Influence of rehydration on short-term recovery from prolonged running and subsequent exercise capacity in humans

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Influence of Rehydration on Short-Term Recovery From Prolonged Running and Subsequent Exercise Capacity in Humans

by

Stephen Heung Sang Wong

A Doctoral Thesis

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

December 1996

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This thesis is dedicated to my wife, Wai-Ling, and to my family, with love and appreciation. Most especially it is dedicated to the memory of my father.
"Unless the Lord builds the house,
is its builders labour in vain.
Unless the Lord watches over the city,
the watchmen stand guard in vain"

Psalms 127: 1

「若不是耶和華建造房屋，
建造的人就枉然勞力。
若不是耶和華看守城池，
看守的人就枉然儆醒。」

詩篇 127:1
ABSTRACT

The aim of this research was to investigate the influence of rehydration with carbohydrate-electrolyte solutions, during a short-term recovery period, on hydration status, physiological responses, and subsequent endurance capacity.

The first study (Chapter 4) examined whether prescribed or ad libitum rehydration with a carbohydrate-electrolyte solution (CHO-E), during 4 h recovery from prolonged, submaximal running would influence the subsequent endurance capacity. Five women and two men performed the "recovery" protocol consisting of a 90 min run at 70% VO₂ max on a level treadmill (T1) followed by 4 h rehydration-recovery (REC), and then an open-ended run to exhaustion at 70% VO₂ max (T2) as a measure of their endurance capacity, on two occasions, at least 7 days apart. During the REC, subjects were allowed to drink a 6.9% CHO-E ad libitum (AL) on one occasion. On the other occasion, the volume of the same fluid was prescribed (PI) from calculations of the body mass lost during T1. During T2, in the PI trial, the run time to exhaustion was 16% longer (P < 0.05) than during T2 in the AL trial (69.9 ± 9.1 vs. 60.2 ± 10.2 min). Thus, ingestion of a prescribed volume of CHO-E after prolonged exercise, calculated to replace the body fluid losses, restored endurance capacity to a greater extent than ad libitum rehydration during the REC.

The second study (Chapter 5) investigated the influence of ingesting 50 g of carbohydrate (CHO) immediately after exercise, either with subsequent serial CHO feeding or water ingestion during the REC from prolonged, submaximal running on rehydration and subsequent endurance capacity. Eight male subjects performed the "recovery" protocol [i.e. 90 min run at 70% VO₂ max (T1), 4 h rehydration-recovery (REC), and open-ended run at 70% VO₂ max (T2)] on two occasions. During the REC, subjects ingested a prescribed volume of fluid equal to the body mass lost during T1 in both conditions. Subjects ingested 50 g of CHO from a 6.9% CHO-E 15 min after T1 on both occasions as their first prescribed fluid intake. Thereafter, subjects drank either the same solution (CE) or water (W) at each hour after T1 during the REC. During T2, the run time to exhaustion was 54.2 ± 9.2 min in the CE trial and 52.2 ± 6.2 min in the W trial, respectively (NS). The volume of fluid retained expressed as a percentage of the volume ingested (% rehydration) during the CE trial was greater than that of the W trial (CE: 73.5 ± 4.2% vs. W: 63.0 ± 5.7%; P < 0.05). Serial CHO feeding during the REC was associated with increased CHO oxidation and suppressed fat oxidation during subsequent exercise. Thus, ingesting ~150 g of CHO in a 6.9% CHO-E over a 4 h period following prolonged running is more effective in terms of rehydration compared to the same volume of fluid containing only 50 g of CHO and water, but does not have a greater effect on subsequent endurance capacity.
The third study (Chapter 6) investigated the effects of rehydration per se and CHO ingestion, during the REC, on subsequent endurance capacity. Nine male subjects performed the "recovery" protocol on two occasions. During the REC, subjects drank either a 6.9% CHO-E (CE) or a CHO-free sweetened placebo (PL) every 30 min after T1 up to the beginning of the 4 h of the REC. Volumes prescribed (ml) were equal to 200% of the body mass lost during T1. However, the total volume of fluid ingested during the REC was only 170.8 ± 12.6% and 172.6 ± 13.8% of the body mass lost after T1 (NS). During T2, in the CE trial, the run time to exhaustion was 54% longer (P < 0.01) than during T2 in the PL trial (69.3 ± 5.5 vs. 45.0 ± 4.2 min). After the REC, subjects were in positive fluid balance by 423 ± 215 ml in the CE trial and 446 ± 239 ml in the PL trial (NS). Thus, positive fluid balance can be achieved by ingesting a prescribed volume of either a 6.9% CHO-E or a placebo solution over the REC, calculated to replace approximately 170% of the body fluid loss. Despite this similar hydration status after the recovery in both conditions, ingesting a CHO-E is more effective in restoring endurance capacity compared to the same volume of placebo solution.

The fourth study (Chapter 7) was intended to examine, and verify, the effects of ingesting different amounts of CHO in the form of a CHO-E during the REC on rehydration and subsequent endurance capacity. Nine male subjects performed the "recovery" protocol on two occasions. During the REC, a fixed volume of fluid equivalent to 150% of the body mass lost during T1 was consumed. Subjects ingested 50 g of CHO from a 6.5% CHO-E 30 min after T1 on both occasions as their first prescribed fluid intake. Thereafter, subjects ingested either the same solution (CE) or a CHO-free sweetened placebo (PL) every 30 min up to the beginning of the 4 h of the REC. During T2, the run times were 56.9 ± 8.1 min in the CE trial and 65.4 ± 7.8 min in the PL trial (NS). After the REC, subjects were almost equally euhydrated (CE: 0 ± 184 ml; PL: -27 ± 120 ml) in both conditions (NS). Serial CHO feeding over the REC was accompanied by enhanced CHO oxidation and suppressed fat oxidation. In conclusion, ingesting a placebo solution containing 50 g of CHO and placebo over a 4 h period following prolonged running, calculated to replace 150% of the body fluid loss, is equally effective in achieving approximate euhydration and restoring endurance capacity compared to the same volume of CHO-E containing ~167 g of CHO.

The studies reported in this thesis suggest that in order to achieve euhydration during recovery, a volume of fluid substantially larger (≥ 150%) than that lost must be ingested. The provision of additional CHO (~150 to 170 g) would be expected to restore the body's CHO stores to a greater extent than a smaller amount of CHO (50 g) during the REC and, thereby, improve the subsequent endurance capacity. However, this was not the case. It appears that the ingestion of large amounts of CHO, during the REC, resulted in disturbances in fat and CHO metabolism which prevented an improvement in endurance capacity during T2, after consumption of the additional CHO.
ACKNOWLEDGEMENTS

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I would like to express my deepest gratitude to my supervisor, Professor Clyde Williams, for his guidance, patience, and encouragement at every step throughout my studies, especially during the final stages of writing up this thesis.

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I would also like to express my sincere thanks to all members of the Sports Nutrition and Exercise Biochemistry Research Group for their invaluable assistance in the experimental work and for their friendship over the past few years.

Special thanks are due to SmithKline Beecham for providing the test drinks throughout this work. I also wish to thank the individuals who kindly volunteered as subjects and gave a tremendous commitment to these demanding experiments. Without their efforts, this thesis would not have been possible.

Finally, I thank my Lord for His unfailing love and guidance over the years.
PUBLICATIONS

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

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<td>ad libitum</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>BM</td>
<td>body mass</td>
</tr>
<tr>
<td>CHO</td>
<td>carbohydrate</td>
</tr>
<tr>
<td>CHO-E</td>
<td>carbohydrate-electrolyte solution</td>
</tr>
<tr>
<td>CV</td>
<td>coefficient of variation</td>
</tr>
<tr>
<td>FFA</td>
<td>free fatty acids</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
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<td>GI</td>
<td>gastrointestinal</td>
</tr>
<tr>
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<td>hour</td>
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<td>maximum heart rate</td>
</tr>
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<td>Na⁺</td>
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<tr>
<td>NADH</td>
<td>reduced nicotine adenine dinucleotide</td>
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<tr>
<td>ORS</td>
<td>oral rehydration solution</td>
</tr>
<tr>
<td>PI</td>
<td>prescribed fluid intake</td>
</tr>
<tr>
<td>RPE</td>
<td>rate of perceived exertion</td>
</tr>
<tr>
<td>RER</td>
<td>respiratory exchange ratio</td>
</tr>
<tr>
<td>REC</td>
<td>4 hours rehydration-recovery period</td>
</tr>
<tr>
<td>SEM</td>
<td>standard error of the mean</td>
</tr>
<tr>
<td>mU</td>
<td>milli units</td>
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<td>VCO₂</td>
<td>carbon dioxide production (volume/time)</td>
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<td>VO₂</td>
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<td>watt</td>
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CHAPTER 1

INTRODUCTION

Nutrition as one of the most important factors influencing physical performance has attracted considerable attention from sports scientists, athletes, and coaches over the last 50 years. Despite the wealth of published information dealing with "proper nutrition" and the advocacy of a healthy, well-balanced diet, numerous athletes still believe that special foods or dietary supplements are necessary for optimal performance. However, it is now firmly established that carbohydrate (CHO) is the most essential nutrient for prolonged, intense training and successful athletic performance (Costill, 1988; Williams, 1993). Yet, its stores within the body are relatively small.

The classical studies of Christensen and Hansen (1939a, 1939b) clearly demonstrated the importance of CHO availability during prolonged exercise and the potential influence of dietary CHO on endurance exercise performance. After the introduction of the percutaneous muscle biopsy technique, it became clear that at exercise intensities higher than 70% VO\(_2\) max, the concentration of muscle glycogen is a determinant of endurance exercise capacity (Bergstrom and Hultman, 1967; Bergstrom et al., 1967; Hermansen et al., 1967).

Although the depletion of the body's CHO reserves is the primary cause of fatigue in prolonged, exhaustive exercise, other factors concurrently exist to limit human performance. One of those physiological perturbations that cause early fatigue is the failure of the body to maintain fluid balance which, in turn, affects the efficiency of thermoregulation during exercise (Coyle and Montain, 1992). The rise in body temperature that normally accompanies exercise stimulates an increase in blood flow to the skin and the onset of sweating. A balanced hydration status is, therefore, a good protection of these thermoregulatory responses, whereas even a slight amount of dehydration results in measurable declines in cardiovascular and thermoregulatory function (Costill and Sparks, 1973; Nadel et al., 1980; Heaps et al., 1994).
Mild to severe dehydration commonly occurs among athletes, even when fluid is readily available (Carter and Gisolfi, 1989; Gonzalez-Alonzo et al., 1992; Nielsen et al., 1986; Noakes et al., 1988). This involuntary dehydration (Greenleaf and Sargent, 1965) not only compromises physiological function, but also impairs exercise performance and increases the risk of heat intolerance and illness.

In view of the importance of substrate availability and fluid balance before or during exercise, it is not surprising to find that numerous studies have been undertaken in these areas over the past decades. The research findings result in a comprehensive understanding of the CHO utilisation during exercise and the physiological consequences of dehydration and, more recently, of the physiological and performance benefits of euhydration (Below et al., 1995; Montain and Coyle, 1992a, 1992b). Since reduction, or depletion, of the body's CHO stores and dehydration are the inevitable results of prolonged, heavy exercise, it is logical to assume that the two main goals of the recovery strategy of athletes, following prolonged exercise, are to achieve complete rehydration and replenish their muscle glycogen stores rapidly. It is particularly true for those individuals involved in activities that elicit large sweat losses, and in which more than one bout of exercise may be completed in a single day.

Rehydration following exercise involves the administration of dilute CHO-electrolyte solutions which can be rapidly emptied from the gastrointestinal (GI) tract. CHO-electrolyte solutions can serve the twin aims of providing a source of CHO to replenish the body's limited stores and supply water and electrolytes to achieve rehydration after exercise. Dehydration does not delay muscle glycogen resynthesis (Neufer et al., 1991), but it may be responsible for an inability to recover completely from prolonged exercise. In a hot environment, rehydration is even more important because the consequences of dehydration are potentially life threatening.

Whilst the benefits and the physiological responses of fluid replacement with CHO before, and during, exercise have been extensively studied by scientists interested in sports nutrition, not much research has been undertaken to investigate the effects of rapid restoration of CHO reserves and rehydration during the post-exercise recovery period, let alone the question of the nutritional strategies on subsequent exercise performance. The majority of those studies investigating the post-exercise recovery period have focused on the influences of CHO ingestion on muscle glycogen resynthesis and the optimal composition of rehydration fluids. Yet, few have examined the effects of the resulting glycogen and hydration levels on the return of functional capacity. It is generally assumed that nutritional strategies stimulating the
high rates of muscle glycogen resynthesis and level of rehydration after exercise will result in a greater restoration of exercise capacity than strategies resulting in slower rates of glycogen resynthesis and rehydration.

Only one study investigating the effect of CHO-electrolyte ingestion, following prolonged exercise on subsequent endurance running capacity, has so far been published in the scientific literature (Fallowfield et al., 1995). Thus, there is a pressing need for more research about the influence of rehydration strategies on metabolic and physiological responses during the recovery period, as well as during the subsequent exercise. Furthermore, this lack of knowledge on the post-exercise recovery period and the preparation for the subsequent exercise performance is an omission in the study of fatigue in particular, and in sports nutrition in general.

Thus, the principal aim of this thesis was to investigate the influence of rehydration with CHO-electrolyte solutions, during a short-term recovery period, on hydration status, physiological responses, and subsequent running capacity. The studies were also designed to generate more useful information which could serve as practical, nutritional guidelines to hasten the recovery process of the practising athlete.

This thesis is presented in a sequence that progresses from a consideration of the influence of the drinking pattern, to the effect of fluid volume on achieving euhydration during short-term recovery. The Review of Literature (Chapter 2) provides a summary of the available evidence regarding the effect of fluid replacement and CHO-electrolyte feedings after exercise on metabolism, restoration of glycogen reserves, and subsequent exercise performance. Factors influencing the fluid balance following exercise are also reviewed. The methodology employed in this thesis is described in Chapter 3. A standardised "recovery protocol" developed by Fallowfield et al. (1995) was continuously used throughout the studies of this thesis in order to investigate the influence of rehydration on subsequent endurance capacity. This experimental protocol basically involved a 90 min endurance run at 70% VO₂ max, followed by a 4 h controlled rehydration-recovery period, and a subsequent open-ended endurance run to exhaustion at the same exercise intensity. The 4 h rehydration-recovery period was selected for comparison with similar periods in previous studies that assessed the effects of various fluid replacement regimens on rehydration and subsequent exercise performance (Lambert et al., 1992; Ivy et al., 1988b; Keizer et al., 1986; Nielsen et al., 1986; Fallowfield et al., 1995).
The first study (Chapter 4) examined the influence of drinking patterns on subsequent endurance capacity after a 4 h rehydration-recovery period (REC). It has been shown that short-term recovery from exhausting exercise is enhanced, and subsequent endurance capacity is improved, by ingesting a CHO-electrolyte solution immediately after exercise (Fallowfield et al., 1995). However, it is unclear how a particular drinking pattern, during such short-term recovery, will in turn influence the level of rehydration and subsequent endurance capacity. Thus, the aim of this study was to investigate whether prescribed or ad libitum rehydration with a CHO-electrolyte solution, during 4 h recovery from prolonged, submaximal running, influenced subsequent endurance capacity.

Rapid restoration of muscle glycogen and rehydration after exercise are of importance for optimal substrate provision, cardiovascular function, and thermoregulation during subsequent bouts of exercise (Costill and Sparks, 1973; Montain and Coyle, 1992a, 1992b; Heaps et al., 1994). In order to hasten the glycogen resynthesis process, it has been recommended that at least 50 g of CHO should be ingested immediately after exercise (Coyle, 1993). Thus, the ability to maximise muscle glycogen stores during restricted recovery periods would give an athlete an advantage during repeated bouts of exercise. As mentioned previously, CHO-electrolyte solution can serve the purposes of providing an energy source to restore the body's limited CHO stores, and also water to offset the negative effects of dehydration. However, it remains unclear whether consuming only 50 g of CHO immediately after exercise, as recommended by Coyle (1993), is sufficient for restoring energy capacity for subsequent exercise. Therefore, the purpose of the second study (Chapter 5) was to investigate the influence of ingesting 50 g of CHO immediately after exercise, either with subsequent serial CHO feeding or water ingestion during the REC from prolonged, submaximal running, on rehydration and subsequent exercise capacity.

Although fluids with different compositions combined with a prescribed drinking pattern have been employed after exercise-induced dehydration, complete rehydration is still not achieved during a recovery period of only 2 to 4 h when the volume of fluid ingested is equal to the body weight loss (Costill and Sparks, 1973; Gonzalez-Alonso et al., 1992; Carter and Gisolfi, 1989; Nielsen et al., 1986). In order to restore body weight as rapidly as possible after dehydration, it has been suggested that the ingestion of a larger fluid volume may be necessary during a short rehydration period (Grandjean et al., 1992). A recent study successfully showed that complete rehydration could occur during a short rehydration period after exercise-induced dehydration when the volume ingested was substantially greater than the fluid loss during exercise (Maughan et al., 1994).
It is also suggested that more effective rehydration could occur with a solution containing a higher sodium content and/or low concentration of CHO (Mitchell et al., 1994). However, the effects of ingesting such a large volume of fluid on subsequent exercise performance remains unknown and no studies so far have been conducted to clarify this issue. Therefore, the purpose of the third study (Chapter 6) was to compare the effects of rehydration with a large volume of fluid per se together with CHO ingestion, during the REC, on subsequent endurance running capacity.

