and 7.8% over R_2 (ie. $\Delta\%$ -3.8) ($p < 0.01$). Whilst plasma potassium increased by 16.9% and 15.9% during the two runs respectively in the CHO group (ie. $\Delta\%$ -1.0) ($p < 0.01$). Resting concentrations were restored prior to R_2 in both groups. There were no differences between the groups in plasma electrolyte responses.

4.3.6 Body weight, plasma volume and heart rate and thermoregulatory responses to exercise

Body weight was reduced by 3.1% and 2.3% during R_1 and R_2 in the CON group. These values take into account fluid ingestion of 248 (± 59) ml and 220 (± 63) ml respectively. Body weight was reduced in the CHO group by 2.7% and 3.2% during the two runs, with fluid intakes equivalent to 385 (± 108) ml and 447 (± 114) ml. Pre- R_1 body weights were restored in both groups over the recovery period.

Plasma volume decreased during R_1 by 5.7 (± 1.7)% and 7.5 (± 1.6)% in the CON and CHO groups respectively, and decreased during R_2 by 7.1 (± 1.2)% and 10.8 (± 1.7)% respectively (NS). Heart rate progressively increased in both groups during exercise, though there were no differences between the groups.

Figures 4.5 and 4.6 illustrate the T_{ty} and T_{sk} profiles of the CON and CHO groups during R_1 and R_2 . Tympanic membrane temperature was maintained relatively stable throughout the two trials, with only minor deviations at the onset and cessation of exercise. Whereas, T_{sk} was less stable at the start of each exercise bout, attaining a plateau during the mid-phase, before deviating non-uniformly at exercise cessation. Thermoregula-

tory responses did not appear to be influenced by the different dietary treatments of the recovery period.

Table 4.5 Plasma electrolyte concentrations (mmol·l⁻¹) in the control (CON) and carbohydrate (CHO) groups (mean±SE)

		Plasma sodium		Plasma potassium	
		Pre	Post	Pre	Post
R ₁	CON	138.6	142.3	3.79	4.23
	±SE	1.3	1.1	0.07	0.09
	CHO	136.9	139.3	3.79	4.43
	±SE	1.9	1.7	0.07	0.05
R ₂	CON	140.3	138.6	3.83	4.13
	±SE	2.7	2.6	0.09	0.17
	CHO	140.9	141.0	3.84	4.45
	±SE	0.9	0.9	0.06	0.08

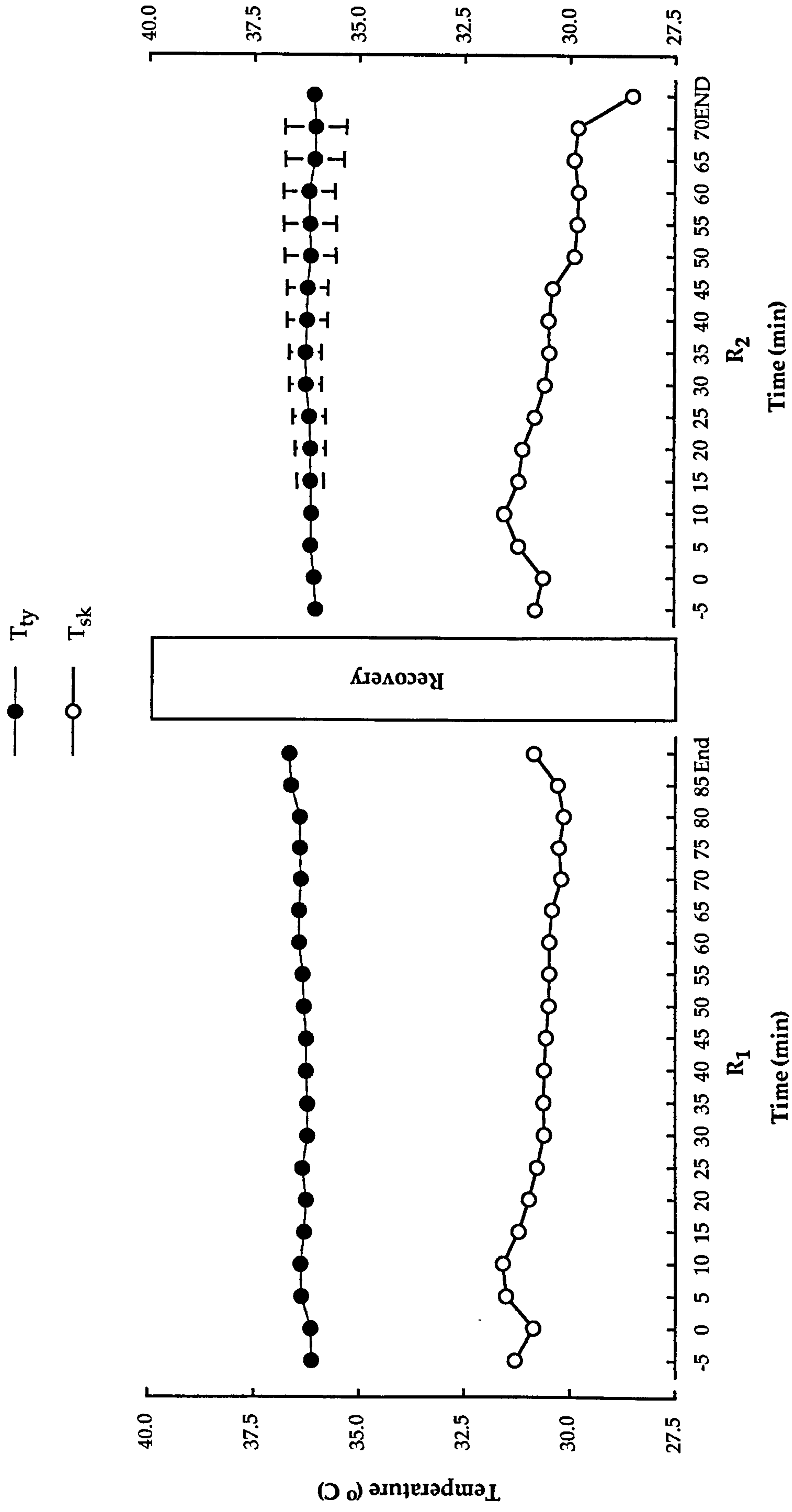


Fig 4.5 Tympanic (T_{ty}) and weighted mean skin (T_{sk}) temperature responses of the control (CON) group (mean \pm SE)

Note: Error bars hidden by symbol

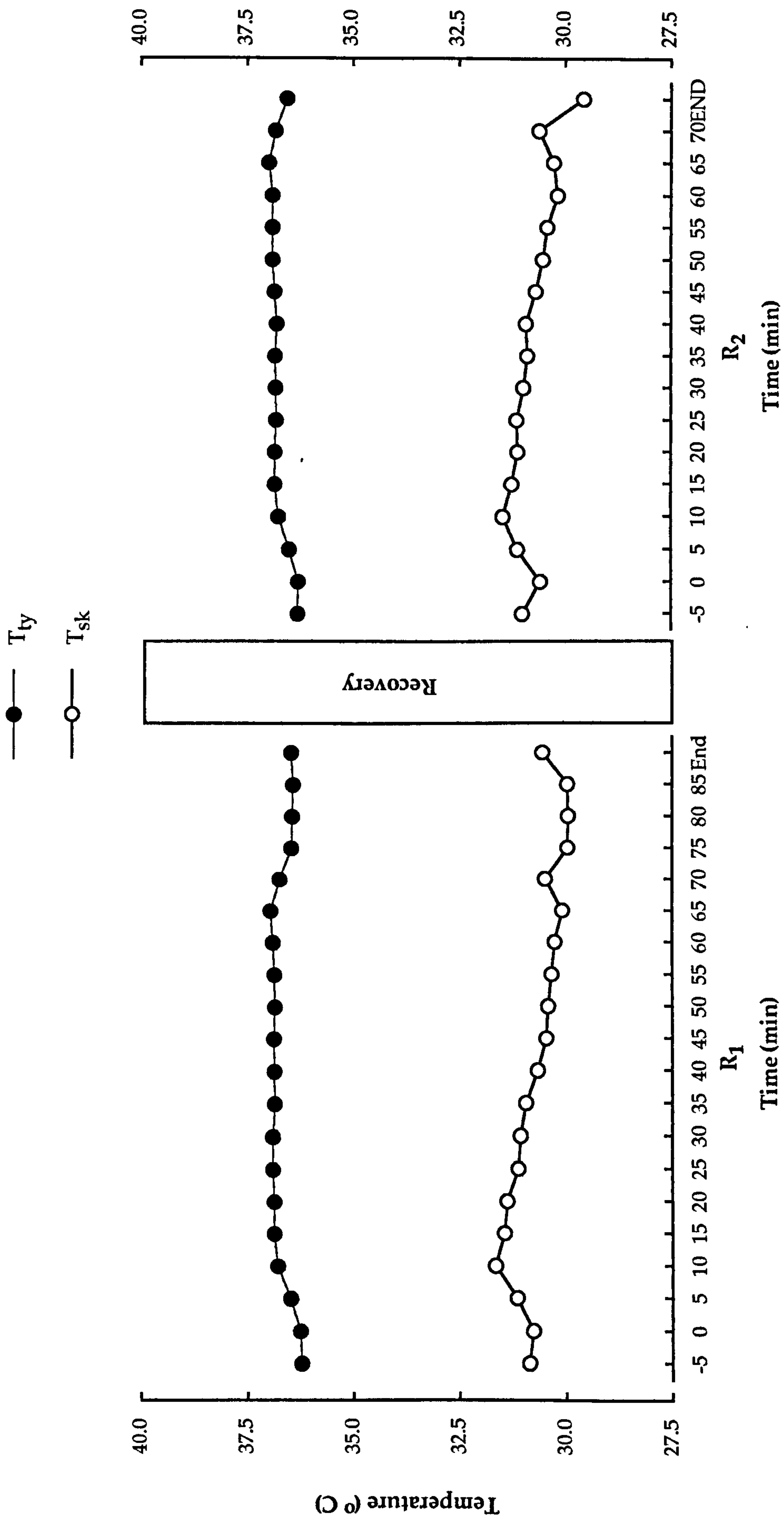


Fig 4.6 Tympanic (T_{ty}) and weighted mean skin (T_{sk}) temperature responses of the carbohydrate (CHO) group (mean \pm SE)

Note : Error bars hidden by symbol

4.4 Discussion

The main finding of this study was that a normal diet supplemented with additional carbohydrate restored endurance capacity following a recovery period of 22.5-h. An isocaloric diet in which additional energy was provided in the form of fat and protein did not result in a similar return in exercise capacity. The groups were well matched in terms of their physiological characteristics, respiratory and cardiovascular responses, running economy, and blood lactate concentrations during submaximal exercise. This was also reflected in their similar R_1 run times.

Keizer et al (1987) observed that a diet providing $\sim 8.0 \text{ g} \cdot \text{CHO} \cdot \text{kg}^{-1} \cdot \text{body wt}$ restored resting muscle glycogen concentrations within 22-h after exhaustive intermittent cycling. This is similar to the level of carbohydrate intake as provided in the present study. However, Keizer et al (1987) report that despite replenishing the immediate energy store of muscle, MPWC was reduced by 7.3%. This contrasts a return in endurance capacity as observed in the present study. The MPWC test provided a measure of the maximal power generating capacity of muscle as opposed to the capacity to sustain constant pace, submaximal exercise. The ability to generate power is largely determined by muscle fiber recruitment patterns. The prior bout of exhaustive cycling possibly evoked an inflammatory response (O'Reilly et al, 1987), which may have interfered with propagation and realisation of action potentials. As a consequence, the number of muscle fibers recruited may have been reduced resulting in impaired MPWC. Whilst muscle biopsies taken during the study may have exacerbated this situation to compound the muscle damage resulting directly from the prolonged, intermittent exercise bout (Costill, Pearson and Fink, 1988).

In the present study, the post-recovery trial (R_2) was a submaximal test of uniform intensity (ie. constant pace running) unlike the incremental MPWC test, which attained a maximal exercise intensity over a relatively short period of time. Thus, the performance tests differ in both their neural demands as well as their energy metabolism demands. Anaerobic metabolism will substantially contribute to a

high rate of energy demand during the MPWC test. Accumulation of metabolic end-products may interfere with anaerobiosis to limit exercise performance. Submaximal exercise on the other hand has a lower rate of energy demand, which is sustainable over a longer period of time through a greater dependency upon aerobic metabolism. Thus, performance of the MPWC test is limited by the rate of energy (ie. ATP) turnover, whereas prolonged, constant pace running is limited by a total capacity for energy production (ie. the magnitude of fuel reserves). Carbohydrate availability is not a major limitation in the case of the former, whilst becoming increasingly so in the case of the latter. A final consideration is the localised nature of the MPWC cycling test in comparison with the whole body activity of R_2 , where the former presents a proportionately greater physiological challenge to active tissue.

In the present study, the CON group consumed 446.5g of carbohydrate during the recovery (ie. $6.3 \text{ g}\cdot\text{kg}^{-1}\text{body wt}$). This is higher than values for the general population but is consistent with their habitual intake and values reported for endurance runners (Costill, 1988). Nevertheless, this was found to be insufficient to restore endurance capacity within 22.5-h. The CHO group consumed 631.8g of carbohydrate (ie. $8.8 \text{ g}\cdot\text{kg}^{-1}\text{body wt}$) during the recovery. This is in agreement with the dietary recommendations of previous authors for optimal muscle glycogen replenishment (Costill et al, 1981; Keizer et al, 1987). The recovery diets of both groups contained more carbohydrate than was estimated to have been oxidised during R_1 . This was equivalent to a normal level of carbohydrate intake for the CON group. However, the CHO recovery diet provided adequate carbohydrate to accommodate both the daily requirements of the body as well as the additional deficit accrued during R_1 , plus an additional amount to cover possible under-estimations.

Although carbohydrate supplementation in the present study was successful in restoring endurance capacity, an intake of $8.0 \text{ g}\cdot\text{kg}^{-1} \text{ body wt}\cdot 24\text{h}^{-1}$ failed to prevent a cumulative glycogen depletion in subjects performing strenuous running on five successive days (Kirwan et al, 1988). Thus, a higher carbohydrate intake may be necessary to maintain fuel reserves during periods of heavy training. Alternatively, a

✓ storage limitation may act as a physiological safety mechanism to restrict further activity (Kirwan et al, 1988).

A difference between the CON and CHO groups with respect to estimated levels of carbohydrate oxidation during R_1 cannot be satisfactorily explained from the available data. The running economy and blood lactate responses of the groups are indicative of a similar training status (Table 4.2). The CHO group was estimated to have a lower rate of oxidation ($1.7 \pm 0.1 \text{ g} \cdot \text{min}^{-1}$), in comparison with the CON group ($2.0 \pm 0.1 \text{ g} \cdot \text{min}^{-1}$). The effect that this could have had on the energy reserves may have been compounded by the marginally, but not significantly, longer run time of the CON group during R_1 .

Muscle glycogen concentrations in subjects on a normal mixed diet (ie. 40-50% carbohydrate) are reported to be $\sim 80 \text{ mmol} \cdot \text{kg}^{-1} \text{ wet wt}$ (Hultman and Sjoholm, 1983). If an average total muscle mass of $\sim 25\text{-}30 \text{ kg}$ is assumed, this would allow $\sim 350\text{g}$ of carbohydrate to be stored as muscle glycogen (Essen, 1977). Thus, Newsholme (1983) estimated total body carbohydrate reserves to be $\sim 440 \text{ g}$, as a further $\sim 90 \text{ g}$ is stored in the liver. Assuming that 60% of the total muscle mass is active during running, the carbohydrate available to fuel exercise will be $\sim 300 \text{ g}$ (Callow, Morton and Guppy, 1986). However, Sherman et al (1981) observed muscle glycogen reserves ranging between 130 and 135 $\text{mmol} \cdot \text{kg}^{-1} \text{ wet wt}$ in trained individuals on diets providing $\sim 50\text{-}55\%$ of the total energy intake as carbohydrates. Thus, it should be noted that the available carbohydrate for running exercise in endurance trained subjects may exceed 300g. Nevertheless, subjects in the present thesis were considered to conform more closely with the observations of Hultman and Sjoholm (1983) with respect to training status. As such, calculations in this thesis will be based upon these values unless otherwise stated.

Muscle glycogen represents the largest carbohydrate store in the body (Newsholme and Leech, 1983). If it is assumed that muscle glycogen provides the majority of carbohydrate oxidised during R_1 , this energy store would be reduced by $\sim 58\%$ in the CON group and by $\sim 47\%$ in the CHO group. These values are in agreement with Costill et al (1981) for running exercise of a similar intensity and duration. Moreover,

Costill et al (1981) report that substantial glycogen stores may remain in muscle following prolonged exhaustive running. Consistent with adequate carbohydrate availability, blood glucose was normally maintained in both groups during R_1 and R_2 , such that hypoglycaemia per se was not thought to be a major contributor to fatigue.

The importance of consuming carbohydrate immediately after exercise in order to achieve a rapid rate of glycogen resynthesis has already been demonstrated (Ivy et al, 1988a). In the present study, the first meal provided in the laboratory for both groups on completion of R_1 contained foods of a high glycaemic index. Thus, a rapid availability of ingested carbohydrate was ensured (Coyle, 1991; Robergs, 1991). The CON meal contained 102.1 (± 15.0) g·CHO, and the CHO meal contained 82.7 (± 9.6) g·CHO; this difference was not significant. The remainder of the prescribed diet was consumed ad libitum over the 12-h between the end of R_1 and the start of the second overnight fast prior to R_2 . The precise timing of ingestion was noted but not controlled. An optimal rate of muscle glycogen resynthesis may be achieved over the initial 4 to 5-h post-exercise by ingesting 1.0 g·CHO $\text{kg}^{-1}\text{body wt}\cdot 2\text{h}^{-1}$ (Ivy, 1991). This is equivalent to 37.0 $\text{g}\cdot\text{h}^{-1}$. Thus, the first meal taken following R_1 provided adequate carbohydrate for optimal glycogenesis over the early recovery phase.

The eccentric component of running is associated with muscle soreness (Newham et al, 1983b), and ultrastructural changes indicative of intracellular lesions (Hikida et al, 1983; O'Reilly et al, 1987). Such localised tissue damage has been found to impair muscle glycogen replenishment (Costill et al, 1990). This may be due to an infiltration of traumatised muscle by inflammatory cells. As well as being oxidatively active, these cells release a factor which stimulates carbohydrate metabolism. Increased competition develops between inflammatory cells and glycogen depleted muscle fibres for the available glucose. Costill et al (1990) observed that a normal level of carbohydrate ingestion of 4.3 $\text{g}\cdot\text{kg}^{-1}\text{body wt}\cdot 24\text{h}^{-1}$ was inadequate for replenishing glycogen reserves when performing strenuous activity on a daily basis. It was suggested that this may be appeased by consuming a high carbohydrate diet (ie. $>8.5 \text{ g}\cdot\text{kg}^{-1}\text{body wt}\cdot 24\text{h}^{-1}$). In the present study, both groups reported joint stiffness and muscle soreness 22.5-h after R_1 ,

which may be indicative of sub-clinical micro-trauma. It would be inappropriate to speculate on the respective levels of glycogen resynthesis in the two groups following the recovery period. However, it might be argued in light of previous authors' findings (Costill et al, 1990) that additional carbohydrate provided in the CHO recovery diet would facilitate the recovery process.

Exercise was associated with plasma ammonia and urea accumulation in both groups. Resting concentrations were restored in the CHO group during the recovery. However, plasma ammonia and urea concentrations remained elevated in the CON group prior to R₂, plasma urea continuing to increase above post-R₁ levels during the 22.5-h recovery. This was probably due to the additional protein prescribed in the CON recovery diet. Elevated resting concentrations of ammonia and urea prior to R₂, both of which represent potential metabolic toxins (Banister et al, 1983), may have impaired the CON group performance (Banister and Cameron, 1990). Plasma ammonia concentrations post-exercise were similar to values previously observed immediately following a 30s bout of maximal sprinting on a cycle ergometer (Bogdanis, unpublished observations). Notably, plasma ammonia 6 min post-sprinting had increased by a further 67%, which probably reflects muscle ammonia efflux. As such, it might be speculated that elevated ammonia concentrations at a muscle fibre level may play a part in limiting performance. Plasma ammonia concentrations immediately following intermittent sprint cycling (ie. ten 6s sprints with 30s recovery periods) were 85% higher than values reported in the present study (Nevill, Lakomy, McKee, Weller and Nevill, 1993). Thus, elevated plasma ammonia concentrations per se are not thought to limit constant pace running. Nevertheless, increasing systemic ammonia levels in combination with a number of other factors may have contributed to fatigue.

The protein intake of both groups prior to R₁ were consistent with current dietary recommendations for active individuals (Lemon, 1991). Lemon (1991) suggests that a daily protein intake equivalent to ~1.2-1.4 g·kg⁻¹body wt will maintain health, and ultimately performance, in endurance athletes. This normally represents ~12-15% of total energy intake, providing that energy intake is adequate to meet

the demands of exercise in addition to the demands of normal living.

Redressing an energy imbalance is not the only concern following exercise, restoring a favourable fluid balance is also of paramount importance (Barr, Costill and Fink, 1991). Both groups experienced decreases in body weight during R_1 and R_2 , which essentially reflected losses in body fluid. These decrements were greater than the 2.0% threshold identified by Armstrong et al (1985), beyond which athletic performance is impaired. Notably, plasma volume was reduced to a greater extent in the CON group over R_2 relative to R_1 . This was despite a shorter run time for the second exercise bout, which suggests a higher rate of fluid loss. Core temperature did not differ between the two groups. Thus, it might be speculated that reduced metabolic efficiency may have elevated heat production in the CON group. This would result in greater fluid losses in the form of sweat in order to maintain a constant body temperature. A higher fluid loss may have contributed to an unfavourable metabolic environment through adversely impinging upon cardiovascular integrity and temperature regulation (Costill and Sparks, 1973). Thus, an elevated rate of fluid loss in the CON group may have been a contributory factor to the onset of fatigue.

In summary, increasing the carbohydrate content of a normal diet to $8.8 \text{ g}\cdot\text{kg}^{-1}\text{body wt}\cdot 24\text{h}^{-1}$ restores endurance capacity within 22.5-h. Additional substrate was provided to facilitate replenishment of endogenous carbohydrate reserves, a process which may have been hampered by localised tissue damage. However, any consideration of the recovery process cannot focus exclusively upon energy status. The restoration of fluid balance is also important, as this will influence the physiological milieu in which metabolism takes place.

Effect of water ingestion during prolonged, constant pace running on endurance capacity

5.1 Introduction

Dehydration impairs prolonged exercise performance (Armstrong et al, 1985). High rates of fluid loss may reduce blood volume, which in turn would compromise cardiovascular integrity. Maintenance of central blood pressure is prioritised over peripheral circulation (Fortney et al, 1984). Whilst a concomitant decrease in sweating rate (Sawka et al, 1985) would further compromise heat dissipation, and result in a rapid increase in core temperature (Gissolfi and Copping, 1974). Fluid ingestion during exercise may attenuate this temperature rise (Costill et al, 1970).

Compartmental fluid shifts in response to transient osmotic imbalances compound a cumulative exercise-induced deficit. Ad libitum fluid ingestion during and after prolonged exercise does not always result in an adequate rate of rehydration (Carter and Gissolfi, 1989).

As well as adversely effecting exercise capacity, dehydration will also impinge upon the body's recovery capacity. Implementing strategies to maintain fluid balance during an event may also offer benefits in improving the recovery process over the immediate post-exercise period. The purpose of this study was to examine whether fluid provision during constant pace running can limit dehydration and influence endurance capacity. Performance benefits of drinking water during exercise were assessed in terms of exercise time to fatigue, whereas post-exercise physiological responses were determined to establish the nature and extent of metabolic disturbances arising from prolonged running. These disturbances must be promptly addressed by the recovery process.

5.2 Methods

5.2.1 Subjects

Four men and four women took part in the study. All were physical education students at Loughborough University, who exercised on a routine bases. Mean (\pm SE) age, height, weight and $\dot{V}O_2$ max were 21.4 (\pm 0.7) yr, 169.4 (\pm 2.2) cm, 63.10 (\pm 2.91) kg, and 51.12 (\pm 1.80) ml \cdot kg $^{-1}$ min $^{-1}$ respectively.

Dietary intake was controlled during the 48-h preceding each trial by dietary recall. A food diary was completed prior to the first trial itemising the foods consumed and estimated portion sizes. Subjects followed the same diet during the 48-h prior to the second trial. A constant training regimen was maintained throughout the study and subjects refrained from heavy exercise for 2-d preceding each trial.

5.2.2 Protocol

Subjects completed three preliminary tests following familiarisation (Section 3.4). Two trials were then performed in a counter-balanced design, separated by an interval of at least 7-d (Figure 3.2). During one trial, no fluid was ingested during the exercise bout (NF). Whilst during the fluid replacement (FR) trial a water bolus equivalent to 3.0 ml \cdot kg $^{-1}$ body wt was ingested immediately prior to the warm-up, followed by serial feedings equivalent to 2.0 ml \cdot kg $^{-1}$ body wt every 15 min during exercise. Each feeding was presented in calibrated plastic syringes, and was maintained at a uniform temperature of \sim 9-10°C. The total fluid ingested was recorded at the end of the FR-trial and accounted for in post-exercise changes in body weight.

Subjects arrived at the laboratory after an over-night fast of 10-h, and maintained a relaxed standing position whilst ECG and temperature electrodes were positioned. This allowed for equilibration of imbalances between body fluid compartments

which arise through changes in posture. Arterialisation of venous blood was achieved by immersing the lower arm in a 42°C water bath. A 10 ml venous blood sample was drawn from an antecubital vein after 20 min, whilst duplicate 20 μ l capillary blood samples were taken for the determination of blood glucose and blood lactate.

Immediately following the standardised warm-up, the treadmill speed was increased to a pace which initially elicited an oxygen consumption equivalent to 70% $\dot{V}O_2$ max. Subjects ran to volitional fatigue, which was defined as the point at which the required running pace could no longer be maintained. Endurance capacity was measured in terms of exercise time to fatigue.

Further capillary samples were taken after 30 and 60 min during each trial. Venous and capillary blood samples were obtained at the end of exercise with subjects maintaining a supported standing position. This was once again to minimise the influence of postural changes on blood volume distribution whilst the sample was drawn (Hagan, Diaz and Horvarth, 1978). Packed cell volume and haemoglobin concentrations were measured in whole blood. Glycerol, FFA (method-ii), sodium, potassium and ammonia concentrations were determined in plasma, and cortisol was determined from serum. Blood samples were collected, treated, stored and analysed as described previously.

Expired air samples were collected over 60s intervals after 5 and 15 min of exercise, and every 15 min thereafter. Simultaneously, subjective ratings of perceived exertion and ratings of muscular effort were obtained. From gas analyses, \dot{V}_E , $\dot{V}O_2$ and $\dot{V}CO_2$ were determined, and R values calculated. Energy expenditure and rates of fat and carbohydrate oxidation were estimated from pulmonary respiratory exchange data.

Performance times of the two trials and blood biochemical responses were compared by Student's T-test for paired data.

Cardiorespiratory responses were compared by two-way (trial-by-time) repeated measures ANOVA and a Tukey post-hoc test.

5.3 Results

5.3.1 Performance

The mean run time of the NF-trial was 77.7 (± 7.7) min, compared to 103.0 (± 12.4) min for the FR-trial. Thus, FR-trial run time exceeded NF-trial run time by 25.3 (± 5.8) min ($p < 0.01$). Performances during the NF-trial ranged between 52 and 115 min, whereas performances ranged between 65 and 150 min during the FR-trial.

5.3.2 Fuel utilisation during exercise

Figure 5.1 illustrates the shift in energy metabolism with respect to changes in R values. A total of 3.7 (± 0.3) MJ of energy were expended during the NF-trial, whereas 5.0 (± 0.4) MJ of energy were expended during the FR-trial ($p < 0.01$). This difference reflects a longer run time with fluid replacement. However, the rate of energy expenditure was similar for both trials. This was equivalent to 47.2 $\text{kJ} \cdot \text{min}^{-1}$ during the NF-trial, and 48.8 $\text{kJ} \cdot \text{min}^{-1}$ during the FR-trial. In the NF-trial, 72.9% of the total energy expenditure was in the form of carbohydrate and 27.1% was in the form of fat. This compares with 63.5% and 36.5% respectively in the FR-trial. Consistent with this pattern of energy metabolism, the FR-trial was associated with a greater $\dot{V}\text{O}_2$ -drift ($p < 0.05$). The rate of oxygen consumption increased by 0.25 (± 0.06) $\text{l} \cdot \text{min}^{-1}$, in comparison with an increase of 0.17 (± 0.05) $\text{l} \cdot \text{min}^{-1}$ in the NF-trial (Figure 5.2). There were no differences between the trials with respect to \dot{V}_E -drift, which represented 11.49 (± 3.23) $\text{l} \cdot \text{min}^{-1}$ in the NF-trial and 13.78 (± 4.25) $\text{l} \cdot \text{min}^{-1}$ in the FR-trial. Thus, carbohydrate metabolism was enhanced and fat metabolism suppressed when fluid was not consumed during exercise ($p < 0.01$). The respective rates of fuel metabolism are illustrated in Figures 5.3 and 5.4.

5.3.3 Blood glucose and blood lactate responses

Blood glucose concentrations were maintained within the normal range during both trials (Figure 5.5). During the FR-trial concentrations were maintained at $\sim 4.25 \text{ mmol}\cdot\text{l}^{-1}$. Whilst during the NF-trial blood glucose concentrations tended to increase from a pre-exercise value of $4.28 (\pm 0.20) \text{ mmol}\cdot\text{l}^{-1}$ to $4.81 (\pm 0.25) \text{ mmol}\cdot\text{l}^{-1}$ at exercise cessation (NS). This difference in response was most evident over the later stages of exercise.

Blood lactate increased to $\sim 4.00 \text{ mmol}\cdot\text{l}^{-1}$ following the onset of exercise (Figure 5.6). This is relatively high in comparison with values reported in Chapters 6 and 7 of this thesis for exercise of the same relative exercise intensity. From preliminary test data (Table 5.1), subjects in the present study were less trained with respect to prolonged, constant pace running in comparison with those taking part in the later studies. This may have contributed in the higher blood lactate concentrations

Stable blood lactate levels were maintained over the initial 60 min of exercise. However, concentrations were elevated over the later stages of the NF-trial in comparison with the FR-trial ($p < 0.05$).