The fourth study (Chapter 7) was to designed to further investigate the findings of previous studies in this thesis. It was achieved by examining and verifying the influence of increasing the fluid volume, during 4 h recovery, on metabolism and subsequent constant pace running performance. Ingesting a volume of fluid significantly larger than the body weight loss was found necessary for achieving complete rehydration (Chapter 6), the question as to whether the additional CHO intake would further enhance the subsequent exercise remained unanswered. In the second study (Chapter 5), no differences were observed in run times during the open-ended run between trials. This finding was unexpected in view of the greater amount of CHO ingested during the recovery period. Thus, the aim of this study was to re-examine the effect of ingesting different amounts of CHO, in a volume of fluid which was greater than the body weight loss, on rehydration and subsequent running capacity.

Finally, in the General Discussion and Conclusions (Chapter 8), the findings of the studies reported in this thesis and the different aspects elaborated in the Review of Literature are drawn together. Emphasis is laid on the relationship between rehydration and CHO ingestion during the short-term recovery and its effects on subsequent exercise capacity.
CHAPTER 2

REVIEW OF LITERATURE

During prolonged, exhaustive exercise, the depletion of muscle glycogen and dehydration are the two main causes of fatigue (Ahlborg et al., 1967a; Armstrong et al., 1985a). Exercise-induced dehydration adversely affects cardiovascular function and temperature regulation (Costill and Fink, 1974; Montain and Coyle, 1992a, 1992b). Besides the body hydration level, CHO availability is also of importance to exercise performance. Prolonged exercise at 60 to 80% \( \dot{V}O_2 \) max is known to reduce the CHO reserves of the body, and contributes to the onset of fatigue (Ahlborg et al., 1967b; Bergstrom and Hultman, 1966; Hermansen et al., 1967; Hultman, 1967).

This thesis investigates whether rehydration with CHO-electrolyte solution during the short-term post-exercise period facilitates the recovery process, so that individuals are able to restore their fluid balance and consequently their homeostasis. This will be referred to as the "functional capacity" of an individual, as reflected by an ability to repeat their previous endurance running capacity. As mentioned in Chapter 1, the reason for this scientific inquiry is because of the limited knowledge of the influence of rehydration strategies on metabolic and physiological responses during the recovery period, as well as during the subsequent running capacity.

To begin with, this review briefly considers energy metabolism during prolonged, submaximal exercise. The effects of depletion of endogenous CHO stores and dehydration on exercise performance are also discussed. The review then examines the factors affecting muscle glycogen resynthesis after exercise and the influence of fluid replacement on subsequent exercise performance. Finally, the factors influencing the effectiveness of fluid replacement during recovery are considered.
2.1. ENERGY METABOLISM DURING PROLONGED EXERCISE

The energy used to sustain steady-state aerobic exercise in humans is derived predominately from the oxidation of CHO and fat (Krogh and Lindhard, 1920). Normally, bodily protein oxidation does not contribute significantly to energy production (Romijn and Wolfe, 1992, Hood and Terjung, 1990). Therefore, the four major sources of energy for exercise are muscle glycogen, blood glucose (from liver glycogen), plasma free fatty acids (FFA), and intramuscular triglyceride (Coyle et al., 1986; Romijn et al., 1993).

During prolonged, strenuous exercise, muscle glycogen and blood glucose are important substrates for contracting muscle and fatigue often coincides with depletion of these CHO sources (Coggan and Coyle, 1987; Constantin-Teodosiu et al., 1992; Sahlin et al., 1990). One cannot oxidise fat at high enough rates to provide all the energy required by moderate to high intensity exercise (Davies and Thompson, 1979). At such high intensities, CHO oxidation must provide the major source of energy not available from fat (Coggan and Coyle, 1991). Consequently, fatigue often occurs when muscle glycogen becomes depleted (Coggan and Coyle, 1991). This is the rationale for the implementation of dietary CHO supplementation before, during, and after exercise.

The amount of energy stored in the form of triglycerides within adipocytes in the body is large, totalling 200 - 625 MJ (~50,000 - 150,000 kcal) in men and women with a normal body composition of between 10 - 30% body fat. Triglycerides stored in adipocytes can be hydrolysed into glycerol and FFA; the latter must bind to the protein carrier, albumin, for transport via the circulation to the exercising muscles (Bulow and Madsen, 1981). Additional triglyceride is stored in droplets within the muscle fibres and is available for oxidation following intramuscular lipolysis.

Despite the large amount of potential energy in the body's fat stores, the rate at which they can be oxidised is limited. Thus, CHO metabolism is needed to provide the additional substrate for oxidation as the intensity of exercise is increased. It has been suggested that increases in the level of lactate in blood may retard FFA release (Fredholm, 1969; Boyd et al., 1974; Issekutz et al., 1975) and thus, reduce the availability of FFA in parallel with increasing work intensity. However, it was found that during moderate intensity exercise in endurance-trained athletes, plasma FFA and intramuscular triglyceride contribute equally to total fat oxidation (Hurley et al., 1986; Martin et al., 1993).
CHO is stored as glycogen, both within the muscle fibres and liver (Bergstrom and Hultman, 1966; Nilsson and Hultman, 1973). Consumption of a high CHO diet is associated with an increased rate of CHO oxidation during exercise (Bergstrom et al., 1967; Galbo et al., 1979; Martin et al., 1978), and increased muscle glycogenolysis (Gollnick et al., 1972). In view of the importance of CHO for exercise performance, the goal of CHO nutritional strategies during exercise is to optimise the availability of muscle and liver glycogen and blood glucose, so as to enhance and maintain CHO oxidation (Costill and Hargreaves, 1992).

Thus, it is clear, through the literature, that during exercise of low to moderate intensity, most of the energy is derived from the oxidative phosphorylation of CHO and the lipolysis of fat. The activation threshold is low in Type I motor neurons and it increases in neurons activating Type IIa to Type IIb. Therefore, during prolonged exercise at intensities of 60 - 75% \( \text{VO}_2 \text{ max} \), muscle glycogenolysis occurs primarily in the Type I muscle fibres (Gollnick et al., 1973, 1974; Vollestad et al., 1984; Tsintzas, 1993; Tsintzas et al., 1995, 1996), although there may be some glycogen degradation in Type IIa fibres (Vollestad et al., 1984).

In a recent study by Ball-Burnett et al. (1990), a 2 h period of dynamic exercise was performed at an intensity of 61% \( \text{VO}_2 \text{ max} \). The glycogen degradation, as estimated in pooled samples of fibres, was most pronounced in Type I fibres. Histochemical analyses showed that the glycogen loss during the first 15 min of exercise was detectable in 75% of Type I fibres, compared with 28% of Type II fibres. These results confirm earlier studies with histochemical analyses of glycogen degradation during exercise which show that glycogen depletion initially occurs in Type I fibres, and then gradually increases, first in Type IIa fibres and then in Type IIb fibres (Gollnick et al., 1973; Essen, 1978; Thomson et al., 1979; Vollestad and Blom, 1985).

More recently, samples of single muscle fibres from the m. vastus lateralis were analysed before and after exhaustive running at 70% \( \text{VO}_2 \text{ max} \) (Tsintzas et al., 1996). Glycogen concentrations in Type I fibres decreased from 317.0 ± 34.2 to 31.6 ± 10.3 mmol·kg\(^{-1}\) dry weight, and in Type II fibres from 443.4 ± 44.9 to 103.9 ± 29.2 mmol·kg\(^{-1}\) dry weight. Tsintzas et al. (1996) concluded that compromised CHO availability specifically in Type I fibres is associated with fatigue during prolonged, constant pace running.
As mentioned previously, muscle glycogen is the most important substrate during prolonged exercise. Its rate of utilisation is most rapid during the early part of exercise and is related to exercise intensity (Gollnick et al., 1974; Saltin and Karlsson, 1971). As muscle glycogen declines with continued exercise, blood glucose becomes more important as a CHO fuel source. Muscle glucose uptake can increase up to 20-40 times the resting level, depending upon the exercise intensity and duration (Hargreaves, 1990, 1991). During the latter stages of prolonged exercise, glucose delivery may become a rate-limiting factor as arterial blood glucose levels decline (Ahlborg et al., 1974; Ahlborg and Felig, 1982; Katz et al., 1991). Accompanying the increased muscle glucose uptake is an increase in liver glucose output; however, during the latter stages of prolonged exercise, when liver glycogen levels are low, gluconeogenesis is an important source of glucose (Felig and Wahren, 1975). Under such circumstances, liver glucose output may fall behind muscle glucose uptake, resulting in hypoglycaemia.

There is increasing evidence that lactate, derived from contracting and inactive muscle, is an important oxidative substrate for contracting skeletal (Stanley et al., 1986) and cardiac (Gertz et al., 1988) muscle, and gluconeogenic precursor for the liver (Wasserman et al., 1991a). Lactate is also a valuable metabolic intermediate, rather than simply being a waste product of anaerobic glycolysis (Brooks, 1986).

To summarise, the relative contribution of CHO and fat will be influenced by a number of factors. These include mainly exercise intensity and duration, preceding diet and substrate availability. Besides, training status, environment, age and gender can also influence the process of energy metabolism (Hargreaves, 1995).
2.2 DEPLETION OF GLYCOGEN AND DEHYDRATION DURING EXERCISE

Muscular fatigue is usually defined as a failure to maintain the required or expected force- or power-generating capacity (Vollestad and Sejersted, 1988; Enoka and Stuart, 1992). Numerous factors have been linked to fatigue resulting from prolonged, endurance exercise. These involve specific impairments within the muscle itself, including transmission of the neural stimulus to the muscle at the motor end plate and propagation of that stimulus throughout the muscle (Sjøgaard, 1987), disruption of calcium release and uptake within the sarcoplasmic reticulum (Fitts and Metzger, 1993), substrate depletion (Coggan and Coyle, 1991), and other various metabolic events that impair energy provision and muscle contraction at the periphery (Green, 1987).

As discussed previously, glycogen storage in skeletal muscle is a major limiting factor in prolonged performance, and accordingly, preservation of these stores during exercise is of great importance. Fatigue during such exercise is often associated with the depletion of glycogen in the contracting muscle, specifically in those fibres recruited during exercise (Hermansen et al., 1967; Vollestad et al., 1984). Thus, the depletion of the glycogen stores in the contracting muscle is regarded as a limiting factor during prolonged strenuous exercise (Ahlborg et al., 1967a). The critical role of CHO, in particular, muscle glycogen, during exercise above 70% \( \dot{V}O_2 \) max. has been demonstrated by patients who lack glycogen phosphorylase (McArdle's disease). These patients are unable to utilise muscle glycogen and, thus, have maximal exercise capacities that are only about 50% of expected normal value (Lewis and Haller, 1986).

Glycogen depletion is reduced during prolonged exercise in trained, compared with untrained, individuals working at the same absolute rate: that is, at the same rate of \( O_2 \) consumption (Fitts et al., 1975), although the rate of glycogen depletion may be similar if the subjects are exercising at the same relative intensity, or the same percentage of \( \dot{V}O_2 \) max (Hermansen et al., 1967; Henriksson, 1991). In contrast, Jansson and Kaijser (1987) demonstrated reduced glycogen utilisation in the muscles of endurance-trained subjects when exercise was performed at the same relative exercise intensity of 65% \( \dot{V}O_2 \) max. These authors, and also Green et al. (1990), found that training exerts its greatest effect in reducing glycogen degradation early in exercise. Green et al. (1990) were also able to show that this effect occurs in both fast- and slow-twitch fibres. The observation that the trained individual can work
longer supports the hypothesis that depletion of body CHO stores is not only correlated with, but is causative of, muscular fatigue during endurance activity.

The close relationship between muscle glycogen depletion and fatigue appears to be due to the inability of glycogen-depleted muscle cells to maintain a sufficient rate of ATP resynthesis (Hargreaves et al., 1992). Another possibility is that a certain level of muscle glycogen metabolism is required for either the optimal production of NADH and electron transport or the maintenance of fat oxidation; perhaps intermediates of the Krebs cycle become limiting without adequate glycogen metabolism. Support for this hypothesis comes from the observation of Sahlin et al. (1990) who found the sum of measured tricarboxylic acid cycle intermediates to first increase and then decline following 40 min exhaustive bicycle exercise.

Recently, it has been suggested that fatigue can also result from alterations within the central nervous system (CNS). This central fatigue theory suggests that increased brain serotonin (5-hydroxytryptamine, or 5-HT) can impair the CNS function during prolonged exercise and, thereby, cause a deterioration in exercise performance (Newsholme et al., 1987), and influence running time to exhaustion (Bailey et al., 1993a, 1993b). Increased brain serotonin synthesis occurs in response to an increased delivery to the brain of blood-borne tryptophan, an amino acid precursor to serotonin. Serotonin is involved in the control of sleep, food intake, mood, pain sensitivity and pituitary hormone release (Newsholme and Leech, 1983). Most of the plasma tryptophan circulates loosely bound to albumin, but is displaced by increasing plasma concentrations of FFA and large neutral amino acids, such as glutamine (Curzon et al., 1973; Newsholme and Leech, 1983). Enhanced competition for albumin binding sites during prolonged exercise results in elevated plasma free tryptophan concentrations. This free tryptophan is then transported across the blood brain barrier. This transport initiated by a specific mechanism that tryptophan shares with the branched-chain amino acids (BCAA). Thus, brain serotonin will increase when there is an increase in the ratio of the concentration of the free tryptophan in blood plasma to the BCAA, i.e. free tryptophan/BCAA rises (Chaouloff et al., 1986). Elevated brain serotonin in specific areas of the brain are speculated as playing a role in the onset of fatigue (Newsholme et al., 1991; McAndrew and Newsholme, 1990).
Apart from the depletion of muscle glycogen, dehydration is another major physiological challenge during prolonged exercise. At rest, the rate of heat production by the body is low, but during exercise, at least 75% of the energy used is released as heat. The evaporation of sweat is the most important mechanism of dissipating this heat and preserving the body's thermal homeostasis. However, the progressive storage of heat within the body will dramatically reduce the capacity for exercise, and in severe cases precipitate physical collapse (Pugh et al., 1967), or heat stroke (Wyndham, 1977).

Sweat rate during exercise is dependent on a number of factors, including the size of the individuals and their degree of acclimatisation, the environmental conditions, the clothing worn, and the intensity of physical activity (Molnar et al., 1946; Adolph, 1947; Strydom et al., 1966; Shapiro et al., 1982). Actual sweat rates may vary greatly and, in some sporting situations, may exceed 1 l·h⁻¹. The highest sweat rate reported in the literature is 3.7 l·h⁻¹, which was measured for Alberto Salazar during the 1984 Olympic marathon (Armstrong et al., 1986).

During physical exercise, the major problem is how to closely match the volume of fluid intake to the volume of sweat loss. However, this is a difficult task to achieve because thirst itself is not a good indicator of body water requirements (Engell et al., 1987). Several classical studies (Pitts et al., 1944; Rothstein et al., 1947; Sohar et al., 1962) have reported that, when working in desert heat, men did not voluntarily replace all of the water losses incurred due to sweating. Recent studies (Phillips et al., 1984; Carter and Gisolfi, 1989) also report the same observation that ad libitum drinking results in incomplete fluid replacement during exercise. This phenomenon has been called "voluntary dehydration" (Rothstein et al., 1947), but it is now usually referred to as "involuntary dehydration" because the dehydrated individual has no volition to rehydrate (Nadel et al., 1990).

The physiological effects of exercise-induced dehydration have been studied by comparing the physiological responses of athletes when they replace either none, some, or all of their fluid lost during prolonged exercise. Plasma volume falls at the beginning of exercise. This fall is influenced by the type and intensity of exercise, and by the posture adopted (Coyle and Hamilton, 1990). Thereafter, a progressive exercise-related fall in plasma volume is reduced in proportion to the amount of fluid ingested during exercise (Barr et al., 1991; Candas et al., 1988; Maughan et al., 1987; Montain and Coyle, 1992a, 1992b). The change is least when most fluid is ingested (Montain and Coyle, 1992b) and can be prevented if the rate of fluid ingestion equals the rate of fluid loss (Hamilton et al., 1991).
Serum osmolality rises if no fluid is ingested during prolonged exercise (Candas et al., 1988; Maughan et al., 1987, 1989). Conversely, this rise is reduced by fluid ingestion (Candas et al., 1988; Maughan et al., 1987) and is least when the rate of fluid ingestion matches the rate of fluid loss (Montain and Coyle, 1992b). Serum sodium concentrations also rise with exercise-induced dehydration but are maintained when fluid is ingested (Noakes, 1991, 1992).

Some studies have shown that the sweat rate falls with increasing levels of dehydration (Ladell, 1955; Ekblom et al., 1970; Greenleaf and Castle, 1971; Strydom et al., 1975), whereas others have not found this effect (Gisolfi and Copping, 1974; Hamilton et al., 1991; Montain and Coyle, 1992a, 1992b). An early study found that sweat rate falls only above a certain level of dehydration (Ladell, 1955).

Prolonged exercise causes "cardiovascular drift", characterised by a progressive increase in heart rate and decrease in stroke volume throughout exercise (Rowell, 1974, 1986). The first factor which most likely contributes to the progressive cardiovascular drift during prolonged exercise is the redistribution of blood volume from the central circulation to the periphery (Rowell, 1986). Reduction of blood volume that occurs when sweat losses are not replaced may be the other factor. Increase in body temperature can also contribute to part of the increase in heart rate during prolonged exercise (Rubin, 1987). Fluid ingestion, however, can attenuate cardiovascular drift (Coyle and Montain, 1993).