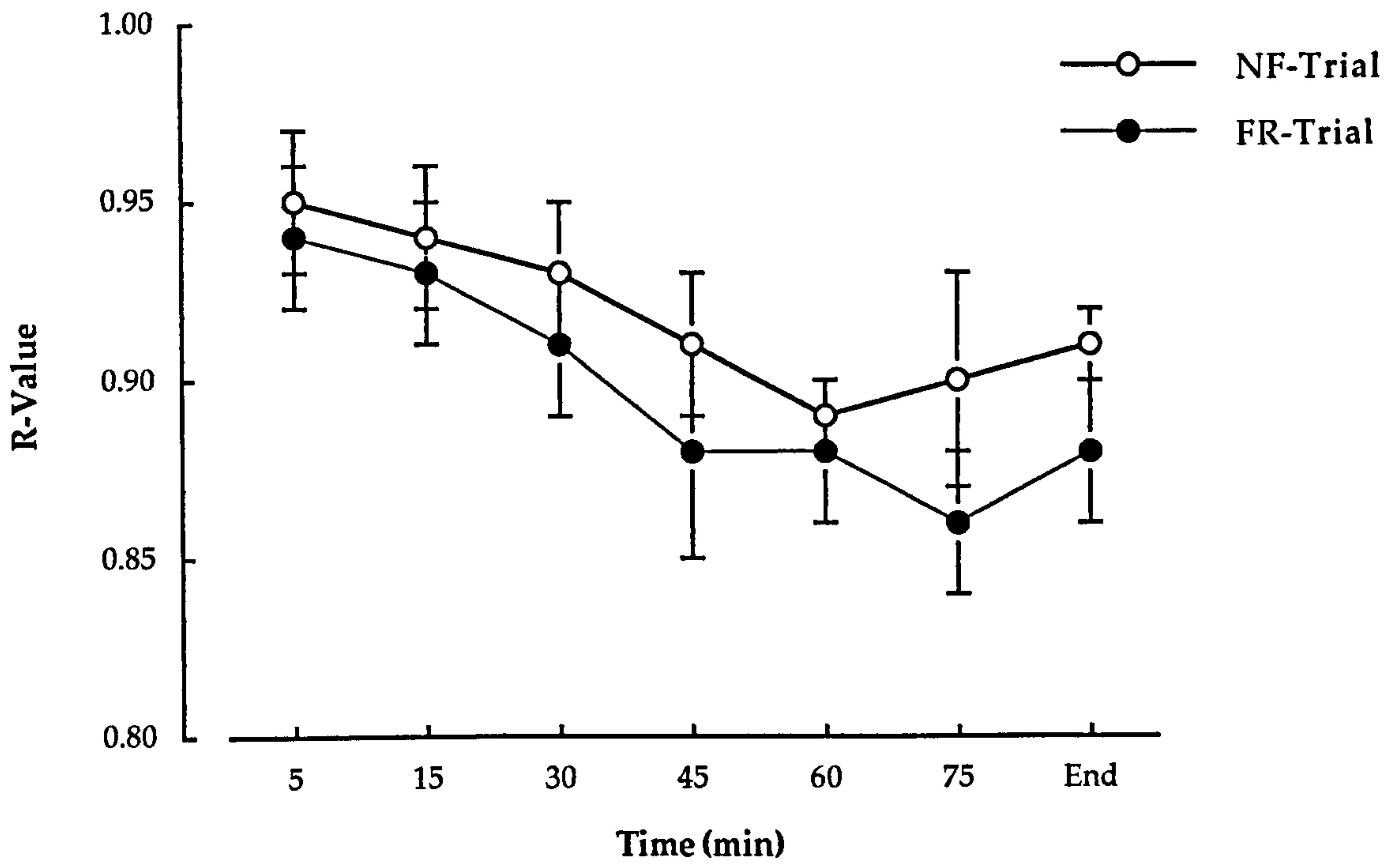


Fig 5.1 R values during the no fluid (NF) and fluid replacement (FR) trials (mean \pm SE)

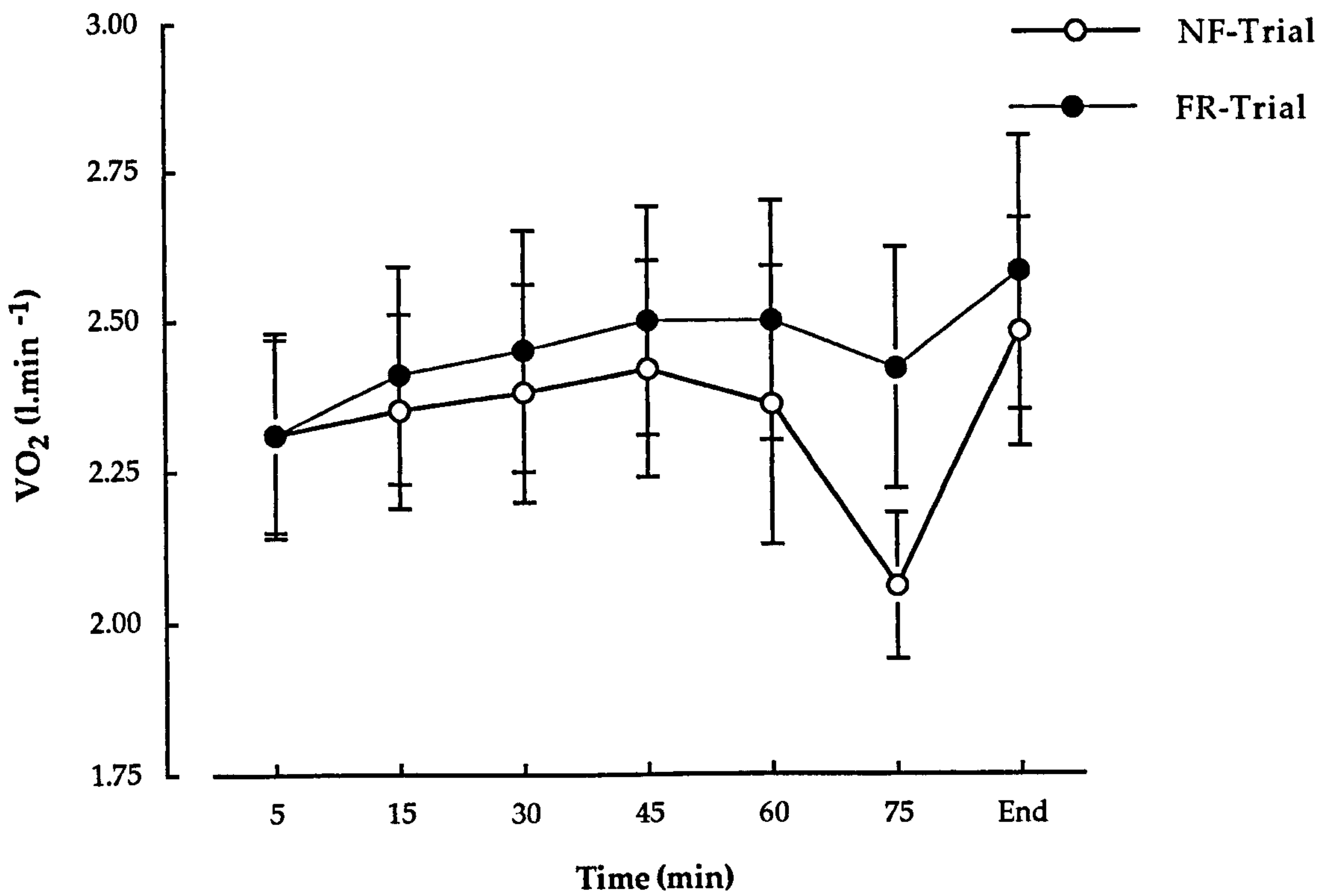


Fig 5.2 Oxygen consumption during the no fluid (NF) and fluid replacement (FR) trials (mean \pm SE)

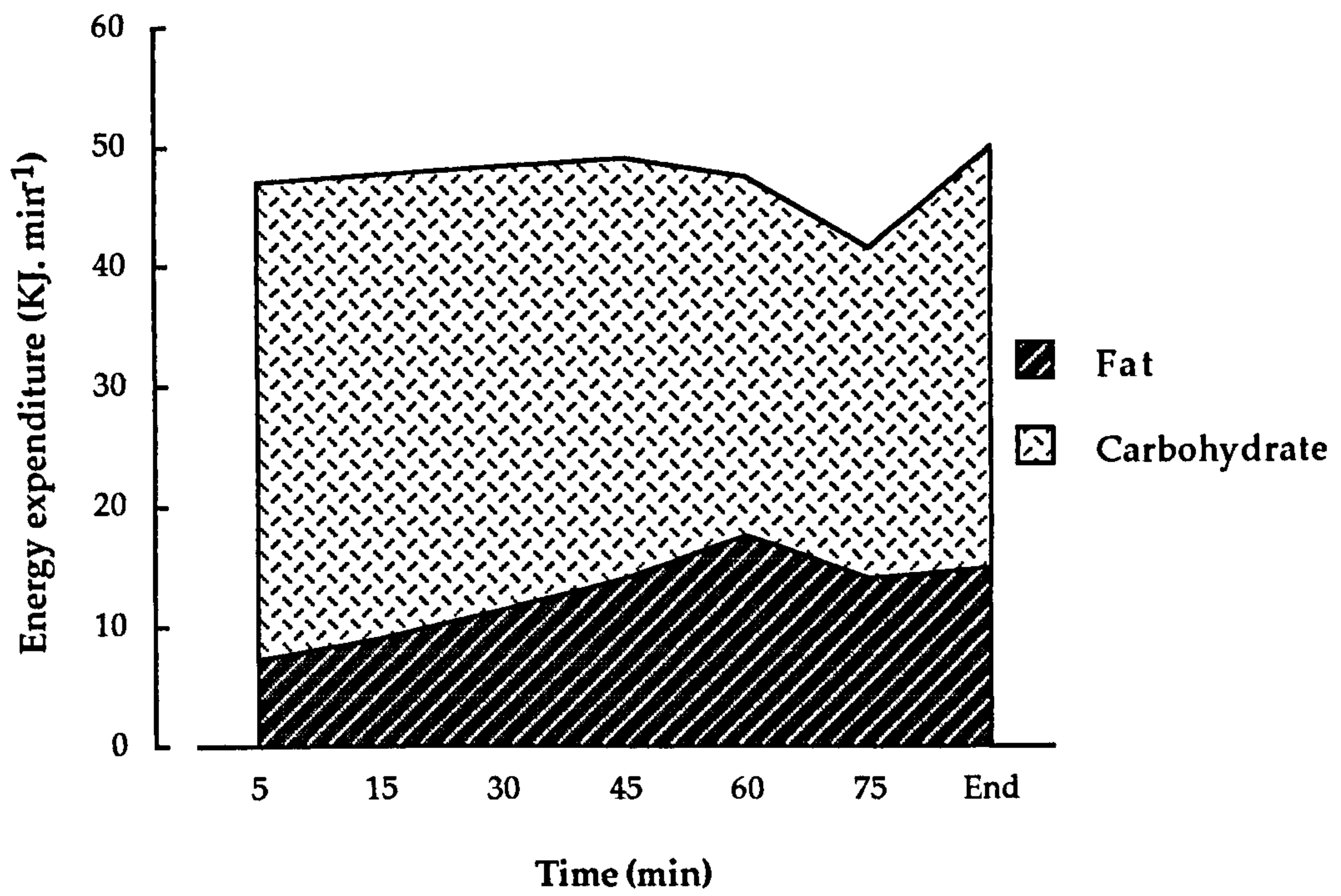


Fig 5.3 Energy expenditure during the no fluid (NF) trial

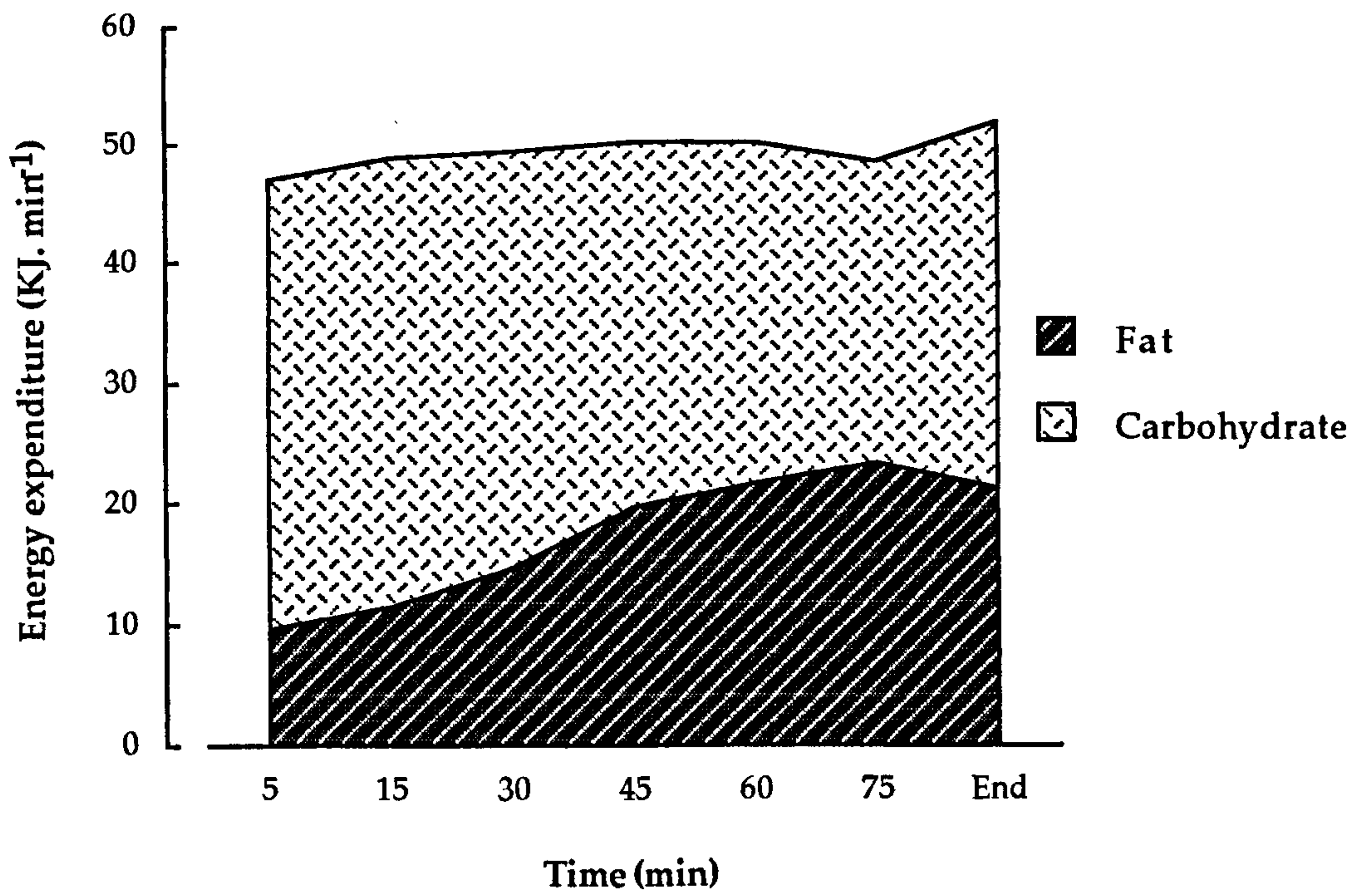


Fig 5.4 Energy expenditure during the fluid replacement (FR) trial

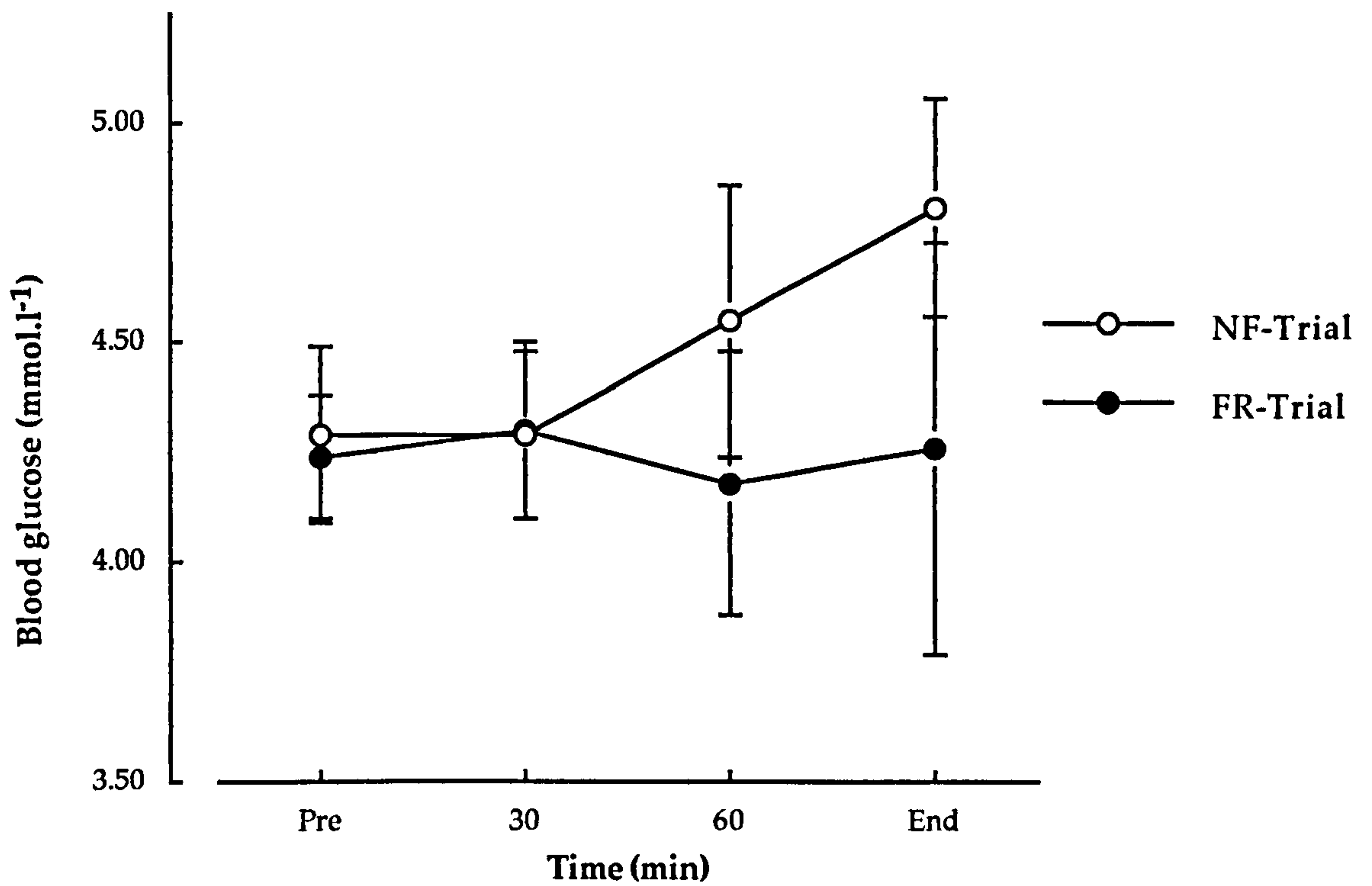


Fig 5.5 Blood glucose concentrations during the no fluid (NF) and fluid replacement (FR) trials (mean±SE)

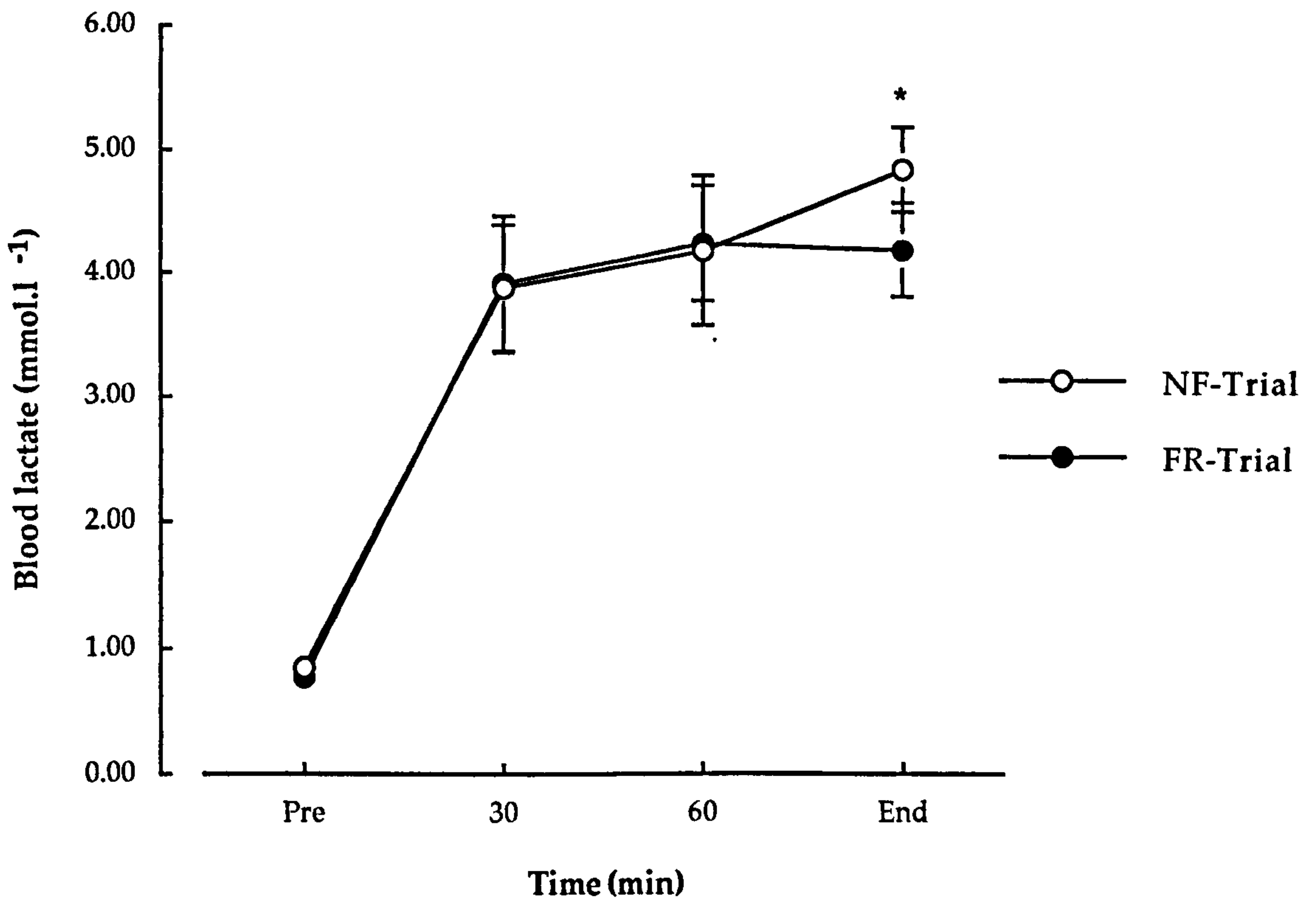


Fig 5.6 Blood lactate concentrations during the no fluid (NF) and fluid replacement trials (mean±SE)

* Denotes NF-trial significantly different from FR-trial (p<0.05)

Anova: Main effect - treatment (p<0.05); time (p>0.05)
 Interaction - treatment * time (p<0.05)

Table 5.1 Running speeds ($\text{m}\cdot\text{s}^{-1}$) and relative exercise intensities ($\% \dot{V}\text{O}_2\text{max}$) at blood lactate concentrations of $2 \text{ mmol}\cdot\text{l}^{-1}$ and $4 \text{ mmol}\cdot\text{l}^{-1}$ (mean \pm SE)

	Speed		$\% \dot{V}\text{O}_2\text{max}$	
	$2 \text{ mmol}\cdot\text{l}^{-1}$	$4 \text{ mmol}\cdot\text{l}^{-1}$	$2 \text{ mmol}\cdot\text{l}^{-1}$	$4 \text{ mmol}\cdot\text{l}^{-1}$
Mean	2.98	4.01	62.6	79.5
\pm SE	0.22	0.17	3.7	3.4

5.3.4 Plasma FFA, glycerol and ammonia responses

Plasma FFA increased by ~100% during both trials, from a pre-exercise level of ~0.4 mmol·l⁻¹ to ~0.9 mmol·l⁻¹ at exercise cessation ($p < 0.01$). Plasma glycerol increased ~seven-fold during the NF-trial, and ~nine-fold during the FR-trial (NS). There were no differences between the trials with respect to changes in plasma lipid metabolites (Figure 5.7).

Plasma ammonia increased by ~150% in both trials (Figure 5.8). This represents a rate of change of 0.79 (± 0.15) $\mu\text{mol}\cdot\text{l}^{-1}\text{min}^{-1}$ during the NF-trial, compared with 0.59 (± 0.12) $\mu\text{mol}\cdot\text{l}^{-1}\text{min}^{-1}$ during the FR-trial (NS).

5.3.5 Serum cortisol responses

There were no differences in serum cortisol responses between the two trials. Concentrations increased by 16% during the NF-trial (ie. 481 \pm 46 to 569 \pm 47 nmol·l⁻¹) at a rate of 0.98 (± 0.74) nmol·l⁻¹min⁻¹. During the FR-trial serum cortisol increased by 22% (ie. 483 \pm 59 to 606 \pm 47 nmol·l⁻¹) at a rate of 1.03 (± 0.57) nmol·l⁻¹min⁻¹.

5.3.6 Plasma sodium and potassium responses

Electrolyte responses did not differ between the two trials. Plasma potassium increased by 21% during the NF-trial (ie. 4.00 \pm 0.18 to 4.83 \pm 0.15 mmol·l⁻¹, $p < 0.01$), and by 23% during the FR-trial (ie. 4.08 \pm 0.10 to 5.00 \pm 0.32 mmol·l⁻¹, $p < 0.01$). Whilst plasma sodium concentrations remained stable during both trials (ie. NF-trial: pre- vs. post-, 140.0 \pm 0.7 to 141.7 \pm 0.8 mmol·l⁻¹; FR-trial: pre- vs. post-, 138.8 \pm 0.7 to 141.5 \pm 1.3 mmol·l⁻¹).

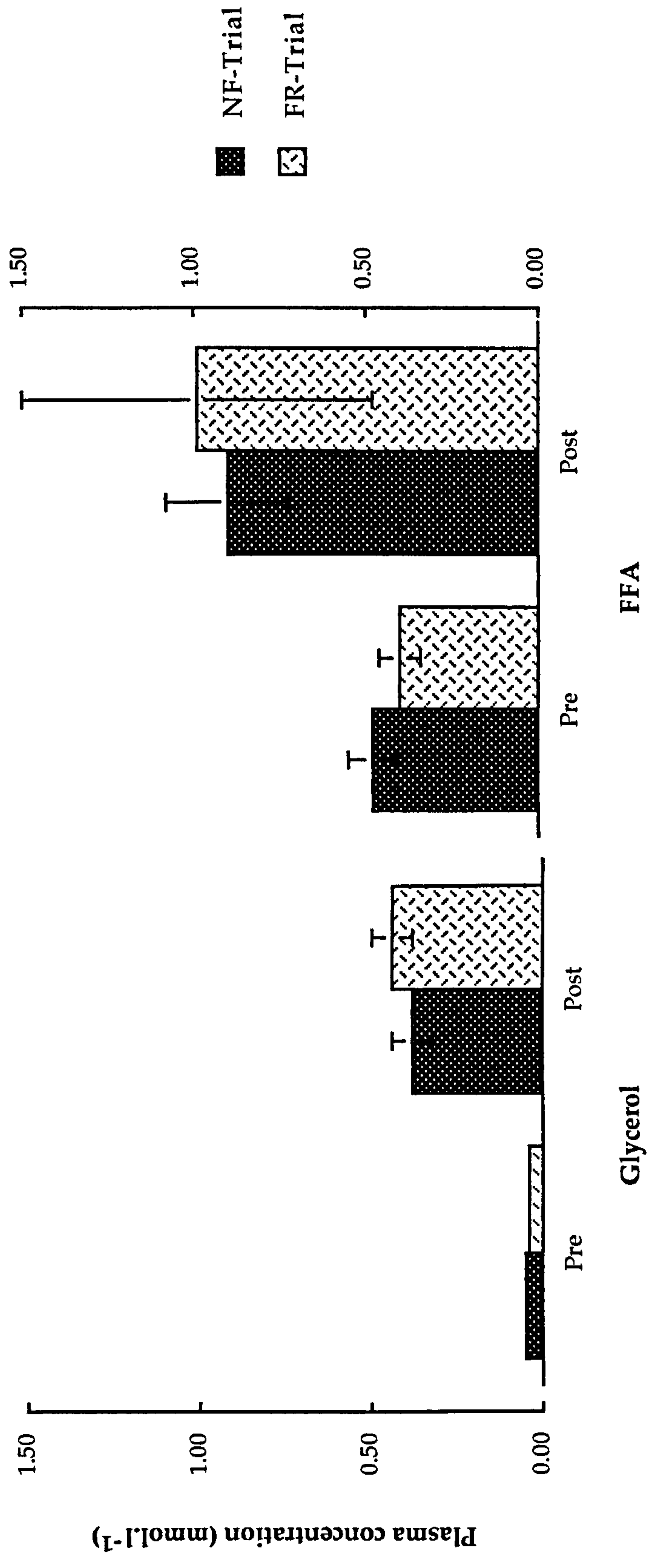


Fig 5.7 Plasma glycerol and FFA concentrations during the no fluid (NF) and fluid replacement (FR) trials (mean±SE)

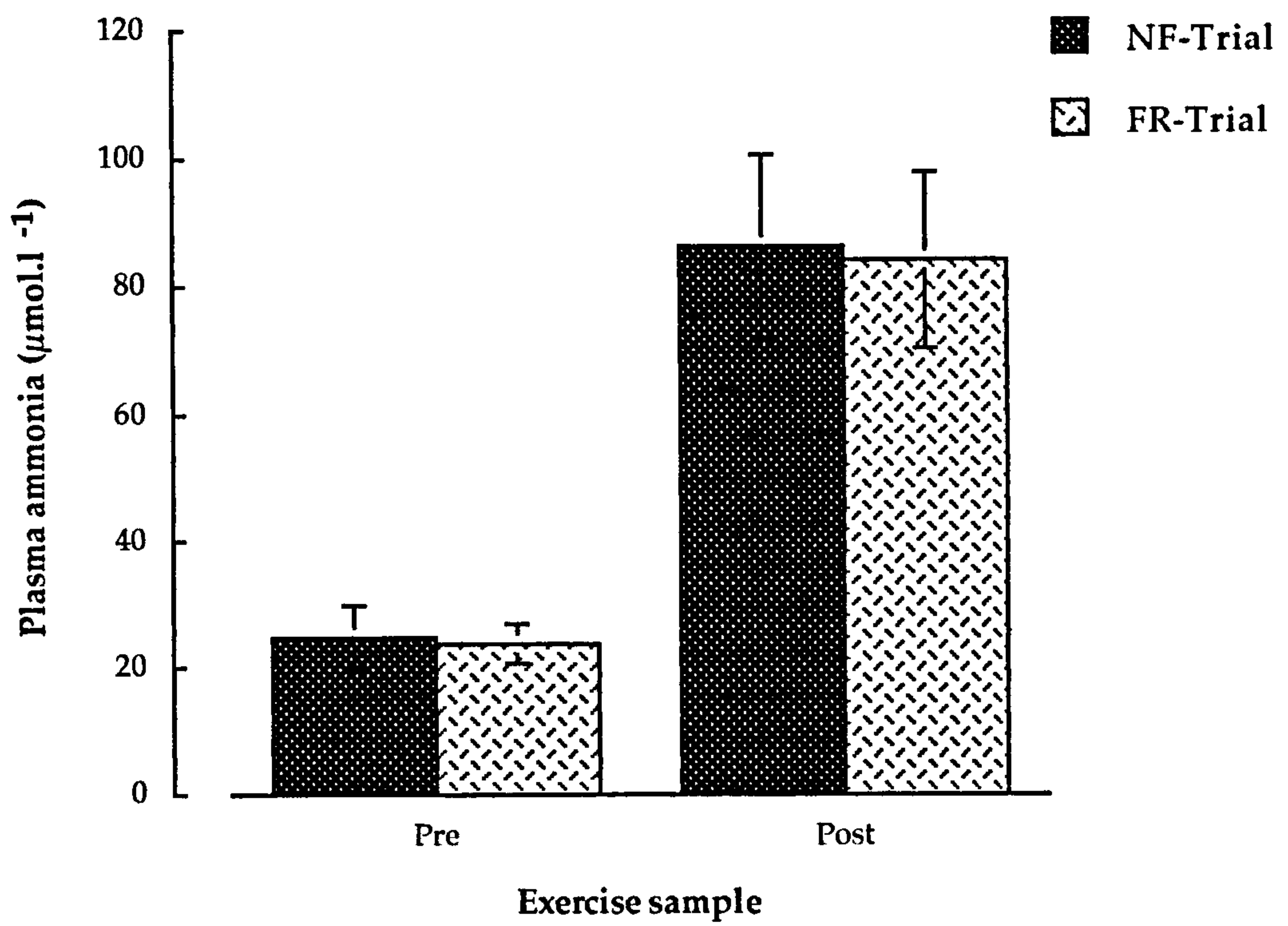


Fig 5.8 Plasma ammonia concentrations during the no fluid (NF) and fluid replacement (FR) trials (mean±SE)

5.3.7 Body weight, plasma volume and heart rate responses

Total water ingestion during the FR-trial was 929 (± 96) ml, which is equivalent to $9 \text{ ml}\cdot\text{min}^{-1}$ or $0.5 \text{ l}\cdot\text{h}^{-1}$. After correcting for fluid intake, body weight decreased by 1.71 (± 0.16) kg in the FR-trial and 1.29 (± 0.17) kg in the NF-trial, which are equivalent to decreases in body weight of 2.7 (± 0.2)% and 2.0 (± 0.2)% respectively ($p < 0.01$). Estimated sweating rates were similar for the two trials (ie. NF-trial: $\sim 0.84 \text{ l}\cdot\text{h}^{-1}$; FR-trial: $\sim 0.83 \text{ l}\cdot\text{h}^{-1}$), which are consistent with a rate of weight loss equivalent to $\sim 1.0 \text{ kg}\cdot\text{h}^{-1}$ for both trials. Plasma volume was reduced by 1.1 (± 1.1) % during the NF-trial, and by 3.5 (± 1.1) % during the FR-trial (NS). This difference was not significant due to a high variance in measures, which was evident despite careful experimental control. Between the 5 min- and End-sampling points, HR increased by 12.8 (± 3.2)% in the NF-trial and by 11.9 (± 3.8)% in the FR-trial.

Perceived exertion increased with run time in both trials. However, scores were higher in the NF-trial at the 30 min ($p < 0.05$), and 45 and 60 min ($p < 0.01$) time points. In agreement, increases in the sensation of muscular fatigue (CRS-scores) paralleled PRE scores, with higher scores during the NF-trial at the 45 min ($p < 0.05$) and 60 min ($p < 0.01$) time points.

5.3.8 Thermoregulatory responses

Weighted mean T_{sk} and T_{rec} were equally well maintained during the two trials (Figures 5.9 and 5.10). An increase in T_{rec} during the initial 35 min of exercise was followed by a period of relative stability. In the NF-trial T_{rec} plateaued at $38.6 (\pm 0.1) ^\circ\text{C}$, whereas a higher plateau of $39.1 (\pm 0.1) ^\circ\text{C}$ was maintained in the FR-trial ($p < 0.01$). Core temperature was again tending to increase at exercise cessation. Mean T_{sk} tended to decrease following the onset of exercise, and was then maintained at $\sim 30.0^\circ\text{C}$ throughout the remainder of both trials.