Hamilton et al. (1991) required endurance-trained cyclists to ingest no fluid or sufficient fluid to fully replace all body water losses during 2 h of exercise at 70 - 76% \( \text{VO}_2 \) max. When no fluid was ingested during exercise, cardiovascular drift developed as stroke volume declined 15%, heart rate increased 10%, and cardiac output declined 7%. Fluid replacement totally prevented the decline in stroke volume, but heart rate still increased by 5%, leading to a 7% increase in cardiac output. The authors concluded that fluid replacement at a rate which prevented dehydration also prevented the decline in stroke volume and allowed heart rate to increase only in proportion to the 6% increase in oxygen uptake.
The exercise-related rise in rectal temperature is also attenuated by fluid ingestion during exercise (Costill et al., 1970; Hamilton et al., 1991; Montain and Coyle, 1992a, 1992b). For each 1.0% decrease in body weight, there is an increase in core temperature of between 0 and 0.1°C (Ekblom et al., 1970; Greenleaf and Castle, 1971; Sawka et al., 1985). The rise is reduced in proportion to the amount of fluid ingested and is least when the rate of fluid ingestion approximates to the sweat rate (Greenleaf and Castle, 1971; Montain and Coyle, 1992b). Fluid replacement reduces the rectal temperature response only after a minimum of 60 - 80 min of exercise (Strydom and Holdsworth, 1968; Barr et al., 1991; Montain and Coyle, 1992a, 1992b). This effect is mediated by maintaining a higher skin blood flow during the second hour of exercise (Montain and Coyle, 1992a). No effect of fluid replacement on rectal temperature has been found in a study where exercise was of shorter duration but higher intensity (Walsh et al., 1994), possibly because exercise terminated before the completion of 80 min (Noakes, 1993).

Montain and Coyle (1992b) also found that the magnitude of hyperthermia and cardiovascular drift during 2 h of moderately intense exercise in a thermally stressful environment was directly related to the magnitude of dehydration which occurred during exercise. These authors suggested that the optimal rate of fluid replacement to attenuate hyperthermia and cardiovascular drift during prolonged exercise is the rate that most closely matches sweat loss, at least up to 80% fluid replacement (Coyle and Montain, 1993).

Plasma concentrations of the fluid- and electrolyte-regulating hormones, specifically, antidiuretic hormone (ADH: also arginine vasopressin), aldosterone, and renin, increase during prolonged exercise (Brandenberger et al., 1989; Altenkirch et al., 1990; Freund et al., 1990). Fluid ingestion during exercise reduces the hormonal activities and concentrations; concentrations are believed to be further reduced when subjects hyperhydrate before exercise (Brandenberger et al., 1989).

Dehydration, beginning with as little as 1% decrease in body weight (Ekblom et al., 1970), is considered to be an important factor impairing exercise performance (Buskirk et al., 1958; Craig and Cummings, 1966; Wyndham and Strydom, 1969; Olsen and Saltin, 1976; Armstrong et al., 1985a). However, this conclusion has been drawn mainly from the results of hypohydration studies in which a body water deficit was induced before the onset of exercise by the use of saunas (Caldwell et al., 1984; Webster et al., 1988), diuretics (Claremont et al., 1976; Nielsen et al., 1981; Armstrong et al., 1985a), or heat (Buskirk et al., 1958; Craig and Cummings, 1966; Candas et al., 1988). Although these experimental models may be appropriate to
examine the effects of pre-exercise dehydration on subsequent exercise performance, they may be less relevant for activities in which dehydration develops during exercise.

This difference is noteworthy because hypohydration before exercise markedly decreases plasma volume, sweat rate, and stroke volume during exercise, contributing to an increase in serum osmolality, heart rate, and body heat storage (Nadel et al., 1980; Fortney et al., 1981, 1988; Sawka et al., 1985). Conversely, these physiological changes occur to a lesser extent when the dehydration develops during exercise (Coyle and Hamilton, 1990; Noakes, 1993).

Armstrong et al. (1985a) demonstrated that dehydration by 2% of body weight using a diuretic drug (furosemide) reduced running performance in 1,500, 5,000, and 10,000 m events by averages of 3.1, 6.7, and 6.3%, respectively. It means that running performance was reduced by dehydration to a greater extent in the longer (5,000 and 10,000 m) than in the shorter (1,500 m) race. These investigators also speculated that hyperthermia may have provided the physiological challenges that caused greater performance decrements in the longer races. In addition, the greatest decrements in exercise performance occur during prolonged exercise in hot environments at high levels of dehydration, even though low levels of dehydration (< 2%) can also have a detrimental effect on performance (Sawka, 1985; Walsh et al., 1994).

The impairing effects of inadequate fluid intake during endurance exercise were also observed by Barr et al. (1991). In this particular study, eight subjects attempted to perform three 6 h cycle bouts at 55% \( \dot{V}O_2 \text{max} \) at 30°C and 50% relative humidity. In two of the trials, subjects ingested either water or saline at rates designed to balance sweat and urinary fluid losses, whereas in the other trial, no fluid was taken. Seven of the eight subjects completed 6 h of cycling with fluid or saline ingestion. However, subjects terminated the ride after ~4.5 h when no fluid was ingested, having lost 6.4% of body mass on average.

High intensity (90% \( \dot{V}O_2 \text{peak} \)) cycling time to exhaustion was also impaired even at low (1.8%) levels of dehydration (Walsh et al., 1994). These investigators had six subjects ride for 60 min at 70% \( \dot{V}O_2 \text{peak} \) and then to exhaustion at 90% \( \dot{V}O_2 \text{peak} \) at 32°C and 60% relative humidity on two occasions. In one trial, subjects ingested fluid before and during the exercise, whereas no fluid was given in the other trial. Weight loss was significantly reduced and high intensity cycling time to exhaustion was increased when fluid was ingested during exercise.
In summary, it appears that dehydration as low as 2% loss of body mass has the capacity to adversely influence physiological responses and exercise performance. Prolonged exercise that primarily requires aerobic metabolism is most likely to be adversely affected by dehydration. Fluid ingestion during prolonged exercise reduces hyperthermia by attenuating changes in some physiological variables associated with dehydration, thus enabling the maintenance of a high skin blood flow (Montain and Coyle, 1992a, 1992b).

2.3 MUSCLE GLYCOGEN RESYNTHESIS AFTER EXERCISE

It has been stated in the previous sections that prolonged endurance exercise can significantly reduce muscle glycogen stores (Coyle et al., 1986; Coggan and Coyle, 1987). The rapid replenishment of body CHO reserves during the hours immediately after exercise is critical to the athlete undertaking multiple activities in a single day and for the daily restoration of those CHO stores. When muscle glycogen reserves are depleted, ingested glucose escapes the splanchnic bed and contributes to muscle glycogen resynthesis rather than to liver glycogen resynthesis (Mæhlum et al., 1977b; Krzentowski et al., 1982; Ferrannini et al., 1985). Therefore, muscle glycogen resynthesis apparently predominates over liver glycogen resynthesis during recovery from exercise. Recently, the muscle glycogen resynthesis during the post-exercise recovery period has been examined in detail (Costill et al., 1981; Blom et al., 1987; Blom, 1989; Ivy et al., 1988a, 1988b; Reed et al., 1989). The results from these studies suggest that the amount, type, and timing of CHO consumption, as well as other factors can all influence muscle glycogen resynthesis after exercise. In the following section, those factors affecting this recovery process during the short-term post-exercise period will be briefly discussed.

2.3.1 Level of Glycogen Depletion

After exercise, the rate of glycogen recovery is thought to depend on the level of the depletion (Mæhlum et al., 1977; Mæhlum and Hermansen, 1978; Fell et al., 1982; Ivy, 1991). The activity of glycogen synthase has been shown to be greater when glycogen is very low (Danforth, 1965; Mæhlum et al., 1977; Mæhlum and Hermansen, 1978; Bak and Pedersen, 1990) and the rate of glucose transport into an exercised muscle is greater under these conditions (Fell et al., 1982). It is possible that glycogen
synthase activity and glucose transport may interact to control the rate of glycogen resynthesis after glycogen-depleting exercise (Ivy, 1991).

Zachwieja et al. (1991) have demonstrated that the rate of muscle glycogen storage is greater in the early hours of post-exercise recovery when the glycogen is low than when it is only lightly depressed. In this study, six cyclists completed a protocol involving both one- and two-legged cycling which resulted in different degrees of glycogen reduction. Following exercise, equal amounts of CHO were ingested over 6 h, biopsies taken during the recovery demonstrated that the rate of muscle glycogen resynthesis was greater in the leg which had the greater reduction in glycogen. The investigators concluded that the glycogenic drive within the muscle is one of the main factors which influences its ability to resynthesise glycogen during post-exercise recovery.

2.3.2 Amount of Carbohydrate

The rate of muscle glycogen resynthesis is linear during the first 6 h after glycogen-reducing exercise (Blom et al., 1987). Thus, most studies have used this, or a shorter time frame, to determine the effects of different amounts, timing, and types of CHO ingested on the rate of muscle glycogen resynthesis. When no CHO is consumed after exercise, very little glycogen resynthesis occurs (MacDougall et al., 1977; Ivy et al., 1988a). Blom et al. (1980) published the first results that compared rates of post-exercise glycogen resynthesis when consuming different amounts of oral CHO following cycling to exhaustion at 75% \( \dot{V}O_2 \) max. Subjects ingested liquid glucose at a rate of 0.35, 0.7, and 1.0 g·kg\(^{-1}\)·h\(^{-1}\). Glycogen resynthesis rates during 8 h of recovery did not differ between the 0.7 and 1.0 g trials but were both significantly different from the 0.35 g trial.

Similar findings were reported by Blom et al. (1987) and Ivy et al. (1988b). These investigators have described a ceiling for CHO intake above which no further glycogen storage is stimulated. From their studies, it appears that providing 0.7, 1.4, 1.5 or 3.0 g glucose·kg\(^{-1}\) body weight at 2 h intervals results in a similar rate of glycogen resynthesis during 4 h of recovery. This plateau in glycogen resynthesis does not appear to simply be due to an accumulation of CHO in the GI tract because Reed et al. (1989) demonstrated that consumption of either solid or liquid CHO (1.5 g
CHO-kg\(^{-1}\) body weight ingested immediately and at 2 h intervals after exercise) produced similar rates of muscle glycogen resynthesis. In contrast, intravenous glucose infusion at \(\sim 100\) g every 2 h failed to increase muscle glycogen resynthesis above 7 - 8 mmol-kg\(^{-1}\)-h\(^{-1}\).

Based on the results reported, it appears that muscle glycogen resynthesis is near optimal (5 - 7 mmol-kg\(^{-1}\)-h\(^{-1}\)) when 0.7 - 1.5 g-kg\(^{-1}\) body weight of CHO, or \(\geq 50\) g of CHO, is consumed every 2 h in the early stages of recovery (Coyle, 1992, 1995). However, it is noteworthy that the CHO feeding rates used by Blom et al. (1980, 1987) and Ivy et al. (1988a, 1988b) provided in excess of 400 g of CHO during a 4 h period. Ivy et al. (1988b) revealed that, based on a 10 kg muscle mass, between 25 and 35\% of the ingested CHO was converted to muscle glycogen. This means that the larger the quantity of ingested CHO, the smaller the glycogen conversion percentage. Consequently, the majority of ingested glucose after exercise is not involved in skeletal muscle glycogen resynthesis. It remains unclear whether this low efficiency is due to a limitation of the gastric emptying, the peripheral utilisation or a limitation of supply, such as that induced by restricted blood flow and muscle perfusion.

However, in a recent study by Doyle et al. (1993), it was demonstrated that the rate of glycogen synthesis might be even further increased when 0.4 g CHO-kg\(^{-1}\) body weight is consumed beginning immediately after exercise at every 15 min for 4 h. That study demonstrated on two different occasions in the same subjects, the rate of muscle glycogen synthesis averaged 8 - 10 mmol-kg\(^{-1}\)-h\(^{-1}\). This feeding schedule produced a much larger and more persistent elevation of blood insulin than did the other feeding schedules.

2.3.3 Timing of Carbohydrate

The pattern of muscle glycogen resynthesis following exercise-induced depletion has found to be bi-phasic (Adolfson and Ahren, 1971; Garetto et al., 1984; Ivy, 1991). The rapid phase is characterised by the fast replenishment of glycogen stores occurring over the first 24 h of recovery, but particularly during the first few hours. It is followed by the slow phase in which muscle glycogen increases very gradually above the pre-exercise level over the next few days, dependent upon CHO consumption and
physical activity pattern. Apart from the increased glycogen synthase activity (Danforth, 1965; Adolfson, 1973), the early hours of glycogen resynthesis are also driven by an increased sarcolemmal permeability to glucose with an increased number of GLUT 4 transporters being recruited to the cell membrane (Ivy and Holloszy, 1981; Fell et al., 1982; Richter et al., 1985; Ivy, 1987) and an increase in the muscle's sensitivity to insulin (Richter et al., 1982, 1984).

In order to examine the influence of the timing of post-exercise CHO ingestion on glycogen resynthesis, Mæhlum et al. (1978a) provided liquid solutions of 1.4 g glucose-kg\(^{-1}\)body weight-h\(^{-1}\) at either 15 min or 14 h post-exercise. Although the focus of the study was on long-term glycogen resynthesis, the results indicated that the rate of muscle glycogen resynthesis was higher when the CHO was ingested 15 min post-exercise rather than 14 h post-exercise.

In another study which focused on the rate of glycogen resynthesis during the more immediate hours of recovery, Ivy et al. (1988a) fed subjects with liquid CHO solution (70% maltodextrin, 15% glucose, and 15% sucrose) of 1.0 g·kg\(^{-1}\)body weight·h\(^{-1}\) immediately after exercise, or at 2 h post-exercise. When CHO ingestion was delayed, the rate of glycogen resynthesis was 47% slower than if CHO was ingested immediately after exercise. Glycogen synthase activity was not different between treatments and could not account for the differences in resynthesis rate. Thus, it is likely that glucose transport was accelerated during the initial 2 h after exercise, accounting for the resynthesis rate (Friedman et al., 1991).

Although no particular studies were undertaken to compare the differences in the rates of glycogen resynthesis between bolus and serial CHO feedings during recovery, feeding schedules employed in various studies investigating muscle glycogen resynthesis can provide some understanding in this area. The work of Ivy et al. (1988a, 1988b), Blom et al. (1980, 1987), and Reed et al. (1989) involved bolus feedings of CHO at 0, 2, and/or 4 h post-exercise. These feeding schedules resulted in a rate of muscle glycogen resynthesis which averaged 5 - 7 mmol·kg\(^{-1}\)·h\(^{-1}\), whereas Zachwieja et al. (1991) and Doyle et al. (1993) provided serial feeding (every 20 and 15 min, respectively) and reported a glycogen resynthesis rate approximating 10 mmol·kg\(^{-1}\)·h\(^{-1}\). Based on these results, it appears that a more frequent CHO feeding schedule that maintains more stable
elevations in blood glucose and insulin concentration after glycogen-reducing exercise may produce a more consistent stimulus for glycogen resynthesis (Villar-Palasi and Larner, 1961; Roch-Norlund et al., 1972).

Recently, the effects of CHO ingestion during exercise on muscle glycogen resynthesis after exercise have been studied. Zachwieja et al. (1993) had 8 subjects pedal for 2 h on a cycle ergometer at 70% \( \dot{V}O_2 \) max while consuming either a 10% CHO solution (CHO) or a placebo (P). During both trials, food intake was withheld for the first 2 h of recovery, but at 2 h post-exercise, a 24% CHO solution was ingested. The rate of muscle glycogen resynthesis during the first 2 h of recovery was similar for the CHO and P trials. Following ingestion of the 24% CHO supplement, the rates of muscle glycogen resynthesis also increased similarly in both trials. These investigators concluded that CHO feedings taken during exercise had little effect on post-exercise muscle glycogen resynthesis.

2.3.4 Type of Carbohydrate

In planning CHO replacement after exercise, the glycemic index of a food should be considered (Roberts et al., 1988; Hargreaves, 1991; Coyle, 1991, 1992; Burke et al., 1993). The glycemic index concept has been developed to define CHO foods according to their actual postprandial glycemic impact (Jenkins et al., 1981). This is measured by monitoring the changes in blood glucose following the ingestion of 50 g of CHO from the food. However, research into the effect of different CHO foods on glycogen storage has taken a simplistic approach to CHO nutrition, dividing foods into "simple" or "complex" types of CHO on the basis of their chemical composition (Costill et al., 1981; Kiens et al., 1990), without considering the glycemic responses to their ingestion. Consumption of simple CHO foods is expected to elicit a large, rapid, and short-lived rise in blood glucose, whereas the response to complex CHO foods will be flatter and more sustained (Burke et al., 1993). In fact, this simplistic model is quite incorrect and these terms have been inconsistently defined in the scientific literature. Frail and Burke (1994) indicated that "simple" and "complex" classification of CHO foods are not synonymous with high glycemic index and low glycemic index because many complex CHO foods have a high glycemic index (e.g. bread,
potatoes) while some simple sugars (e.g. fructose) present a low glycemic index. Thus, a clearer classification of CHO foods is needed in order to avoid the confusion in interpreting the resultant metabolic and physiological responses (Frail and Burke, 1994).

Different types of CHO and CHO foods appear to have different effects in promoting the resynthesis of muscle glycogen after exercise. As mentioned previously, the rate of glycogen resynthesis after exercise and the ingestion of glucose, or food with a high glycemic index, is 5 - 7 mmol·kg⁻¹·h⁻¹ (Blom et al., 1987; Ivy et al., 1988b; Reed et al., 1989). However, when fructose is ingested alone, the rate of muscle glycogen resynthesis is only 3 mmol·kg⁻¹·h⁻¹ because of its low glycemic index (20 - 30% of that of glucose) (Jenkins et al., 1984; Blom et al., 1987). Fructose may be a better CHO source to replenish liver glycogen than glucose. Nilson and Hultman (1974) infused or fed glucose or infused fructose in the post-absorptive state. The rate of liver glycogen synthesis was similar for infused and ingested glucose; however, fructose infusion increased liver glycogen synthesis 3.7 fold above that for glucose. The greater rate of liver glycogen synthesis from fructose compared with that from glucose may be due to a higher liver fructose kinase activity than glucose kinase activity (Heinz, 1972; Newsholme and Start, 1973). Nevertheless, in comparing fructose with glucose ingestion, more recent research in both humans and rats suggests that fructose is less effective in promoting a rapid post-exercise muscle glycogen resynthesis (Conlee et al., 1982; Blom et al., 1987).

Blom et al. (1987) also demonstrated that the rate of muscle glycogen resynthesis was similar for feedings of glucose or sucrose, in spite of the fact that the glycemic index of sucrose is 60 - 70% of that of glucose (Jenkins et al., 1984; Blom et al., 1987). Because sucrose is broken down into equimolar amounts of glucose and fructose, it is less clear why the rate of muscle glycogen resynthesis would be equivalent to that of glucose. However, blood glucose and insulin responses were similar for the glucose and sucrose feedings, therefore, a possible explanation is that the fructose released from the breakdown of sucrose depressed hepatic glucose uptake, which allowed glucose to bypass the liver for muscle uptake and synthesis to glycogen (Coyle, 1991).
Kiens et al. (1990) investigated post-exercise muscle glycogen resynthesis following the ingestion of simple and complex CHO diets that differed in glycemic index. Subjects exercised until muscle glycogen stores were exhausted, and were then fed either a complex or simple isocaloric diet. Both diets provided 70% of energy from CHO. Results indicated that the simple CHO diet was accompanied by a 98% higher blood insulin concentration, despite similar blood glucose concentrations. After 6 h of recovery, muscle glycogen resynthesis during the simple CHO trial had occurred at almost double the rate of the complex CHO trial. However, there was no difference in glycogen resynthesis between the simple and complex CHO diet 20, 32, or 44 h after exhaustive exercise.

More recently, Burke et al. (1993) demonstrated that the most rapid increase in muscle glycogen content during the first 24 h of recovery was achieved by consuming food with a high glycemic index. Five cyclists undertook an exercise trial to deplete muscle glycogen (2 h at 75% \( \dot{V}O_2 \) max followed by four 30-s sprints) on two occasions. For 24 h after each trial, subjects consumed a high CHO diet, with one trial providing foods with a high glycemic index, and the other providing foods with low glycemic index. Muscle biopsy results showed that intake of high glycemic index CHO foods after prolonged exercise produced significantly greater glycogen storage than consumption of low glycemic index CHO foods.

**2.3.5 Influence of Other Nutrients**

The digestible CHO content of liquid and/or solid glucose feedings can consist of either glucose, fructose, galactose, sucrose, starch, or a synthetic multidextran polymer. Furthermore, the glycemic index of a feeding containing these CHO structures can vary due to the delaying influence of fats and proteins on gastric emptying, and by increased gastric motility from dietary fibre (Hunt et al., 1985). The combination of these CHO and other nutrients may, therefore, influence the process of glycogen resynthesis. However, the effects of the co-ingestion of other nutrients on glycogen resynthesis have not been well studied.
Zawadzki et al. (1992) investigated the effect of CHO and protein ingestion on muscle glycogen resynthesis after exhaustive exercise. These researchers reported that co-ingestion of protein (40.7 g) increased the rate of glycogen storage during 4 h of recovery in glycogen-depleted subjects who consumed a CHO supplement (112 g) immediately after exercise and again 2 h later. It was concluded that an increased plasma insulin response to the CHO-protein supplementation enhanced the glycogen resynthesis. However, when the composition of the supplements received by the subjects was scrutinised, it revealed that the three supplements (CHO, Protein, and CHO and Protein) ingested were not isocaloric and this may have confounded the results.

Recently, Burke et al. (1995) demonstrated that co-ingestion of moderate amounts of fat and protein does not appear to have a direct effect on muscle glycogen storage during 24 h of recovery from prolonged exercise, provided CHO intake is adequate. However, consumption of large amounts of protein and fat may displace CHO foods within the athlete's energy requirements of eating comfort, and may thus indirectly interfere with glycogen storage by causing inadequate total CHO intake (Burke et al., 1995).

There are other substances which may also contribute towards muscle glycogen resynthesis either directly or indirectly via hepatic conversion, e.g. glycerol tricarboxylic acid intermediates and amino acids. There has been much interest lately in the role of amino acids in glycogen resynthesis and glutamine is one of those which has received much attention. Glutamine is the most abundant free amino acid in the circulation and can stimulate hepatic glycogen synthesis (Katz et al., 1976). Skeletal muscle is a major source of plasma glutamine at rest (Ruderman, 1975) and skeletal muscle output of glutamine represents active synthesis since stored concentrations of muscle glutamine are too low to account for the output of glutamine alone. During moderate intensity exercise, ammoniagenesis increases and muscle glutamine output increases with increasing workload (Babij et al., 1983; Henriksson, 1991), but during high intensity exercise, or during prolonged exercise, plasma glutamine concentrations will eventually decrease ( Henriksson, 1991). This is presumably partly because precursors (i.e. muscle glutamine) become depleted (Wagenmakers et al., 1991), and partly because of
increased uptake by other organs, such as the intestine and liver (Wasserman et al., 1991b).

Leese (1994) investigated the effects of infused glutamine on muscle glycogen resynthesis after exhaustive cycling exercise. In this study, three study groups each comprising 6 subjects exercised for 90 min at 70% $\dot{V}O_2$ max. Subjects were then randomly allocated to receive a primed constant infusion of glutamine (50 mg·kg$^{-1}$·h$^{-1}$), alanine and glycine (30.5 and 25.7 mg·kg$^{-1}$·h$^{-1}$, respectively) or 0.9% w/v saline over 2 h. Subjects who received glutamine or saline were also infused with a primed constant infusion of glucose (10 mg·kg$^{-1}$·h$^{-1}$). During the glutamine infusion, plasma glutamine and muscle glutamine concentrations increased (from $590 \pm 38$ to $967 \pm 23$ μmol·l$^{-1}$ and from $11.8 \pm 1.7$ to $13.8 \pm 0.5$ mmol·kg$^{-1}$ respectively). Muscle glycogen concentrations increased more during the glutamine infusion than during saline or alanine/glycine infusions. The authors concluded that intravenous glutamine infusion after exercise, sufficient to increase the concentration of muscle glutamine, promoted muscle glycogen resynthesis. Their data would also support previous work in rats which suggested that glutamine stimulates glycogen synthase (Mouterde et al., 1992; Scislowski et al., 1989).

2.3.6 Muscle Damage

It has been repeatedly demonstrated that post-exercise muscle glycogen resynthesis is impaired during the days following eccentric exercise (Sherman et al., 1983; Kuipers et al., 1985; O'Reilly et al., 1987; Costill et al, 1988b, 1990) which may be due to the ultra-structural damage interfering with glycogen resynthesis and the increased competition between the inflammatory and muscle cells for blood glucose (Hikida et al., 1983; Shearer et al., 1988; Forster et al., 1989). However, it has also been suggested that rest and daily low intensity exercise during the week after the marathon may have constituted an insufficient stimulus for glycogen resynthesis. This is supported by the low activation state of glycogen synthase on the days after the marathon (Sherman et al., 1983). Nevertheless, no study has elucidated the specific mechanisms by which damage from eccentric contractions affects muscle glycogen resynthesis in humans. Research using animal models has clearly demonstrated that eccentric exercise or forced muscle lengthening results in muscle damage.
and impaired glycogen synthesis (Armstrong et al., 1983). However, due to the extreme efforts used to invoke damage in these models, the results may have limited applications to human athletes.

Another concern that should be considered when evaluating studies of muscle glycogen levels after eccentric exercise is the reduced rate of glycogen resynthesis in regions previously traumatised by repeated muscle biopsy sampling (Costill et al., 1988b; Constantin-Teodosiu et al., 1996). Because the biopsy sampling is traumatic, it is possible that the biopsy itself may induce inflammation similar to that resulting from eccentric exercise and, if the muscle biopsies during eccentric studies are not obtained from alternative legs or from a site some distance away from the previous biopsy, there may be an effect of the biopsy itself on muscle glycogen (Robertson, 1991). In fact, Costill et al. (1988b) suggested that ".... alterations in muscle glycogen storage persists for 10 days after the first biopsy, suggesting that care must be taken in selecting the site for repeated biopsies from the same muscle".

The influence of muscle damage induced by different types of exercise on post-exercise muscle glycogen resynthesis during the initial hours of recovery has not been widely researched. However, Doyle et al. (1993) still observed evidence consistent with the hypothesis that muscle damage may adversely affect muscle glycogen resynthesis. They compared the rate of muscle glycogen resynthesis during initial hours of recovery following concentric or eccentric exercise 2 and 48 h earlier. The rate of post-exercise glycogen resynthesis was not different between concentric and eccentric exercise during the first 4 h of recovery. However, glycogen replenishment was 25% lower in muscle that had been subjected to eccentric exercise 48 h earlier than in concentrically exercised muscle.

2.3.7 Methods of Recovery

Although the topic of passive or active recovery has been extensively studied, an evaluation of their effects on muscle glycogen resynthesis after prolonged, exhaustive exercise has been limited. Continuing exercise during the recovery period from prior exercise, at intensities requiring CHO oxidation, may jeopardise the process of rapid glycogen resynthesis. Bonen et al. (1985) compared between post-exercise glycogen resynthesis
with or without an active recovery following 30 min of cycling at 75% VO₂ max. Subjects ingested 1.5 g glucose-kg⁻¹ body weight 10 min after exercise and again 2 h later. During the total 4 h recovery period, the subjects either rested one leg or they exercised one leg to elicit an energy expenditure equivalent to 20% VO₂ max. With an active recovery, muscle glycogen was further degraded, yet during the first 2 h of a passive recovery, the glycogen resynthesis occurred at a rate of 5 mmol·kg⁻¹ wet weight·h⁻¹. Thus, it appears that even low-intensity exercise after a prolonged exercise trial could impair the recovery of muscle glycogen levels, perhaps by maintaining elevated blood epinephrine levels (Bonen et al., 1985).

2.4 REHYDRATION-RECOVERY AND SUBSEQUENT EXERCISE PERFORMANCE

Although many studies addressed the important attributes of rehydration with CHO solution after exercise to optimise fluid replacement and the rate of glycogen resynthesis, few studies have determined whether there is any relationship between the resultant fluid and muscle glycogen levels and the recovery of exercise performance, particularly in the endurance type of exercise.

Keizer et al. (1987) determined the relationship between glycogen repletion and short-term maximal physical working capacity (MPWC). 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 CHO-rich diets (i.e. ~590 g or 8.0 g·kg⁻¹ body weight·24 h⁻¹) were consumed. No differences existed between the rate of muscle glycogen resynthesis when fed either solid or liquid meals containing approximately 70% kcal from CHO during the first 5 h of recovery. However, despite almost complete restorage of muscle glycogen after 22 h of recovery in the liquid and solid CHO trials, the MPWC was significantly lower (~7%) after the recovery when compared to an identically administered MPWC prior to the initial glycogen depletion exercise. The reduced exercise capacity at normal glycogen levels supports the idea that factors other than muscle glycogen influence muscle function (Young and Davies, 1984).
Nielsen et al. (1986) required subjects to perform an exercise test (supramaximal work at 105% \( \dot{V}O_2 \) max) before dehydration and after rehydration. During a 2 h period after exercise-induced dehydration (3% of body weight), subjects ingested one of the four test drinks at 15 min intervals. The solutions were control, high potassium, high sodium or high sugar. The largest increase in plasma volume was found after the sodium-rich solution, while the potassium-rich solution produced the smallest and slowest increase in plasma volume. The work capacity during the supramaximal exercise test was reduced by 20% after 2 h rest and rehydration. The reduction was similar for, and independent of, the composition of all drinks. The investigators concluded that this reduction of the work capacity might be due to the decrease in the muscle glycogen stores.

The efficacy of rehydration with water following 24 h of dehydration on body fluid balance and rowing performance has also been examined (Burge et al., 1993). Eight international rowers performed a maximal trial on a rowing ergometer while euhydrated and following partial rehydration. Body weight was reduced using exercise together with food and fluid restriction over 24 h. It was followed by rehydration with 1.5 l of water over 2 h. Although rehydration restored plasma volume to 50% of the decreased value, maximal rowing performance was impaired due to a decrease in plasma volume and decreased muscle glycogen utilisation. These data demonstrated that low muscle glycogen and incomplete rehydration caused a significant decrease in the ability to sustain work at a high intensity.

Recently, the effects of ingestion pattern on partial rehydration and exercise performance subsequent to passive dehydration were investigated (Melin et al., 1994). Using the same protocol as in a previous experiment (Melin et al., 1990), this study is probably the only one in the scientific literature undertaken to compare the effects between bolus and serial feedings on prolonged exercise performance. After dehydration by passive heating (2.6% of body weight), the subjects rested for 1 h in a thermoneutral environment before they started the subsequent exercise which required them to march on a treadmill until exhaustion at 50% \( \dot{V}O_2 \) max. During the rest period, 50% of the fluid lost in the dehydration session was replaced by drinking mineral water given either in one drink immediately before the onset of exercise or divided into four equal portions before the exercise and on three occasions at 15 min intervals during exercise. Although no differences were found in exercise duration between trials, it was concluded that the swift replacement of the fluid loss in the dehydrated subjects was beneficial to exercise performance by rapidly correcting the disturbances in body fluid balance.
Using a 4 h recovery model, Fallowfield et al. (1995) examined the influence of ingesting a CHO-electrolyte (CE) solution following prolonged running, on subsequent exercise capacity. Sixteen subjects were divided into two matched groups which were randomly assigned to either a placebo (P) or CHO condition. Both groups ran at 70% $\text{VO}_2$ max on a treadmill for 90 min and at the same speed to exhaustion 4 h later to assess endurance capacity. The CHO group ingested a 6.9% CE solution providing 1.0 g CHO-kg$^{-1}$ body weight immediately after the 90 min run and again 2 h later, whereas the P group ingested an equal volume of a placebo solution. Fluid losses reduced body weight ~2.6% in both trials during the 90 min run. During the recovery, subjects ingested 1.98 and 2.06 kg of fluid in the P and CHO group and neither group restored pre-exercise body weight after the 4 h recovery. Although similar levels of rehydration were achieved in both groups (P: 65.9%; CHO: 62.6%), the CHO group ran 22.2 min longer than the P group during the subsequent endurance run. The investigators concluded that the difference in post-recovery performance appears to be related to CHO availability rather than inadequate rehydration. One interesting point to note in this particular study is that, similar to the findings of the previous studies (Costill and Sparks, 1973; Carter and Gisolfi, 1989; Gonzalez-Alonso et al., 1992; Lambert et al, 1992), subjects in both groups could not achieve rehydration after the recovery period, even though 116% and 109% of fluid loss were ingested in the P group and CHO group, respectively.

Using a 2 h rehydration-recovery protocol, 2 similar studies were undertaken by the same group of researchers to investigate the effect of a CHO-electrolyte solution (CES) on rehydration efficacy and subsequent cycling performance (Singh et al, 1996; Kovacs et al., 1996). In the first study, subjects exercised for 90 min in the heat until 3% of body weight was lost. Following exercise, subjects rested in a neutral environment for 30 min prior to beginning the 2 h rehydration period. During the rehydration period, subjects were fed the CES or a water placebo drink (P) as 120% of previous body mass loss at 0, 30, and 60 min, in 50%, 40%, and 30% boluses, respectively. After the rehydration, subjects were asked to perform a certain amount of work with no further fluid intake as fast as possible, which was equivalent to about 1 h of exercise. Rehydration with CES was higher than with P (70% vs. 60%). However, despite this more effective rehydration with CES, the performance results did not differ between trials. The authors speculated that the insulin-mediated rebound effect on the CHO metabolism during the performance, in which no further CHO was ingested, had been counterproductive to the rehydration benefit.
The protocol of the second study (Kovacs et al., 1996) was exactly the same as the first one just described. The only difference was that the subjects were allowed to ingest 2 ml·kg\(^{-1}\) of high CES (152.6 g CHO·L\(^{-1}\)) or P at 1 min into warm-up and on achieving 25% and 50% of the amount of work during the cycling performance. Similarly, rehydration with CES was higher than with P (71% vs. 63%). However, unlike the first study, higher power output (301 vs. 281 Watts) and faster performance time (61.2 vs. 65.7 min) were observed when subjects ingested the CES. The investigators attributed the improvement to the increased blood glucose concentrations and better fluid balance.

Recently, the possible interactions between the replacement of utilised substrate and the fluid lost during exercise was investigated (Below et al., 1995). In this study, subjects consumed either an electrolyte (Na\(^{+}\): 619 mg; K\(^{+}\) 141 mg) or CHO-electrolyte (CHO: 79 g; Na\(^{+}\): 619 mg; K\(^{+}\),141 mg) solution during a first 50 min exercise bout then immediately undertook a cycle ergometer performance test. They either received these in a large (1330 ml) or small (200 ml) volume. These researchers found that the fluid and CHO each improved performance independently and each by approximately 6%; performance times were 6.5% faster when the large beverage volume was consumed as opposed to the small volume and were 6.3% faster when CHO-containing beverages were consumed as opposed to the CHO-free beverages.

To summarise, based on the limited research, it appears that the replenishment of CHO stores during the short-term recovery is still the major determinant for the success of subsequent exercise. However, the relationship between rehydration and CHO ingestion during such a short recovery period remains unclear.

### 2.5 FACTORS INFLUENCING REHYDRATION AFTER EXERCISE

As discussed previously, dehydration during exercise is known to result in decreased plasma volume, increased plasma osmolality, decreased sweat rate and skin blood flow, and eventually raised core temperature (Harrison, 1985, 1986; Hamilton et al., 1991; Montain and Coyle, 1992a, 1992b). High sweat rates accompanying prolonged exercise may also compromise the body's electrolyte balance, as electrolytes, especially sodium (Na\(^{+}\)) and chloride (Cl), are lost with sweat. Thus, after prolonged exercise, rapid restoration of the body's water and electrolyte balance, as well as energy stores, are the major considerations of the recovery process, particularly when repeated bouts of exercise have to be performed. The effectiveness of the rehydration process is multifactorial but mainly influenced by the nature and composition of the
fluid, the physiological responses of ingestion, and the behavioral response to fluid replacement. Nevertheless, due to the specific needs of each individual under different circumstances, a single formulation of an "optimal" fluid replacement beverage which will serve all individuals in all situations is impossible (Gisolfi and Duchman, 1992; Gisolfi et al., 1995). In the following section, factors influencing fluid replacement after exercise will be discussed.