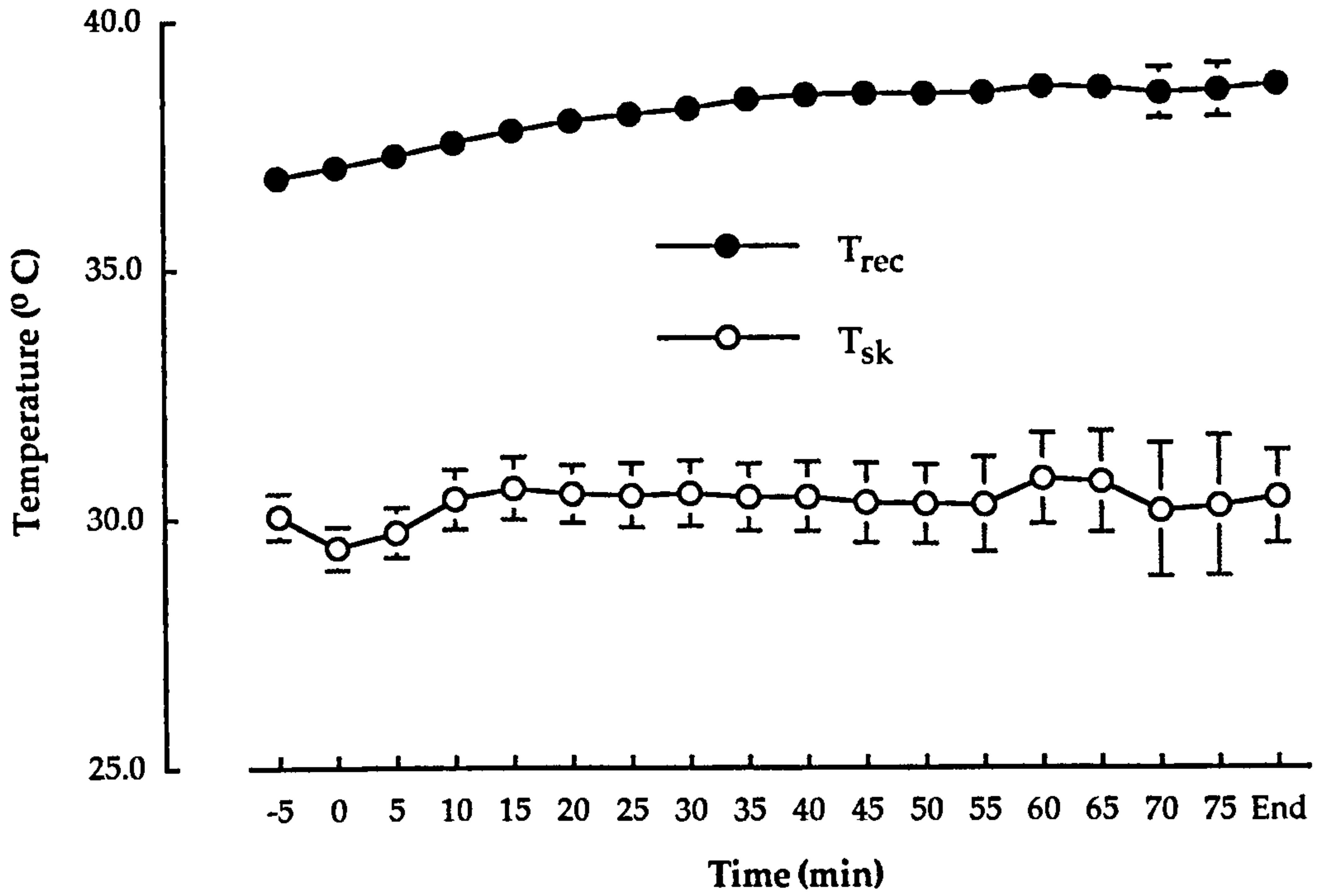


Fig 5.9 Rectal (T_{rec}) and weighted mean skin (T_{sk}) temperature responses during the no fluid trial (mean \pm SE)

Note : Error bars hidden by symbol

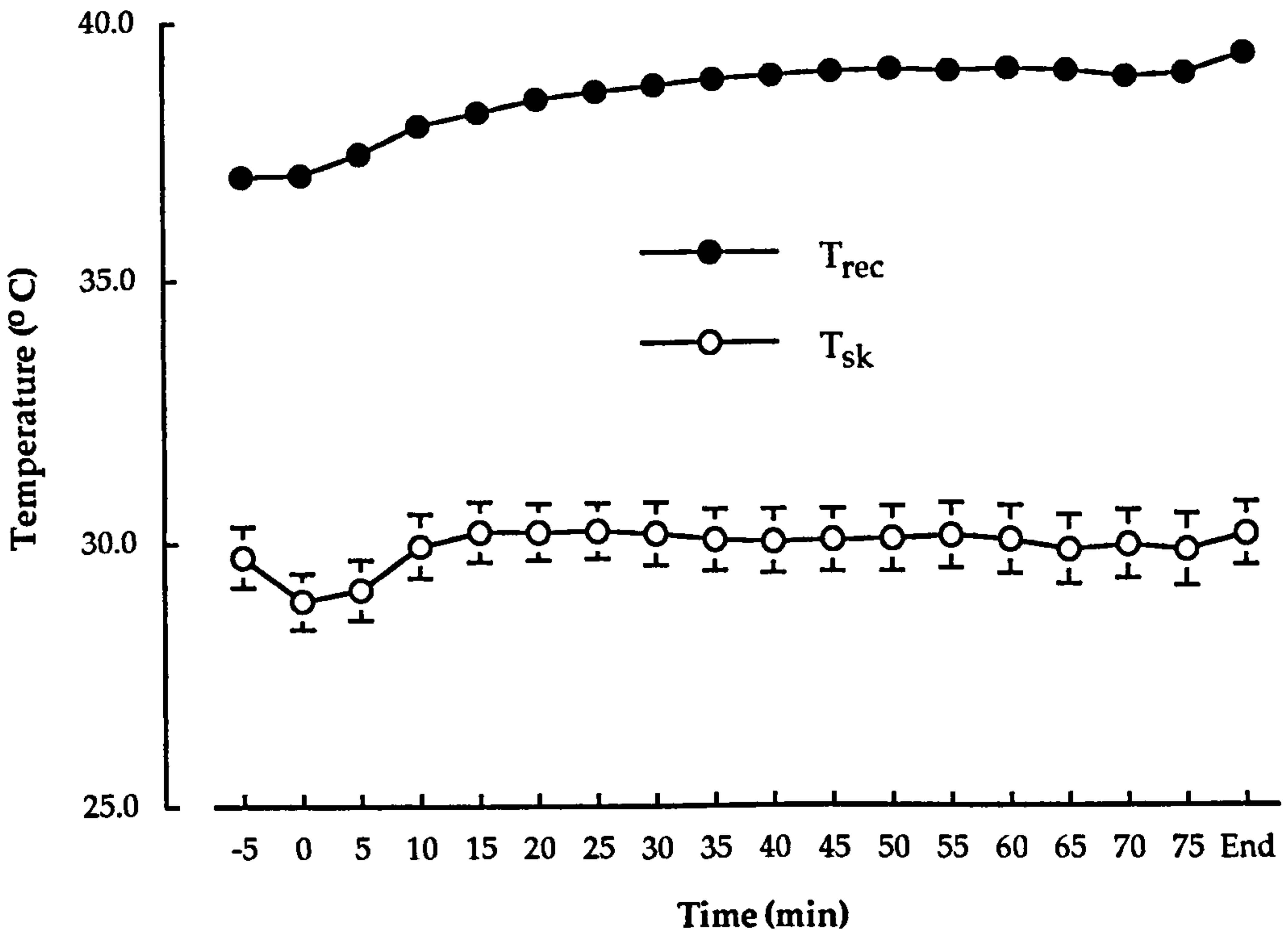


Fig 5.10 Rectal (T_{rec}) and weighted mean skin (T_{sk}) temperature responses during the fluid replacement trial (mean \pm SE)

5.4 Discussion

The main finding of the study was that the ingestion of $3.0 \text{ ml}\cdot\text{kg}^{-1}$ body wt of water prior to prolonged running, and further feedings of $2.0 \text{ ml}\cdot\text{kg}^{-1}\text{body wt}\cdot 15\text{min}^{-1}$ during the run, improved endurance capacity. A difference in performance between the NF and FR trials was evident despite similar decreases in body weight, which provided an index of exercise-induced fluid loss. The prescribed rate of water ingestion is consistent with the American College of Sports Medicine position statement for The Prevention of Thermal Injuries During Distance Running (1987). This rate of fluid ingestion appeared to maintain performance by off-setting whole body thermoregulatory fluid losses.

During 60 min of exercise at $70\% \dot{V}O_2\text{max}$, T_{rec} , V_s and HR were observed to remain relatively stable (Hamilton et al, 1991). However, these responses began to diverge when exercise was continued for a further 60 min. In a NF-trial, T_{rec} and HR rapidly increased, possibly in response to a reduction in V_s . Thus, water ingestion during prolonged exercise attenuates hyperthermia over the later stages (Costill et al, 1970; Hamilton et al, 1991). Costill et al (1970) reported a plateauing of T_{rec} after ~ 45 min of running ($70\% \dot{V}O_2\text{max}$). Consistent with this observation, a plateau in T_{rec} was observed in the present study after ~ 35 min of exercise. There were no differences in HR response between the two conditions, where a secondary rise in HR following an initial increase during both trials was symptomatic of an underlying cardiovascular drift (Nielsen et al, 1984). Weighted mean T_{sk} and T_{rec} reflected similar levels of thermoregulatory control during the two trials. Plasma cortisol concentrations were further indicative of comparable exercise-distress tolerances. This failure to differentiate between the NF and FR trials in the present study is possibly a function of the exercise time and training status of the subjects. Unlike the previously cited studies, subjects in this study were routinely exercising but were not highly trained endurance athletes. Thus, factors other than circulatory dysfunctioning (eg. compromised energy substrate availability in muscle) may have precipitated the earlier onset of fatigue in the NF-trial.

A tendency towards a greater decrease in plasma volume during the FR-trial cannot be explained from the available data. It is possible that changes in osmotic balance between body compartments as a consequence of drinking during exercise may have influenced fluid distribution (Nielsen et al, 1986). Though a high variance in the measurements make further speculation tenuous. Moreover, it remains open to debate whether the small changes in plasma volume reported in the present study are of any physiological significance (Harrison, 1985).

Plasma sodium concentrations were maintained within the normal range in both trials (ie. 135 to 145 mmol·l⁻¹, Nottingham City Hospital, Nottingham, UK, normative data, personal communication). This is in contrast with previous findings where plasma sodium concentrations decreased during prolonged exercise (Barr et al, 1991). Plasma potassium increased ~22% in response to prolonged running. This change will partly reflect enhanced mobilisation of muscle glycogen (Hultman, 1967), whilst potassium conductance is enhanced in fatigued, glycogen-deficient muscle fibres (Fink and Luttgau, 1976). Notably, an elevated cellular efflux of potassium accompanying exercise did not increase plasma concentrations beyond the normal physiological range (ie. 3.5 to 5.3 mmol·l⁻¹, Nottingham City Hospital, Nottingham, UK, normative data, personal communication). Changes in plasma osmolality are implicated in reduced cutaneous blood flow (Horstman and Horvarth, 1972), and a diminished sweating response (Costill et al, 1976; Greenleaf and Castle, 1971; Nielsen, 1974). Thus, an impaired capacity to dissipate heat may have contributed to fatigue.

Montain and Coyle (1992a) examined fluid ingestion during prolonged cycling. A carbohydrate-electrolyte solution was prescribed before and during a FR-trial. Exercise responses to the FR-trial were compared with a NF and a control (CON) trial. Blood volume was maintained during the CON-trial, without disturbing serum osmolality, through intravenous infusion of a blood volume expander. Fluid replacement maintained skin blood flow such that hyperthermia was reduced during the later stages of prolonged exercise. As core temperature was lower in the FR-trial in comparison with the

CON-trial, this effect was not simply due to the maintenance of systemic blood volume. Montain and Coyle (1992a) suggest that fluid ingestion during exercise stabilises skin blood flow, which in turn will influence the ability to thermoregulate, by preventing an increase in serum osmolality.

Fluid ingestion in the present study was associated with a more favourable energy balance with respect to sustaining prolonged exercise. Fat oxidation was suppressed during the NF-trial as carbohydrate oxidation was enhanced. This shift in energy metabolism was reflected in elevated blood glucose and blood lactate concentrations over the later stages of exercise. Conversely, VO_2 -drift was greater during the FR-trial, which indicates an increasing oxygen cost of energy metabolism in the face of parallel minute ventilation rates. Thus, dependency upon fat metabolism to fuel activity was enhanced in the FR-trial, whilst a dependency upon carbohydrate metabolism decreased.

Elevated blood lactate concentrations, which are associated with more rapid rates of glycogen depletion, have previously been reported during dehydrating exercise (Kozłowski et al, 1985; Nadel et al, 1980). In the present study, anecdotal reports of localised fatigue in the quadriceps and gastrocnemius muscles, possibly arising from such selective substrate depletion, were associated with higher CRS- and PRE-scores during the NF-trial.

Differences in carbohydrate metabolism between the two trials may have thermoregulatory implications if it is assumed that water liberated from glycogen breakdown becomes physiologically available (Olsson and Saltin, 1970). As discussed previously, temperature regulation was similar during the two trials, as were estimated rates of sweating and body weight loss. Carbohydrate oxidation during the FR-trial was equivalent to $1.8 (\pm 0.1) \text{ g}\cdot\text{min}^{-1}$, which is potentially associated with a liberation of $\sim 4.9 \text{ ml}\cdot\text{min}^{-1}$ of water (ie. assuming 2.7 g of water is bound with 1.0 g of glycogen). Water absorption in the small intestine takes place at a rate of $\sim 1.8 (\pm 2.8) \text{ ml}\cdot\text{cm}^{-1}\cdot\text{h}^{-1}$ (Leiper and Maughan, 1986) or $\sim 8 \text{ ml}\cdot\text{min}^{-1}$. Thus, $\sim 13 \text{ ml}\cdot\text{min}^{-1}$ of water would theoretically be made physiologically available during the FR-

trial. A carbohydrate oxidation rate equivalent to $2.0 (\pm 0.2) \text{ g}\cdot\text{min}^{-1}$ during the NF-trial would potentially be associated with only $\sim 5.5 \text{ ml}\cdot\text{min}^{-1}$ of water. Accepting that these calculations are largely speculative, it is apparent that $\sim 130\%$ more fluid might be made available during the FR-trial in comparison with the NF-trial. This is despite possible increases in water availability arising from shifts in energy metabolism. Nevertheless, an incidental thermoregulatory role of metabolic water cannot be discounted. Where body fluid compartment stores combine with the small amount of water liberated during exercise energy metabolism, to contribute to the maintenance of a thermic homeostasis in the NF-trial. Plyley et al (1980) observed that during 45 min of exercise at $65\% \dot{V}O_{2\text{max}}$ in a hot, moderately humid environment, glycogen-bound water appears to function in the maintenance of plasma volume rather than playing a direct role in temperature regulation. However, it might be argued that this role in ensuring cardiovascular integrity indirectly assists thermoregulation.

In conclusion, the ingestion of water during prolonged running improves endurance capacity. Fluid ingestion during exercise was associated with a more favourable fuel balance with respect to meeting the body's energy requirements. In contrast, abstaining from fluid ingestion was accompanied by enhanced carbohydrate oxidation and suppressed fat oxidation. This shift became manifest in elevated blood lactate concentrations and localised muscle fatigue over the later stages of exercise. Thus, fluid ingestion during exercise plays a role in optimising performance, but may also hold implications for post-exercise recovery.

The influence of liquid carbohydrate ingestion on short-term recovery from prolonged, constant pace running

6.1 Introduction

The recovery process is concerned with reinstating a normal physiological balance such that further activity is made possible. The ability to sustain prolonged exercise is limited by several factors. Chapter 4 examined the effect of increased carbohydrate provision in alleviating one possible factor (Bergstrom and Hultman, 1967), whilst Chapter 5 examined the factors limiting performance arising through exercise-induced dehydration (Armstrong et al, 1985). Ingesting carbohydrate-electrolyte (CE) beverages may alleviate symptoms of fatigue associated with such physiological disturbances (Carter and Gisolfi, 1989).

The biological availability of an orally ingested solution is jointly determined by gastric emptying, intestinal absorption, and subsequent fluid retention (Mitchell and Voss, 1991). All three processes are influenced by the solute content of a fluid (Costill and Saltin, 1974; Nose et al, 1988a; Nose et al, 1988b). The addition of carbohydrate provides energy, and in small amounts, does not appear to compromise fluid replacement (Costill and Sparks, 1973). An adequate carbohydrate intake following exercise is essential for a rapid repletion of endogenous reserves (Bergstrom et al, 1967). Optimal rates of muscle glycogen resynthesis are achieved when $1.0 \text{ g}\cdot\text{CHO}\cdot\text{kg}^{-1}\cdot\text{body wt}\cdot 2\text{h}^{-1}$ is consumed over the initial 4 to 6-h post-exercise (Ivy, 1991). This in turn will enhance energy availability during further exercise.

Ingesting CE solutions during exercise improves prolonged, exercise performance (Maughan et al, 1989; Tsintzas et al, 1993a; Williams et al, 1990), whilst dehydration is reduced (Carter and Gisolfi, 1989). However, it remains unclear whether provision of CE solutions during a short recovery period similarly promotes rehydration and carbohydrate availability, such that endurance capacity is improved during a later bout of exercise.

Thus, the aim of this study was to compare the influence of ingesting either sweetened water or an optimum quantity of a carbohydrate (glucose-polymer) solution during 4-h recovery, on rehydration and subsequent exercise capacity.

6.2 Methods

6.2.1 Subjects

Twelve men and four women took part in the study. Subjects were allocated to two matched groups which were randomly assigned to either a control (P) or a carbohydrate (CHO) trial. Subjects were matched according to age, height, weight, $\dot{V}O_2\text{max}$, $\dot{V}_E\text{max}$, HRmax, and training status.

Mean age, height, weight and $\dot{V}O_2\text{max}$ of the P group were 27.9 (± 1.9) yrs, 173.9 (± 2.5) cm, 68.8 (± 3.4) kg and 57.6 (± 2.1) ml·kg⁻¹ min⁻¹ respectively, and of the CHO group were 26.1 (± 1.3) yrs, 174.3 (± 2.4) cm, 68.8 (± 4.6) kg and 59.5 (± 1.7) ml·kg⁻¹min⁻¹ respectively.

6.2.2 Protocol

Weighed-food intake dietary analyses were completed before the start of the study (Section 3.3), from which subjects were prescribed their normal diet over the 48-h prior to the experimental trial.

After familiarisation subjects completed the three preliminary tests (Section 3.4), allowing training status to be ascertained (Table 6.1), and appropriate running speeds for the experimental trials determined.

The first run (R_1) was performed early in the morning after a 10-h overnight fast (Figure 3.1). Subjects were prepared for the trial as described previously.

Whilst maintaining a relaxed seated position on the treadmill, a pre-exercise expired air sample was collected using the Douglas bag technique. A 10 ml venous blood sample was drawn from an antecubital vein after ~15 min of rest. Simultaneously, duplicate 20 μ l capillary blood samples were taken from the thumb of a pre-warmed hand. These procedures were repeated immediately prior to R₂. Subjects then performed the standardised warm-up and first treadmill run (R₁).

Expired air samples were collected at 15 min intervals during R₁ and R₂, and subjective ratings of perceived exertion and muscular effort obtained. Further capillary samples were taken after 30 and 60 min of each run, from which blood glucose and blood lactate were determined. Venous and capillary blood samples were similarly obtained at the end of R₁ and R₂. Immediately following post-exercise blood sampling, sweat was sampled in the male subjects from the scapula region of the back as described in Section 3.8. Wet sponges and drinking water were available *ad libitum* during exercise. The total fluid ingested was recorded at the end of each run and accounted for in post-exercise changes in body weight. Only a prescribed fluid was ingested during the recovery.

The standardised endurance task R₁ was followed by a controlled 4-h recovery. After which, R₂ provided a measure of the efficacy of the prescribed recovery in terms of exercise time to fatigue.

An isotonic sports drink (6.9% GP solution; Lucozade Sport, Smithkline Beecham Plc) was ingested during the CHO trial immediately following R₁, and 2-h later (Appendix L). Each feeding provided a carbohydrate load equivalent to 1.0 g·kg⁻¹ body wt. A placebo drink was provided in the form of a low-calorie orange cordial diluted in water. Equal volumes of the placebo drink were ingested at the same time points during the control (P) trial.

Packed cell volume and haemoglobin concentrations were determined in whole blood, and FFA (method-i), glycerol, sodium, potassium, ammonia and urea concentrations determined in plasma. Cortisol and insulin concentrations were determined in serum. Blood samples were collected, treated, stored and analysed as previously described (Section 3.7).

Performance times were compared by analysis of covariance, where R_1 represented the covariate. Blood biochemical responses within each trial were examined using Student's T-test for paired data. Differences between trials were examined by an independent T-test. All other physiological responses were analysed by two-way ANOVA with repeated measures on one factor (treatment-by-time), and a Tukey post-hoc test.

6.3 Results

6.3.1 Performance

The run times of the P and CHO trials were not different for R_1 (P: 86.3 ± 3.8 min, CHO: 87.5 ± 2.5 min), whereas the CHO group exercised for $22.2 (\pm 3.5)$ min longer than the P group during R_2 ($p < 0.05$). Mean run times over this second exercise bout were $39.8 (\pm 6.1)$ min for the P trial (range 20.0 to 66.5 min), and $62.0 (\pm 6.2)$ min for the CHO trial (range 42.5 to 90.0 min).

Table 6.1 Running speeds ($\text{m}\cdot\text{s}^{-1}$) and relative exercise intensities ($\% \dot{V}\text{O}_2\text{max}$) at blood lactate concentrations of $2 \text{ mmol}\cdot\text{l}^{-1}$ and $4 \text{ mmol}\cdot\text{l}^{-1}$ of the control (P) and carbohydrate (CHO) groups (mean \pm SE)

	Speed		$\% \dot{V}\text{O}_2\text{max}$	
	$2 \text{ mmol}\cdot\text{l}^{-1}$	$4 \text{ mmol}\cdot\text{l}^{-1}$	$2 \text{ mmol}\cdot\text{l}^{-1}$	$4 \text{ mmol}\cdot\text{l}^{-1}$
P	3.71	4.56	67.3	86.3
\pm SE	0.21	0.18	2.8	1.3
CHO	3.57	4.65	68.3	85.1
\pm SE	0.25	0.15	4.1	3.1

6.3.2 Blood glucose, blood lactate, serum insulin and serum cortisol responses

Blood glucose was maintained within the normal range during R₁ (Figure 6.1), whilst serum insulin concentrations decreased by ~50% (Table 6.2). Serum insulin was higher in the CHO trial following the 4-h recovery, and then decreased by 68% during R₂. Nevertheless, blood glucose remained stable in the face of these fluctuations in serum insulin concentrations. Serum cortisol increased during exercise in both trials ($p < 0.05$) (Table 6.2). This change in systemic concentrations was greater over R₂ in both trials (ie. P: R₂Δ% +107%; CHO: R₂Δ% +85%).

Blood lactate increased with the onset of exercise (Figure 6.1). Pre-R₁ concentrations were restored in the P trial during the recovery, whereas blood lactate remained elevated prior to R₂ in the CHO trial (pre-R₁ vs. pre-R₂ - ΔP: 0.21 mmol·l⁻¹; ΔCHO: 0.39 mmol·l⁻¹, $p < 0.05$). This finding could not be attributed to differences in training status, as the two groups were well matched with respect to running economy and blood lactate responses to submaximal graded exercise (Table 6.1). There were no differences between the trials in blood lactate concentrations during the second exercise bout.

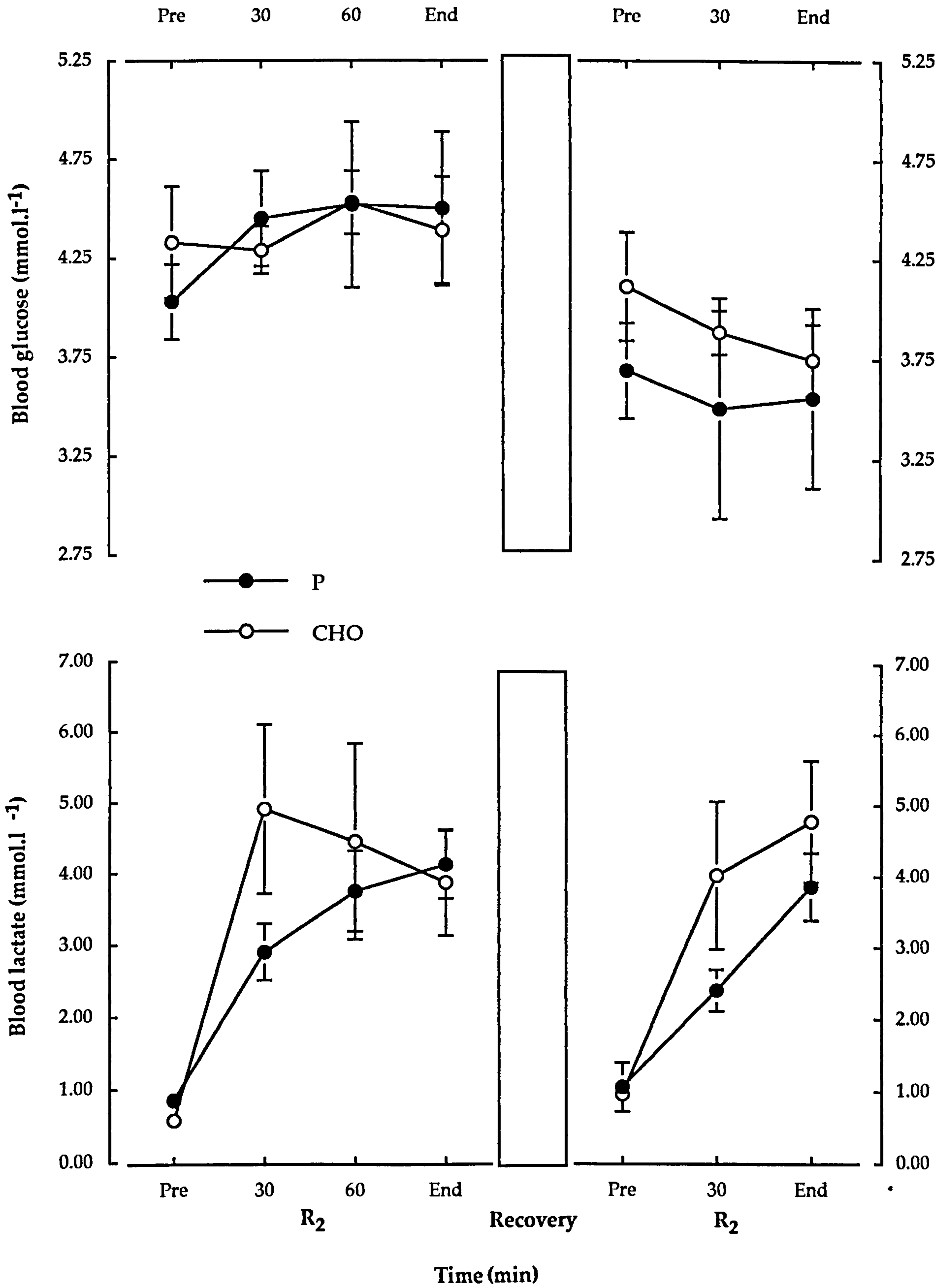


Fig 6.1 Blood glucose and blood lactate concentrations of the control (P) and carbohydrate (CHO) trials during R₁ and R₂ (mean±SE)

Table 6.2 Serum insulin (mU·l⁻¹) and serum cortisol (nmol·l⁻¹) concentrations of the control (P) and carbohydrate (CHO) trials over R₁ and R₂ (mean±SE)

		Serum insulin		Serum cortisol	
		Pre	Post	Pre	Post
R ₁	P	13.54	6.54	596.9	*748.9
	± SE	6.16	1.89	44.5	26.8
	CHO	11.72	5.98	589.0	*621.3
	± SE	4.42	2.21	63.6	39.5
R ₂	P	6.83	4.32	325.6	*675.5
	± SE	1.45	1.10	29.0	45.3
	CHO	10.57	3.34	358.3	*665.7
	± SE	3.68	0.18	56.7	62.0

* Denotes significantly different from pre-exercise values (p<0.05)

6.3.3 Pre-exercise energy intake and fuel utilisation during exercise

The carbohydrate intake of the P and CHO groups over the 48-h prior to R₁ represented 53 (±3)% and 55 (±4)% of the total energy intake respectively (Table 6.3). The P group expended 4.8 (±0.3) MJ of energy during R₁, compared with 5.0 (±0.3) MJ expended by the CHO group. The fluid ingested by the P group during the recovery contained 15.5 (±0.8) g of carbohydrate providing 0.3 MJ, whereas the fluid ingested by the CHO group contained 138.0 (±9.0) g of carbohydrate and provided 2.3 MJ.

The nature of energy metabolism did not differ between the two trials during R₁ (Figure 6.2), though R values were higher in the CHO trial compared with the P trial after 15 min of R₂ (p<0.05). Carbohydrate oxidation contributed 41% of the total energy requirement of the P group during R₁, compared with 32% during R₂. Whilst 47% of the energy requirement was provided by carbohydrate oxidation during R₁ in the CHO group, compared with 44% during R₂. Thus, the P group relied less on carbohydrate as a fuel for exercise over the later stages of R₂ in comparison with the CHO group (p<0.05).

The oxygen cost of constant pace running during R₁ and R₂ for the P and CHO trials are given in Table 6.4. There was evidence of $\dot{V}O_2$ -drift during R₁ (p<0.01) in both trials, though there were no differences between the trials. This was associated with an increase in the estimated energy cost of running equivalent to ~3.9 KJ·min⁻¹ during exercise.

Table 6.3 The daily energy and nutrient intakes of the subjects in the control (P) and carbohydrate (CHO) groups (mean \pm SE)

	Energy	Protein		Carbohydrate		Fat
	MJ	g	g \cdot kg ⁻¹ body wt	g	g \cdot kg ⁻¹ body wt	g
P	13.4	109.2	1.6	443.1	6.4	113.9
\pm SE	1.2	9.7	0.1	53.1	0.8	12.0
CHO	12.3	97.3	1.4	413.7	6.0	95.7
\pm SE	0.8	9.2	0.1	30.1	0.4	14.5

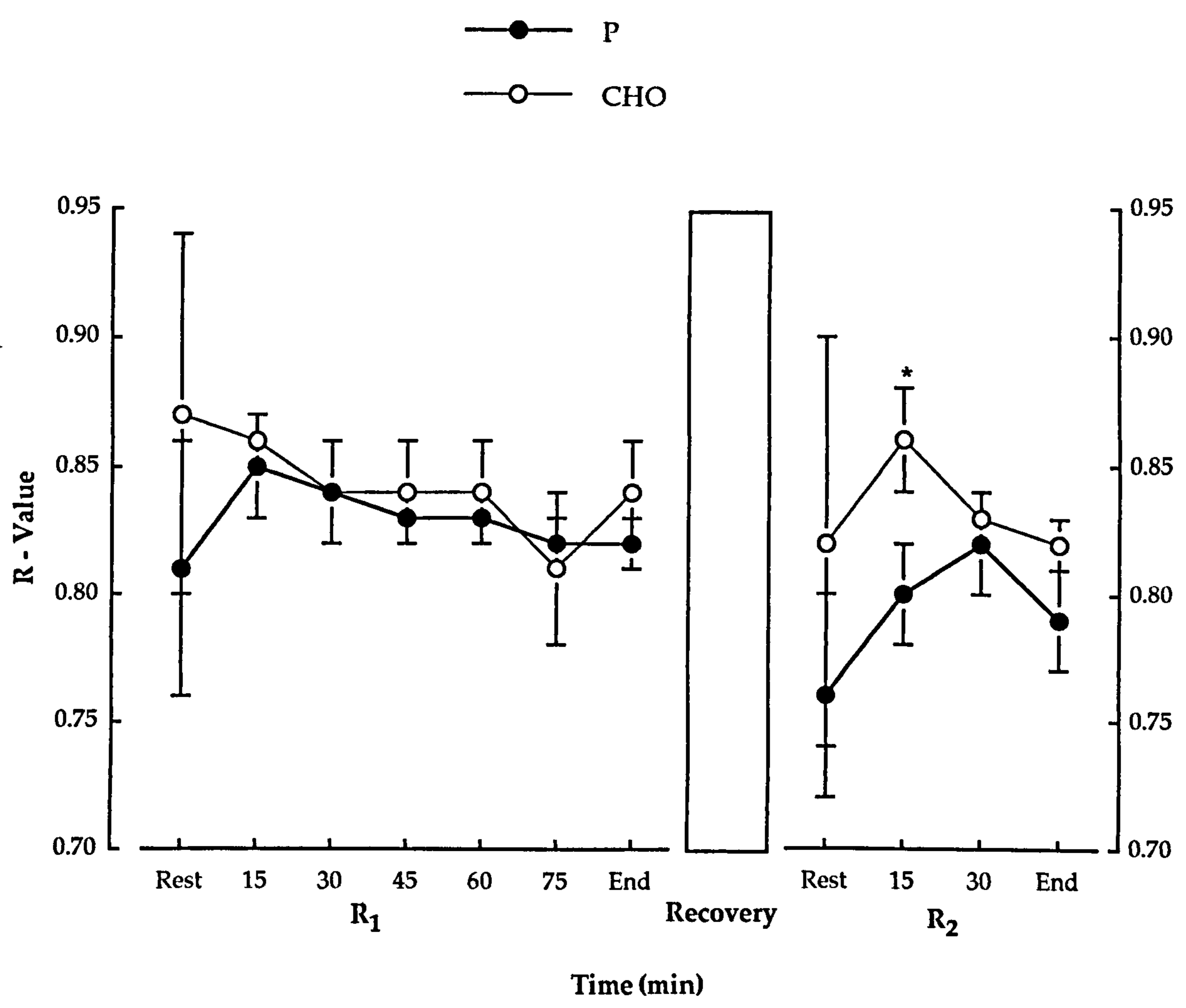


Fig 6.2 R values of the control (P) and carbohydrate (CHO) trials during R₁ and R₂ (mean±SE)

* Denotes P-trial significantly different from the CHO-trial (p<0.05)

Anova : Main effect (treatment) (p<0.05)

Table 6.4 Oxygen consumption ($l \cdot \text{min}^{-1}$) of the control (P) and carbohydrate (CHO) trials during R₁ and R₂ (mean \pm SE)

	R ₁						R ₂				
	Rest	15	30	45	60	75	End	Rest	15	30	End
P	0.22	2.83	2.90	2.94	2.98	2.88	3.01	0.24	3.04	2.94	3.01
\pm SE	0.02	0.19	0.19	0.20	0.22	0.23	0.23	0.01	0.19	0.29	0.23
CHO	0.25	2.96	2.99	3.02	3.04	3.11	3.12	0.28	2.99	3.05	3.07
\pm SE	0.02	0.17	0.18	0.18	0.18	0.25	0.21	0.03	0.19	0.20	0.21

6.3.4 Plasma FFA, glycerol, ammonia and urea responses

Plasma FFA concentrations doubled during R_1 ($p < 0.01$), and plasma glycerol increased approximately nine-fold ($p < 0.01$) (Figure 6.3). Plasma glycerol remained elevated in the P trial following the recovery ($p < 0.01$), whilst FFA concentrations had increased by a further 15% ($p < 0.01$). In contrast, pre-exercise plasma glycerol and FFA concentrations were restored in the CHO trial prior to R_2 . ←

Resting plasma ammonia concentrations (Figure 6.4) were higher in the CHO trial than in the P trial prior to R_2 ($p < 0.05$), though the rate of change of plasma ammonia during the second exercise bout was greater in the P trial ($p < 0.01$). Thus, absolute concentrations were the same in both trials at the end of exercise. Plasma urea increased with exercise during both trials (Figure 6.5), though notably concentrations tended to be elevated over R_2 in the P trial in comparison with the CHO trial (NS).