2.5.1 Gastric Emptying

The primary objective of fluid replacement after prolonged exercise is to restore the state of euhydration (Murray, 1987). The availability of ingested fluid and exogenous CHO depends mainly on the rates at which these fluids are emptied from the stomach into the small intestine together with the rate of intestinal absorption. Thus, gastric emptying is the rate-limiting step for the absorption of CHO in solution (Costill and Saltin, 1974). The contribution of different factors to the overall rate of gastric emptying is still unclear in detail; however, volume (Mitchell and Voss, 1991; Noakes et al., 1991; Mitchell et al., 1994), energy content and osmolality (Costill and Saltin, 1974; Foster et al., 1980; Brener et al., 1983; Hunt et al., 1992; Vist and Maughan, 1995), temperature (Fone et al., 1990), physical activity (Marzio et al., 1991; Gisolfi et al., 1992) are all known to influence the rate. In addition, dehydration also reduces the gastric emptying rate (Neufer et al., 1989a; Rehrer et al., 1990a) and increases the risk of GI distress (Rehrer et al., 1990a).

Most of the information about the rate of gastric emptying is derived from studies on resting subjects. However, due to the variation of the methodology used, there is much confusion and limitation as to the usefulness of many studies in the published literature. Most of the previous studies quoted have relied on gastric aspiration techniques in which the stomach contents are recovered at a fixed time point after fluid ingestion. It has been usual to report results as the volume remaining in the stomach after a fixed time period. However, use of scintigraphic techniques to assess emptying rates has demonstrated that the rate of gastric emptying of most solutions follows an exponential time course (Hunt and Spurrell, 1951; Leiper and Maughan, 1988; Rehrer et al., 1989; Vist and Maughan, 1995). That is, the volume of fluid emptied per unit of time is directly proportional to the volume present in the stomach. The
interpretation of the results is, therefore, strongly influenced by the time at which measurements are taken. This means that the results of a single time point measurement may be misleading because different investigators have chosen different time points of sampling following ingestion. These differences make the comparisons between studies extremely difficult. Although several investigators have used the calculated rate of gastric emptying in millilitres per minute in an attempt to avoid this confusion, their results are still strongly influenced by the time of sampling (Vist and Maughan, 1995).

There are other complicating factors; for instance, the gastric emptying rates of any drink, even when measured under strict laboratory conditions, can be extremely variable. Furthermore, gastric emptying can be influenced by one’s emotional state, menstrual cycle, environmental conditions, and a number of other factors beyond voluntary control (Murray, 1987). Recently, techniques using stable isotopes have been developed to measure gastric emptying. $^{13}$C acetate has been found to be useful in measuring gastric emptying of liquids (Meyer-Wyss et al., 1991) because it is not absorbed through the stomach but only in the small intestine via $\text{Na}^+, \text{K}^+$ and pH-independent mechanisms (Watson et al., 1991). Once ingested, it is rapidly metabolised with $^{13}$CO$_2$ production which is released in the breath. It has recently been shown that in the post-exercise state, the $^{13}$C acetate breath test can be used to differentiate the gastric emptying rates of water and CHO solutions of different properties (Leese et al., 1995).

The two main factors that stimulate gastric emptying are the nerve impulses that act in response to stomach distension (Brener et al., 1983), and the action of gut hormones (Minami and McCallum, 1984). The composition of the fluid ingested does, however, strongly influence gastric emptying rate. Receptors embedded in the gastric musculature, and in the walls of the duodenum and jejunum, are sensitive to changes in volume, osmolality, pH, fat and amino acid levels (Murray, 1987). However, the two primary determinants of the emptying of fluids from the stomach are volume (Nose et al., 1988a; Noakes et al., 1991; Mitchell and Voss, 1991; Mitchell et al., 1994) and the CHO content (Mitchell et al., 1988, 1989).
When a fluid is ingested, one determinant of gastric emptying is the influence of volume on intragastric pressure (Brouns et al., 1987). Receptors in the gastric musculature respond to the increasing gastric distension and pressure by increasing the rate of emptying (Hunt and MacDonald, 1954; Minami and McCallum, 1984). The exponential nature of the emptying curve indicates the crucial importance of the volume of the stomach contents in controlling the rate of emptying. As fluid is emptied and the stomach volume falls, so the rate of emptying is decreased. Therefore, volume is a powerful regulator of the rate of gastric emptying and maintaining a large volume in the stomach by repeated drinking will enhance the rate of emptying (Rehrer et al., 1990b; Noakes et al., 1991).

Recent studies have shown that even with forced fluid administration during relatively short post-exercise periods (2 - 4 h), complete rehydration is not achieved even though 100% of the fluid lost via sweating is ingested (Costill and Sparks, 1973; Nielsen et al., 1986; Gonzalez-Alonzo et al., 1992). In view of these unsuccessful rehydration regimens, Mitchell et al. (1994) increased the volume ingested and examined the effects of ingesting 100% versus 150% of fluid lost on rehydration during a 3 h period after exercise-induced dehydration. The final rehydration was 48.1% and 67.9% for the 100% and 150% trials, respectively. The gastric emptying results indicated that the cumulative volume of fluid emptied by the stomach was significantly greater in the 150% condition at each hourly measurement throughout the 3 h rehydration period.

In another study which also examined the effect of the volume of fluid intake on rehydration, Maughan and Shirreffs (1994) showed that positive fluid balance can be achieved after exercise-induced dehydration when the volume consumed is substantially greater than the loss (150 - 200% of lost). However, no study has been undertaken to investigate the influence of ingesting such a large volume on subsequent exercise performance.
Another important determinant of gastric emptying rate is the CHO content of the ingested fluid. In general, more concentrated solutions leave the stomach at a slower rate than less concentrated solutions, and as CHO content of an ingested fluid rises, the gastric emptying rate declines (Hunt and Knox, 1968; Barker et al., 1978; Foster et al., 1980). Some previous studies have indicated that the rate of gastric emptying is decreased if the CHO content of the drink exceeds 2.5% (Costill and Saltin, 1974; Foster et al., 1980), but more recent studies have demonstrated that emptying of solutions containing up to 10% glucose or glucose polymer is not delayed relative to water (Owen et al., 1986; Rehrer et al., 1989). Maughan and Leiper (1990) have examined the effect of increasing glucose concentration on the time course of gastric emptying. Their results clearly showed that the emptying rate is slowed in proportion to the glucose content and slows as the volume of fluid remaining in the stomach decreases. From these results, it appears that even a 5% glucose solution will delay gastric emptying. Although increasing the glucose content of the ingested fluid does slow the rate at which fluid leaves the stomach, it results in a faster delivery of glucose, in agreement with the findings of Hunt et al. (1985).

A study by Brener et al. (1983) measured the gastric emptying rates of 400 ml of 5%, 12.5% and 25% glucose solutions and isotonic saline. As expected, the gastric emptying rate was fastest for the isotonic saline solution and gastric emptying slowed as the glucose concentration of the ingested solution increased. However, when the gastric emptying was expressed as the rate at which calories were emptied from the stomach (in kcal·min⁻¹), the glucose solutions emptied at similar rates regardless of the concentration of the ingested solution. It was then suggested that the rate of energy delivery to the intestine is constant for all solutions used in this study. However, the non-linear nature of the time course of emptying makes it difficult to accept this interpretation (Maughan, 1991).

Previous research indicated that the osmolality of the ingested solution played a key role in determining the gastric emptying rate (Barker et al., 1978; Foster et al., 1980). An increasing osmolality of the ingested fluid has been suggested to delay gastric emptying. There is some evidence that the use of glucose polymers, instead of free glucose, may be effective in enhancing the emptying rate (Foster et al., 1980). Glucose polymers (maltodextrins) possess significantly lower osmolality when compared to
isocaloric glucose solutions (Murray, 1987). Sole and Noakes (1989) reported that a 15% glucose polymer solution emptied faster than the corresponding 15% free glucose solution, whereas the 5% and 10% solutions of polymer and free glucose appeared to be emptied at similar rates. Other researchers (Hunt and Stubbs, 1975) have reported that the gastric emptying rates of CHO solutions vary as a function of the caloric content of the ingested beverage and are uninfluenced by beverage osmolality.

Recently, using the double sampling gastric aspiration technique, Vist and Maughan (1995) measured the rate of gastric emptying of isocaloric and isosmotic solutions of glucose and glucose polymer. Their results confirmed that both osmolality and CHO content influence gastric emptying, but the CHO content has a much greater influence on the rate of gastric emptying of liquids than osmolality. The results of this study also demonstrated that dilute (40 g·l⁻¹) solutions of glucose and glucose polymer empty from the stomach at similar rates, whereas concentrated (188 g·l⁻¹) solutions of glucose polymers empty faster from the stomach than isocaloric monomeric glucose solutions.

In the study by Costill and Saltin (1974), it was suggested that beverage temperature may influence gastric emptying. These authors stated that there is an advantage in ingesting chilled drinks (5°C) as this accelerates gastric emptying and thus improves the availability of ingested fluids. The more recent studies (Sun et al., 1988; McArthur and Feldman, 1989), however, suggest that the gastric emptying rate of cold and warm beverages is not different. In spite of this, there may be advantages in taking cold drinks because the palatability of most CHO-electrolyte drink is improved at low temperature (Maughan, 1992).

The effects of exercise intensity on gastric emptying rate have been studied by numerous investigators (Fordtran and Saltin, 1967; Costill and Saltin, 1974; Owen et al., 1986; Neufer, 1986, 1989b). The general consensus appears to be that exercise intensity at about 70% \( \dot{V}O_2 \) max has no significant influence on gastric emptying rates (Burke, 1994). Recent studies of cycling and running have also found gastric emptying and intestinal absorption of isotonic and hypertonic CHO solutions to be independent of exercise mode (Houmard et al., 1991; Rehrer et al.,
Similarly, mode of exercise is not significant with respect to the post-exercise recovery period.

It has been suggested that other factors, such as anxiety and emotional distress, the time and contents of the previous meal, environmental temperature, hormonal responses and pH, may also influence gastric emptying (Owen et al., 1986; Fone et al., 1990); however, the most important factors are the CHO content and osmolality. Increasing the CHO content of fluids will delay gastric emptying, whereas substitution of glucose polymers for free glucose appears to increase the rate of delivery of fluid and substrate to the small intestine.

2.5.2 Carbohydrate, Electrolytes and Intestinal Absorption

After prolonged exercise, replenishment of energy and provision of water are of crucial importance. Absorption rates of water, electrolytes, and CHO are interrelated determinants of how effectively the rehydration fluid replenishes these losses. Two major factors governing net water transport in the small intestine are osmolality (Hunt et al., 1991; Wapnir and Lifshitz, 1985; Wapnir et al., 1991) and solute flux (Binder, 1988; Malawer, 1965). Solutions hypertonic to human plasma (> 280 mOsm·kg⁻¹) stimulate less water absorption and more secretion while hypotonic solutions (< 280 mOsm·kg⁻¹) promote the fastest rates of water absorption (Farthing, 1990; Hunt et al., 1992), with the optimal osmolality being in the range of 200 to 250 mOsm·kg⁻¹ (Wapnir and Lifshitz, 1985; Leiper and Maughan, 1986). A fluid of high CHO content, if rapidly emptied from the stomach and quickly absorbed, maximizes energy repletion. However, high CHO content usually produces high osmolality, which can delay the rehydration processes. A negative correlation between water absorption and osmolality has been repeatedly observed in studies involving only a single transportable substrate (Wapnir and Lifshitz, 1985; Farthing, 1988; Hunt et al., 1991; Wapnir et al., 1991).

Fluid absorption and secretion are also related to solute transport, which, in turn, is related to the type of CHO in solution. For instance, mole per mole, glucose stimulates more net water and Na⁺ absorption than fructose (Fordtran, 1975). In humans (Wheeler and Banwell, 1986), less water
absorption was found from sucrose than glucose, or when sucrose replaced a portion of the glucose or maltodextrin. However, a recent study (Gisolfi et al., 1992) showed that water absorption was independent of CHO type in solutions containing up to 6% of CHO with the same osmolality and caloric concentration. Increasing CHO concentration to 8% significantly reduced water absorption from isocaloric solutions of glucose and corn syrup solids, but not from 8% solutions of sucrose or maltodextrin. In another study (Gisolfi et al., 1990), increasing the concentration of glucose in the lumen to 10% caused fluid secretion and even GI distress.

Carbohydrates are available as monosaccharides, disaccharide, and oligomers. The two readily available monosaccharides, glucose and fructose, have the advantage of being direct substrates for transport by separate non-competitive pathways; both used simultaneously in the same solution might give additive CHO absorption. Since they are monosaccharides, their inherent disadvantage is a rapid increase in osmolality as their concentration is raised, thereby limiting water absorption.

Disaccharide and oligomers have the advantage of providing a high CHO load with minimal effects on osmolality, especially with oligomers. The potential limitations of these CHO is digestion rate, which can be tested experimentally, and may exceed absorption rate. Monosaccharides, disaccharide, and oligomers may be combined to provide two substrates for transport with only a modest increase in osmolality at high CHO loads which would, in turn, be absorbed more effectively than simple glucose. A recent study (Shi et al., 1995) examined this hypothesis and demonstrated that solutions with multiple transportable CHO substrates stimulate several different solute absorption mechanisms, yielding greater water absorption than solutions with only a single transportable substrate. In this particular study, the 6% CHO-electrolyte solutions containing a combination of free glucose and fructose are found to maximise water and CHO absorption.

Sodium is the major ion in the extracellular fluid space and replacement of Na⁺ is necessary to restore the extracellular fluid volume lost. Nose et al. (1988a, 1988b, 1988c) have undertaken a series of detailed studies on the role of Na⁺ during dehydration and rehydration. The essential conclusion from these studies is that the Na⁺ content of the extracellular space must regulate the extracellular fluid volume (Nadel et al., 1990; Nose et al.,
1988a). As a result, the extracellular fluid volume must contract whenever a Na⁺ deficiency develops.

Nose et al. (1988b) also observed that rehydrating with plain water during the post-exercise period will dilute the plasma volume rapidly as the water is absorbed and, in turn, diminish both the volume-dependent and osmotic drive for drinking and increase urine output. Thus, a more effective body fluid volume restoration following dehydration is achieved by providing NaCl with the water during rehydration. Nose et al. (1988b) reported that net fluid retention after 3 h of rehydration was 51% of that lost when drinking water alone, but 71% of that lost when salt capsules were given in conjunction with the drinking water. When Na⁺ was taken with water, plasma Na⁺ concentration remained elevated throughout a longer duration of the rehydration period and was significantly higher than when drinking water alone for the entire 3 h period of recovery. Thus, the salt-dependent thirst drive was maintained and the stimulation of urine production was delayed, leading to a more complete restoration of body water content within the 3 h recovery period.

Costill and Sparks (1973) also showed that ingestion of a glucose-electrolyte solution after dehydration resulted in a smaller urine production and a more complete restoration of plasma volume than did ingestion of plain water. These researchers also suggested that those who attempt rapid rehydration must give serious consideration to the electrolyte composition of the ingested fluid. Similar findings were reported by Gonzalez-Alonso et al. (1992). They compared the effectiveness of two common rehydration beverages (a caffeinated diet cola and a 6% CHO-electrolyte solution) with water on rehydration. Their results showed that when the subjects drank the diet cola or water, they replaced only 55% and 65% of their fluid losses, respectively. However, when they ingested the CHO-electrolyte solution, over 75% of the lost volume was replaced. These researchers concluded that ingestion of a dilute CHO-electrolyte solution is more effective in promoting rehydration than either plain water or a low electrolyte diet cola.

Therefore, the reason for addition of Na⁺ in an rehydration fluid is: First, to enhance intestinal absorption of water and glucose via the glucose-Na⁺ linked carrier mechanism. The combined active transport of glucose and Na⁺ across the gut membrane is very fast and stimulates water absorption
due to the osmotic action of these solutes (Schedl and Clifton, 1963; Levinson and Schedl, 1965; Crane, 1968; Ferrannini et al., 1982; Gisolfi et al., 1990). This will lead to a more rapid restoration of the extracellular volume and plasma volume during recovery (Costill and Sparks, 1973; Candas et al., 1986; Nose et al., 1988b; Noakes, 1992), but not necessarily increase the contribution of the oxidation of ingested glucose to the total energy yield (Massicotte et al., 1996).

Second, to maintain the osmotic drive to drink (Nose et al., 1988b). Third, to increase palatability (Murray, 1987) because improving the taste of a beverage has been shown to increase voluntary consumption (Boulze et al., 1983; Barnes et al., 1984; Hubbard et al., 1984; Carter and Gisolfi, 1989). Fourth, to replace Na⁺ lost in sweat (Nadel et al., 1990, 1993). Hypotonic hyponatremia may be associated with consumption of plain water or other electrolyte-free solutions (Noakes et al., 1985; Noakes, 1988), or with large NaCl losses (Hiller and Laird, 1986).