6.3.5 Plasma electrolyte responses

Plasma sodium concentrations did not differ between the two trials (Table 6.5). Plasma potassium increased by ~19% in both the P ($p < 0.01$) and CHO ($p < 0.05$) trials during R_1 . Pre- R_1 levels were restored in both trials prior to R_2 , after which potassium levels increased by 8.7% in the P trial (NS) and 13.5% in the CHO trial ($p < 0.05$) during the second exercise bout.

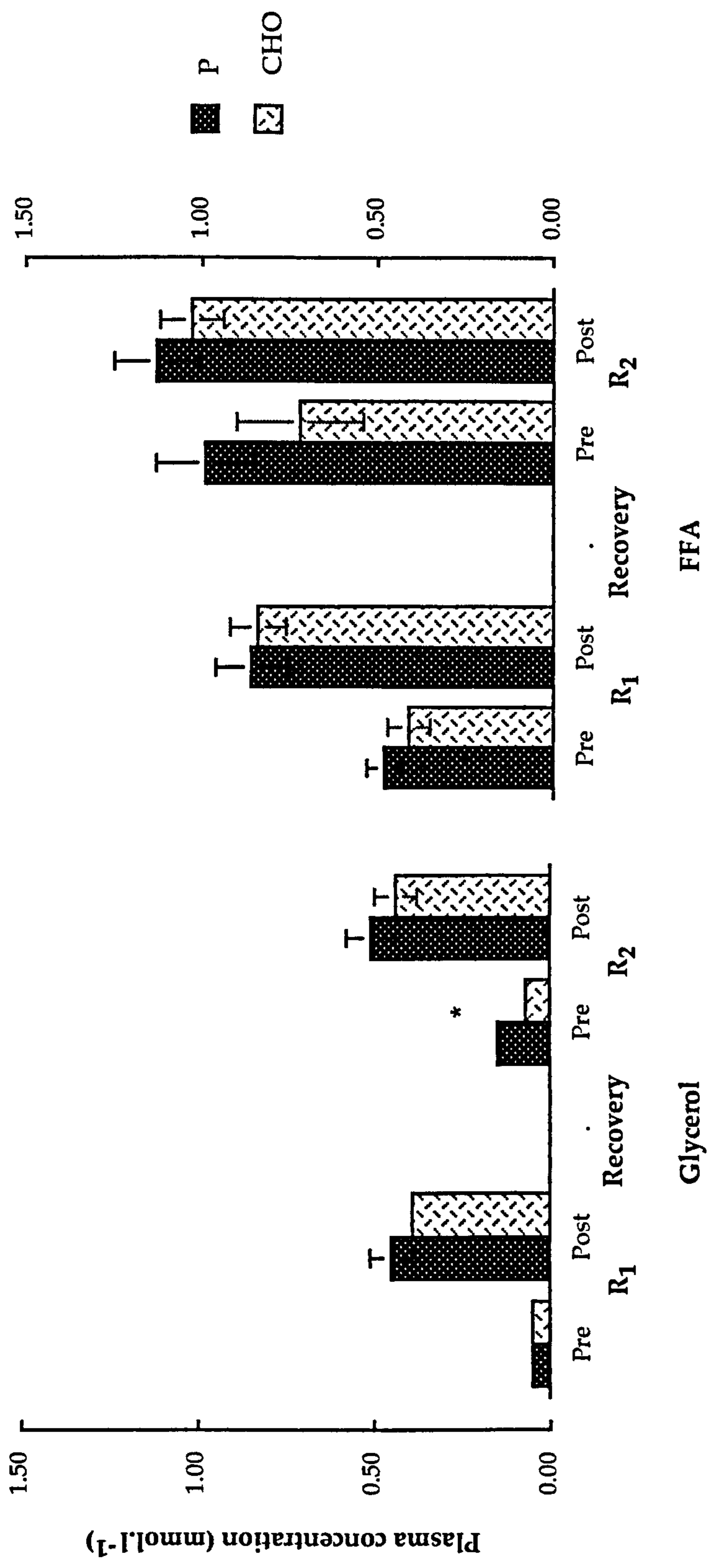


Fig 6.3 Plasma glycerol and FFA concentrations of the control (P) and carbohydrate (CHO) trials fo R₁ and R₂ (mean±SE)

* Denotes P-trial significantly different from the CHO-trial (p<0.05).

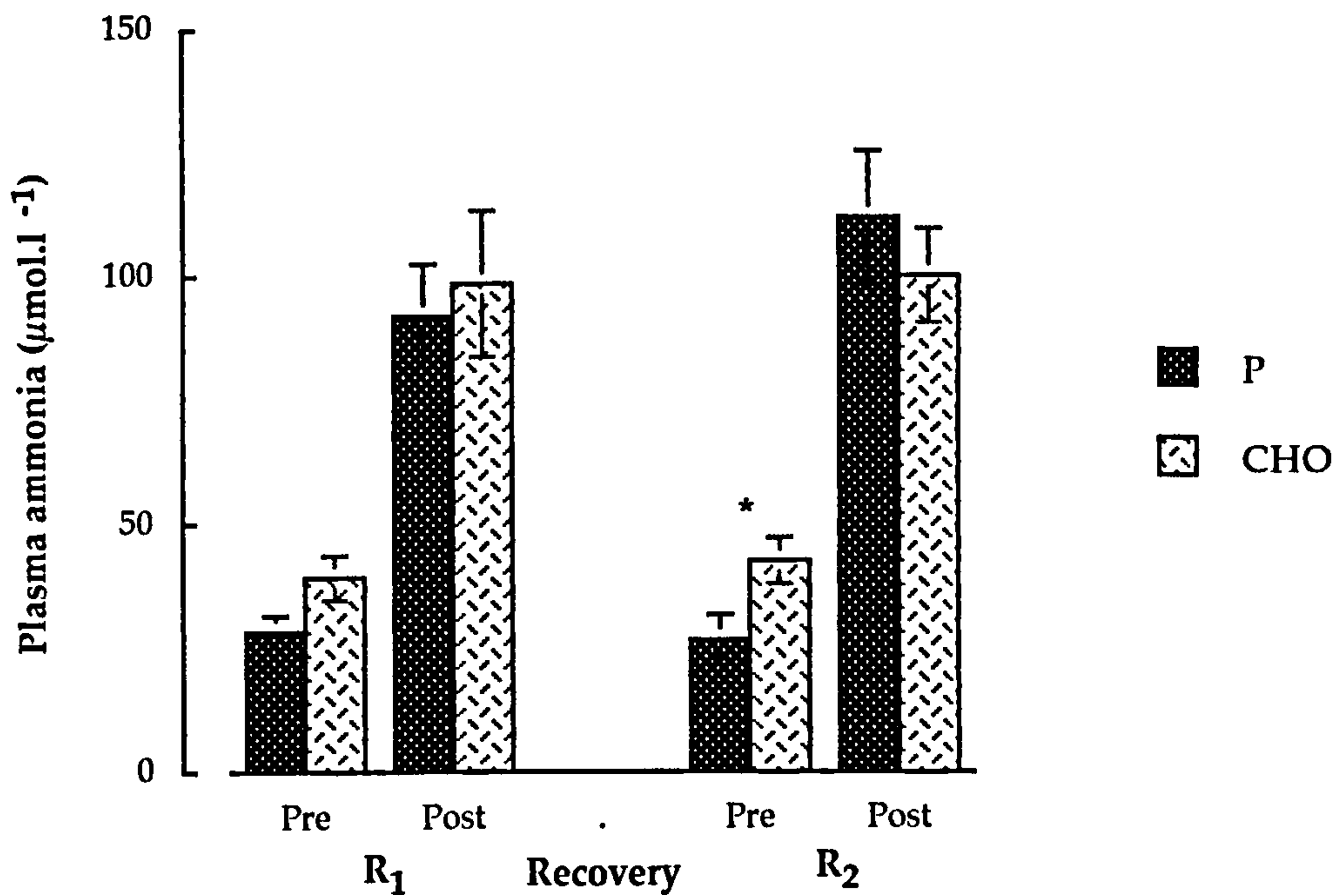


Fig 6.4 Plasma ammonia concentrations of the control (P) and carbohydrate (CHO) trials for R₁ and R₂ (mean±SE)

* Denotes P-trial significantly different from the CHO-trial (p<0.05)

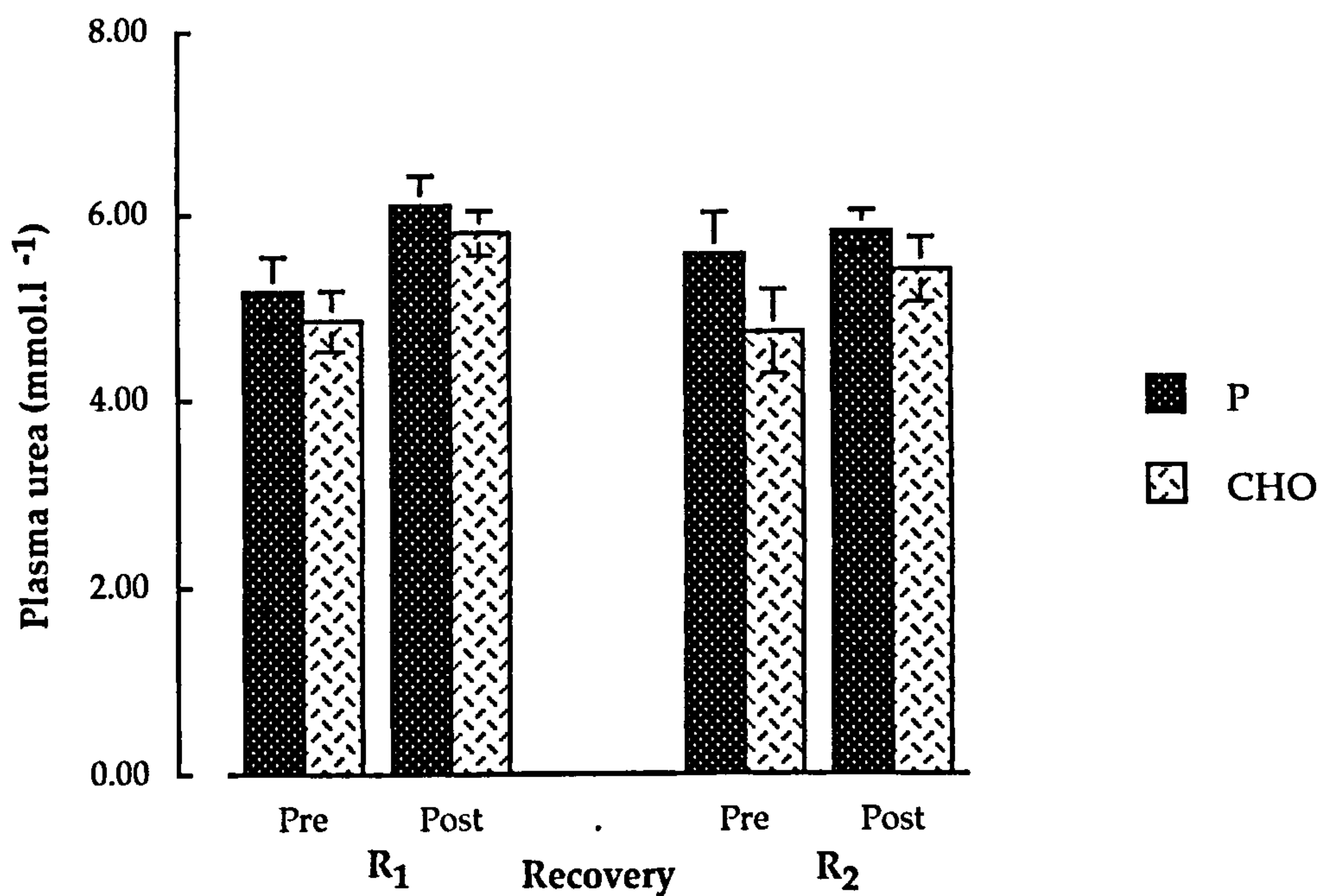


Fig 6.5 Plasma urea concentrations of the control (P) and carbohydrate (CHO) trials for R₁ and R₂ (mean±SE)

Table 6.5 Plasma electrolyte concentrations (mmol·l⁻¹) of the control (P) and carbohydrate (CHO) groups over R₁ and R₂ (mean±SE)

		Plasma sodium		Plasma potassium	
		Pre	Post	Pre	Post
R ₁	P	141.4	145.0	3.84	**4.59
	± SE	2.3	3.4	0.15	0.18
	CHO	141.1	141.1	4.08	*3.95
	± SE	2.3	3.2	0.16	0.07
R ₂	P	139.0	138.6	3.90	4.24
	± SE	3.1	2.6	0.13	0.18
	CHO	142.0	144.0	3.95	*4.48
	± SE	1.7	2.9	0.07	0.16

** Denotes post-run value significantly different from pre-run value (p<0.01)

* Denotes post-run value significantly different from pre-run value (p<0.05)

6.3.6 Plasma volume and heart rate responses, and subjective ratings of fatigue

Plasma volume decreased by 8.7 (± 1.4)% during R₁ ($p < 0.01$). Following restoration of pre-exercise levels during the 4-h recovery, plasma volume then decreased by 5.4 (± 1.3)% in the P trial and by 7.2 (± 1.7)% in the CHO trial during R₂ (NS).

There were no differences with respect to heart rate responses between the two trials.

Ratings of perceived exertion and muscular effort were higher in both trials during R₂ in comparison to R₁ ($p < 0.01$). It is worth noting that subjective ratings of PRE ($p < 0.05$) and CRS ($p < 0.01$) were higher in the P trial in comparison with the CHO trial after 15 min of R₂, and for the remainder of the second exercise bout.

6.3.7 Changes in body weight, %-Rehydration and thermoregulatory responses

Fluid losses resulted in a reduction in body weight of $\sim 2.6\%$ during R₁. During the recovery, a total of 1.98 (± 0.09) kg of fluid was ingested in two feedings in the P trial, and a total of 2.06 (± 0.13) kg of fluid was ingested in the CHO trial (NS). Neither trial restored pre-R₁ body weight following the 4-h recovery ($p < 0.05$). Estimated rehydration in the P trial was 65.9 (± 6.3)%, compared with 62.6 (± 7.3)% in the CHO trial (NS). These values take into account the weight of energy substrate metabolised during R₁, as well as the potential liberation of metabolic water. Thus, despite the two treatments providing adequate fluid to cover dehydratory weight losses incurred during R₁, subjects in both trials may have been relatively hypohydrated prior to R₂.

Nevertheless, despite this incomplete rehydration during the recovery, weighted mean T_{sk} followed similar profiles during R₁ and R₂ (Figures 6.6 and 6.7), though notably T_{sk} remained

elevated in both trials at the start of R₂ ($p < 0.01$). In contrast with the periphery, pre-R₁ T_{rec} was restored during the P trial recovery, but was still elevated at the start of R₂ in the CHO trial ($p < 0.05$).

6.3.8 Post-exercise sweat secretion

Post-exercise sweat secretion rates were similar in the two trials. Secretion followed an exponential pattern, with values ranging from 20 (± 9) ml·m⁻²·min⁻¹ at the end of exercise to 6 (± 2) ml·m⁻²·min⁻¹ after 5 mins of recovery. Sodium secretion following R₁ decreased from 2.5 (± 0.5) mmol·m⁻²·min⁻¹ to 1.1 (± 0.3) mmol·m⁻²·min⁻¹ by the fifth minute of sampling. A similar decrease was observed in the CHO trial following R₂. Sodium secretion was depressed following R₂ in the P trial reaching a peak of 1.6 (± 0.5) mmol·m⁻²·min⁻¹. However, in contrast to changes in plasma potassium levels, sweat potassium secretion was higher following R₂ in the P trial than in the CHO trial ($p < 0.05$).

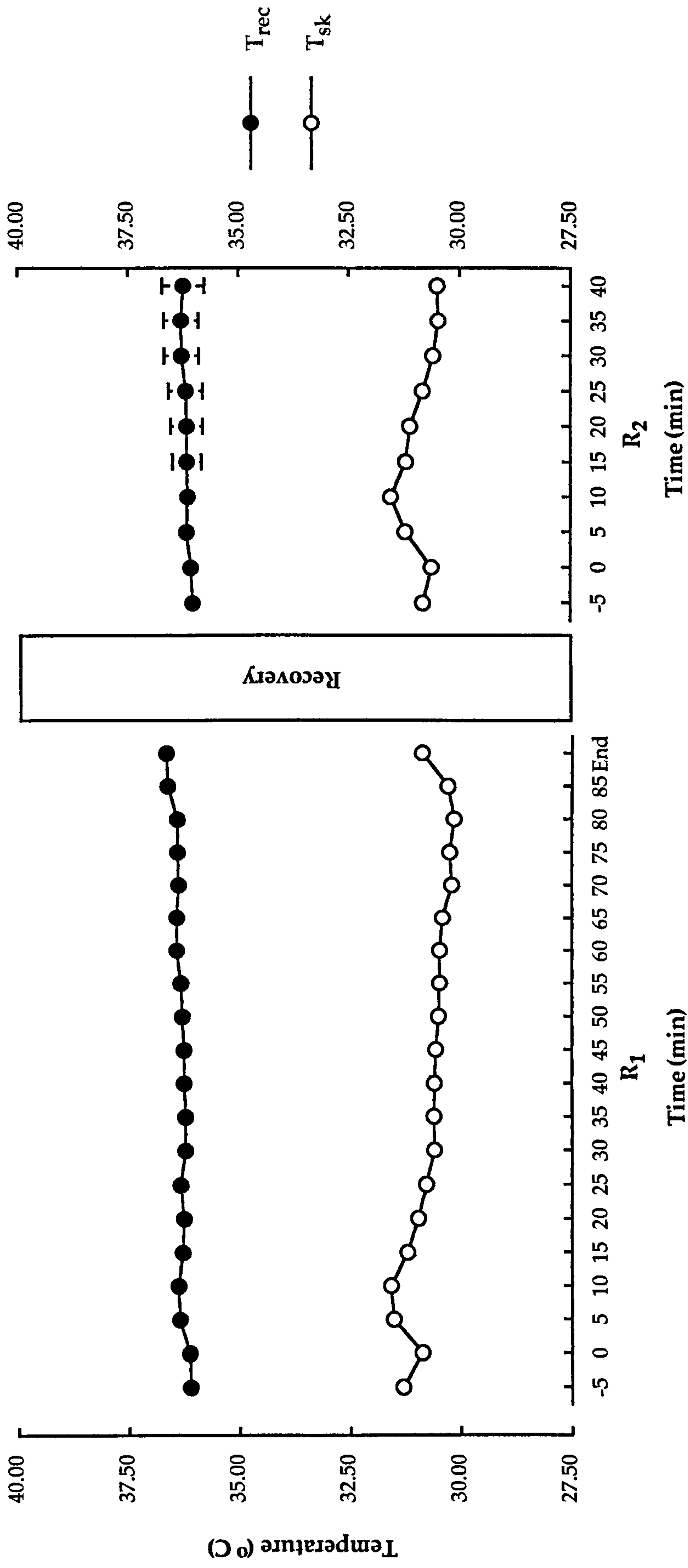


Fig 6.6 Rectal (T_{rec}) and weighted mean skin (T_{sk}) temperature responses during the control (P) trial (mean \pm SE)

Note: Error bars hidden by symbol

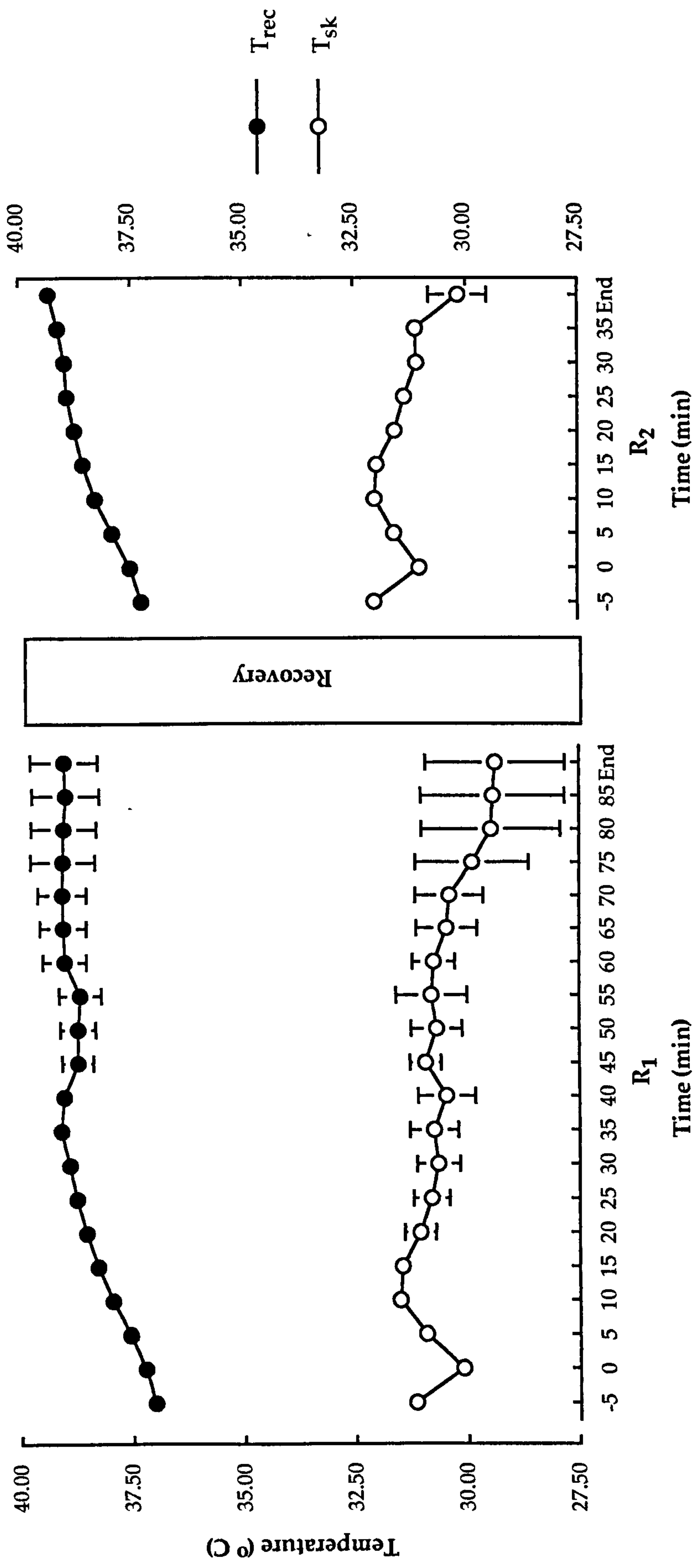


Fig 6.7 Rectal (T_{rec}) and weighted mean skin (T_{sk}) temperature responses during the carbohydrate (CHO) trial (mean \pm SE)

Note : Error bars hidden by symbol

6.4 Discussion

The main finding of this study was that ingesting $1.0 \text{ g} \cdot \text{CHO} \cdot \text{kg}^{-1} \text{body wt} \cdot 2\text{h}^{-1}$ following prolonged running improved endurance capacity after 4-h recovery. As similar levels of rehydration were achieved when either sweetened water or a 6.9% glucose-polymer drink was ingested, the difference in post-recovery performance appears to be related to the provision of carbohydrate and electrolytes. The two groups were well matched in terms of their physiological characteristics, respiratory and cardiovascular responses, running economy and blood lactate profiles. This was reflected in equal run times for R_1 . Whereas, the CHO group exercised for $22.2 (\pm 3.5)$ min longer than the P group during R_2 .

An estimated $152 (\pm 16)$ g of carbohydrate were oxidised during R_1 . Tsintzas (1993) performed single fibre analyses on biopsy samples obtained from the m.vastus lateralis before and after exhaustive running ($70\% \dot{V}O_2\text{max}$). Muscle glycogen concentration decreased from $317.0 (\pm 34.2)$ to $31.6 (\pm 10.3)$ $\text{mmol} \cdot \text{kg}^{-1} \text{dry wt}$ in type-I fibres, and from $443.4 (\pm 44.9)$ to $103.9 (\pm 29.2)$ $\text{mmol} \cdot \text{kg}^{-1} \text{dry wt}$ in type-II fibres. When expressed as means for mixed fibre samples, glycogen concentration decreased from $380.2 (\pm 35.2)$ to $67.8 (\pm 15.2)$ $\text{mmol} \cdot \text{kg}^{-1} \text{dry wt}$, which is equivalent to a utilisation rate of $\sim 2.85 \text{ mmol} \cdot \text{kg}^{-1} \text{dry wt min}^{-1}$ for treadmill running. Extrapolating from these observations, it might be speculated that R_1 reduced muscle glycogen concentration by $\sim 67\%$. This is in general agreement with levels of carbohydrate oxidation estimated from indirect calorimetry.

The placebo solution ingested during the 4-h recovery in the P trial contained $15.5 (\pm 0.8)$ g of carbohydrate (provided by a sweetening agent) which would be sufficient to replace $\sim 10\%$ of that estimated to have been metabolised during R_1 . This compares with $138.0 (\pm 9.0)$ g of carbohydrate contained in the sports drink ingested during the CHO trial, which would be sufficient to replace $\sim 91\%$ of that estimated to have been metabolised. During the second exercise bout, R values indicated that carbohydrate oxidation was reduced by 40% in the P trial, but was increased by 3% in the CHO trial. This reduction in carbohydrate metabolism during the P trial was associated with a

compensatory increase in fat metabolism, as reflected in elevated systemic concentrations of lipid metabolites. A large increase in plasma glycerol concentration relative to changes in FFA concentrations probably reflects increased FFA oxidation (Ahlborg et al, 1974), and decreased splanchnic uptake of glycerol (Havel et al, 1964). During R_1 , a maximum of 61% of the total energy expenditure was derived from fat. In contrast, during R_2 76% of energy expenditure in the P trial was derived from fat, compared with 60% in the CHO trial.

The maintenance of stable blood glucose concentrations during R_2 in both groups may have been associated with reduced carbohydrate availability within active muscle cells. Systemic glucoregulation represents a potent homeostatic mechanism, whereby blood glucose is maintained in order to provide a readily available fuel supply for the brain and CNS (Reichard et al, 1961). As a consequence, cellular glucose transport may be compromised as circulatory demands are prioritised over muscle tissue needs. Thus, reduced whole body carbohydrate availability during R_2 in the P trial may have been associated with restricted blood glucose supply at a muscle cell level. Recent evidence indicates that decreased carbohydrate availability specifically in type-I fibres is associated with fatigue during prolonged, constant pace running (Tsintzas, 1993). The required exercise intensity could not be sustained despite the presence of adequate glycogen stores in adjacent type-II fibres. Elevated fat oxidation stimulates mobilisation of FFA, which may directly interfere with cellular glucose transport (Hargreaves et al, 1991). Alternatively, FFA may suppress the activity of PFK (Randle et al, 1963; Hargreaves and Richter, 1988). In either case, fatigue arises despite apparently adequate fuel provision, as an obligatory requirement for carbohydrate intermediates in both glycolysis and fat oxidation is not fulfilled. Thus, enhanced fat metabolism during the P trial may have indirectly played a role in limiting endurance capacity through substrate inhibition of carbohydrate metabolism (Costill et al, 1977).

The importance of timing post-exercise carbohydrate ingestion in optimising the recovery process has already been demonstrated (Ivy et al, 1988a). Insulin increases muscle membrane permeability and promotes glucose uptake (Narahara and Ozand, 1963). Contractile

activity per se also stimulates sarcolemma glucose transport. This is achieved through enhancing the number of glucose carriers and their intrinsic levels of activity (Goodyear et al, 1990b; DeFronzo et al, 1981). In the present study, plasma insulin concentrations decreased during exercise. Following the recovery, insulin concentrations tended to be higher in the CHO trial relative to the P trial. This is in agreement with the thesis whereby enhanced carbohydrate availability in the form of blood glucose stimulates pancreatic insulin secretion (Ivy et al, 1988b).

The nature of the carbohydrate ingested also influences post-exercise glycogen resynthesis (Blom et al, 1987b). Supplements made up of simple carbohydrates with a high GI are most effective over this period (Burke et al, 1993a; Coyle, 1991; Kiens et al, 1990). Glucose and sucrose appear to be twice as effective as fructose in replenishing muscle glycogen (Blom et al, 1987b), whereas fructose is more rapidly metabolised in the liver (Zakim et al, 1969). Blom et al (1987b) speculated that the presence of fructose would reduce hepatic glucose uptake, thereby rendering a greater proportion of the total glucose absorbed available to muscle tissue. In the present study, the sports drink contained a mixture of sucrose and maltodextrins, potentially providing substrate for both liver and muscle carbohydrate replenishment (Blom et al, 1987b).

Increases in plasma ammonia during prolonged exercise observed in the present study were consistent with previous findings (Broberg and Sahlin, 1988). Protein turnover is enhanced, which in turn is associated with increased muscle ammonia production (Lemon and Mullin, 1980; Lemon and Nagle, 1981). Splanchnic uptake of ammonia from the systemic circulation during heavy exercise is the same as at rest (Eriksson et al, 1985). Thus, an increase in the rate of plasma ammonia appearance is probably due to increased release from active muscle. A higher rate of plasma ammonia accumulation during the P trial may have contributed to a shorter R₂ run time (Banister et al, 1983; Banister and Cameron, 1990).

As discussed in Chapter 5, endurance exercise disturbs the body's fluid balance as well as disturbing the body's energy balance.

Decreases in body weight during R_1 were greater than the 2.0% threshold (Armstrong et al, 1985). Neither group achieved euhydration during the 4-h recovery, where euhydration represents pre- R_1 body fluid levels which were assumed to equate with a normal fluid balance. This was despite the P group ingesting 116% of R_1 fluid loss and the CHO group ingesting 109%. The levels of rehydration estimated in the present study are consistent with previously reported values (Costill and Sparks, 1973; Gonzalez-Alonso et al, 1992). Costill and Sparks (1973) estimated ~74% rehydration following full fluid replacement with either water or a CE solution, though normalisation of exercising HR was achieved after only ~62% rehydration. Gonzalez-Alonso et al (1992) reported 64% rehydration with water and 69% rehydration with a CE solution. Lambert, Costill, McConell, Benedict, Lambert, Robergs and Fink (1992) examined fluid replacement over a 4-h post-exercise period, observing incomplete rehydration with both carbohydrate and non-carbohydrate solutions. These cited studies administered fairly intensive feeding patterns, as rehydration per se was being examined. However, post-exercise ingestion of fluid and nutrients needs to be carefully monitored if the recovery period between repeated bouts of physical activity is relatively short. In the present study, the feeding pattern represented a compromise between the provision of fluid and carbohydrate on the one hand, and the practical constraints with respect to further exercise and potential G-I discomfort on the other. Optimal post-exercise rehydration will be achieved when a prescribed feeding pattern is consonant with both maximal rates of gastric emptying and intestinal absorption, as well as the body's capacity to then retain the fluid.