More recently, the effects of systematic variations in the Na⁺ content of ingested fluids on the effectiveness of fluid replacement after exercise has been examined (Maughan and Leiper, 1995). In this study, subjects were dehydrated by intermittent cycle exercise in a warm, humid environment until 2% body mass was lost. Subjects then ingested a volume of glucose drink equal to 1.5 times the sweat loss. Sodium content of the drinks was 2, 26, 52, 100 mmol·l⁻¹, respectively. From the 1.5 h sample onwards, a significant treatment effect on cumulative urine output was observed, with the volume excreted being inversely related to the Na⁺ content of the drink ingested. Their results clearly demonstrated that rehydration after exercise can only be achieved if the Na⁺ lost in sweat is replaced with the water. Based on the results of these studies, it might be suggested that a Na⁺ concentration similar to that of sweat should be present in the rehydration drinks (Maughan, 1992). Thus, if palatability were not an issue, the ideal fluid replacement beverage should contain a Na⁺ concentration between 40 and 60 mmol·l⁻¹ (Nadel et al., 1993). Rehydration with a dilute, low Na⁺ solution is not an effective method of rapid rehydration and may be detrimental to subsequent performance (Mitchell et al., 1994).
Contrary to the previous studies, Gisolfi et al. (1995) reported that adding Na\(^+\) to rehydration fluid does not enhance fluid absorption. Using the segmental perfusion technique, intestinal absorption during infusion of a 6\% CHO (2\% glucose and 4\% sucrose) solution containing either 0, 25 or 50 mmol\cdot\text{l}^{-1} Na\(^+\) was measured. Surprisingly, doubling the Na\(^+\) concentration of the 6\% CHO solution from 25 to 50 mmol\cdot\text{l}^{-1}, or reducing it to zero, failed to alter the absorption rate of water, Na\(^+\), K\(^+\), or glucose. It was suggested that glucose in the infused solution was the more important factor for enhancing intestinal water absorption compared with plain water, because glucose alone was as effective as glucose plus Na\(^+\). These investigators further speculated that oral consumption of these beverages, rather than intestinal perfusion, would probably produce similar results.

Potassium (K) is the major intracellular cation and the serum K\(^+\) concentration is normally around 4.2 (3.5 - 4.8) mmol\cdot\text{l}^{-1}. This value must be tightly regulated across a very narrow range because the heart is possibly injured below 3 mmol\cdot\text{l}^{-1} (Hubbard et al., 1990). Nadel et al. (1990) speculated that addition of K\(^+\) to a rehydration fluid would enhance the replacement of intracellular water after exercise and thus promote rehydration. This hypothesis was first tested by Maughan et al. (1994) when they investigated the effect of the addition of K\(^+\) salt on the effectiveness of post-exercise fluid replacement. After being dehydrated by approximately 2\% of body mass by intermittent cycle exercise, their subjects ingested one of the four test drinks of a volume equal to their body mass lost. Drink A was a 90 mmol\cdot\text{l}^{-1} glucose solution; drink B contained 60 mmol\cdot\text{l}^{-1} sodium chloride; drink C contained 25 mmol\cdot\text{l}^{-1} potassium chloride; drink D contained 90 mmol\cdot\text{l}^{-1} glucose, 60 mmol\cdot\text{l}^{-1} sodium chloride and 25 mmol\cdot\text{l}^{-1} potassium chloride. The results showed that inclusion of K\(^+\) is as effective as Na\(^+\) in retaining water ingested after exercise-induced dehydration. There appeared, however, to be no additive effects of including both Na\(^+\) and K\(^+\) as would be expected if they acted independently on different body fluid compartments.
2.5.3 Palatability

The palatability of fluid replacement beverages also contributes significantly to rehydration, because quality, flavour, and temperature are important factors determining consumption (Adolph, 1947; Boulze et al., 1983; Hubbard et al., 1984; Armstrong et al., 1985b; Szlyk et al., 1987, 1989a, 1989b). A beverage can be perfectly balanced in energy and electrolytes but it may have little benefit for rehydration if the athlete does not like its taste. During both rest and exercise, people tend to drink more of what tastes good to them. Flavouring water, regardless of its temperature, increases fluid intake (Szlyk et al., 1987, 1989a). Sohar et al. (1962) reported that soldiers marching in the desert heat preferred cold flavoured drinks when large quantities of fluid had to be consumed rapidly (15 - 20 min). Similarly, successful rehydration after exercise will be more likely to be achieved with a drink that tastes good because voluntary fluid intake increases with the perceived palatability of the drink (Boulze et al., 1983).

Boulze et al. (1983) also observed that when rating the preference for water ranging in temperature from 0°C to 50°C, maximum intake for 15 sec of drinking occurred between 15° - 22°C, whilst consumption of cooler and warmer water was reduced. Whilst very cold water (0°C) was rated as the most pleasurable, cool water (15°C) was consumed in greater quantities. Their results are in agreement with the earlier findings (Adolph and associates, 1947; Sohar et al., 1962) that 15°C is the preferred temperature for consumption, particularly when large quantities must be drunk to reduce dehydration.

Most studies quoted above, which have examined fluid palatability and voluntary fluid consumption during dehydrating exercise, have been conducted on adults. Bar-Or and his colleagues have undertaken a series of studies to determine whether the same findings on adults applied to children (Bar-Or et al., 1980; Meyer et al., 1994, 1995; Wilk et al., 1994, 1995, 1996). The general conclusion of these studies confirm the observations that, like adults, children who exercise in the heat develop progressive dehydration when water is given to them ad libitum and they preferred the flavoured drink to water. However, contrary to the findings on adults, most children spontaneously overcompensated their body water
losses during recovery from exercise-induced mild dehydration (Meyer et al., 1994).

2.5.4 Other Factors

Apart from the factors described in the previous section, the effectiveness of rehydration after exercise is also influenced by other variables. Drinking behaviour is known to be one of those important factors for effective rehydration. As indicated previously, the human thirst mechanism is relatively insensitive, and the phenomenon of "involuntary dehydration", or the inability to match fluid ingestion to water loss, is common (Armstrong et al., 1985a; Gisolfi et al., 1992; Greenleaf, 1992). \textit{Ad libitum} drinking is notoriously inadequate as a means for rehydration and this fluid intake pattern can be influenced by a number of factors, including drink palatability, drink temperature, sensations of stomach fullness, and oropharyngeal cues associated with the sensations of thirst (Adolph et al., 1954; Andersson, 1978; Rolls et al., 1980; Engell et al., 1987; Szlyk et al., 1989a). The results of Carter and Gisolfi (1989) have demonstrated that 3 h of \textit{ad libitum} drinking is insufficient to achieve complete rehydration following prolonged exercise in the heat. Forced (prescribed), serial drinking is now considered to be necessary for rapid restoration of the body's fluid balance after exercise, especially during a short-term recovery period.

In addition, during the post-exercise recovery period, the simultaneous consumption of food while drinking enhances fluid consumption (Szlyk et al., 1990). In a more recent study, Maughan et al. (1996a) investigated the effects of post-exercise rehydration with fluid alone or with a meal plus fluid. After being dehydrated by 2.1% of body mass by intermittent cycle exercise, their subjects ingested a commercially-available sports drink (6.4 g·l⁻¹ CHO, 21 mmol·l⁻¹ Na⁺, 3.4 mmol·l⁻¹ K⁺, 12 mmol·l⁻¹ Cl⁻) on two trials. On the third trial, a standard meal [63 kJ·kg⁻¹ body mass (53% CHO, 28% fat, 19% protein, 0.118 mmol·kJ⁻¹ Na⁺, 0.061 mmol·kJ⁻¹ K⁺)] plus drink (1 mmol·l⁻¹ Na⁺, 0.4 mmol·l⁻¹ K⁺, 1 mmol·l⁻¹ Cl⁻) were consumed. The water intake was 1.5 times of the mass lost. Rehydration data showed that, in the third trial, subjects remained in positive fluid balance until 2 h after the end of the rehydration period; at the end of the study period (6 h), the subjects were essentially euhydrated. The authors
concluded that post-exercise fluid replacement could be achieved by ingestion of water if consumed in sufficient volume together with a meal providing significant amounts of electrolytes.

In another study, Maughan et al. (1996b) examined whether fluid replacement after exercise-induced dehydration varied over the normal menstrual cycle. They found that acute restoration of fluid balance after exercise-induced hypohydration is unaffected by the normal menstrual cycle in healthy untrained eumenorrheic young women.

In conclusion, fatigue during prolonged, submaximal exercise ultimately results from the depletion of endogenous CHO reserves and dehydration. A rapid replacement of the body's CHO stores and fluid balance following exercise is essential if functional capacity is to be restored. As mentioned previously, the majority of studies investigating the post-exercise recovery period have focused on the rates of muscle glycogen resynthesis and the effectiveness of rehydration fluids. There is little research to examine the effects of the resulting glycogen and hydration levels on subsequent exercise performance. Therefore, the following studies have been undertaken in an attempt to investigate the influence of rehydration with CHO-electrolyte solution on physiological responses and subsequent exercise capacity.
CHAPTER 3

GENERAL METHODS

This chapter is divided into four sections. The first section (3.1) describes the preliminary measurements employed by all of the studies in this thesis. It includes the selection of subjects, equipment used, and the preliminary testing procedures. The second section (3.2) clarifies the particular standardised experimental model which was used to investigate the influence of rehydration on short-term recovery from prolonged running. The third section (3.3) deals with the collection, treatment, and analysis of blood samples, whilst the final section (3.4) provides details regarding the statistical techniques employed. The specific rehydration-recovery procedures used in each study are described in the method section of each experimental chapter (Chapter 4 to 7).

The research proposals and all experimental procedures of this thesis were approved by the Ethical Advisory Committee of Loughborough University. Experiments were carried out in accordance with the "Code of Practice for Persons Having Contact with Human Body Fluids" and conducted in the Sports Science Research Laboratory of Loughborough University.

3.1 PRELIMINARY MEASUREMENTS

3.1.1 Subjects

Thirty-eight (33 male and 5 female) well-trained runners volunteered for the studies described in this thesis. Twenty were recruited from the university population and eighteen were enlisted from running clubs in the local area. However, due to the intense nature of the experimental protocol and the unexpected GI discomfort following rehydration, 5 subjects eventually dropped out from the studies. The age, body mass, height, and VO$_2$ max of those 33 subjects (28 male and 5 female) who completed the experiments were (mean ± SD) 28.4 ± 7.8 yr, 69.0 ± 9.4 kg, 176.2 ± 7.0 cm, and 58.3 ± 5.8 ml·kg$^{-1}$·min$^{-1}$ (range: 50.4 to 72.4 ml·kg$^{-1}$·min$^{-1}$), respectively.
Middle to long distance running and related training were the primary year-round physical activities for all subjects. One of the criteria for inclusion in the studies was that the subjects were able to run for at least 1 h continuously at about 70% \( \dot{V}O_2 \) max. Before giving a statement of informed consent (Appendix A), all subjects were fully informed about the experimental procedures as well as the potential risks and discomforts which might incur. Subjects were also required to complete a medical history questionnaire (Appendix B) and provide general information on their eating habits. None of the subjects had any relevant past medical histories or were taking any medication. Only self-motivated subjects who were non-smokers, free of any disease and well-trained were included.

### 3.1.2 Equipment and Instrumentation

A motor-driven treadmill (Quinton, Model 24-72, Seattle, USA) was used in all the studies in this thesis. The treadmill 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 performed in this laboratory. The treadmill elevation ranged from a gradient of 0 to 40%, which fulfilled the requirements of all experimental testings.

Prior to the start of each study, the treadmill was calibrated by measuring both the treadmill belt length (in metres) and the time taken (in seconds) to complete fifty revolutions at various speeds spanning the experimental range. Using the relationship between distance, time, and speed, the actual speed of the treadmill was validated and the reliability of both the analogue speedometer and the speed shown on the interfaced computer confirmed.

The treadmill was connected to a microcomputer (BBC Master series), which was in turn interfaced with a two channel 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.
3.1.3 General Procedures

3.1.3.1 Body mass, height and heart rate

Body mass was measured using a balance scale (Avery Ltd., Model 3306ABV) with a capacity of 120 kg and accurate to ± 0.05 kg. Subjects were weighed wearing running shorts before each preliminary test, whereas nude body mass was measured before and after each run in the main trials. Body height was measured using a wall mounted stadiometer (Holtain Ltd.) with a maximum range of 200.00 cm and accurate to ± 0.01 cm. Throughout the treadmill running in each study (Chapter 4 to 7) and during the 4 h rehydration-recovery period (REC) in study 3 (Chapter 6) and 4 (Chapter 7), each subject's heart rate (HR) was continuously monitored using a short-range radio telemeter (Sports Tester PE4000, Polar Electro, Finland).

3.1.3.2 Skin and rectal temperature

Four interchangeable skin temperature thermocouples (Edale, Type EU) were placed on the posterior calf, quadriceps, upper arm, and chest as described by Mitchell and Wyndham (1969). Weighted mean skin temperature (T_{sk}) was calculated according to the equation of Ramanathan (1964) as follows:

\[ T_{sk} = 0.3 \left( T_{chest} + T_{arm} \right) + 0.2 \left( T_{calf} + T_{thigh} \right) \]

In the athletic setting, body temperature can be measured at several locations, including the rectum, tympanic membrane, aural canal, oral cavity, axilla, and by means of urine. Oral and axillary readings are easily accessible, but they do not always reflect core temperature accurately. Tympanic membrane temperature changes rapidly with skin temperature and may underestimate hyperthermia (Roberts, 1994). On the other hand, the rectal temperature measure is regarded as a good indicator of core temperature because of its independence from environmental influence (Livingstone et al., 1983; Deschamps et al., 1992). Thus, in all studies reported in this thesis, rectal temperature (T_{rec}) during exercise was measured to reflect the core temperature of subjects. The temperature was measured with a rectal probe (Edale) inserted 10 cm beyond the anal...
sphincter. Both the $T_{sk}$ and $T_{rec}$ were monitored and recorded continuously throughout each run by connecting all five thermistor probes into a six input electronic thermometer (Edale Instruments, Model C). The thermometer was calibrated and the temperature range was $25^\circ C$ to $45^\circ C$ (increments of $0.2^\circ C$).

3.1.3.3 Expired air collection and analysis

Expired air samples were collected in 150 l capacity Douglas bags (Harvard Equipment Ltd.) for determination of oxygen uptake and carbon dioxide production. During the collection, subjects wore a nose clip (Harvard Equipment Ltd.) and a snorkel type mouthpiece (Harvard Equipment Ltd.). Subjects breathed through a lightweight two-way low resistance respiratory valve (Jakeman and Davies, 1979), which in turn was attached to a 1.5 m section of wide bore (30 mm diameter) lightweight tubing (Falconia: Baxter, Woodhouse and Taylor). The tubing was connected to a two-way tap (Harvard Equipment Ltd.) which was used to open and close the Douglas bag. Thus, a closed-circuit was created when the nose clip and mouthpiece were correctly worn, allowing expired air to be collected over a measured time period.

Oxygen ($O_2$) and carbon dioxide ($CO_2$) content were measured using a paramagnetic oxygen analyser (Taylor Servomex, Model 570A) and an infra-red $CO_2$ analyser (Lira: Mines Safety Appliances Ltd., Model 303). Both analysers were calibrated against certified reference gases (CryoService Ltd., Worcester, UK) and room air immediately before each series of gas analyses. All reference gases were in turn calibrated against a "gold standard" reference gas (CryoService Ltd., Worcester, UK). The volume of expired air withdrawn for analysis was measured using a flow meter.
After the O₂ and CO₂ analysis had been completed, the volume of air left in the Douglas bag was measured by evacuation (Moulinex vacuum pump 237) through a Harvard digital dry gas meter. This had previously been calibrated against a 600 l Tissot spirometer (Collins Ltd., USA). The air temperature was determined during evacuation by a thermistor placed in the air outlet pipe of the dry gas meter and this thermistor was connected to a thermometer (Edale, Type 2984, Model C). The analogue readout of the thermometer was calibrated before each set of analyses against two standard settings of 25°C and 50°C.

Using the Haldane transformation formula (Consolazio et al., 1963) and software developed in the department (© Dr. HKA Lakomy, Department of Physical Education, Sports Science and Recreation Management, Loughborough University), the measured gas volumes were then corrected to standard temperature and atmospheric pressure for a dry gas. This allowed the calculation of oxygen uptake (\( \text{VO}_2 \)), carbon dioxide production (\( \text{VCO}_2 \)), minute ventilation (\( \text{VE} \)), and respiratory exchange ratio (RER).

### 3.1.3.4 Estimation of energy expenditure by indirect calorimetry

Much of the work performed in this thesis used the principle of indirect calorimetry to measure substrate utilization and energy expenditure. The usefulness of indirect calorimetry is dependent upon two main principles. Firstly, most ATP is generated by CHO and fat oxidation requiring O₂ utilization, with only a small proportion being generated by glycolysis and protein oxidation (Consolazio et al., 1963). Thus, the proportions of energy derived from CHO and fat were estimated from the non-protein RER value. Secondly, O₂ reserves within the body are very small in comparison with the rate of O₂ consumption, and arterial blood O₂ concentrations remain constant both at rest and during exercise. This means that whole body \( \text{VO}_2 \), which is calculated from expired air analyses, is a direct reflection of O₂ consumption in the tissues. As a result, indirect calorimetry can be used to calculate total energy expenditure and fuel utilization from measurements of \( \text{VO}_2 \) and \( \text{VCO}_2 \). The details of such calculations have been described by McArdle et al. (1991) and these authors' table was used to evaluate the quantities of CHO and fat consumed during the experimental trials.
There are, however, some limitations in the use of indirect calorimetry. During high intensity exercise, glycolysis proceeds more rapidly than substrate oxidation and hence ATP generation does not relate closely to oxygen consumption, which in turn, invalidates calculations based on indirect calorimetry. The amount of \( \text{O}_2 \) used and \( \text{CO}_2 \) produced per gram of glucose oxidised is less than that per gram of glycogen oxidised, thus having a small influence on calculations of substrate utilization. The amount of \( \text{O}_2 \) consumed and \( \text{CO}_2 \) produced is, fortunately, similar among different types of fat or amino acids oxidised (Jequier and Felber, 1987).