The volumes of fluid prescribed in the present study can be emptied from the stomach and are available for absorption within 2-h (Gonzalez-Alonso et al, 1992). This suggests that incomplete rehydration in this instance may not totally be due to a G-I limitation. It is possible that further body fluid losses during the recovery period through substrate metabolism and urine formation, as well as insensible losses through respiration and sweating, add to the dehydrating effect of prior exercise (Lambert et al, 1992). A movement of electrolytes out of intra- and extra-cellular spaces may also contribute to incomplete rehydration (Nose et al, 1988b).

Exercise-induced dehydration impairs cardiovascular function, exacerbating an underlying challenge presented by physical activity (Barr et al, 1991). Heart rate increased throughout exercise in both trials. This possibly reflects an attempt to maintain central blood pressure in the face of changes in blood volume distribution (Saltin and Stenberg, 1964). Subjects effectively thermoregulated during R₁ and R₂ in both trials despite disturbances to cardiovascular integrity. Similar observations are reported by Sawka, Knowlton and Critz (1979) for repeated bouts of prolonged running (ie. two 80 min bouts at 70% $\dot{V}O_2$ max, with a 90 min recovery period). Though notably in the present study, core temperature was higher in the CHO trial over the early stages of R₂ relative to R₁, whilst this was not evident in the P trial. Lambert et al (1992) similarly reported elevated T_{rec} in carbohydrate versus non-carbohydrate trials. This 'thermic effect of food' (TEF) following carbohydrate ingestion may result from the increased energy requirements of digestion, absorption, transport, and storage (Acheson, Schutz, Bessard, Ravussin, Jequier and Flatt, 1984). Whilst increased blood glucose availability stimulates insulin secretion, which in turn stimulates skeletal muscle thermogenesis (Balon et al, 1984).

Plasma potassium concentrations increased during exercise in the present study, most probably due to elevated potassium ion efflux from exercising muscle (Lindinger and Sjogaard, 1991; Sjogaard, 1990). Systemic potassium is removed primarily by the kidneys, but extrarenal tissues such as the liver and skeletal muscle also play a part (Bia and DeFronzo, 1981). Extrarenal potassium metabolism is enhanced by insulin and adrenaline, though there is also evidence that aldosterone is an essential prerequisite for maintaining normal potassium tolerance (Bia and DeFronzo, 1981). More than 10% of the total muscle potassium content may be released during 2-h of prolonged submaximal exercise (Sjogaard, 1986). In this instance, non-active muscle tissue plays an important role in preventing excessive plasma potassium accumulation (Lindinger and Sjogaard, 1991). The plasma-to-intracellular potassium concentration gradient favours cellular uptake in non-contracting muscle fibres. This potassium is then slowly released during the recovery, as whole body homeostatic mechanisms come into play (Lindinger and Sjogaard, 1991).

Resting plasma electrolyte concentrations were restored in both the P and CHO trials over the 4-h recovery. However, electrolyte losses from the body during exercise in sweat secretions and urine production were not measured in this study. Thus, the possibility of a whole body electrolyte deficit cannot be ignored, such a change in osmotic balance may adversely influence rehydration (Gonzalez-Alonso et al, 1992). In addition, large potassium effluxes across the sarcolemma are reported to interfere with excitation-contraction coupling in muscle fibres, reducing their force generating capacity (Fink and Stephenson, 1987; Moussavi, Carson, Boska, Weiner and Miller, 1989; Sjogaard, 1986; Sjogaard, Adams and Saltin, 1985). It has been suggested that such potassium fluxes associated with the development of muscle fatigue are involved in a safety mechanism, protecting the muscle cell against mechanical overload (Sjogaard, 1989). Hyperkalemia also has a vasodilatory effect, which promotes the delivery of substrates to active tissue and the removal of metabolic waste (Lindinger and Sjogaard, 1991). Whilst elevated plasma potassium concentrations stimulate ventilation via activation of arteriole chemoreceptors (Paterson, 1989; Sjogaard, 1990). Thus, feedback systems operate to limit the potentially harmful effects of extracellular potassium accumulation. The exercise-induced potassium efflux is not reversed until after exercise cessation. This necessitates a passive recovery phase if previously active muscle is to restore a potassium homeostatic balance (Sjogaard, 1989).

A movement of sodium ions into the muscle cell to counter the movement of potassium ions is less pronounced, as extracellular fluid appears to buffer sodium ion movements more effectively than the movement of potassium ions (McKenna, 1992). In addition, the potassium efflux depolarises the cellular membrane which inactivates fast sodium channels. The combined effect of these phenomena may account for the relatively stable plasma sodium concentrations in the face of increasing plasma potassium concentrations observed in the present study.

Thus, ingesting $1.0 \text{ g} \cdot \text{CHO} \cdot \text{kg}^{-1} \text{body wt}$ at 2-h intervals following prolonged, constant pace running improves endurance capacity 4-h later. The provision of carbohydrate in a 6.9% solution facilitated rehydra-

tion as effectively as water. Thus, failure to maintain adequate carbohydrate availability, rather than a failure to adequately rehydrate, appeared to hasten the onset of fatigue.

The influence of a high carbohydrate intake on recovery from prolonged, constant pace running

7.1 Introduction

A carbohydrate intake equivalent to $1.0 \text{ g}\cdot\text{kg}^{-1}\text{body wt}\cdot 2\text{h}^{-1}$ maintains optimal muscle glycogen resynthesis rates over the initial 4 to 6-h post-exercise (Ivy, 1991). The functional advantage of ingesting this amount of carbohydrate during 4-h recovery from prolonged, constant pace running was demonstrated in Chapter 6. However, this level of carbohydrate intake is associated with transient shifts in blood glucose and plasma insulin (Ivy et al, 1988b). Doyle et al (1993) suggest that a feeding pattern which maintains elevated systemic glucose and insulin concentrations over the immediate post-exercise period will further enhance recovery.

The volume of a solution rather than its carbohydrate content is the more important factor regulating gastric emptying during rest and moderate exercise (Noakes et al, 1991). Thus, the rate of carbohydrate delivery to the small intestine is higher with ingestion of concentrated rather than dilute glucose-polymer (GP) solutions. As such, if concentration is not limiting carbohydrate absorption and assimilation, this method might then be applied during recovery to provide the body with carbohydrate at a greater rate.

The fate of carbohydrate ingested in excess of $1.0 \text{ g}\cdot\text{kg}^{-1}\text{body wt}\cdot 2\text{h}^{-1}$ still remains unclear. If not incorporated into muscle glycogen nor remaining within the vascular system (Ivy et al, 1988b), then the other main storage site is the liver. However, direct assessment of liver glycogen content cannot be undertaken routinely in healthy humans. Nevertheless, elevated liver glycogen might contribute to energy metabolism during repeated exercise bouts, such that performance is maintained. Thus, the present study examined whether increasing post-exercise carbohydrate intake to $3.0 \text{ g}\cdot\text{kg}^{-1}\text{body wt}\cdot 2\text{h}^{-1}$ during 4-h recovery, provides any additional benefits in terms of exercise capacity.

7.2 Methods

7.2.1 Subjects

Nine men and eight women took part in this study (Table 7.1). As in the previous studies, this sample included a wide range of performers, though all were of a similar endurance trained status.

7.2.2 Protocol

Dietary intake was controlled during the 48-h prior to each trial, subjects having completed weighed-food intake dietary analyses from which individual dietary prescriptions were prepared (Section 3.3).

Subjects completed the three preliminary tests following an initial familiarisation period (Section 3.4). Thus, training status was assessed (Table 7.2) and appropriate running speeds for the experimental trials were determined. Also during this period, a practice run was conducted to verify, and if necessary adjust, the work rate for the trials. This consisted of running for 5 min at 60% $\dot{V}O_2\text{max}$, followed by a maximum of 60 min at 70% $\dot{V}O_2\text{max}$. No blood sampling was carried out during the run and the recovery period was not monitored.

Two experimental trials, separated by at least 7-d, were completed in a counter-balanced, single blind design (Figure 7.1). On the day of each trial, subjects arrived at the laboratory after a 10-h overnight fast, and proceeded through the preparations as described previously (Chapter 6). Subjects maintained a relaxed standing position for ~20 min prior to R_1 , after which a 10 ml venous blood sample was drawn from the antecubital vein. Duplicate 20 μl capillary blood samples were simultaneously taken from the thumb. Further capillary blood samples were taken after 30 and 60 min of each exercise bout, and at 30 min intervals during the 4-h recovery. Venous and capillary blood samples were obtained at the end of R_1 , and before and after R_2 .

Post-exercise venous blood samples were drawn with the subject maintaining a standing position with support. This was to minimise postural influences on the composition of pre- and post-exercise blood samples (Hagan et al, 1978).

The blood samples were collected, treated, stored, and subsequently analysed as described previously. In brief, packed cell volume and haemoglobin concentrations were determined in whole blood, FFA (method-ii), glycerol, ammonia, sodium and potassium concentrations were determined in plasma, whilst insulin and cortisol concentrations were determined in serum.

Before commencing R_1 , a 6 min expired air sample was collected for determining pre-exercise metabolic rate. Subjects then performed the standardised 5 min warm-up before commencing R_1 . A standardised warm-up was similarly performed prior to R_2 . The treadmill speed was then increased to the test pace. The first run was a standardised endurance task in which all subjects completed 90 min of exercise at 70% VO_2 max. The second run was a performance test, in which endurance capacity provided a measure of the efficacy of the prescribed recovery.

Further expired air samples were collected over 1 min intervals at 5 and 15 min of R_1 and R_2 , and every 15 min thereafter. A final expired air collection was taken over the last minute of exercise, or part thereof if a subject was unable to complete a further 60 s of work. Simultaneously, subjective ratings of perceived exertion and muscular effort were obtained.

Wet sponges and drinking water were available *ad libitum* during R_1 and R_2 , but drinking water was not taken during the recovery period. The total fluid ingested during exercise was recorded at the end of each run and accounted for in post-exercise changes in body weight.

The two trials were separated by a 4-h controlled recovery during which subjects remained within the laboratory and their activity levels were minimal. A prescribed fluid was consumed immediately on cessation of R_1 , and then 2-h later. During one trial, a dilute GP solution (6.9% carbohydrate, Appendix L; Lucozade Sport, Smithkline Beecham Plc) was ingested providing $1.0 \text{ g}\cdot\text{CHO}\cdot\text{kg}^{-1}\text{body wt}$ (D-trial). An equal volume of a concentrated GP solution (19.3% carbohydrate, Appendix M; Lucozade Original, Smithkline Beecham Plc) was ingested during the other trial providing $3.0 \text{ g}\cdot\text{CHO}\cdot\text{kg}^{-1}\text{body wt}$ (C-trial). Both solutions were non-carbonated and caffeine free, and were maintained at a uniform temperature of $\sim 6\text{-}9^\circ\text{C}$.

Resting expired air samples were collected for 6 min intervals after 30, 60, 90, 150, 180, and 210 min of recovery. Mood states were examined after the 180 min collection using the Bipolar Profile of Mood States (POMS-BI), which measures six bipolar (positive vs. negative) mood states (Lorr, 1984).

Run times were compared using Student's T-test. Cardio-respiratory responses were examined by two-way ANOVA with repeated measures (trial-by-time). Blood biochemical responses, mood-state (POMS-BI) scores, dietary data, energy intake and energy expenditure data were examined using T-tests for paired data. Similar comparisons were made between males and females by two-way ANOVA and independent T-tests respectively. A Tukey post-hoc test was applied to identify the nature of differences.

7.3 Results

7.3.1 Performance

All subjects completed the 90 min endurance task (R_1). The mean R_2 run time for the D-trial was $58.5 (\pm 5.2)$ min, compared with $57.6 (\pm 6.3)$ min for the C-trial (NS). The male and female R_2 run times did not differ (male subjects ($n=9$) D-trial: $56.7 (\pm 6.5)$ min, C-trial: $53.4 (\pm 9.5)$ min; female subjects ($n=8$) D-

trial: 60.5 (± 8.7), C-trial: 62.5 (± 8.4) min) (Figure 7.2).

7.3.2 Blood glucose and serum insulin responses, 'Gut Fullness' ratings and mood states

Blood glucose was equally well maintained over R_1 in both trials, increasing from 3.87 (± 0.14) to 4.22 (± 0.21) mmol·l⁻¹ ($p < 0.01$) (Figure 7.3), whereas plasma insulin decreased by 33% in the D-trial and by 31% in the C-trial (Table 7.3; Figure 7.4). During the recovery, blood glucose peaked 30 min after the first feeding, reaching 6.44 (± 0.20) and 6.40 (± 0.33) mmol·l⁻¹ in the D and C trials respectively (NS). After 90 min of recovery, blood glucose remained higher in the C-trial ($p < 0.01$). However, 30 min following the second feeding, blood glucose was higher in the D-trial ($p < 0.01$). Concentrations were again similar after 180 min, but remained higher in the C-trial after 210 min of recovery ($p < 0.01$). Thus, blood glucose followed different patterns of response over the recovery phases of the two trials, but remained within a similar concentration range. This is despite an increase in plasma insulin of 431% during the recovery in the C-trial compared to 16% in the D-trial ($p < 0.01$). Thus, plasma insulin was higher prior to R_2 in the C-trial ($p < 0.01$).

Drowsiness, lethargy and nausea apparent in subjects during the recovery in the C-trial was perhaps associated with the large shift in plasma insulin concentrations. These subjective observations reflected less positive mood states (POMS-BI) in subjects prior to R_2 ($p < 0.01$). The male and female subjects differed in their mood states responses to the experimental treatments. The male subjects were less confident, less energetic and less clearheaded following the recovery in the C-trial in comparison with the D-trial ($p < 0.05$). The female subjects were similarly less confident ($p < 0.05$), but also felt less congenial and less elated, though more composed during the C-trial ($p < 0.05$).

Higher responses to the 'Gut Fullness Scale' during the C-trial ($p < 0.01$) may reflect slower absorption of the concentrated solu-

tion during the 4-h recovery. Nevertheless, a greater insulin response would suggest enhanced glucose absorption and assimilation in the C-trial. During R_2 , blood glucose was maintained at a higher concentration in the C-trial, and this was most noticeable in the male subjects ($p < 0.01$). Consistently, serum insulin also remained higher in the C-trial at the end of R_2 ($p < 0.01$) despite a 57% decrease in concentration. Whereas, serum insulin decreased by 16% over R_2 in the D-trial ($p < 0.01$).

7.3.3 Serum cortisol responses

A high variance between subjects in serum cortisol concentration made interpretation of the data difficult (Figure 7.4). Serum cortisol tended to increase during R_1 in both trials (NS). During the recovery, cortisol decreased by 36% in the D-trial and 22% in the C-trial (NS). Concentrations remained stable over R_2 in the D-trial, but increased in the C-trial ($p < 0.05$).

7.3.4 Blood lactate and plasma ammonia responses

Blood lactate responses did not differ between the trials during R_1 and R_2 (Figure 7.5). Similarly, there were no differences in plasma ammonia responses, with concentrations increasing during exercise in both trials ($p < 0.01$) (Figure 7.6). Post-exercise blood lactate concentrations peaked 150 min after carbohydrate ingestion, and remained elevated thereafter in the C-trial ($p < 0.05$) whilst pre- R_1 values were restored in the D-trial. There were no differences in blood lactate response between the male and female subjects during R_1 and R_2 , although it is worth noting that the female subjects maintained higher lactate concentrations than the male subjects throughout the recovery phase of both trials ($p < 0.05$).

Table 7.1 Physiological characteristics of the male and female subjects
(mean \pm SE)

	Age (yrs)	Height (cm)	Weight (kg)	$\dot{V}_{E\max}$ (l \cdot min $^{-1}$)	HRmax (b \cdot min $^{-1}$)	$\dot{V}O_{2\max}$ (ml \cdot kg $^{-1}$ min $^{-1}$)
Male	31.3	175.0	69.9	116.1	186	57.9
\pm SE	2.9	2.7	1.2	3.6	4	1.5
Female	30.4	**164.1	**57.5	**84.5	186	**47.4
\pm SE	1.3	1.8	1.3	2.8	2	1.8

** Denotes female data significantly different from male data ($p < 0.01$)

Table 7.2 Running speeds ($\text{m}\cdot\text{s}^{-1}$) and relative exercise intensities ($\% \dot{V}\text{O}_2\text{max}$) at blood lactate concentrations of $2 \text{ mmol}\cdot\text{l}^{-1}$ and $4 \text{ mmol}\cdot\text{l}^{-1}$ of the male and female subjects (mean \pm SE)

	Speed		$\% \dot{V}\text{O}_2\text{max}$	
	$2 \text{ mmol}\cdot\text{l}^{-1}$	$4 \text{ mmol}\cdot\text{l}^{-1}$	$2 \text{ mmol}\cdot\text{l}^{-1}$	$4 \text{ mmol}\cdot\text{l}^{-1}$
Male	3.49	4.74	62.2	84.7
\pm SE	0.39	0.29	4.9	2.9
Female	3.13	4.03	72.1	89.9
\pm SE	0.23	0.17	2.3	2.7

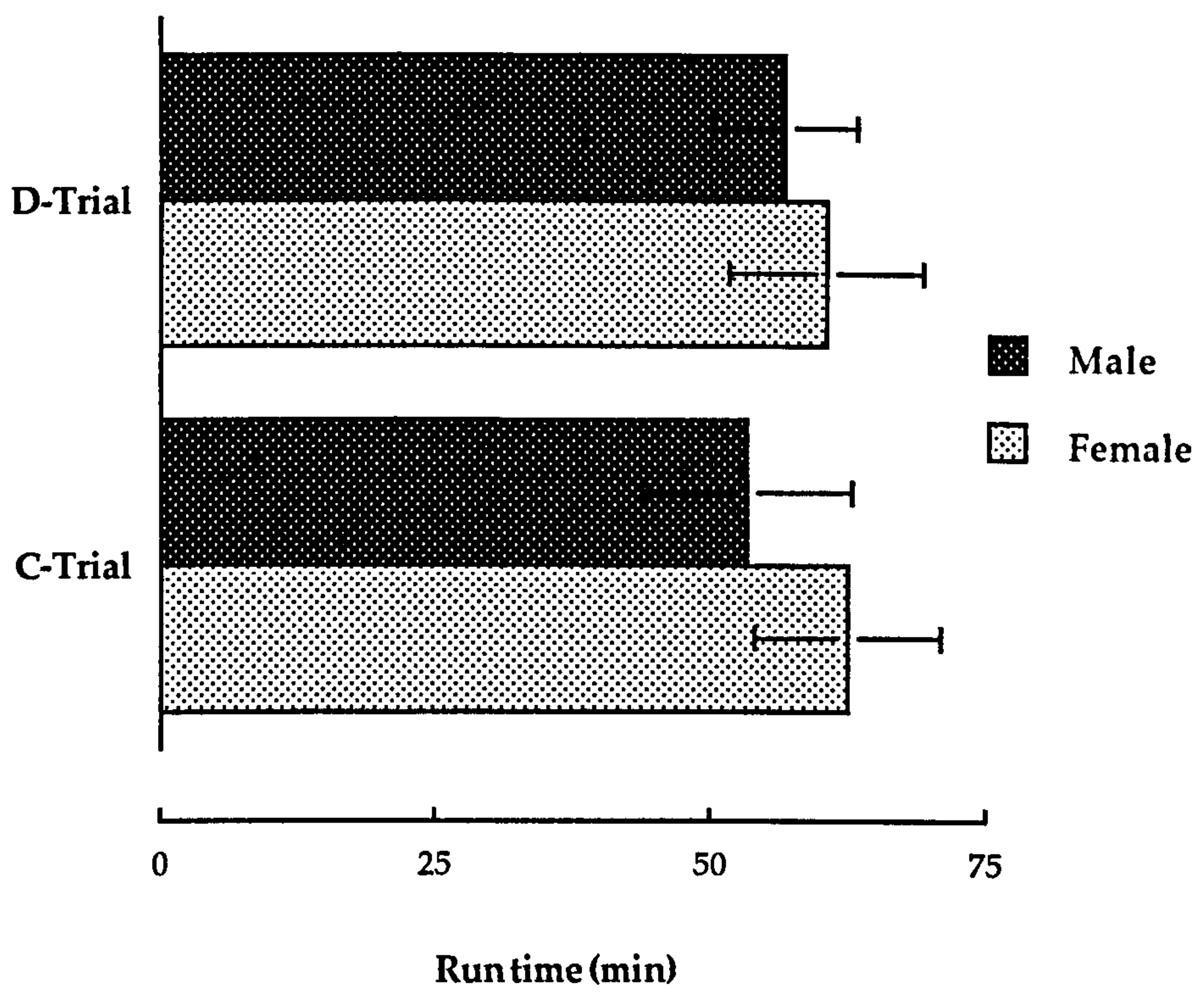


Fig 7.2 Male and female R₂ run times for the dilute (D) and concentrated (C) trials (mean±SE)

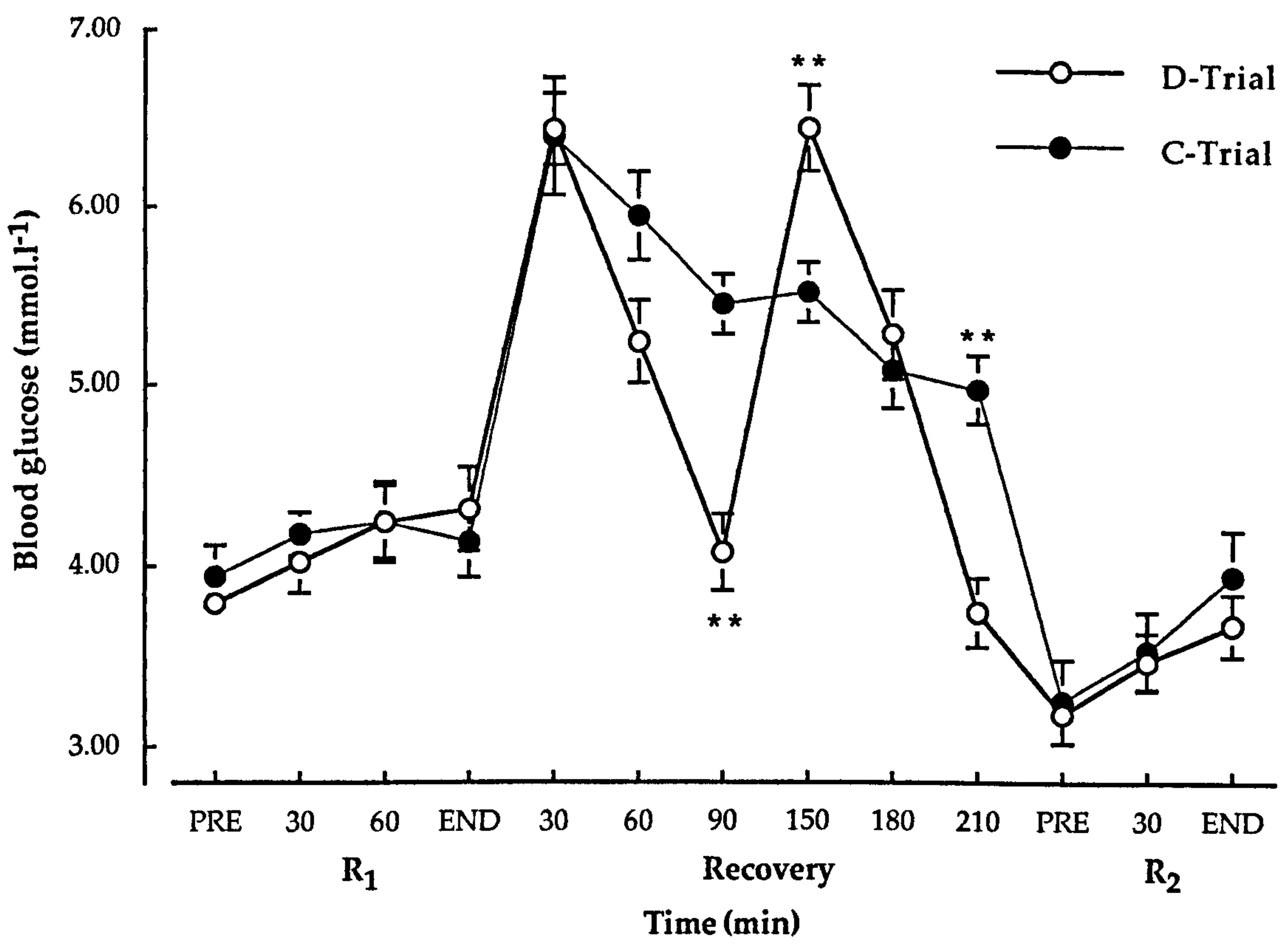


Fig 7.3 Blood glucose concentrations of the dilute (D) and concentrated (C) trials during R₁, the 4-h recovery, and R₂ (mean±SE)

** Denotes D-trial significantly different from C-trial (p<0.01)
 Anova : Main effect - treatment (p<0.05); time (p<0.01)
 Interaction - treatment * time (p<0.01)

Table 7.3. Serum insulin ($\text{mU}\cdot\text{l}^{-1}$) concentrations of the the male and female subjects during the dilute (D) and concentrated (C) trials over R_1 and R_2 (mean \pm SE)

		Male		Female	
		Pre	Post	Pre	Post
D	R_1	6.00	3.14	7.27	5.44
	\pm SE	0.55	0.40	0.74	1.08
	R_2	5.68	3.28	5.15	4.23
	\pm SE	1.63	0.59	0.71	0.38
C	R_1	5.83	3.57	6.56	5.50
	\pm SE	0.34	0.49	0.85	0.90
	R_2	**19.21	*5.49	**26.22	5.48
	\pm SE	2.74	0.62	9.26	1.13

** Denotes C-trial significantly different from D-trial ($p < 0.01$)

* Denotes C-trial significantly different from D-trial ($p < 0.05$)

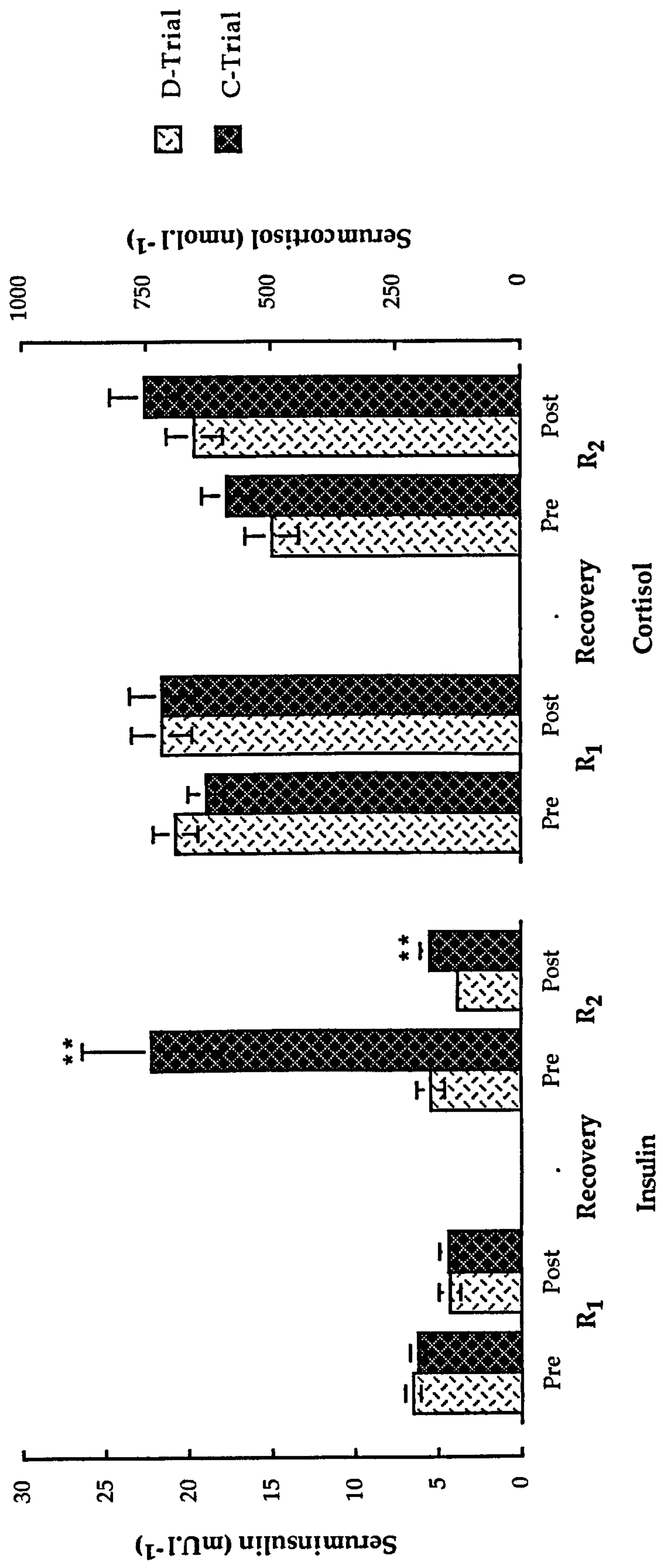


Fig 7.4 Serum insulin and cortisol concentrations of the dilute (D) and concentrated (C) trials for R₁ and R₂ (mean±SE)

** Denotes C-trial R₂ values significantly different from D-trial values (p<0.01)

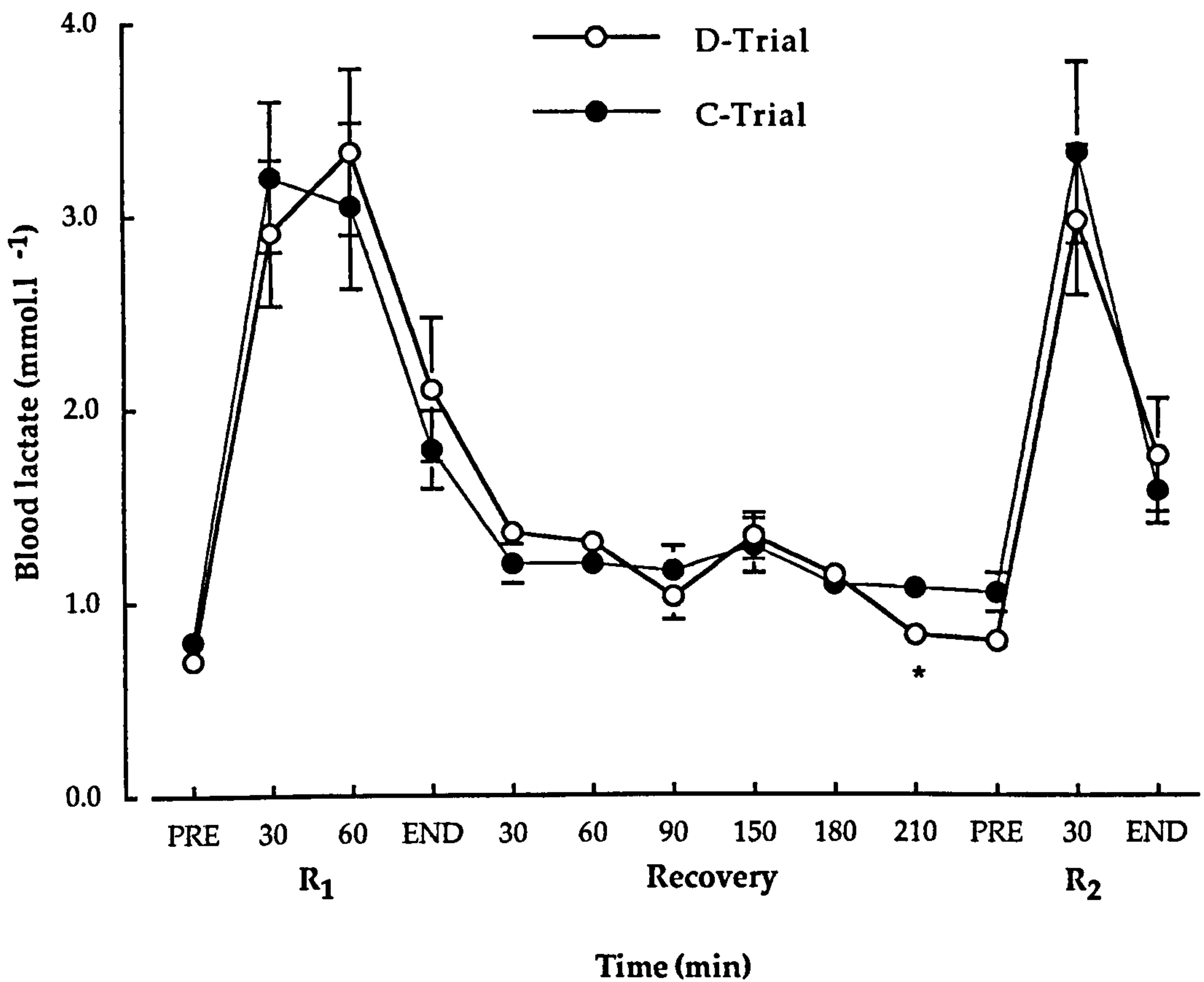


Fig 7.5 Blood lactate concentrations of the dilute (D) and concentrated (C) trials during R₁, the 4-h recovery, and R₂ (mean±SE)

* Denotes D-trial significantly different from C-trial (p<0.05)

Anova : Main effect - time (p<0.01)

Interaction - treatment * time (p<0.01)

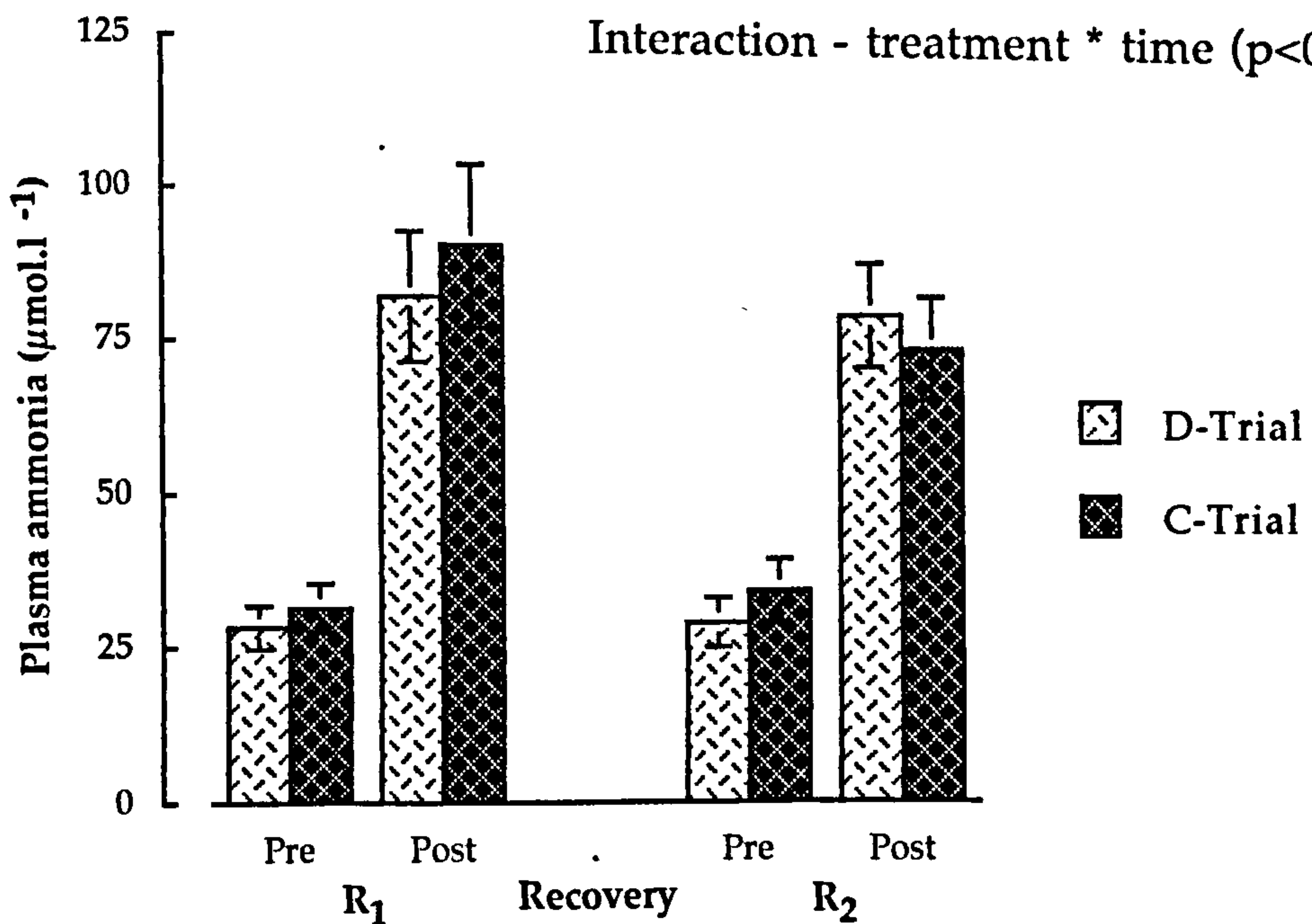


Fig 7.6 Plasma ammonia concentrations of the dilute (D) and concentrated (C) trials for R₁ and R₂ (mean±SE)

7.3.5 Pre-exercise energy intake and fuel utilisation during exercise

The daily carbohydrate intake of the male and female subjects over the 48-h prior to R_1 were 520.6 (± 52.5) g and 382.6 (± 24.1) g respectively ($p < 0.05$) (Table 7.4). This represents 56.2 (± 2.2)% of the total energy intake of the male subjects, and 64.3 (± 2.3)% for the female subjects ($p < 0.05$). When expressed in relation to body weight, there were no differences in carbohydrate intake of the male and female subjects. The energy cost of R_1 was 4.54 (± 0.02) MJ, of which 71 (± 2)% was provided by carbohydrate metabolism. The total volume of GP solution ingested during the D-trial provided 127.3 (± 2.5) g of carbohydrate (2.1 MJ), compared to 382.0 (± 7.4) g of carbohydrate (6.4 MJ) in the C-trial.