Although the pool of \( \text{O}_2 \) in the body is limited, the \( \text{CO}_2 \) pool is not. Thus, hyperventilation will result in falsely elevated \( \dot{\text{V}}\text{CO}_2 \) measurements, which are not due to increased peripheral \( \text{CO}_2 \) production as a result of increased substrate oxidation. The measurement of \( \dot{\text{VO}}_2 \), however, will be unaffected by hyperventilation. In an attempt to minimise this potential error during the REC, subjects breathed into the mouthpiece for 2 min before their air samples were collected into the Douglas bag.

### 3.1.3.5 Calculation of body fluid balance

In Chapter 4 and 5, body fluid balance after the recovery period was estimated according to the method of González-Alonso et al. (1992). The percent gain in body weight over the recovery period relative to weight loss during the previous exercise provided an index of rehydration. Percentage of rehydration represented the amount of ingested fluid that was retained within the body after the REC. In Chapter 6 and 7, the fluid volume ingested during the REC was calculated to replace 200% and 150% of the subject’s body weight loss, respectively. Due to this increased fluid volume, the previous method was unsuitable for calculating the body fluid balance. Therefore, net fluid balance in these two studies was calculated based on body weight loss, volume of fluid ingested and urinary volume. Subjects provided urine samples into clean, inert plastic cups for measurements.
3.1.3.6 Environmental conditions

All experimental trials reported in this thesis were conducted in the laboratory under neutral environmental conditions (18-23°C). Wet and dry bulb temperatures were measured, and adjusted where possible, throughout the tests using a whirling thermohygrometer (Brannan Thermometer Ltd.) at approximately 1.5 m above the ground surface in close proximity to the treadmill. Relative humidity was subsequently calculated from these values using a conversion scale. Barometric pressure was obtained from a wall mounted barometer (Griffen and George Ltd.).

3.1.3.7 Perceptual variables

During the preliminary tests and the experimental trials, the twenty point Borg scale (1973) was used to estimate the subjects' rate of perceived exertion (RPE). In order to monitor the subjects' thirst perception and abdominal discomfort, two ten point scales were used during exercise and recovery. Both of these scales are linear rating scales ranged from 1 to 10. In the thirst scale, 1 is anchored by the expression "Not Thirsty" and 10 is anchored by the expression "Very Very Thirsty", whereas in the abdominal discomfort scale, 1 denotes "Completely Comfortable" and 10 denotes "Unbearable Pain". For the study reported in Chapter 7, a similar ten point scale was designed to measure the subjects' taste perception during the REC. In this scale, 1 is anchored by the expression "Unpleasant" and 10 is anchored by the expression "Very Very Pleasant".

3.1.3.8 Rehydration beverages

Three different experimental drinks have been used in the studies of this thesis, namely a CHO-electrolyte solution (6.5% and 6.9%) (Appendix C), a glucose- and electrolyte-free artificially sweetened placebo, and tap water. Sodium and potassium concentrations in tap water were undetectable by flame photometry and the osmotic activity was negligible. The drinks were served cool (10 to 15 °C) in plastic opaque squeeze bottles in all experiments. Since the CHO and placebo drinks were specifically formulated and flavored by a sponsoring manufacturer,
subjects could not distinguish between the solutions according to their questionnaires completed after each experimental trial.

Both ad libitum drinking and prescribed drinking were employed in this thesis to investigate the influence of rehydration. Subjects were prescribed the specified volume of fluids during the REC based on their body mass losses incurred during previous exercise (Chapter 4 to 7). The overall time allocated for each prescribed ingestion was 10 min. On one occasion in Study 1, subjects were allowed to drink the specified fluid ad libitum. To avoid bias in this particular study, the specific purpose related to drinking behaviour was not disclosed. Consequently, during the ad libitum session, subjects were shown the drink bottle and were told "Here is your drink. You can drink whenever you want". From that moment on, there was no more mention of "drinking", "fluid", etc. and the subjects were not encouraged by the investigators to drink at anytime. The bottle was placed within an arm's distance such that the subjects could reach it easily during rest. Unknown to the subjects, the volume of fluid intake was monitored periodically with a measuring cylinder.

3.1.4 Dietary Analyses and Training Control

Prior to the experimental trials, a three-day weighed food record diary (Appendix D) was obtained from each subject. Subjects were given a weighing scale and precise written and verbal instructions on how to weigh (to the nearest gramme) and measure all food and fluid consumed over the three-day period. They were also requested to note the frequency of feedings and any ingestion of extra vitamin or mineral supplements. In order to clarify these dietary records, they were encouraged to include labels from packaged and "sports" foods consumed. Analyses of the nutritional content of their normal diets were made from these food diaries (Comp-Eat 4.0, Lifeline Nutritional Services Ltd., London). The food data base of this software was based on the Royal Society of Chemistry Food Composition Tables (Holland et al., 1991). Subjects were then instructed to repeat the same diet before the following trials in order to minimise variation in muscle and liver glycogen concentrations. Subjects were also advised to maintain their normal training programme during the study and were asked to incorporate the experimental test into their training schedule as a "hard work out".
Haemoconcentration caused by heavy sweating (Beaumont et al., 1973) can theoretically increase serum and plasma concentrations, whereas muscle glycogen is reduced or depleted after heavy training, both of these factors will subsequently affect the performance in the experimental trials. Therefore, in order to avoid any residual effects of prior physical training on the experimental treatments and to obtain normal baseline blood samples, subjects were required to refrain from strenuous exercise 2 days before each test. An almost identical procedure has been shown to result in similar pre-test glycogen concentrations (Miller et al., 1983). In addition to exercise, food and fluid intake could affect the level of hydration and plasma volume. Thus, on each experimental trial, subjects reported to the laboratory after a 10 to 12 h overnight fast. In order to increase the likelihood of euhydration before each testing session, subjects were also instructed to ingest approximately 500 ml of water the night before.

3.1.5 Preliminary Testing

3.1.5.1 Familiarisation

During the first visit to the laboratory, subjects were introduced to running on a motorised treadmill, the laboratory environment, and the experimental protocols. Subjects were also invited to observe the experimental protocol "in vivo". This was found to be a valuable aid in helping to alleviate any anxiety as to what was required and whether any trial could be completed efficaciously. Since treadmill running was the exercise mode in all testing sessions, subjects were thoroughly familiarised with running on the specified treadmill. They were also introduced to the methods of collecting expired air and blood samples.
3.1.5.2 *Speed - Oxygen uptake test*

The purpose of the first preliminary test was to determine the relationship between \( \dot{V}O_2 \) and submaximal running speed on a level treadmill. The speeds were chosen with reference to each subject's training status and set between 60 and 70% \( \dot{V}O_2 \) max. Actual speed ranged from 2.75 to 4.50 m·s\(^{-1}\) on a continuous test with subjects running for 4 min at four different speeds. Expired air samples were collected during the last minute of each 4 min period and analysed as described previously. Heart rate and responses to the Borg scale were monitored and recorded throughout the run. By applying linear regression analysis relating to \( \dot{V}O_2 \) and the running speed, individual relationships were established for each subject.

3.1.5.3 *Maximal oxygen uptake test*

Maximal oxygen uptake was determined using a continuous, incremental graded uphill treadmill running test to volitional exhaustion. The protocol used was modified from Taylor et al. (1955). The submaximal running speed was kept constant throughout the test, whereas the inclination of the treadmill was increased by 2.5% every 3 min from an initial gradient of 3.5%. Maximal exertion was expected in this test and subjects were required to run for as long as possible. Expired air samples were collected over the third minute of each stage. A final expired air collection was taken when subjects indicated that they were only able to sustain the required exercise intensity for one more minute. The \( \dot{V}O_2 \) value obtained during the last expired air sample was considered as the \( \dot{V}O_2 \) max value. For subjects who were unsure whether they had reached the maximal stage during the test, their \( \dot{V}O_2 \) max was verified after about 10 min of recovery. This was achieved by having them run for 2 min at the maximal grade and same speed previously attained, and at the next grade the subjects would have attained if they could have continued. Strong verbal encouragement was given throughout the test, especially in the last minute. Heart rate was continuously monitored, and the responses to the Borg scale were recorded every 3 min. It was judged that subjects had reached \( \dot{V}O_2 \) max when the following criteria were met: (a) a plateau of \( \dot{V}O_2 \) with increasing work rate, (b) an RER greater than 1.15, and (c) a HR within 5 beats/min of age-predicted maximum (Astrand and Rodahl, 1986). From the results of the submaximal speed - \( \dot{V}O_2 \) test and the \( \dot{V}O_2 \) max test, the running speed for
each subject required to elicit 70% \( \dot{V}O_2 \) max was computed by means of linear regression analysis.

3.1.5.4 One hour familiarisation run

The purpose of this 1 h familiarisation run was to enable the subjects to further familiarise themselves with all the instrumentation and experimental procedures employed within the studies. In addition, the familiarisation run provided the subjects with a training opportunity to experience a long duration treadmill run, and at the same time, helped the investigators to assess the suitability of each subject for the specified study. The run also enabled the authors to verify the predetermined exercise intensity relative to the experimental subjects' running abilities.

Following a 5 min warm-up at 60% \( \dot{V}O_2 \) max, subjects ran for 60 min at a speed corresponding to 70% \( \dot{V}O_2 \) max. Expired air samples were collected during the last minute of each 15 min exercise period. Heart rate, RPE, sensations of thirst and abdominal discomfort were also recorded during the gas collection. All the procedures were standardised and identical to that during the main trials.

3.2 STANDARDISED EXPERIMENTAL PROCEDURES

A standardised "recovery" protocol was continuously used throughout the studies of this thesis to investigate the influence of rehydration on subsequent endurance capacity. This procedure basically involved a 90 min constant pace run at 70% \( \dot{V}O_2 \) max (T1), followed by the 4 h rehydration-recovery period, and a subsequent open-ended run to exhaustion at the same exercise intensity (T2) (Figure 3.1)
Fig. 3.1 Schematic representation of the standard "recovery" experimental model
After the preliminary tests, subjects were required to take part in two experimental trials in which two treadmill runs at 70% $\mathrm{\dot{V}O_2}\max$ were performed in each experimental condition. These trials were separated by at least seven days. The order of these experiments was randomised (Chapter 4 to 7) and administered in a double-blind, cross-over design (Chapter 6 and 7). To avoid bias in Chapter 4 and 5, the specific purpose related to drinking behaviour and effect was not disclosed and subjects were left uninformed as to the fluid they ingested.

The purposes of the T1 (i.e. 90 min run at 70% $\mathrm{\dot{V}O_2}\max$) were twofold: firstly, to reduce muscle glycogen concentration in both fibre types (Ivy et al., 1988a; Tsintzas, 1993; Tsintzas et al., 1996); secondly, to induce significant body fluid loss (> 2.5% body mass) which, if not replaced, impairs subsequent exercise performance. In addition, the REC was selected for comparison with similar periods in previous studies that assessed the effects of various fluid replacement regimens on rehydration and subsequent exercise performance (Nielsen et al., 1986; Keizer et al., 1986; Ivy et al., 1988b; Lambert et al., 1992; Fallowfield et al., 1995). It has been demonstrated that the standardised protocol adopted in the studies of this thesis (i.e. a standardised endurance run, a controlled rehydration-recovery period, and an open-ended steady state endurance capacity test) provided a reliable measure for assessing short-term recovery from an endurance exercise bout (Fallowfield, 1994).

On the day of the experimental trials, subjects reported to the laboratory at about 08:00 am after a 10 to 12 h overnight fast. They then rested for 15 min before emptying their bladders. Nude body weights were measured before and after each run. The subject then lay on an examination couch and pre-exercise capillary, and venous, blood samples were taken as described later in Section 3.3. Further capillary samples were taken during each exercise bout, and venous and capillary samples were obtained at the end of the exercise bout as well as the REC (Chapter 5 to 7).

After the collection of the baseline blood samples, a standardised 5 min warm-up at 60% $\mathrm{\dot{V}O_2}\max$ was then performed. The treadmill speed was increased to that required to elicit 70% $\mathrm{\dot{V}O_2}\max$ immediately following the warm-up. As mentioned previously, T1 required the subjects to run for 90 min, or until volitional fatigue, whichever came first. Volitional fatigue was defined as the point at which the subject could no longer maintain the required running speed.
During T2, subjects were required to run for as long as possible, such that their endurance capacity could be measured in terms of exercise time to fatigue. To ensure maximal effort in T2, subjects were given strong verbal encouragement throughout the run and encouragement was given only by experimenters who were unaware of which of the treatments had been administered. No external time clues (i.e. clocks or radio) were provided, but the subjects were allowed to listen to recorded music. To enable subjects to more accurately assess their level of fatigue towards the end of each run, they were allowed to reduce the treadmill speed to walking pace once during T1 and twice in T2 for a duration of 2 min. Immediately after each reduction, the prescribed speed was resumed and subjects continued to run to exhaustion. However, the time of reduction of the speed was not counted in the final run time during T2.

The 90 min run and T2 were separated by the REC. During this short-term recovery period, subjects ingested a certain amount of fluid according to the specified drinking schedules in different studies. The fluid was prepared and administered as described in Section 3.1.3.8. To avoid the sensation of repletion or gut fullness which could be accompanied by GI discomfort during T2, fluid ingestion was ceased in the fourth hour of the REC in all studies.

During each treadmill run, subjects were cooled by electric fans and wet sponges were available for use. Heart rate, skin temperature, and rectal temperature were continuously monitored and recorded throughout the treadmill runs as described previously. Expired air collections were taken during each exercise bout (Chapter 4, 5, 6 and 7) and REC (Chapter 6 and 7). Simultaneously, subjective ratings of perceived exertion, thirst, and abdominal discomfort were obtained. Resting expired air collections were also taken in Study 4 (Chapter 7) which allowed pre-exercise metabolic rate to be determined. At the end of each trial, subjects were requested to complete a questionnaire (Appendix E), so that their general comments about the ingested fluid, the occurrence of GI discomfort and muscle soreness could be evaluated.
3.3 COLLECTION AND ANALYSIS OF BLOOD SAMPLES

3.3.1 Capillary Blood Samples

The haemodynamic responses of postural changes are well documented (Harrison, 1985; Shirreffs and Maughan, 1994). On going from an upright to supine position, a marked increase in plasma volume occurs as a result of the altered capillary filtration pressure, and this effect is reversed on standing. These changes are essentially complete within about 15 to 20 min. Therefore, before each resting sample was taken in all studies of this thesis, subjects were required to maintain a standing position for 20 min. After this 20 min, pre-exercise duplicate 20 µl arterialised capillary blood samples were collected from the thumb of a pre-warmed hand using an Auto-clix automatic lancet (Boehringer Mannheim, UK Ltd.) and micro-pipettes (Acupette Pipettes, Scientific Industries Ltd.) (Chapter 4, 5, 6, and 7). Further capillary samples were obtained during and after exercise (Chapter 4, 5, 6, and 7), as well as during the REC (Chapter 5, 6, and 7). Samples were immediately deproteinised in 200 µl of 0.4 mol·l⁻¹ (2.5%) perchloric acid and subsequently centrifuged (Eppendorf, Model 5414) at 12,000 rev·min⁻¹ for 30 sec before being stored at -20°C. These were later analysed for blood glucose and blood lactate.

Blood lactate concentration was determined by fluorimetric analyses (Locarte, London, Model 8-9) (Chapter 4, 5, and 6) on 20 µl aliquots of perchloric acid extract using an enzymatic method adapted from Maughan (1982) (Appendix F), whereas in Chapter 7, blood lactate concentration was determined by an automatic photometric analyser (Cobas Bio). Blood glucose concentration was determined using a spectrophotometer (Boehringer Mannheim Glucose test combination, GOD-Perid method) (Chapter 4) (Appendix G), or the automatic photometric analyser with a commercial kit (Roche, Unimate 7 Glucose GDH) (Chapter 5, 6, and 7).
3.3.2 Venous Blood Samples

In Chapter 4, venous blood samples (10 ml) were drawn from an antecubital vein in the forearm before, and immediately after, each exercise bout. In Chapter 5, 6, and 7, venous blood samples (10 ml) were obtained before and immediately after exercise, as well as during the REC, from an antecubital vein using an indwelling catheter (Venflon 2, 18G) which was connected to a 3-way stopcock (Connecta, Sweden) with 10 cm extension tube for blood sampling. The catheter was inserted under local anaesthesia (0.5 ml of 1% lignocaine) after the subject lay on an examination couch. The catheter was kept patent by infusion of sodium chloride solution (0.9% w/v) and remained in place throughout the experimental trial. As noted previously, subjects were required to remain standing for a period of 20 min prior to the collection of each resting blood sample in order to minimise the effect of hydrostatically induced changes to haemoconcentration (Section 3.3.1.).

In all studies reported in this thesis, a 5 ml venous blood sample was dispensed into a lithium heparin plastic tube to prevent coagulation. Two 20 µl aliquots of blood were drawn from each sample using the micropipettes and mixed with 5.0 ml of Drabkins Reagent (Boehringer Mannheim, GmbH Diagnostica). Thus, the haemoglobin concentration was photometrically determined by the cyanomethaemoglobin method (Appendix H). Triplicate 20 µl aliquots of blood were drawn from each sample using heparinised pipettes. Following micro-centrifugation for 15 min at 11,000 rev·min⁻¹ (Hawksley Ltd.), packed cell volume was measured using a haematocrit reader (Hawksley Ltd.). Percentage changes from rest in plasma volume were estimated from the haemoglobin and haematocrit values as described by Dill and Costill (1974).