Respiratory exchange ratio (R) values were similar during R_1 in both trials (Figure 7.7), though notably this was associated with an increase in the oxygen cost of constant pace running ($p < 0.01$) (Table 7.5). Fat metabolism was elevated to accommodate the enhanced energy demand of exercise, whilst the rate of carbohydrate oxidation remained relatively constant. During the recovery, R values were higher in the C-trial indicating a decrease in fat oxidation and enhanced carbohydrate oxidation ($p < 0.05$). Consistently, post- R_1 oxygen consumption rates were elevated above pre- R_1 values. This was especially apparent over the initial 60 min following each feeding in both trials ($p < 0.01$) and after 90 min in the C-trial ($p < 0.05$). Carbon dioxide production was higher in the C-trial relative to the D-trial after 90 min ($p < 0.01$) and 210 min ($p < 0.05$) of recovery (Table 7.6). However, oxygen consumption remained elevated 30 min prior to R_2 in both trials ($p < 0.05$). Comparing R_1 and R_2 revealed that fat metabolism was suppressed and carbohydrate metabolism enhanced during the second run of the C-trial ($p < 0.01$). In contrast, there were no differences in the balance of energy metabolism between R_1 and R_2 in the D-trial

Notably the total rates of exercise energy expenditure did not differ between the trials.

There were no differences in the nature of energy metabolism between the male and female subjects.

7.3.6 Plasma FFA and glycerol responses

Plasma FFA doubled over R_1 in both trials ($p < 0.01$), whereas plasma glycerol increased by eight-fold ($p < 0.01$) (Figure 7.8). During the recovery, decreases in plasma FFA ($p < 0.01$) and glycerol ($p < 0.05$) were greater in the C-trial, whilst concentrations remained elevated 4-h later in the D-trial ($p < 0.01$). Throughout R_2 , plasma glycerol increased in the C-trial at twice the rate as in the D-trial. Whilst plasma FFA increased by 300% in the case of the former, compared with 30% in the latter ($p < 0.01$).

7.3.7 Plasma electrolyte responses

Plasma sodium increased during R_1 in both trials ($p < 0.01$) (Tables 7.7a and 7.7b), but remained elevated in the C-trial following the recovery ($p < 0.05$). However, the rate of plasma change during R_2 was greater in the D-trial ($p < 0.05$), such that values were the same in both trials at the end of exercise. Plasma potassium also increased during R_1 and R_2 ($p < 0.01$), though there were no differences between the trials.

Resting and exercise plasma electrolyte concentrations were similar in the male and female subjects.

Table 7.4 The daily energy and nutrient intakes of the male and female subjects (mean \pm SE)

	Energy	Protein		Carbohydrate		Fat
	MJ	g	g \cdot kg ⁻¹ body wt	g	g \cdot kg ⁻¹ body wt	g
Male	14.6	120.0	1.7	520.6	7.5	109.7
\pm SE	1.2	8.9	0.1	52.5	0.8	11.0
Female	**9.5	**86.5	1.5	*382.6	6.7	**49.6
\pm SE	0.5	8.0	0.1	24.1	0.5	7.9

** Denotes female data significantly different from male data (p<0.01)

* Denotes female data significantly different from male data (p<0.05)

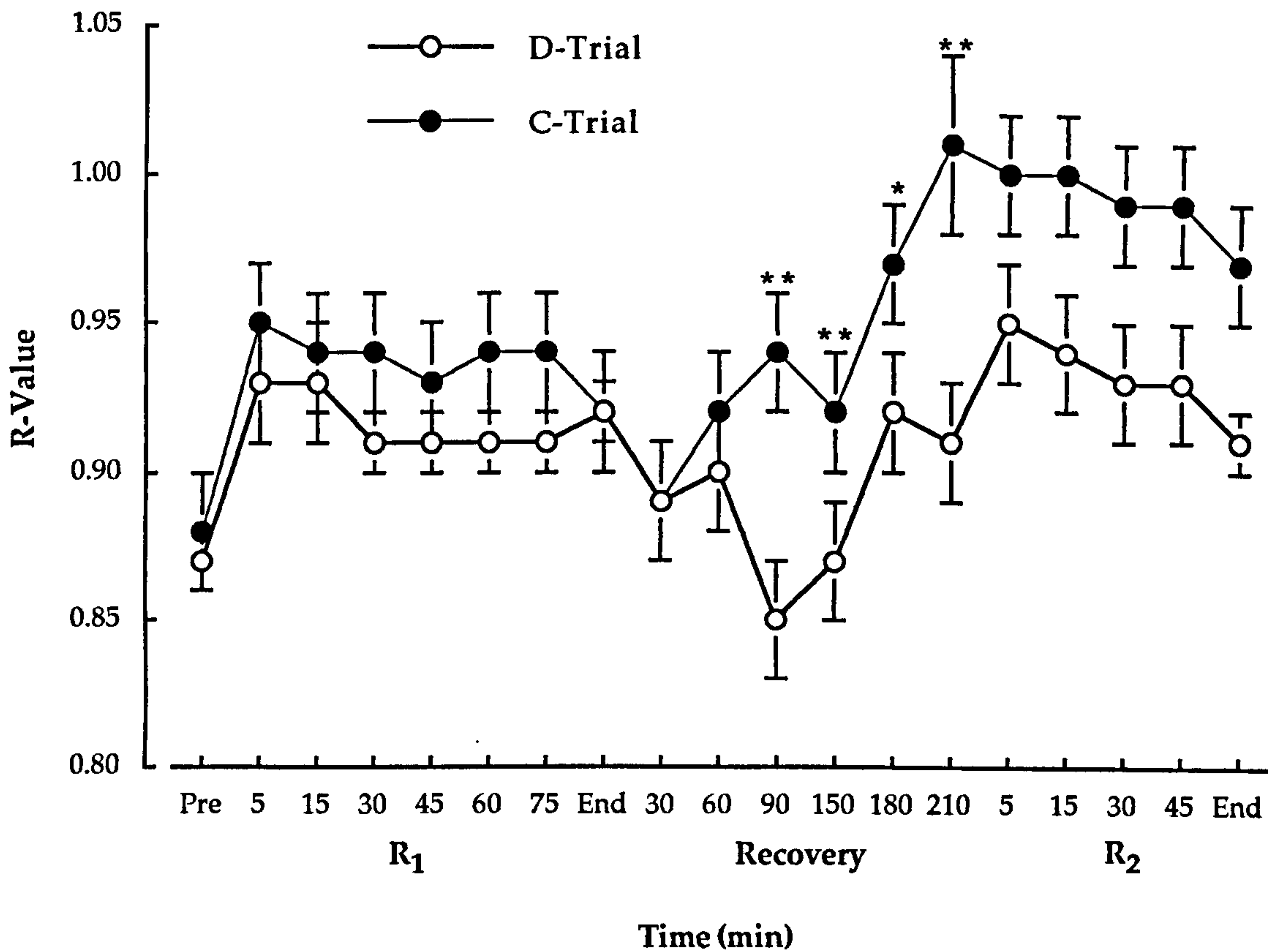


Fig 7.7 R values of the dilute (D) and concentrated (C) trials during R₁, the 4-h recovery, and R₂ (mean±SE)

** Denotes D-trial significantly different from C-trial (p<0.01)

* Denotes D-trial significantly different from C-trial (p<0.05)

Anova : Main effect - treatment (p<0.05); time (p<0.01)

Interaction - treatment * time (p<0.01)

Table 7.5 Oxygen consumption ($l \cdot \text{min}^{-1}$) of the dilute (D) and concentrated (C) trials during R_1 , the Recovery, and R_2 (mean \pm SE)

	R_1										Recovery					R_2			
	Rest	5	15	30	45	60	75	End	30	60	90	150	180	210	5	15	30	45	End
D	0.23	2.42	2.51	2.48	2.48	2.51	2.52	2.52	0.29	0.26	0.25	0.29	0.27	0.26	2.41	2.43	2.44	2.50	2.46
\pm SE	0.01	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.01	0.01	0.02	0.01	0.01	0.02	0.12	0.12	0.12	0.16	0.12
C	0.23	2.37	2.42	2.44	2.45	2.45	2.47	2.48	0.29	0.27	0.26	0.27	0.26	0.26	2.41	2.43	2.44	2.50	2.46
\pm SE	0.01	0.12	0.13	0.13	0.14	0.13	0.14	0.14	0.02	0.02	0.02	0.02	0.02	0.02	0.13	0.13	0.14	0.17	0.12

Table 7.6 Carbon dioxide production ($l \cdot \text{min}^{-1}$) of the dilute (D) and concentrated (C) trials during R_1 , the Recovery, and R_2 (mean \pm SE)

	Recovery										R_2							
	R_1										5	15	30	45	End			
Rest	5	15	30	45	60	75	End	30	60	90	150	180	210	5	15	30	45	End
D	0.22	2.25	2.28	2.25	2.27	2.29	2.30	2.32	0.26	0.24	0.21	0.25	0.24	2.28	2.29	2.27	2.31	2.25
\pm SE	0.02	0.13	0.13	0.12	0.13	0.13	0.13	0.13	0.01	0.01	0.01	0.01	0.02	0.13	0.13	0.12	0.16	0.12
C	0.21	2.26	2.28	2.28	2.27	2.30	2.31	2.28	0.26	0.25	0.24	0.25	0.26	2.33	2.34	2.32	2.35	2.31
\pm SE	0.01	0.13	0.12	0.12	0.13	0.13	0.12	0.12	0.01	0.02	0.02	0.02	0.02	0.14	0.14	0.15	0.19	0.13

** Denotes D-trial significantly different from C-trial ($p < 0.01$)

* Denotes D-trial significantly different from C-trial ($p < 0.05$)

Anova Main effect - treatment ($p < 0.05$); time ($p < 0.01$)

Interaction - treatment * time ($p < 0.01$)

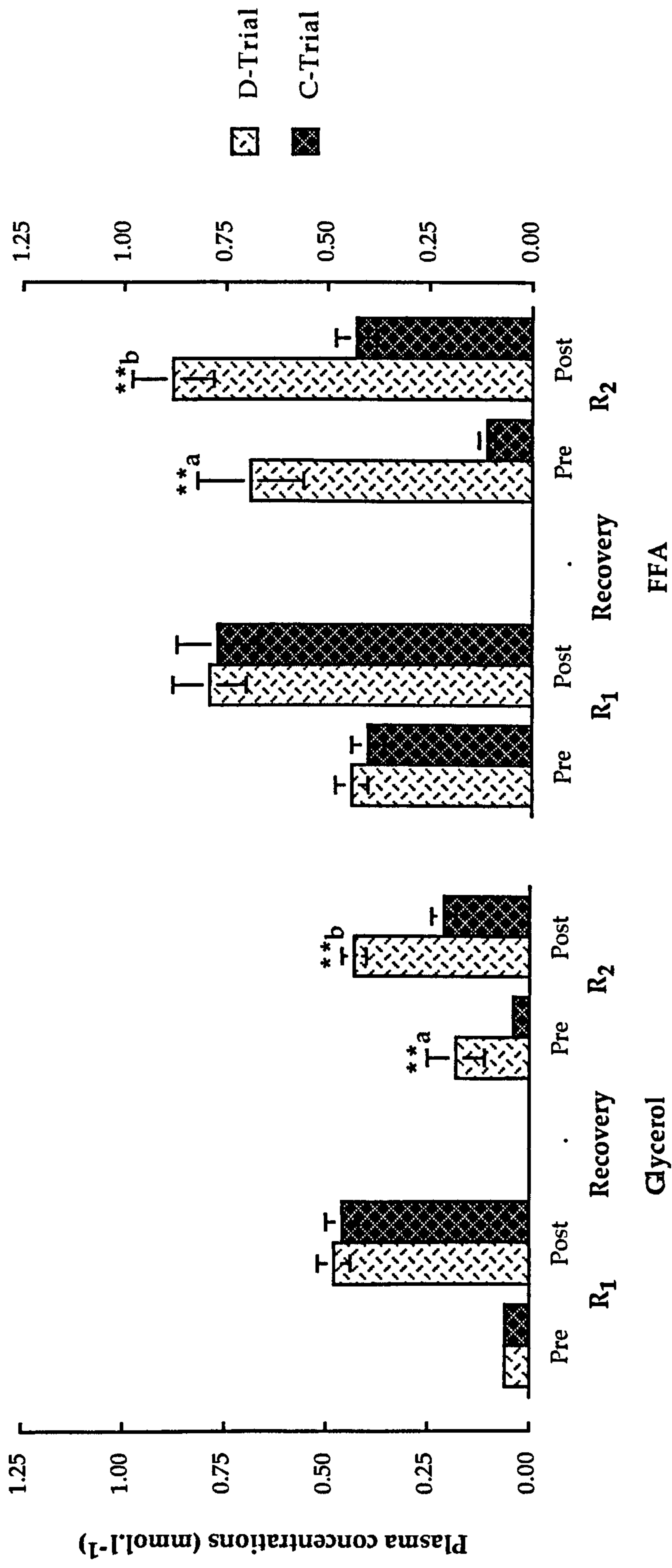


Fig 7.8 Plasma glycerol and FFA concentrations of the dilute (D) and concentrated (C) trials for R₁ and R₂ (mean±SE)

****a** Denotes D-trial pre-R₂ significantly different from pre-R₁, and respective C-trial values (p<0.01)

****b** Denotes D-trial significantly different from C-trial (p<0.01)

Table 7.7a. Plasma electrolyte concentrations (mmol·l⁻¹) of the male subjects during the dilute (D) and concentrated (C) trials over R₁ and R₂ (mean±SE)

		Plasma Sodium		Plasma Potassium	
		Pre	Post	Pre	Post
D	R ₁	139.8	142.1	4.25	4.97
	± SE	0.4	0.6	0.08	0.14
	R ₂	139.5	142.0	3.92	4.57
	± SE	0.2	0.6	0.09	0.14
C	R ₁	139.6	142.0	4.33	4.90
	± SE	0.4	0.7	0.06	0.15
	R ₂	*141.3	141.8	3.98	4.46
	± SE	0.5	0.7	0.06	0.12

* Denotes D-trial significantly different from C-trial (p<0.05)

Table 7.7b. Plasma electrolyte concentrations ($\text{mmol}\cdot\text{l}^{-1}$) of the female subjects during the dilute (D) and concentrated (C) trials over R_1 and R_2 (mean \pm SE)

		Plasma Sodium		Plasma Potassium	
		Pre	Post	Pre	Post
D	R_1	138.4	140.3	4.24	5.35
	\pm SE	0.6	1.1	0.11	0.09
	R_2	138.7	140.4	3.84	4.91
	\pm SE	0.6	0.7	0.10	0.14
C	R_1	138.8	141.0	4.18	5.03
	\pm SE	0.7	0.8	0.08	0.21
	R_2	139.7	141.1	3.67	4.83
	\pm SE	1.0	0.6	0.17	0.12

7.3.8 Plasma volume, heart rate and changes in body weight

Plasma volume was reduced by 3.1 (± 2.4)% during R₁, which was associated with a 10% increase in HR ($p < 0.01$). Following the recovery, plasma volume had increased by 6.6 (± 1.4)% on post-exercise levels in the D-trial and by 6.4 (± 2.7)% in the C-trial (NS). Pre-exercise HR was elevated prior to R₂ ($p < 0.01$) and was higher in the C-trial than in the D-trial ($p < 0.01$). During R₂, plasma volume decreased by 3.3 (± 1.9)% and 6.2 (± 0.9)% in the D and C trials respectively ($p < 0.05$). However, HR responses did not differ between the two trials, but within trial values increased by 7% ($p < 0.01$).

Body weight decreased by 2.8 (± 0.1)% during R₁. Following the recovery period, this loss in body weight had been restored in the C-trial but not in the D-trial ($p < 0.01$). However, there were no differences between the trials by the end of R₂. This was probably due to a greater volume of water ingested by subjects during of the D-trial (D-trial: 170 (± 45) ml; C-trial: 95 (± 29) ml), and reflects higher responses to the 'Gut Fullness Scale' in the C-trial prior to R₂ ($p < 0.01$).

7.3.9 Subjective ratings of fatigue

Ratings of perceived exertion and muscular effort in the D-trial were higher at the start of R₂ ($p < 0.05$) and throughout the exercise bout ($p < 0.01$) in comparison with R₁ values (Figures 7.9 and 7.10). This was also the case during the C-trial ($p < 0.01$). Notably, muscular effort was rated more highly during R₂ in the C-trial relative to the D-trial ($p < 0.01$). Whereas, ratings of perceived exertion did not differ during the second bout of exercise.

7.3.10 Thermoregulatory responses

Rectal temperature followed similar profiles during R₁ and R₂, whilst weighted mean skin temperature tended to be higher during R₂ in comparison with R₁, this difference being most

evident at the onset of exercise ($p < 0.01$).

There were no differences between male and female subjects with respect to T_{rec} and T_{sk} (Figures 7.11 to 7.14).

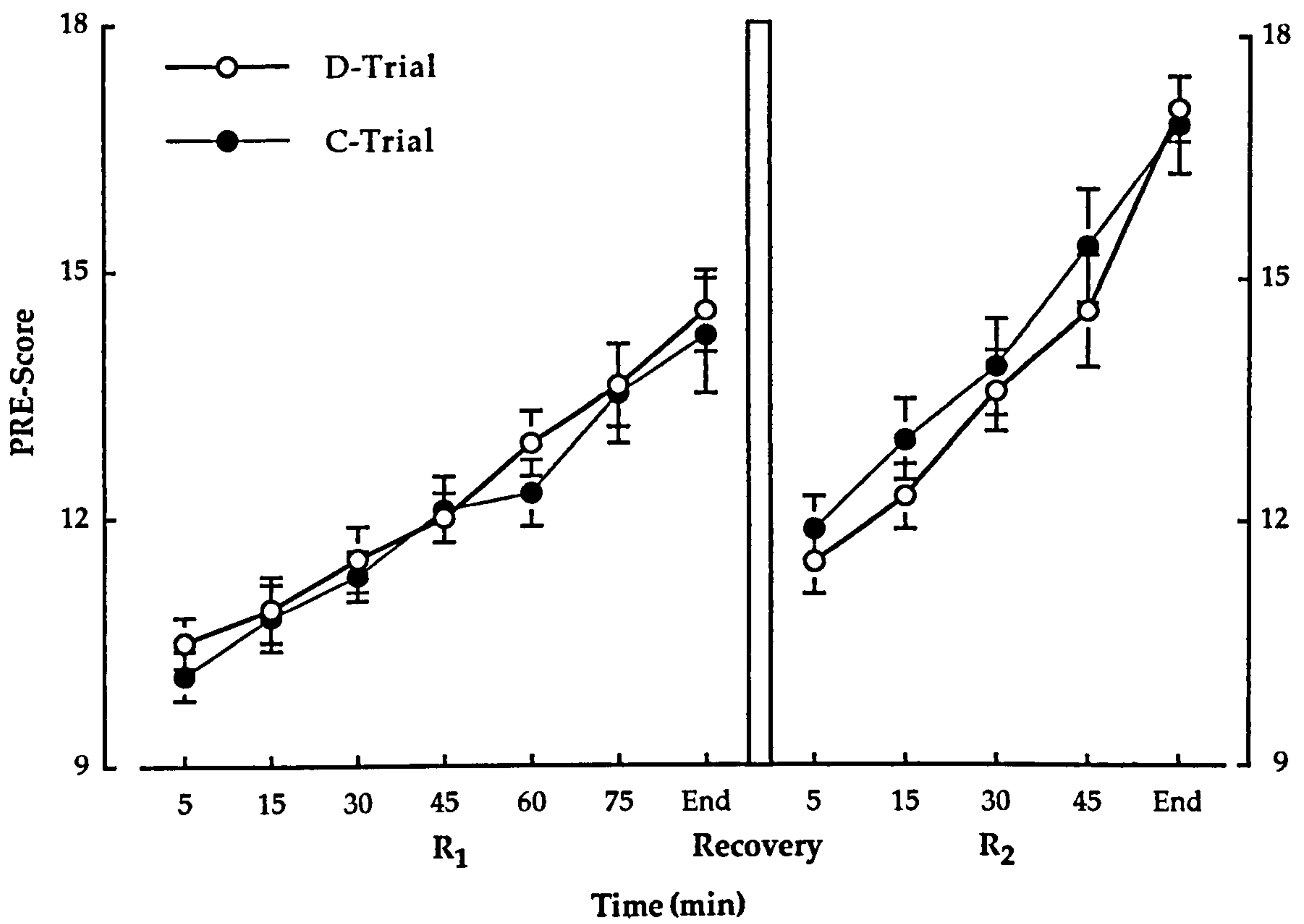


Fig 7.9 Perceived rate of exertion (PRE) of the dilute (D) and concentrated (C) trials during R₁ and R₂ (mean±SE)

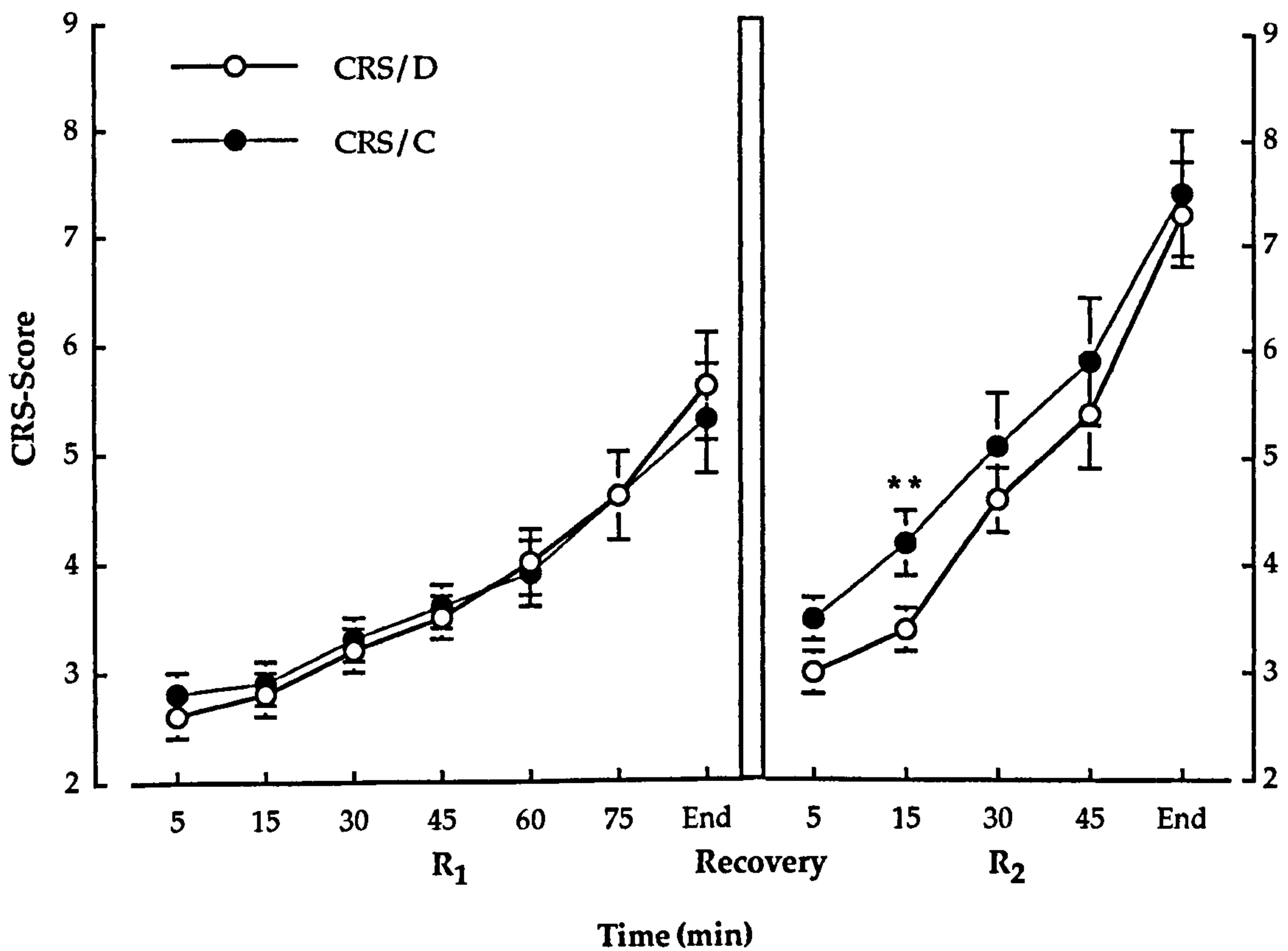


Fig 7.10 Rating of muscular effort (CRS) of the dilute (D) and concentrated (C) trials during R₁ and R₂ (mean±SE)

** Denotes D-trial significantly different from C-trial (p<0.01)

Anova Main effect - treatment (p<0.01); time (p<0.01)

Note : Error bars hidden by symbol

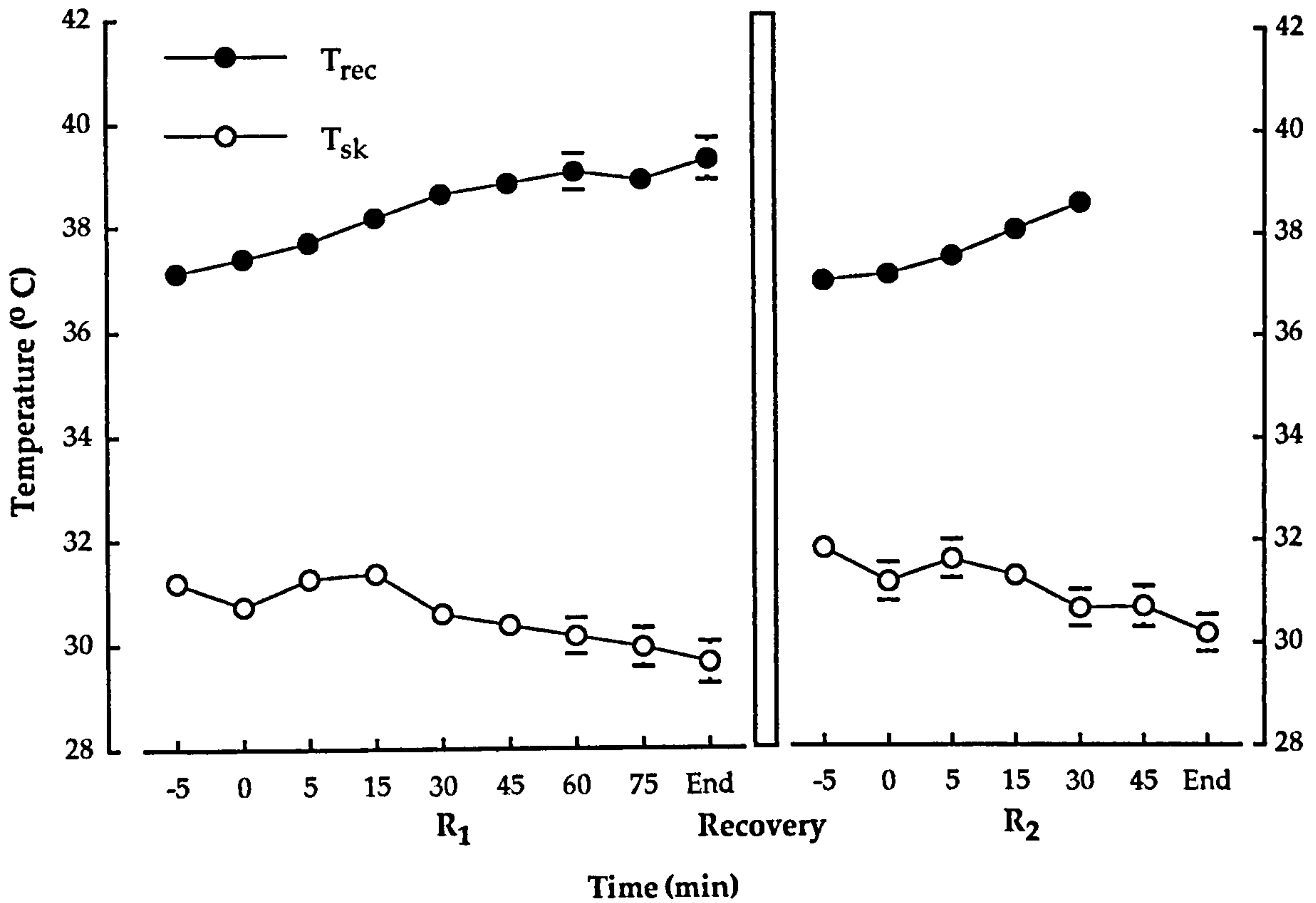


Fig 7.11 Rectal (T_{rec}) and weighted mean skin (T_{sk}) temperature responses of male subjects during R_1 and R_2 of the dilute (D) trial (mean \pm SE)

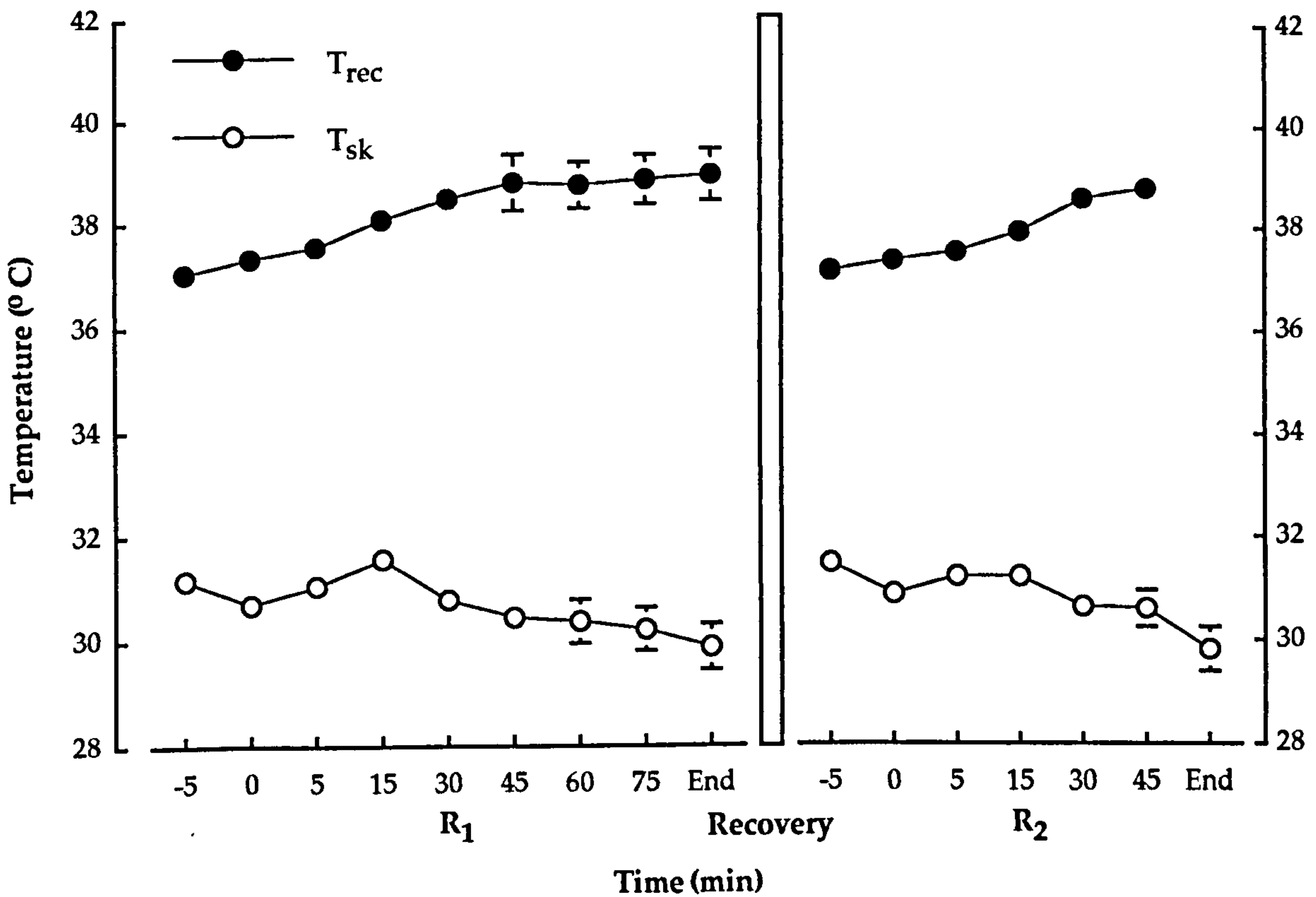


Fig 7.12 Rectal (T_{rec}) and weighted mean skin (T_{sk}) temperature responses of male subjects during R_1 and R_2 of the concentrated (C) trial (mean \pm SE)

Note : Error bars hidden by symbol

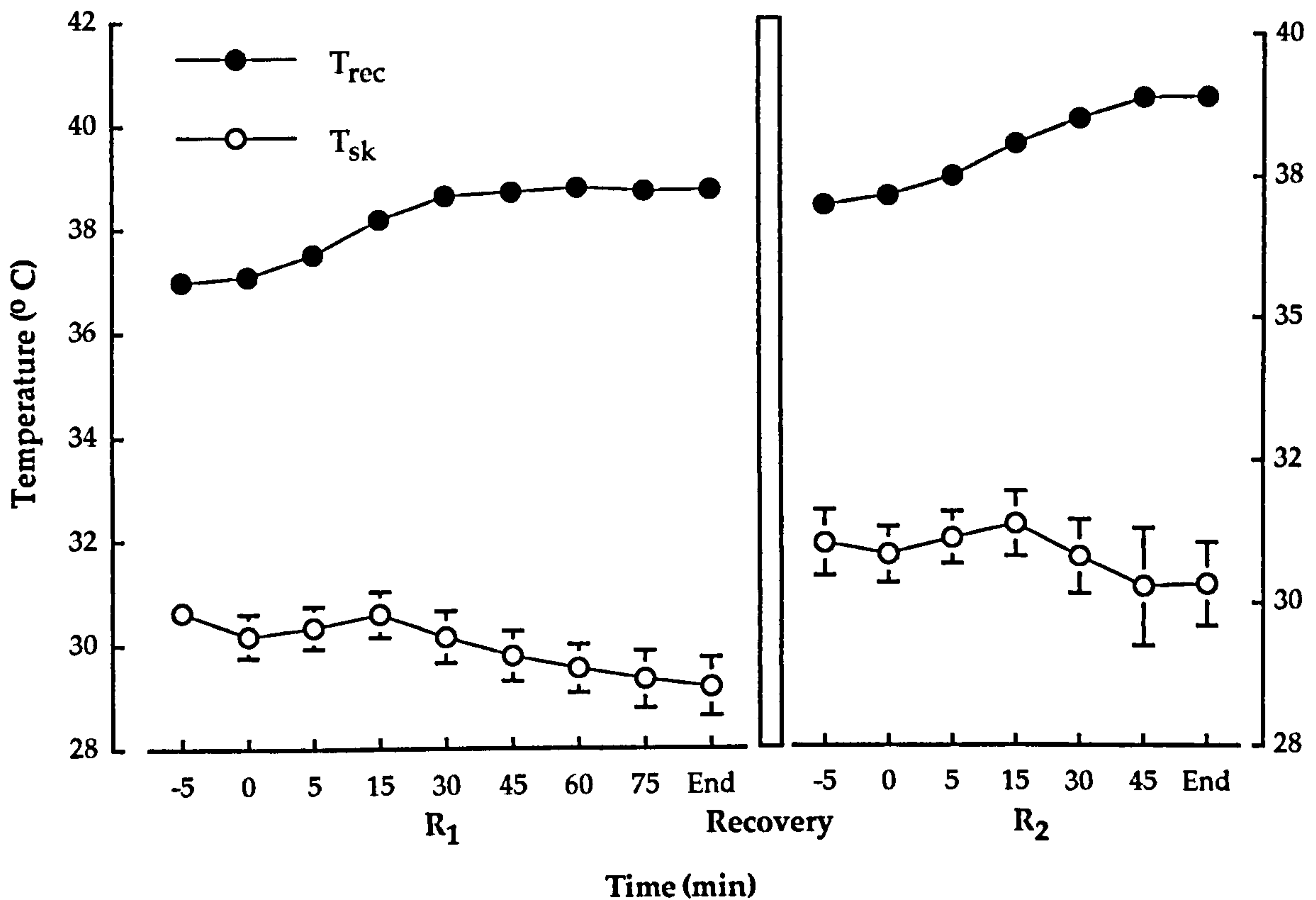


Fig 7.13 Rectal (T_{rec}) and weighted mean skin (T_{sk}) temperature responses of female subjects during R_1 and R_2 of the dilute (D) trial (mean \pm SE)

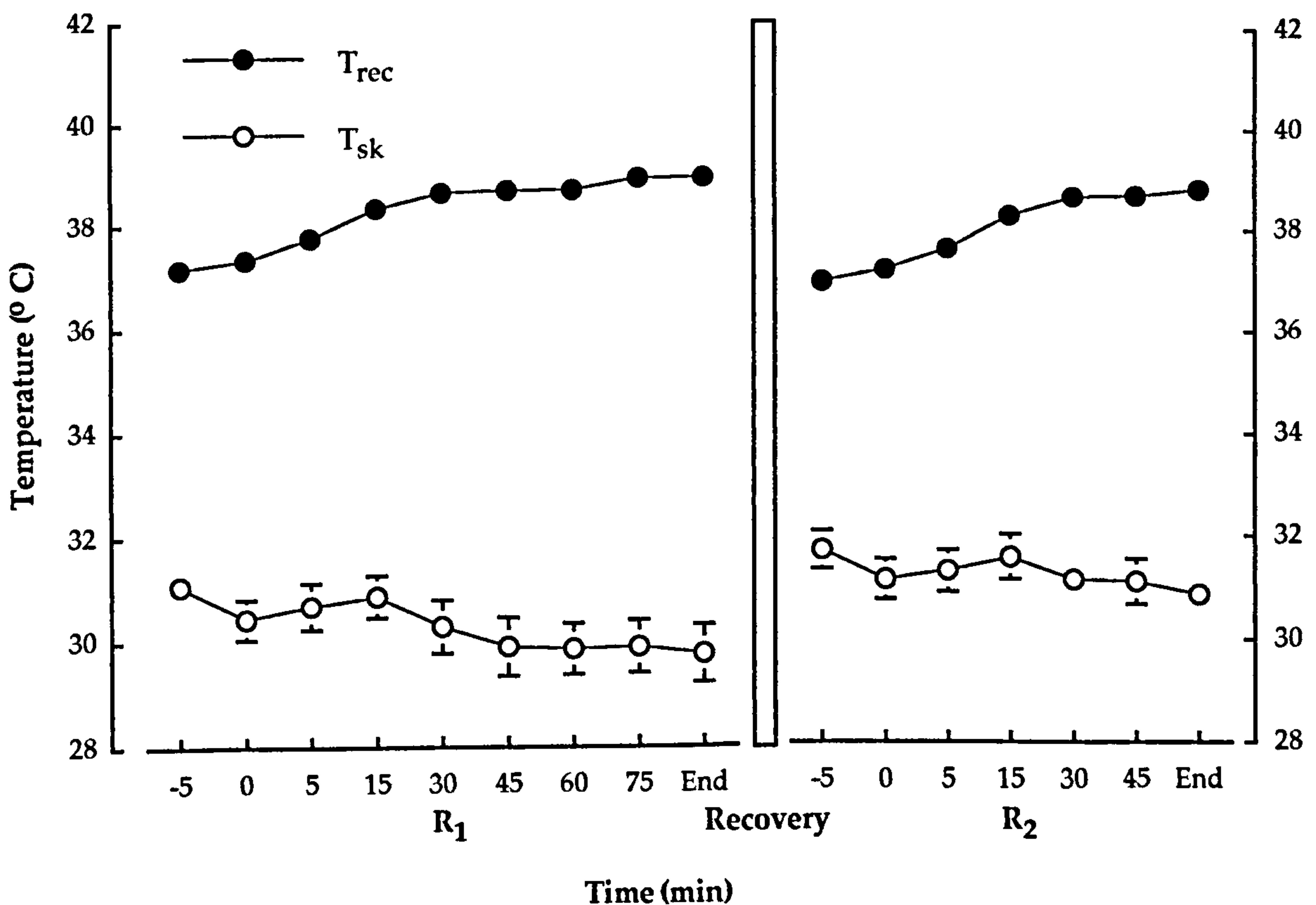


Fig 7.14 Rectal (T_{rec}) and weighted mean skin (T_{sk}) temperature responses of female subjects during R_1 and R_2 of the concentrated (C) trial (mean \pm SE)

7.6 Discussion

The main finding of this study was that ingesting 3.0 g·CHO·kg⁻¹body wt²h⁻¹ during 4-h post-exercise recovery does not further improve endurance capacity, in comparison with ingesting 1.0 g·CHO·kg⁻¹body wt²h⁻¹. Despite additional carbohydrate being provided, it did not appear to contribute to 'useful' energy metabolism during a further bout of exercise. The high carbohydrate dose was associated with lethargy, nausea, and a less positive attitude to exercise. Moreover, incomplete absorption of the concentrated solution resulted in greater G-I discomfort during the recovery and second run.

Post-exercise energy repletion with carbohydrate solutions may be limited through gastric-emptying, intestinal absorption, circulatory transport and cellular uptake, or intracellular processes (Blom et al, 1987b; Ivy et al, 1988b). Of these factors gastric-emptying is not thought to restrict the recovery process (Reed et al, 1989). Rehrer et al (1989) demonstrated that the rate of gastric-emptying (ie. percentage emptied per unit time) is regulated by the carbohydrate content of a solution. However, the volume of solution emptied is determined by the gastric volume, which in turn is dependent upon the volume of fluid consumed. In this study, subjects ingested the same volume of solution during both trials and followed identical feeding regimens. Theoretically, this would result in a greater carbohydrate delivery to the small intestine during the C-trial (Noakes et al, 1991).

Radzuik and Bondy (1982) estimated an upper limit of glucose absorption from the intestine in normal subjects equivalent to $\sim 1.0 \text{ g} \cdot \text{min}^{-1}$. Thus, a maximum of 120 g of glucose could be absorbed during 2-h post-exercise recovery. Assuming complete gastric emptying, 64 g of carbohydrate would be provided per feeding in the D-trial utilizing $\sim 53\%$ of the estimated intestinal absorption capacity. Each feeding in the C-trial provided 191 g of carbohydrate, which exceeds the upper limit of intestinal absorption by 71 g. Thus, as much as 142 g of carbohydrate may remain within the G-I tract after 4-h recovery. Once absorbed from the small intestine, $\sim 15\%$ of the carbohydrate will be incorporated in the liver to leave $\sim 85\%$ available for general metabolism (Ivy et al, 1988b). This reflects the prioritisation of muscle

glycogen resynthesis over liver glycogen resynthesis following exercise (Fell et al, 1980; Mæhlum et al, 1978). The CNS requires ~ 5 g glucose \cdot h $^{-1}$. As such, 88 g of carbohydrate would be available for energy repletion during the 4-h recovery of the D-trial. In contrast, a maximum of ~ 240 g would be absorbed in the C-trial of which 36 g would be captured by the liver. This would leave ~ 184 g available for energy repletion after CNS requirements are taken into account. Thus, intestinal absorption per se would not have been limiting, there being adequate capacity to cope with the carbohydrate delivery of the D-trial, and as now will be discussed, would ensure sufficient carbohydrate uptake during the C-trial.

During R₁, an estimated 190 (± 2) g of glucose were metabolised in fueling exercise. The available carbohydrate provided during the D-trial would cover $\sim 40\%$ of that oxidised during R₁, whereas $\sim 97\%$ of that estimated to have been oxidised during R₁ would be covered by the C-trial feeding regimen. However, only $\sim 30\%$ of glucose theoretically available to the body is actually stored as muscle glycogen (Ivy et al, 1988b). Reed et al (1989) encountered difficulties in accounting for the fate of glucose absorbed from the intestine. It is possible that prior exercise may stimulate enhanced liver glycogen storage (Costill et al, 1983; Terjung et al, 1974) and triglyceride formation (Bahr et al, 1990) above levels previously predicted under basal conditions (Nilsson and Hultman, 1974). Alternatively, muscle not directly recruited in the activity being undertaken may play a part in disposing of the ingested carbohydrate (Reed et al, 1989). Glycogenolysis appears to take place in relatively 'non-active' muscle during exercise, which results in the conversion of glucose to lactate. It follows that these glycogen stores will also need replenishing following exercise.

Despite the high carbohydrate loads, especially during the C-trial, blood glucose concentrations were effectively regulated towards normal values throughout the recovery. Similar patterns of response to post-exercise carbohydrate ingestion are reported by Acheson et al (1984) and Ivy et al (1988b), where blood glucose is down-regulated such that concentrations were the same after 240 min of recovery for both high and low carbohydrate loads (Ivy et al, 1988b). Notably, more stable systemic glucose levels were maintained with a high carbohyd-

ate intake during the C-trial in the present study. This observation is consistent with the findings of Doyle et al (1993), where $0.4 \text{ g}\cdot\text{CHO}\cdot\text{kg}^{-1}$ body wt was administered at 15 min intervals following exercise. This was equivalent to a bi-hourly intake of $3.2 \text{ g}\cdot\text{kg}^{-1}$ body wt during 4-h recovery. Maintaining a steady glucose flow optimises cellular glucose transport (Schultz et al, 1977), which in turn would enhance replenishment of energy reserves and the return of exercise capacity. Vissing et al (1988) demonstrated a mechanism by which stable blood glucose concentrations are maintained through regulated hepatic glucose production. Under basal conditions and during low level work, the mechanism is controlled by feed-back inhibition. This control is modulated by humoral factors (eg. increasing plasma insulin concentrations inhibits hepatic glucose release) (Felig et al, 1975; Zawadzki, Yaspelkis and Ivy, 1992). In contrast, exercise may trigger a feed-forward mechanism, as motor centres in the brain respond to an increase in energy demand.

A high carbohydrate diet has been associated with increased rates of carbohydrate oxidation, whilst fat oxidation is suppressed and protein oxidation remains approximately constant (Acheson et al, 1988; Blom, 1989b). Following prior muscle and liver glycogen depletion through exercise and dietary restriction, glycogen storage initially provided the major avenue for disposing of excess dietary carbohydrate (Acheson et al, 1988). The contribution of de novo lipogenesis to this process increases over time (Bjorntorp and Sjostrom, 1978), but represents a major contributor to carbohydrate disposal only when muscle and liver glycogen stores become saturated (Acheson et al, 1988). Oxidation and storage start to become inadequate for disposing of excess dietary carbohydrate after 2-d of carbohydrate over-feeding (ie. mean \pm SD intake of $836\pm 52 \text{ g}\cdot\text{CHO}\cdot 24\text{h}^{-1}$, equivalent to $12.3\pm 0.8 \text{ g}\cdot\text{CHO kg}^{-1}$ body wt $\cdot 24\text{h}^{-1}$) by which time glycogen stores had accommodated an additional $\sim 500 \text{ g}$. A 'resting' R-value (ie. non-exercising) greater than 1.00 indicates net lipid synthesis (Acheson et al, 1988). R-values greater than 1.00 were observed in the C-trial after 4-h recovery with a high carbohydrate intake. However, elevated carbon dioxide production provided evidence to suggest that this was probably a respiratory response to elevated blood lactate concentrations, where excess carbon dioxide is 'blown off' in an attempt to restore acid base balance

(Astrand and Rodahl, 1986). Notably, lower R-values were maintained during the recovery period of the D-trial, where subjects were prescribed carbohydrate at the recommended rate of ingestion.

During prolonged cycling blood glucose concentrations decline and carbohydrate oxidation becomes compromised (Ahlborg and Felig, 1982; Ahlborg et al, 1974; Felig et al, 1982). Coyle et al (1986) suggests that the provision of carbohydrate during exercise improves endurance capacity by supplementing blood glucose and maintaining carbohydrate oxidation rates, rather than through a glycogen sparing effect. However, Tsintzas (1993) observed that carbohydrate ingestion during prolonged, constant pace running was associated with a sparing of muscle glycogen specifically in type-I fibres. In the present study, blood glucose concentrations were elevated and the rate of carbohydrate oxidation enhanced prior to R₂, but running performance was not improved during this second exercise bout. Thus, constant pace running capacity is not primarily determined by whole body carbohydrate oxidation rates nor by the maintenance of stable blood glucose concentrations. A principal limitation appears to be the availability of carbohydrate in type-I fibres.

Post-exercise oxygen consumption did not differ between treatments despite differences in R-values. The major fraction of excess post-exercise oxygen consumption (EPOC) is believed to arise from the resynthesis of glycogen and enhanced rates of 'futile' energy cycling (Bahr and Mæhlum, 1986). The ingestion of nutrients also stimulates increased oxygen consumption (Bielinski et al, 1985; Gore and Withers, 1990). Elevated 'resting' blood lactate concentrations after 210 min of recovery in the C-trial are possibly indicative of 'futile' carbohydrate cycling. Ivy et al (1988b) similarly observed enhanced post-exercise carbohydrate oxidation with a high carbohydrate intake. Blom et al (1987b) suggest that glucose in excess of immediate requirements is either oxidised, or converted to lactate and released from the cell (Blom, 1989b).

An increased serum insulin response in the C-trial prior to R₂ is consistent with a higher rate of glucose absorption. As discussed previously, this is in agreement with a thesis whereby elevated carbohydr-

rate availability in the form of blood glucose stimulates pancreatic insulin secretion (Ivy et al, 1988b; Kirwan, Bourey, Kohrt, Staten and Holloszy, 1991). Insulin facilitates cellular glucose transport (Berger et al, 1975; Richter et al, 1982a), as well as glycogen synthase activity (Larner and Villar-Palasi, 1971). However, exercise induced catecholamine release inhibits insulin secretion (Galbo et al, 1977). This effect is countered by increased tissue sensitivity to low insulin levels (Mikines et al, 1988; Richter et al, 1982a), whereas contractile activity further increases sarcolemma glucose transport by enhancing the number of glucose carriers and their intrinsic levels of activity (DeFronzo et al, 1981; Goodyear et al, 1990a; Goodyear et al, 1990b; Richter et al, 1984). Thus, transport and cellular uptake of glucose do not appear to limit the recovery process in terms of energy repletion (Blom, 1989b).

The blood glucose profiles possibly reflect a greater level of glucose storage during the C-trial, though serum insulin concentrations per se do not necessarily reflect glucose storage rates in muscle tissue (Blom et al, 1987b; Reed et al, 1989). Young et al (1988) reported that total glucose disposal increased with increasing plasma insulin concentrations. However, at relatively low plasma insulin concentrations increased disposal was achieved through increased glucose oxidation. Only when plasma concentrations were extreme of the normal physiological range was insulin-induced glucose disposal achieved through increased storage (Kelley, Reilly, Veneman and Mandarino, 1990). Insulin elicits a maximum stimulatory effect at plasma concentrations equivalent to $\sim 21.0 \text{ mU}\cdot\text{l}^{-1}$ (Blom et al, 1987b), which is similar to systemic concentrations at the end of the C-trial recovery phase. The findings of Blom et al (1987b) and Ivy et al (1988b) highlight the 'permissive' role of insulin in glucose transport and glycogen synthesis, as opposed to a direct regulatory role (Berger et al, 1975).

Elevated post-exercise plasma FFA and glycerol concentrations reflect mobilisation of adipose fat depots under the influence of sympathetic nervous activity (Bahr et al, 1991; Havel et al, 1964; Paul and Holmes, 1975). Increased oxidation of FFA may spare limited muscle glycogen stores, and hence increase endurance capacity. The proportionately

greater increase in plasma glycerol concentrations in relation to plasma FFA concentrations may reflect decreased splanchnic glycerol uptake due to a reduced hepatic blood flow during exercise (Havel et al, 1964). The low post-recovery plasma concentrations of FFA and glycerol during R₂ of the C-trial reflect the suppression of lipid metabolism (Acheson et al, 1988). Plasma glycerol provides precursors for gluconeogenesis, which may then supplement exogenously provided carbohydrate. Gluconeogenic precursors produced during exercise (eg. lactate, glycerol and BCAA) may be recycled into the glycogen synthetic pathway. This mechanism will partly be responsible for the post-exercise glycogen resynthesis evident under fasting conditions (Mæhlum and Hermansen, 1978). In contrast, the availability of carbohydrate and elevated plasma insulin concentrations during the C-trial may inhibit gluconeogenesis (Zawadzki et al, 1992).

Prolonged exercise and carbohydrate ingestion both influence plasma BCAA concentrations (Davis, Bailey, Woods, Galiano, Hamilton and Bartoli, 1992; Decombaz et al, 1979; Wagenmakers et al, 1991), which in turn influences the tryptophan-to-BCAA ratio (Newsholme et al, 1991). Increasing plasma FFA concentrations during prolonged exercise displaces bound tryptophan from albumin (Curzon et al, 1973). Thus, elevated plasma concentrations of free tryptophan increase the tryptophan-to-BCAA ratio (Blomstrand et al, 1988). This in turn increases 5-HT levels in the brain, and may contribute to the onset of fatigue (Newsholme et al, 1991; Parry-Billings et al, 1990). Carbohydrate ingestion during exercise attenuates systemic BCAA and free tryptophan concentrations, and hence suppresses changes in the tryptophan-to-BCAA ratio (Davis et al, 1992; Wagenmakers et al, 1991). However, high plasma insulin concentrations associated with carbohydrate ingestion under resting conditions, on the one hand may be accompanied by changes in plasma concentrations of large neutral amino acids, whilst on the other hand insulin inhibits BCAA mobilisation. A direct consequence of both of these responses is an increase in plasma free tryptophan concentrations, which in turn will favour enhanced 5-HT production in the brain (Anderson, 1981). This may account for the lethargy and drowsiness of subjects during the C-trial recovery period, where a high post-exercise carbohydrate intake would stimulate insulin secretion.

The importance of timing post-exercise carbohydrate ingestion and the nature of the supplement in optimising the recovery process has already been demonstrated and previously discussed (Blom et al, 1987b; Burke et al, 1993a; Costill et al, 1981; Ivy et al, 1988a; Ivy et al, 1988b; Kiens et al, 1990). The D-trial solution represented a combination of sucrose (2.9%), maltodextrin (2.7%) and fructose (1.3%), whilst the C-trial solution was based upon a monosaccharide glucose syrup (19.3%). Glucose and sucrose appear to be twice as effective as fructose in replenishing muscle glycogen (Blom et al, 1987b), whereas fructose is more rapidly metabolised by the liver (Zakim et al, 1969). However, differences in carbohydrate composition of the two trial solutions were not thought to play an instrumental role with respect to the experimental findings.

In conclusion, increasing carbohydrate intake from 1.0 to 3.0 g·kg⁻¹ body wt·2h⁻¹ does not appear to be beneficial in improving endurance capacity after 4-h recovery. Indirect respiratory and blood biochemical evidence is consistent with the rate-limiting step for repleting cellular carbohydrate stores as being the conversion of glucose to glycogen. Free glucose resulting from a high rate of carbohydrate ingestion, which is not directly incorporated in muscle and liver, may largely be disposed of through enhanced oxidation as elevated lipogenesis initially plays a relatively minor role.

General Discussion

The principal aim of the present thesis was to examine the influence of post-exercise carbohydrate ingestion on the capacity of an individual to reproduce their previous running performance.

From the Review of Literature, the consensus suggests that providing in excess of $8.0 \text{ g} \cdot \text{CHO} \cdot \text{kg}^{-1} \text{body wt} \cdot 24\text{h}^{-1}$ replenishes muscle glycogen reserves following daily bouts of endurance exercise (Bergstrom and Hultman, 1966a; Costill et al, 1981; Keizer et al, 1987; Kochan et al, 1979). This process is optimised if feedings providing $\sim 1.0 \text{ g} \cdot \text{CHO} \cdot \text{kg}^{-1} \text{body wt} \cdot 2\text{h}^{-1}$ are consumed over the immediate post-exercise period (Blom et al, 1987b; Ivy et al, 1988b). However, it remained unclear if the restoration of exercise capacity paralleled this replenishment of muscle glycogen.

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A 90 min bout of constant pace running at $70\% \dot{V}O_{2\text{max}}$ was initially performed in the studies reported in Chapters 4, 6 and 7 (R_1). Previously, Brewer et al (1988) who adopted similar procedures measured mean treadmill run times at $70\% \dot{V}O_{2\text{max}}$ to exhaustion ranging between $105.9 (\pm 24.4)$ to $119.2 (\pm 19.5)$ min. Similarly, Tsintzas et al (1993b) observed a mean run time of $109.6 (\pm 9.6)$ min for exhaustive running at $70\% \dot{V}O_{2\text{max}}$ in subjects of a similar training status. Extrapolating from these observations, it might be cautiously speculated that the 90 min run (R_1) taxed $\sim 85\%$ of the endurance capacity of subjects in the studies reported in this thesis. Tsintzas (1993) further reports that such exhaustive exercise bouts resulted in decreases in muscle glycogen concentration from $317.0 (\pm 34.2)$ to $31.6 (\pm 10.3) \text{ mmol} \cdot \text{kg}^{-1} \text{dry wt}$ in type-I fibres, and from $443.4 (\pm 44.9)$ to $103.9 (\pm 29.2) \text{ mmol} \cdot \text{kg}^{-1} \text{dry wt}$ in type-II fibres. Expressing these values as a mean for mixed fibre samples muscle glycogen concentration decreased from $380.2 (\pm 35.2)$ to $67.8 (\pm 15.2) \text{ mmol} \cdot \text{kg}^{-1} \text{dry wt}$. This is equivalent to a utilisation rate of $\sim 2.85 \text{ mmol} \cdot \text{kg}^{-1} \text{dry weight} \cdot \text{min}^{-1}$ for treadmill running at $70\% \dot{V}O_{2\text{max}}$. Extrapolating once again from these previously reported observations, R_1 would reduce muscle glycogen concentration by $\sim 67\%$. This is in general agreement with estimated levels of carbohydrate oxidation obtained from indirect calorimetry reported in Chapters 4, 6 and 7.

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Blood lactate concentrations increased with the onset of exercise. A plateau was then maintained until ~75 mins of exercise, after which lactate levels tended to fall. Systemic concentrations reflect a difference between the respective rates of lactate production and lactate removal (Wahren, 1977). Thus, a fall in concentrations over the later stages of exercise would result from either a decrease in the rate of production or an increase in the rate of removal. The former may be associated with declining muscle glycogen concentrations, whereas the latter might reflect a contribution of lactate to energy metabolism (Rowell et al, 1966). Blood glucose concentrations and estimated rates of carbohydrate oxidation were consistently maintained throughout R₁, whilst plasma FFA, glycerol, ammonia and urea concentrations increased. It has been suggested that FFA progressively contribute a greater proportion to the total substrate requirements of aerobic metabolism (Ahlborg et al, 1974; Havel et al, 1964). Evidence of this shift in energy metabolism was provided from indirect calorimetry, where protein was assumed to play a relatively minor role.

An increase in fat oxidation would contribute to an upward drift in both ventilation and oxygen consumption (Kirwan et al, 1988). Conversely, enhanced respiration may reflect an increasing energy cost of exercise. A decrease in muscle glycogen concentration is accompanied by a reduction in running economy (Kirwan et al, 1988). Nicol, Komi and Marconnet (1991) report impaired contractile efficiency and decreased force generation following prolonged, submaximal running. This in turn would increase the energy cost of maintaining a constant level of work output. In the studies reported in this thesis, it was estimated that more than 90% of the additional energy requirement late in exercise during R₁ was provided by enhanced fat metabolism, rather than through changes in carbohydrate oxidation.

Elevated fat metabolism is associated with enhanced mobilisation of FFA. Increasing systemic FFA concentrations have been observed to inhibit membrane glucose transport (Randle et al, 1963; Rennie and Holloszy, 1977). Thus, a peripheral limitation to performance may result from compromised cellular carbohydrate uptake at a time of low muscle glycogen reserves. Increasing systemic FFA concentrations are also speculated to elicit a central effect which may limit prolonged exercise performance. The amino acid tryptophan is carried bound to albumin in plasma, but is

-didn't take it!

displaced by FFA (Curzon et al, 1973). Free tryptophan is then able to penetrate the blood-brain barrier, whereupon it is hydroxylated to 5-HT (Newsholme and Leech, 1983). Increased 5-HT concentrations in some areas of the brain are suggested to play a role in fatigue (Newsholme et al, 1991; Parry-Billings et al, 1990).

From the above, fatigue during a second exercise bout (R_2) may have resulted from a combination of mechanical and metabolic factors, which ultimately become incapacitating. The former directly influence muscle fibre contractility and reduces the efficiency by which force is generated. Whereas, the latter may influence events both at a muscle fibre level in terms of substrate availability, as well as events within the central nervous system in terms of initiating a motor action. Further research is required to determine the proportional contribution of these factors to fatigue. Thus, the role of carbohydrates in alleviating the symptoms of fatigue may be assessed in terms of provision both during exercise, as well as over the immediate post-exercise period.

Nevertheless, carbohydrate availability per se does not limit performance during submaximal, constant pace running (Tsintzas, 1993). Rather, decreased carbohydrate availability within specific muscle fibres appears to precipitate fatigue (Kirwan et al, 1988; Tsintzas, 1993). Glycogen resynthesis is prioritised in previously active tissue (Mikines et al, 1988; Richter et al, 1989). As such, it might be argued that post-exercise carbohydrate ingestion would facilitate a return in exercise capacity. Ingesting 8.8 g $\text{CHO} \cdot \text{kg}^{-1} \text{body wt}$ following 90 min of constant pace running at 70% $\dot{V}\text{O}_2\text{max}$ restored endurance capacity within 22.5-h (Chapter 4). An isocaloric diet in which additional energy was provided in the form of fat and protein did not result in the same return of exercise capacity. Thus, the application of dietary recommendations which result in the replenishment of muscle glycogen reserves during ~22-h of recovery (Costill et al, 1981; Keizer et al, 1987), also result in a return in the capacity to perform prolonged, constant pace running. Both recovery diets provided more carbohydrate than was estimated to have been oxidised during the previous exercise bout. However, a mixed diet containing the normal level of carbohydrate intake was associated with impaired running performance 22.5-h later.

Kirwan et al (1988) observed that merely matching carbohydrate intake with carbohydrate expenditure was inadequate for maintaining muscle glycogen stores during 5-d of intense training, (ie. ~80 min of running at ~80% $\dot{V}O_2$ max). It was estimated that a carbohydrate intake equivalent to $8.0 \text{ g}\cdot\text{kg}^{-1}\text{body wt}$ was required to meet the demands of exercise (ie. $\sim 240 \text{ g}\cdot 24\text{h}^{-1}$) as well as accommodating the demands of daily living (ie. $\sim 260 \text{ g}\cdot 24\text{h}^{-1}$). A further 30g was provided in the recovery diet in order to cover any additional requirements. This intake is consistent with the recommendations of Costill et al (1981) (ie. $\sim 530\text{g}\cdot 24\text{h}^{-1}$ cf. $\sim 600\text{g}\cdot 24\text{h}^{-1}$). It was suggested that a greater level of carbohydrate intake may be required by athletes participating in such intensive training programmes. Alternatively, there may be a physiological limitation in the capacity of muscle to store glycogen (Kirwan et al, 1988).

The provision of apparently adequate amounts of carbohydrate is not the only factor to be considered with respect to the recovery process. The ability to perform prolonged exercise is also influenced by fluid balance (Armstrong et al, 1985). There appears to be a limitation to post-exercise rehydration, such that ~60-70% rehydration is achieved during the initial 4-h (Costill and Sparks, 1973; Gonzalez-Alonso et al, 1992). It is suggested that this limitation, which will delay the recovery process, is associated with the distribution of fluid and electrolytes between intra- and extra-cellular spaces (Nose et al, 1988a; 1988b). This possibly represents a safety mechanism (Sjogaard, 1989), whereby further activity is restricted until cardiovascular integrity has been restored. Thus, preserving a favourable internal environment with respect to fluid balance and circulatory osmolality during exercise may benefit the recovery process over the immediate post-exercise period.

Water intake during exercise benefits prolonged, constant pace running performance. Ingesting $3.0 \text{ ml}\cdot\text{water kg}^{-1}\text{body wt}$ before exercise, with further feedings of $2.0 \text{ ml}\cdot\text{kg}^{-1}\text{body wt}\cdot 15 \text{ min}^{-1}$ during exercise, improved endurance capacity by 33% (Chapter 5). Total energy expenditure during prolonged, constant pace running was not influenced by the provision of water, though fluid ingestion was associated with a more favourable balance with respect to energy metabolism. Abstaining from water ingestion was accompanied by increased carbohydrate oxidation and suppressed fat oxidation. This shift in metabolism was associated with elevated blood

lactate concentrations and localised fatigue over the later stages of exercise, whilst blood glucose concentrations remained unchanged. The addition of carbohydrate and electrolytes to a solution ingested during exercise spares muscle glycogen (Tsintzas, 1993). Thus, fluid ingestion may reduce the disturbance of energy, osmotic and temperature balances during exercise, which would also assist the recovery process following exercise.

Ellen Field

Ingesting 1.0 g·CHO·kg⁻¹body wt on cessation of prolonged, constant pace running, with a second carbohydrate feeding after 2-h recovery, improved endurance capacity 4-h later (Chapter 6). A 6.9% glucose-polymer solution achieved a similar level of rehydration over this period as was evident following ingestion of a non-carbohydrate placebo solution. Thus, differences in performance during the second exercise bout were due to the provision of carbohydrate and electrolytes, as opposed to the body merely attaining an adequate level of rehydration. Increasing carbohydrate ingestion to 3.0 g·kg⁻¹body wt·2h⁻¹ did not result in a more complete return in endurance capacity (Chapter 7). A high carbohydrate intake was associated with elevated carbohydrate metabolism before and during the second exercise bout, though exercise time to exhaustion was not improved. The processes by which exogenous carbohydrate is absorbed from the G-I tract, transported in the systemic circulation, and eventually carried across the sarcolemma into the muscle cell are not considered to be limiting (Reed et al, 1989). Thus, providing 3.0 g·CHO·kg⁻¹body wt·2h⁻¹ would be sufficient to replace ~97% of that estimated to have been oxidised during R₁. If this was completely incorporated into muscle tissue, pre-R₂ glycogen stores would be adequate to fuel ~106 min of exercise, (predicted from changes in muscle glycogen during constant pace running at 70% $\dot{V}O_2$ max reported by Tsintzas, 1993). However, run time during a second 'open-ended' bout of exercise represented ~57% of this theoretical end point. Indirect respiratory and blood biochemical evidence suggests that one rate-limiting step in post-exercise energy repletion lies in the conversion of glucose to glycogen. Whilst the entry of pyruvate into the TCA cycle may also be limiting. This would partly account for elevated blood lactate concentrations observed prior to the second exercise bout (Blom, 1989b).

Further research is required to confirm the fate of carbohydrate ingested in excess of current recommendations. Intestinal absorption and assimilation of exogenous carbohydrate may in fact become limiting during carbo-

hydrate overfeeding. Whereas, the role of the liver in the disposal of an oral glucose load has largely been estimated due to difficulties in obtaining direct measurements, as has the contribution of extra-hepatic tissue other than previously active muscle. Thus, ~50% of the carbohydrate ingested following exercise remains unaccounted (Blom, 1989b).

Ivy et al (1988a) demonstrated that the rate of glycogen resynthesis in muscle decreases over time, with the most rapid phase occurring immediately post-exercise. Previous feeding patterns have either failed to take full advantage of this rapid-phase-of-glycogen-resynthesis (Burke et al, 1993a; Sherman et al, 1993; Simonsen et al, 1991), or have provided serial carbohydrate feedings of equal size at 1- or 2-h intervals as in Chapters 6 and 7 (Blom et al, 1987; Burke et al, 1993b; Ivy et al, 1988b; Keizer et al, 1987; Reed et al, 1989). However, a higher rate of glycogen resynthesis is achieved during the initial ~4- to 6-h post-exercise by administering carbohydrate feedings at intervals of ~15-20 min (Doyle et al, 1993; Zachwieja et al, 1991). As such, research is also required to establish the maximal capacity of muscle to recover in terms of replenishing endogenous carbohydrate stores. This research needs to focus upon both the quantity of carbohydrate administered per feeding and the timing of post-exercise ingestion, with intake being prescribed in order to maximise the benefit of favourable physiological conditions. It might be speculated that frequent feedings of variable size (ie. larger feedings on exercise cessation to 'prime' blood glucose availability and stimulate insulin secretion, followed by smaller feedings after ~2 to 4-h recovery) may result in a more rapid replenishment of muscle glycogen. Thus, an upper limit of training load may be determined which is consonant with both an athlete's exercise capacity, as well as their capacity to recover. The practical application of this information may assist in alleviating one contributory factor to overtraining.

In conclusion, the replenishment of endogenous carbohydrate reserves plays a central role in post-exercise recovery, though it is not the only factor determining a return in exercise capacity. The restoration of favourable fluid and osmotic balances, which ultimately define the physiological environment in which metabolism takes place, must also be addressed.

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Appendix A : Health History Questionnaire

NAME SS// AGE DATE
ADDRESS TELEPHONE (home)
..... (office)
..... DATE OF BIRTH
OCCUPATION PLACE OF EMPLOYMENT
MARITAL STATUS: MARRIED SINGLE
DOCTOR ADDRESS

CHECK YES OR NO

PAST HISTORY
(Have you ever had?)

Rheumatic fever/heart murmur [] []
High blood pressure [] []
Any heart trouble [] []
Disease of arteries [] []
Varicose veins [] []
Lung disease [] []
Asthma [] []
Kidney disease [] []
Gout [] []
Diabetes [] []
Epilepsy [] []
Thyroid disease [] []

FAMILY HISTORY
(Have any of your immediate family or grandparents had?)

Heart attacks, who age... [] []
High blood pressure [] []
High cholesterol [] []
Stroke [] []
Diabetes [] []
Congenital heart disease [] []
Heart operations [] []
Early death
Other family illnesses
.....
.....

PRESENT SYMPTOMS REVIEW
(Have you recently had?)

Chest pain/discomfort [] []
Shortness of breath [] []
Heart palpitations [] []
Skipped heart beats [] []
Cough on exertion [] []
Coughing of blood [] []
Dizzy spells [] []
Frequent headaches [] []
Frequent colds [] []
Recurrent sore throat [] []
Back pain [] []
Arthritis/swollen, stiff, painful joints [] []
Orthopaedic problems [] []
Unexplained weight loss (> 5 lb.) [] []
Are you presently taking any medications? [] []

HOSPITALIZATIONS
Year Reason Yes No

.....
.....
Any other medical problems not already indicated? Yes No
.....
.....

LIST ALL CURRENT PRESCRIPTION AND NON-PRESCRIPTION MEDICATIONS (include birth control pills)

Medication Reason for Taking For How Long?
.....
.....
.....

Do you currently smoke?: Yes No
 If so, what? How much?

Have you ever quit smoking? Yes No How many years did you smoke?.....

How much alcoholic beverage do you consume in one week?

What type? Beer Wine Hard Liquor
 cans glasses drinks

How much caffeinated beverage do you consume per day?

What type? Coffee Tea Soft Drinks
 cups cups cans

ACTIVITY LEVEL EVALUATION

Do you engage in regular physical activity? Yes No

If so what type?

How many days per week?
 How much time per day?(check one) Less than 15 minutes 15 to 30 minutes
 30 to 60 minutes More than 60 minutes

Do you ever experience shortness of breath during exercise? Yes No

Do you ever experience chest discomfort during exercise? Yes No

If so, does it go away with rest? Yes No

How would you describe your state of well-being at this time?

Very, very good	<input type="checkbox"/>	Poor	<input type="checkbox"/>
Very good	<input type="checkbox"/>	Very poor	<input type="checkbox"/>
Good	<input type="checkbox"/>	Very, very poor	<input type="checkbox"/>
Neither good nor poor	<input type="checkbox"/>		

EMOTIONAL WELL-BEING (Circle the response which most appropriately describes you):

	<u>NEVER</u>	<u>SELDOM</u>	<u>SOMETIMES</u>	<u>FREQUENTLY</u>	<u>CONSTANTLY</u>
I feel sad or depressed	1	2	3	4	5
I am under considerable stress	1	2	3	4	5
I feel tense and anxious	1	2	3	4	5
I worry about things	1	2	3	4	5
I have an intense desire to achieve	1	2	3	4	5
I am a restless sleeper	1	2	3	4	5
I am worried about my health	1	2	3	4	5
I feel like I cannot cope with daily stress	1	2	3	4	5
I feel like I need to get away	1	2	3	4	5

FOR OFFICE USE ONLY

.....

Appendix B : Glucose Assay

A colourimetric method was applied (Werner, Rey and Wielinger, 1970) based upon the following principles:



Normal Values ⁸

Blood: 3.89 - 5.55 mmol.l⁻¹

Solutions*

- (a) Perchloric acid : 2.5 % w/v
- (b) Phosphate buffer : 100.0 mmol.l⁻¹, pH 7.0
- (c) P.Od : > 0.8 U.ml⁻¹
- (d) G.Od : < 10.0 U.ml⁻¹
- (e) ABTS : 1.0 mg.ml⁻¹
- (f) Standard : 5.55 mmol.l⁻¹

* A Boehringer Mannheim GmbH Diagnostica kit was used for the solutions and standard in this assay

Deproteinisation

A 20 μl capillary blood sample was deproteinised in 200 μl of 0.38 mM perchloric acid. This was mixed thoroughly (Fisons Scientific Apparatus Whirlimixer, Model WM/250/F), centrifuged (Eppendorf, Model 5414), and stored at -20°C until the assay was performed.

⁸ Cited Boehringer Mannheim GmbH Diagnostica

Procedure

1. The samples, standard and reaction mixture (RxM) were removed from the freezer and refrigerator respectively, and allowed to warm to room temperature for at least 1-h.
2. The samples were then mixed thoroughly and centrifuged.
3. 20 μ l of standard or supernatant was placed in a test tube with 1.0 ml of RxM and mixed well (use RxM for blank).
4. The tubes were then left to incubate for at least 20 min at room temperature.
5. An Eppendorf photometer (Model 1101M) was then used to measure the absorbance of the standard and samples at Hg 436 nm, in a cuvette of 1.0 cm light path.
6. The glucose concentration of each sample was calculated using the following equation:

$$c = 5.55 * \frac{A_{\text{sample}}}{A_{\text{standard}}} \dots (\text{mmol}\cdot\text{l}^{-1})$$

Coefficient of Variation (n=20)

Blood Glucose: 1.4%

Note : The coefficient of variation (CV) was determined in this, and subsequent biochemical analyses reported in this thesis, on multiple aliquots drawn from a single sample, (where n refers to the number of aliquots from which the CV was calculated).

Appendix C : Lactic Acid Assay

The method adapted from Maughan (1982) was based upon the release of NADH during the following reaction:



Solutions

- (a) Perchloric acid: 2.5 % w/v
- (b) Hydrazine buffer: (1.1 M, pH 9.36)
 - 1.3 g hydrazine sulphate
 - 5.00 g hydrazine hydrate
 - 0.20 g disodium ethylenediaminetetra acetic acid (EDTA)
 - in 100 ml of distilled water (DW)
- (c) Reaction mixture (RxM): 2.0 mg NAD⁺
 - 10.0 μ l LDH
 - for each ml of hydrazine (200 μ l of hydrazine buffer required per tube)

NB The RxM was always prepared immediately prior to use

Standards

These were made from 1.0 M Sodium L-Lactate stock solution providing concentrations of 0.5, 1.0, 5.0, 10.0, and 15.0 mmol.l⁻¹

Deproteinisation

The capillary blood samples were deproteinised as described in the glucose assay.

Procedure

1. Samples and standards were removed from the freezer and allowed to thaw at room temperature for at least 1-h.
2. Samples were then mixed thoroughly using a whirlimixer and centrifuged for 3 min.
3. 20 μ l of either the standard or supernatant was then transferred into a glass fluorimeter tube, whereupon 200 μ l of the RxM was added.
4. The tubes were mixed thoroughly and allowed to incubate for 30 min.
5. 1.0 ml of Lactate Diluent (0.07 M HCl) was then added to each tube in order to stop the reaction and the contents of the tubes were once again mixed thoroughly.
6. The samples were then read against the blanks and standards with a Locarte (Model 8-9) fluorimeter.
7. The lactate concentrations were then calculated on a BBC Master Series microcomputer using software developed in the department.

Coefficient of Variation (n=20)

Blood Lactate: 1.9%

Appendix D : Ammonia Assay

A colourimetric method was applied (Da Fonseca-Wollheim, 1973) based upon the following principle:



Normal Values⁹

Men: 25-94 mg·dl⁻¹; 14.7-55.3 μmol·l⁻¹

Women: 19-82 mg dl⁻¹; 11.2-48.2 μmol·l⁻¹

Solutions*

(a) NADPH

(b) Buffer/substrate: triethanolamine buffer

α-oxoglutarate

ADP

(c) GLDH

* A Boehringer Mannheim Diagnostica kit was used for the solutions of this assay

Preparation of Reagent Solution

Dissolve contents of bottle 1 in 2.0 ml of solution 2 and store in a closed bottle. Stable for 24-h at +15°C to +25°C.

⁹ Cited Boehringer Mannheim GmbH Diagnostica

Procedure

Store plasma samples at -70°C. Perform assay as soon as possible.

Read on wavelength Hg 365 nm in a cuvette of 1.0 cm light path; measure against air. This is a disappearance assay, thus set zero, ie. R_B reading A_1 , as 2.00.

NB Always keep vessels containing plasma and all solutions firmly closed, since ammonia is readily taken up from the air.

1. Centrifuge samples on thawing.
2. Pipette 200 μ l of plasma and 400 μ l of reagent (combination of 1 and 2) into plastic lactate tubes. Cap tubes and mix.
3. Use 600 μ l of reagent for reaction blank (R_B).
4. Read after 10 min (A_1).
5. Add 4.0 μ l of GLDH using a positive displacement pipette. Recap tubes and mix.
6. Read after 10 min (A_2).
7. Add 4.0 μ l GLDH using a positive displacement pipette. Recap tubes and mix.
8. Read after 10 min (A_3).
9. The ammonia concentration of each sample is calculated using the following equations:

$$(A_1 - A_2) - (A_2 - A_3) = \Delta A R_B \text{ or } \Delta A \text{ sample}$$

$$c = 863 * (\Delta A \text{ sample} - \Delta A R_B) \dots \mu\text{mol}\cdot\text{l}^{-1}$$

Coefficient of Variation (n=21)

Plasma Ammonia: 2.1%

Appendix E : Electrolytes (Na⁺, K⁺)

Performed on plasma using flame photometry (Ciba Corning, Model M435)

Normal Values ¹⁰

Plasma Na⁺: 135 - 145 mmol.l⁻¹

Plasma K⁺: 3.5 - 5.3 mmol.l⁻¹

Solutions

R_B : 3M Lithium diluted 1:200 to give 15 mmol.l⁻¹
(5.0 ml 3M Lithium in 1.0 litre of DW)

Standard : 140 mmol.l⁻¹ Na⁺; 5 mmol.l⁻¹ K⁺ diluted 1:200.
(0.5 ml in 100 ml of 15 mmol.l⁻¹ lithium 'working' solution)

Calibration

A zero base-line was achieved against R_B, whereas the one point standard solution (ie. 140 mmol.l⁻¹ Na⁺; 5 mmol.l⁻¹ K⁺) established the working range. Repeat until readings are stable.

NB Re-calibrate once every 20 samples

Procedure

1. Pipette 30 μ l of sample or standard into bijou bottles.
2. Add 6 ml of 15 mmol.l⁻¹ lithium solution, mix and read.

Coefficient of Variation (n=15)

Plasma Sodium: 0.9%

Plasma Potassium: 0.8%

¹⁰ Nottingham City Hospital, Nottingham, UK, normative data, personal communication

Appendix F : Haemoglobin Assay

A cyanmethaemoglobin method was used (Van Kampan and Zijlstra, 1961) which is a colourimetric method based on the following principle:



Normal Values¹¹

Men: 14-18 g·dl⁻¹; 8.7 - 11.2 mmol·l⁻¹

Women: 12-16 g·dl⁻¹; 7.5 - 9.9 mmol·l⁻¹

Solutions*

Drabkins reagent: 1.63 mmol·l⁻¹ phosphate buffer
 0.75 mmol·l⁻¹ potassium cyanide
 0.60 mmol·l⁻¹ potassium ferricyanide
 5.0 % detergent

The above were dissolved in 1000 ml of DW. Stable for 6 months at +15°C to 25°C if stored in a brown glass bottle.

* The reaction mixture for this assay was provided by a Boehringer Mannheim GmbH Diagnostica kit

¹¹ Cited Boehringer Mannheim GmbH Diagnostica

Procedure

1. 20 μ l of blood was added to 5.0 ml of Drabkins reagent and mixed well to avoid clumping.
2. The solution was allowed to incubate at room temperature for at least 3 min, but not longer than 24-h.
3. The absorbance (A) of the samples was measured using an Eppendorf photometer (Model 1101M) at Hg 546 nm, in a cuvette with a 1.0 cm light path. Drabkins reagent was used as a blank to zero the photometer.
4. Haemoglobin concentrations (c) of the samples were calculated using the following equation:

$$c = (37.2 * A) + 0.06 \dots (\text{g}\cdot 100 \text{ ml}^{-1})$$

Coefficient of Variation (n=10)

Blood Haemoglobin: 0.7%

Appendix G : Free Fatty Acids Assay (method-i)

Method-i was based upon extraction of FFA from plasma using inorganic solvents, as modified from Chromy, Gergel, Voznicek, Krombholzova and Musil (1977).

Solutions

- (a) Extraction solvent : 500 ml volumetric flask
(CHM) 280 ml chloroform
210 ml n-heptane
10 ml methanol
- (b) Stable copper reagent: 500 ml volumetric flask
1.878 g sodium citrate (3.756 g·500 ml⁻¹)
16.775 g triethanolamine (33.55 g·500 ml⁻¹)
8.125 g copper nitrate (16.25 g·l⁻¹)
62.500 g sodium chloride (125.0 g·500 ml⁻¹)
- Make up to 250 ml (500ml), with
DW, and keep refridgerated. Stable for 6
months
- (c) TAC¹²: Dissolve 50 mg 2-thiozolyazo-P-cresol (2-
TPC) in 500 ml of ethanol. Filter if
necessary
- (d) Palmitic acid: mw 256.43. For a 1.0 M solution make up
to 1000 ml 256.43 g of palmitic acid with
CHM
Thus, 4.0 mM= (0.25643 g * 4) in 1000 ml =
1.0257 g in 1000 ml =(1.0257 / 10) g for 100
ml of CHM (0.10257 g)

¹² Noma, Okabe and Kita (1973)

Standards

From 4 mM stock solution of palmitic acid

		Stock solution (ml)	CHM (ml)	mM
in triplicate ...	S1	0.25	4.75	0.2
	S2	0.50	4.50	0.4
	S3	1.00	4.00	0.8
	S4	1.25	3.75	1.0

Keep refrigerated in glass, screw-topped bottles. Seal over caps with parafilm

Procedure

1. Use Oxford pipettor to deliver 3.0 ml of CHM into screw-capped glass tubes.
2. Add 50 μ l of plasma or standard, CHM for blank.
3. Add 1.0 ml of stable copper reagent.
4. Shake vigorously for 10 min, and then centrifuge at 5 rpm for 10 min.
5. Transfer 1.0 ml of upper phase into 5.0 ml glass tube, containing 0.25 ml of TAC. Cap tube and mix well.
6. Read at Hg 578 nm.
7. The FFA concentrations were then calculated on a BBC Master Series microcomputer using software developed in the department.

NB Acid washed tubes give more consistent results.

Coefficient of Variation (n=20)

Plasma FFA: 2.6%

Appendix H : Free Fatty Acids Assay (method-ii)

Method-ii applied a colourimetric approach (Shimizu, Yasui, Tani and Yamada, 1979; Mulder, Schouten and Popp-Snijders, 1983) based upon the following principle:

- (i) $R\text{COOH} + \text{ATP} + \text{CoA} \xrightarrow{\text{ACS}} \text{Acyl-CoA} + \text{AMP} + \text{PP (FFA)}$
- (ii) $\text{Acyl-CoA} + \text{O}_2 \xrightarrow{\text{ACOD}} 2,3 \text{ trans-Enoyl-CoA} + \text{H}_2\text{O}_2$
- (iii) $4\text{-Aminoantipyrine} + \text{MEHA} + 2\text{H}_2\text{O}_2 \xrightarrow{\text{Peroxidase}} \text{RQD} + 4\text{H}_2\text{O}$

Where, PP ... pyrophosphate
 ACS ... acyl-CoA synthetase
 ACOD ... acyl-CoA oxidase
 MEHA ... 3-methyl-N-ethyl-N-(b-hydroxyethyl)-alanine
 RQD ... red quinoneimine dye

Normal Values ¹³

The plasma concentration of FFA is subject to large physiological variations and is particularly high after eating.

Range: 0.3 - 1.0 mmol.l⁻¹ (0.3 - 1.0 mEq.l⁻¹; 0.1 - 0.23 g.l⁻¹)

Solutions*

- (a) Colour reagent A: Acyl-CoA synthetase (300 U.l⁻¹)
 (R_A) Ascorbate oxidase (1500 U.l⁻¹)
 Coenzyme A (8.5 mmol.l⁻¹)
 Adenosine triphosphate (5.0 mmol.l⁻¹)
 4-Aminoantipyrine (1.5 mmol.l⁻¹)

¹³ Cited Wako Chemicals GmbH

-
- | | |
|-----------------------|--------------------------------------------------------------------------------------------------------------------|
| (b) Solvent: | Phosphate buffer (50 mmol·l ⁻¹ ; pH 6.9); |
| (for R _A) | Magnesium chloride (3.0 mmol·l ⁻¹); Triton X-100 (0.2%) |
| (c) Colour Reagent B: | Acyl-CoA oxidase (6600 U·l ⁻¹) |
| (R _B) | Peroxidase (7500 U·l ⁻¹)
3-Methyl-N-ethyl-N-(p-hydroxyethyl-aniline)
(1.2 mmol·l ⁻¹) |
| (d) Solvent: | Maleimide (10 mmol·l ⁻¹) |
| (for R _B) | Phenoxyethanol (0.3% v/v)
Triton X-100 (0.1%). |
| (e) FFA Standard: | Oleic acid (1.0 mmol·l ⁻¹) |

* A Wako Chemicals GmbH kit was used for the solutions in this assay

Preparation of Reagent Solutions

1. Dissolve one bottle of R_A in 10 ml Solvent for R_A, and mix well. Stable for 5 days at 2-8°C; stable for 6 to 8-h at room temperature up to 25°C.
2. Dissolve one bottle of R_B in 20 ml Solvent for R_B, and mix well. Stable for 5 days at 2-8°C; stable for 6 to 8-h at room temperature up to 25°C.

Procedure

1. Centrifuge samples on thawing. Bring the colour reagent solutions to room temperature before use. Protect from sunlight.
 2. Pipette 0.015 ml of standard (e) and sample into test tube.
 3. Add 0.375 ml of R_A (a). Add 0.015 ml of DW. Mix well, and incubate for exactly 10 min at 37°C or 15 minutes at 25°C.
 4. Add 0.750 ml of R_B (3). Mix well, and incubate for exactly 10 min at 37°C or 15 min at 25°C.
 5. Read on wavelength Hg 546 nm, the absorbance of the standard (A_{standard}) and samples (A_{sample}) against a reagent blank. Exceeding the incubation times will give falsely elevated values. The reaction colour is stable for 30 min at room temperature.
-

-
6. The FFA concentration (Conc.) of each sample is calculated using the following equation:

$$\text{Conc.} = \frac{A_{\text{sample}}}{A_{\text{standard}}} * \text{Standard concentration (mmol}\cdot\text{l}^{-1}\text{)}$$

Coefficient of Variation (n=20)

Plasma FFA: 1.5%

Appendix I: Glycerol Assay

The glycerol assay applied a method as modified from Laurell & Tibling (1966).

Solutions

- (a) Zinc sulphate: 6.25 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (mw 287.54) in 250 ml of DW
0.087 M
- (b) Barium hydroxide: 6.55 g $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ (mw 315.4) in 250 ml of DW
0.083 M
- (c) Cysteine: 35.0 mg cysteine in 1.0 ml of 0.4 M NaOH (prepared daily)
0.2 M
- (d) Hydrazine-HCl buffer: 1.0 M hydrazine, ie 19.0 ml hydrazine hydrate ($\text{wt} \cdot \text{ml}^{-1}$ 1.03 g) in 250 ml DW (64 % solution), with 1.5 mM MgCl_2 , ie 76.2 mg in 250 ml of DW.
Adjust pH with HCl to 9.4
- (e) RxN mixture: 100 μl per tube (prepared daily)
-12 mg ATP, 20 mg NAD dissolved in 0.2 ml DW per ml of RxN mixture
-Add 100 μl cysteine 0.2 M
700 μl Hz-HCl buffer 1.0 M
1.0 μl glycerokinase
5.0 μl glycerine-3-phosphate dehydrogenase
- (f) Diluent: 0.01 M NaOH with 1.0 mM EDTA
-ie 0.4 g NaOH with 372.24 mg EDTA
made up to 1000 ml with DW
-

Standards

1. Prepare approximately 4.0 mM solution, ie about 36.8 mg in 100 ml DW. Calculate exact molarity from weight.
2. Dilute ten-fold to give approximately 0.4 mM.
3. Take approximately 0.4 mM as 100 %, then ...

	0.4 mM (ml)	distilled water (ml)
10% ... is ...	0.25	2.25
20 %	0.5	2.0
40 %	1.0	1.5
60 %	1.5	1.0
80 %	2.0	0.5

Procedure

1. Pipette 0.25 ml zinc sulphate into small centrifuge tubes.
2. Add 50 μ l of sample or standard.
3. Add 0.25 ml of barium hydroxide. Mix immediately.
4. Chill in freezer at -20°C , for 5 min. After which, centrifuge for 5 min.
5. Pipette 200 μ l of supernatant into acid-washed fluorimetric tubes.
6. Add 100 μ l of RxN mixture. Mix, cap and incubate for 60 min.
7. Add 1.0 ml of diluent to stop the reaction, and read on medium slit width.
8. The glycerol concentrations were then calculated on a BBC MasterSeries microcomputer using software developed in the department.

Coefficient of Variation (n=20)

Plasma Glycerol: 2.5%

Appendix J : Urea Assay

An enzymatic colourimetric method was used (Fawcett and Scott, 1960), being a modified version of Bertholt's reaction. The test principle is as follows:



Ammonium ions react with sodium hydroxide and hypochlorite to yield a coloured complex.

Normal Values ¹⁴

Serum: 10-50 g·dl⁻¹; 1.7-8.3 mmol·l⁻¹

Solutions*

- | | |
|-----------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| (a) Solution 1: | Phosphate buffer (120 mmol·l ⁻¹ ; pH 7.0)
Urease (>5000 U·l ⁻¹)
Sodium salicylate (62.5 mmol·l ⁻¹)
Sodium nitroprussiate (5.00 mmol·l ⁻¹)
EDTA (1.48 mmol·l ⁻¹) |
| (b) Solution 2: | Sodium Hypochlorite (6.00 mmol·l ⁻¹)
Sodium hydroxide (150.00 mmol·l ⁻¹) |
| (c) Standard: | Urea (30 mg·100 ml ⁻¹) |

* A Boehringer Biochemia Robin kit was used for the solutions of this assay

¹⁴ Cited Boehringer Biochemia Robin

Preparation of Reagent Solutions

1. To ensure optimal dissolution of the reagent, empty the contents of one bag into a vessel, add 50 ml of DW as a single portion and mix thoroughly. Stable for 4 weeks at +2 to 8°C, 8-h at +15 to 25°C, when stored in a dark bottle.
2. Dilute contents of bottle (50 ml), with 450 ml of DW. Stable for 6 months at +2 to 8°C, 3 months at +15 to 25°C, when stored in a dark bottle.
3. Use solution undiluted. Stable up to the expiry date specified when stored at +2 to 8°C.

Procedure

NB The reaction is extremely sensitive to ammonium salts; use perfectly clean glass only.

1. Centrifuge samples on thawing.
2. Pipette 0.010 ml of standard and sample into test tubes.
3. Add 1.25 ml of Solution 1. Mix, and incubate for at least 5 min at +20 to 25°C, or 3 min at +37°C.
4. Add 1.25 ml of solution 2. Mix, and incubate for at least 10 min at +20 to 25°C, or 5 min at +37°C.
5. Read on wavelength Hg 578 nm in a cuvette of 1.0 cm light path, the absorbance of the standard (A_{standard}), and samples (A_{sample}), against a reagent blank. The reaction colour is stable for at least one hour.
6. The urea concentration (c), of each sample is calculated using the following equation:

$$c = \frac{A_{\text{sample}}}{A_{\text{standard}}} * 30 \dots \text{mg urea} \cdot 100 \text{ ml}^{-1}$$

Coefficient of Variation (n=15)

Plasma Urea: 1.0%

Appendix K : Replay - Composition**Replay (BASS Plc)****Ions (meq·ml⁻¹)**

Sodium	3.00
Potassium	1.10
Calcium	0.80
Magnesium	0.35
Chloride	1.10
Sulphate	0.40
Phosphate	0.25

Sugars (g·100ml⁻¹)

Dextrins	6.6
Maltotriose	0.0
Maltose	0.0
Glucose	2.6
Sucrose	0.7
Fructose	6.6
	—
Total	16.5
	—

Fruit juice content = 20%

Energy value = 262.1 kJ·100ml⁻¹

Glucose content equivalent = 804 mmol·l⁻¹

Appendix L : Lucozade Sport - Composition

Lucozade Sport (Smithkline Beecham Plc)

Ions (mg ·100ml⁻¹)

Sodium	52
Potassium	14
Calcium	6
Magnesium	1

Sugars (g·100ml⁻¹)

Sucrose	2.9
Maltodextrin	2.7
Orange Fruit	1.3
	—
Total	6.9
	—

Energy value = 110 kJ·100ml⁻¹Osmolality = 269 mosmol·kg⁻¹Glucose content equivalent = 384 mmol·l⁻¹

Note : Non-carbonated

Appendix M : Lucozade Original - Composition

Lucozade Original (Smithkline Beecham Plc)

Sugars (g·100ml⁻¹)

Dextrose	5.8
Maltose	3.3
Maltotriose	2.6
Tetrasaccharides	1.8
Pentasaccharides	1.3
Higher sugars	4.0
	—
Total	18.8*
	—

Energy value = 309 KJ·100ml⁻¹

Osmolality = 630 mosmol·kg⁻¹

Glucose content equivalent = 916 mmol·l⁻¹

(Note: * 19.3 g·100ml⁻¹ as monosaccharides)

Note : Non-carbonated
 Caffeine free