Plasma samples were removed by centrifugation of the remnant whole blood for a period of 15 min at 6,000 rev·min⁻¹ at a temperature between 3° and 4°C (Burkard µP Koolspin). The plasma obtained was then stored at -20°C before being analysed for FFA in all studies using the automatic photometric analyser with a commercially available kit (Wako Chemical GmbH), and for glycerol (Laurell and Tibbling, 1966) (Appendix I) using the flourimeter (Chapter 5 and 7).
Another 5 ml aliquot of whole blood was placed into a non-heparinised plastic tube and left to clot at room temperature for 1 h. Serum was then obtained following centrifugation at a temperature between 3° and 4°C for 15 min at 6,000 rev·min⁻¹. Aliquots were stored at -70°C and later analysed for insulin in all studies (¹²⁵I radioimunoassay; Coat-A-Count Insulin, DPC kit) using a gamma counter (Packard, Cobra 5000), and for sodium and potassium concentrations by flame photometry (Ciba Corning, Model 435 with Dilutor 800) (Chapter 5, 6, and 7) (Appendix J), and for osmolality by freezing point depression (Osmomat 030, Gonotec, YSI, Farnborough, UK) (Chapter 6 and 7).

All commercial kits used in the biochemical analysis of blood samples were initially checked in the laboratory over a physiological range of standard concentrations. From these procedures, the reliability and validity of each method were ensured. Greater precision was then obtained in all assays. With the exception of the assays where their analyses were automated (Lactate, Glucose and FFA: automatic photometric analyser; Sodium and Potassium: flame photometry with automated sample dilution), an automatic dispenser (Hamilton MicroLab 1000) was used for aspirating and dispensing various volumes of samples and chemical mixture during the assay analyses. In addition, the pH of the various buffers was measured using a pH meter (Corning, Model 240) which was calibrated with appropriate buffer solutions (Fisons Ltd.). All of the biochemical procedures and assays described took place in the biochemistry laboratory at the Department of Physical Education, Sports Science and Recreation Management, Loughborough University.

The coefficient of variation [(S.D./mean) x 100%] of the blood, plasma, and serum metabolite assays in shown in Table 3.1. The coefficient of variation (n=15) for haemoglobin and haematocrit were 0.8% and 0.7%, respectively.
3.4 Statistical Analysis

The performance times and rehydration responses (e.g. total volume ingested, % rehydration, etc.) were compared using Student's *t*-tests for paired data. A two-way analysis of variance (ANOVA) for repeated measures on both factors was used to analyse overall differences between the physiological and blood biochemical responses in both trials. Where significant F ratio were found (P < 0.05), the means were compared using a Tukey post hoc test. Results are presented as means ± standard error (S.E.M.).

Table 3.1 Coefficient of variation (C.V.) of blood, plasma, and serum metabolite assays (n=15)

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>C.V. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood lactate</td>
<td>1.9</td>
</tr>
<tr>
<td>(Fluorimetrically)</td>
<td></td>
</tr>
<tr>
<td>(Automated photometric analysis)</td>
<td>1.9</td>
</tr>
<tr>
<td>Blood glucose</td>
<td>1.4</td>
</tr>
<tr>
<td>(Spectrophotometrically)</td>
<td></td>
</tr>
<tr>
<td>(Automated photometric analysis)</td>
<td>1.2</td>
</tr>
<tr>
<td>Plasma FFA</td>
<td>1.3</td>
</tr>
<tr>
<td>Plasma glycerol</td>
<td>2.7</td>
</tr>
<tr>
<td>Serum insulin</td>
<td>8.7</td>
</tr>
<tr>
<td>Serum sodium</td>
<td>0.4</td>
</tr>
<tr>
<td>Serum potassium</td>
<td>0.4</td>
</tr>
<tr>
<td>Serum osmolality</td>
<td>0.8</td>
</tr>
</tbody>
</table>
CHAPTER 4

INFLUENCE OF FLUID INTAKE PATTERN ON SHORT-TERM RECOVERY FROM PROLONGED, SUBMAXIMAL RUNNING AND SUBSEQUENT EXERCISE CAPACITY

4.1 INTRODUCTION

Fatigue during prolonged, submaximal exercise is associated with the depletion of endogenous CHO stores and dehydration (Bergstrom et al., 1967; Armstrong et al., 1985a). A CHO-electrolyte solution can serve as a significant source of the energy and fluid needs during the restoration process (Carter and Gisolfi, 1989; Gonzalez-Alonso et al., 1992), especially for those athletes whose appetites are suppressed by exercise. However, when left to their own choices, athletes tend to drink insufficient amounts of fluid to rehydrate following exercise (Noakes et al., 1988), a phenomena known as "involuntary dehydration" (Greenleaf and Sargent, 1965).

In order to restore fluid balance after exercise-induced dehydration, prescribed drinking according to the previous fluid loss, has been employed in numerous studies (Costill and Sparks, 1973; Nielsen et al., 1986; Lambert et al., 1992; Gonzalez-Alonso et al., 1992). The levels of rehydration reported in these studies vary considerably and mainly depend on the composition of the test drinks and the drinking regimens of the experiments. Unfortunately, incomplete rehydration has repeatedly been reported during recovery periods of only 3 to 6 h when the volume of fluid ingested is equal to the fluid loss. Prescribed drinking, however, is continually employed, and recommended, for rapid rehydration because it is believed to be more effective in restoring fluid balance than ad libitum drinking. Nevertheless, there is no evidence to support this suggestion in the scientific literature.

Although short-term recovery from exhaustive exercise was enhanced, and subsequent endurance capacity was improved, by ingesting a prescribed CHO-electrolyte solution immediately after exercise (Fallowfield et al., 1995), it is unclear whether similar improvement on subsequent exercise capacity would occur when ad libitum drinking with the same solution is allowed following exercise. Therefore, the aim of this study was to compare the effects of either prescribed or ad libitum
drinking with a CHO-electrolyte solution, during 4 h recovery from prolonged, submaximal running, on rehydration and subsequent exercise capacity.

4.2 METHODS

Subjects

Seven subjects (five female and two male) took part in this study. The subjects' mean (± SEM) age, height, weight, \( \dot{V}O_2 \text{ max} \) and heart rate max were 19.8 ± 0.3 yr, 168.1 ± 2.8 cm, 58.2 ± 1.9 kg, 57.5 ± 3.3 ml·kg\(^{-1}\)·min\(^{-1}\) and 199 ± 4 beat·min\(^{-1}\).

Protocol

Dietary Analysis

Subjects completed weighed-food intake dietary analyses before the start of the study (Section 3.1.4) and were instructed to repeat the same diet before the second trial.

Preliminary Measurements

After subjects became familiar with treadmill running and the experimental procedures, they performed a series of preliminary tests as previously described (Section 3.1.5)

Experimental Design

The standardised "recovery" protocol (Section 3.2) was used in this study. The order of these experimental trials was randomized and no order effect was observed between experimental treatments.

The first treadmill run (T1) required the subjects to run for 90 min at 70% \( \dot{V}O_2 \text{ max} \), or until volitional fatigue, whichever came first. Immediately after T1, the 4 h rehydration-recovery period (REC) started and fluid intake was confined to the REC. During the second treadmill run (T2), subjects were required to run at the same speed for as long as possible, such that their endurance capacity could be measured in terms of subsequent exercise time to fatigue (Fig. 4.1).
On both occasions, the pre-exercise body masses (Prescribed: 58.3 ± 2.0 kg vs. Ad libitum: 58.6 ± 2.1 kg) were not different indicating that subjects started both experiments at the same hydration status. All trials were performed under similar experimental and environmental conditions (Temperature: 19.8 ± 0.4 and 20.6 ± 0.2 °C; Relative Humidity: 53.4 ± 1.7 and 55.6 ± 2.3% for Prescribed and Ad libitum trials).

During the REC, subjects were allowed to drink a 6.9% CHO-electrolyte (CE) solution (Lucozade-Sport; Na+: 24 mmol·l⁻¹; K⁺: 2.6 mmol·l⁻¹; Osmolality: 300 mOsm·l⁻¹) ad libitum (AL) on one occasion. The volume of fluid ingested was recorded every 30 min and the subjects were unaware of these measurements (Section 3.1.3.8). On the other occasion, the volume of the same fluid was prescribed (PI) from calculations of the loss of body weight during T1. Aliquots of the prescribed fluid intake were ingested 15 min after, and at each hour after, T1 during the REC. Subjects drank 725 ml of CE as their first prescribed intake in order to consume 50 g of CHO as recommended (Coyle, 1993). This volume of fluid was subtracted from body mass losses during T1 so as to calculate the remaining hourly fluid intake. To avoid GI distress during T2, fluid ingestion was not continued at the last 50 min of the REC in both conditions.

Whole body rehydration was estimated according to the method of Gonzalez-Alonso et al. (1992). The percent gain in body weight over the REC relative to weight loss during T1 provided an index of rehydration. As such, percent rehydration represented the amount of ingested CE that was retained within the body after the REC. In order to calculate, more accurately, the fluid retention and the change of body weight after each trial, urine voided during the REC was collected and measured.

**Analyses**

Before T1, after the subject had stood quietly for about 20 min, a 10 ml pre-exercise sample of venous blood was taken from an antecubital vein in the forearm and duplicate 20 µl samples of capillary blood from the thumb of a pre-warmed hand were also obtained. Similarly, venous and capillary blood samples were taken at the cessation of each run. Further capillary blood samples were also taken at 30 min intervals during each trial and at 1 h intervals during the REC. Packed cell volume and haemoglobin concentration were measured in whole blood, whilst plasma was analysed for FFA. Lactate and glucose were determined in capillary samples and insulin was measured in serum. All blood samples were collected, treated, and analysed as previously described (Section 3.3).
Throughout each run, expired air samples were collected at 15 min intervals. Simultaneously, subjective ratings of perceived exertion were obtained using the Borg scale (Borg, 1973). All air samples were collected and analysed as described in Section 3.1.3.3.

Data were found to be normally distributed and parametric statistical procedures were applied. Performance times were compared by use of Student's *t*-tests for paired data. Data obtained over time were tested for a Treatment x Time interaction using a two-way analysis of variance (ANOVA) for repeated measures. When a significant interaction was obtained, a Tukey *post hoc* analysis was used to identify differences between means. Total volume consumed, urine volume and change of body mass were analysed by paired *t*-tests. Separate analyses were performed for the exercise and recovery periods. Differences were considered significant at $P < 0.05$. 

Fig. 4.1 Schematic representation of the experimental procedures
4.3 RESULTS

All subjects completed the 90 min run during T1 on both trials, whereas during T2 in the PI trial, the exercise time to exhaustion was 16% longer (P < 0.05) than during T2 in the AL trial (69.9 ± 9.1 vs. 60.2 ± 10.2 min). All subjects were able to run longer during T2 in the PI trial and the run times ranged from 51.9 to 114.0 min, whereas run times during T2 in the AL trial ranged from 35.3 to 112.2 min.

During the REC, the subjects drank a total volume of 1499 ± 155 ml in the PI trial and 1405 ± 215 ml CE in the AL trial (NS). Although there was no difference in the total volume ingested between trials, the volume ingested in the fourth hour of the REC was greater in the PI trial than in the AL trial (PI: 258 ± 52 vs. AL: 78 ± 34 ml; P < 0.05) (Table 4.1). Because of greater fluid intake at this period in the PI trial, the amount of glucose ingested was also greater than that during the AL trial (PI: 17.8 ± 3.6 vs. AL: 5.4 ± 2.4 g; P < 0.05) (Table 4.1).

During the REC, the subjects ingested 103 ± 10 g of CHO in the PI trial, whereas, in the AL trial, subjects ingested 97 ± 18 g of CHO (NS). The contributions of fat and CHO to the total energy expenditure during T1 in the PI and AL trial were both 40% and 60%, respectively. This compares with corresponding values of 41% and 59% during T2 in the PI trial and 39% and 61% in the AL trial (NS).

After exercising for 90 min in T1, the subjects lost 2.6 ± 0.2% and 2.5 ± 0.3% of their initial body mass (BM) in the PI trial and the AL trial, respectively. These decreases of body mass were not different. At the end of the REC in both trials, the subjects had not restored their body mass to pre-T1 values (range, PI: 0.15 - 0.96 kg; AL: 0.45 -1.45 kg below the BM). This was in spite of the fact that in the PI trial, the CE ingested was calculated to replace the total fluid loss during T1. However, the subjects tended to retain more ingested fluid after the REC in the PI trial (0.8 ± 0.2 kg) than in the AL trial (0.6 ± 0.2 kg), for which urine formation was accounted and corrected. Incomplete rehydration resulted from fluid lost in urine, sweat and respiration during the REC.
At the end of the experiments, the subjects lost a total $3.5 \pm 0.4\%$ and $3.4 \pm 0.4\%$ of their pre-T1 BM in the PI trial and the AL trial, respectively. Although the percent of body mass loss that was regained at the end of the REC, i.e., the percent rehydration, during PI trial was approximately 11% greater than that for the AL trial, these differences were not statistically significant (PI: $53.6 \pm 7.6\%$; AL: $42.7 \pm 7.6\%$) (Fig. 4.2). Total urine volume did not differ between experimental treatments due to high individual variance (PI: $447 \pm 82$ ml; AL: $520 \pm 153$ ml).

Figure 4.3 shows the changes in plasma volume as a result of exercise-induced dehydration in T1 and T2 and after the REC. All values are expressed as a percent change from the resting levels. The change in plasma volume was similar between two experimental treatments immediately after T1. After the REC, plasma volume returned to the initial level in the AL trial, whereas plasma volume did not restore in the PI trial. However, plasma volume returned to resting level at the end of T2 in both trials. No differences were observed between trials at any time due to the great variability between subjects.

Blood glucose concentrations were equally well maintained within the normal range during T1 and T2 in both trials and no differences were found between trials. Serum insulin decreased during T1 in both trials and further decreased after the REC (Table 4.2). Blood lactate increased during T1 and T2 in both trials following the onset of exercise. Plasma FFA increased during T1 in both trials, whereas concentrations decreased during T2 in the AL trial but rose in the PI Trial. However, these changes were not significantly different between trials. The respiratory exchange ratio (RER) did not differ between trials during T1 and T2 (Fig. 4.4).

Perceived rates of exertion increased similarly during T1 and T2 in both trials and no differences were observed between trials. The average heart rates of the runners during T1 in the PI and AL trials were $169 \pm 5$ and $168 \pm 4$ beat-min$^{-1}$, respectively (Fig. 4.5). At the end of T2, the heart rates were $177 \pm 5$ in the PI and $176 \pm 5$ b-min$^{-1}$ in the AL trial (NS).
There were no differences in mean skin temperature between the PI and AL trials during T1 and T2. Core temperature was similarly regulated during the two trials. During T1 in the PI trial, values ranged from $37.0 \pm 0.2^\circ C$ at the start of exercise to a maximum of $38.6 \pm 0.4^\circ C$ ($P < 0.05$), and in the AL trial from $37.0 \pm 0.2^\circ C$ to $38.7 \pm 0.5^\circ C$ ($P < 0.05$). In T2, values ranged from $37.1 \pm 0.2^\circ C$ to $38.7 \pm 0.4^\circ C$ in the PI trial ($P < 0.05$), and from $37.1 \pm 0.1^\circ C$ to $38.3 \pm 0.4^\circ C$ in the AL trial ($P < 0.05$). No differences were observed between trials. No GI discomfort was reported during treadmill running nor during the REC.
The volume of CHO-electrolyte solution (ml) and the amount of glucose (gram) per kg body weight ingested during the REC in the PI and AL trials; values represent means (± SEM)

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**Table 4.2**  Blood glucose, serum insulin, blood lactate, plasma FFA concentration before and after the T1 and T2 in the PI and AL trials; values represent means (± SEM)
Fig. 4.2 Fate of the ingested volume during the 4 h recovery period in the PI and AL trials. The height of the graph represents the total amount of fluid consumed (ml). The stacked bars represent the fate of the ingested volume: The ingested fluid was either retained in the body or lost in the form of urine and other insensible losses. The percent of the body weight loss that was regained, percent rehydration, was used as indicative of the volume retained.
Fig. 4.3  Plasma volume responses after T1, 4 h recovery and T2 during ad libitum (AL) and prescribed (PI) trials (mean±SEM)

*P < 0.01 from Post T1
Fig. 4.4  Respiratory exchange ratio (RER) during T1 and T2 in the ad libitum (AL) and prescribed (PI) trials (mean±SEM)
Fig. 4.5 Heart rate (beat·min\(^{-1}\)) during T1 and T2 in the ad libitum (AL) and prescribed (PI) trials (mean±SEM)
4.4 DISCUSSION

The primary purpose of this investigation was to examine the influence of two different fluid intake patterns on the level of rehydration and subsequent endurance capacity. The results show that prescribed rehydration with a 6.9% CHO-electrolyte solution, calculated to replace the body fluid losses after prolonged running, restores endurance capacity following a 4 h recovery period to a greater extent than ad libitum rehydration. This difference in running time to exhaustion occurred even though the total volume of fluid ingested was the same between trials.

Following the rehydration period in the PI and AL trials, the subjects remained hypohydrated, with percent rehydration being 53.6 ± 7.6% and 42.7 ± 7.6%, respectively. This amount of rehydration is less than the values reported by other researchers using prescribed (controlled) (Costill and Sparks, 1973; Nielsen et al., 1986; Gonzalez-Alonso et al., 1992; Lambert et al., 1992) and ad libitum rehydration protocols (Nose et al., 1988). By employing prescribed rehydration after exercise-induced or thermal dehydration, which decreased body weight by 2.5% to 4.1%, Costill and Sparks (1973), González-Alonso et al. (1992) and Lambert et al. (1992) also found that subjects were hypohydrated after ingestion of a fluid equal to their weight loss. Carter and Gisolfi (1989) found that 3 h of ad libitum drinking was inadequate to completely rehydrate their subjects following prolonged exercise. The dissimilarities in degree of dehydration and percentage rehydration between the present study and those studies in the literature may be due to the utilisation of different experimental protocols and rehydration solutions. For example, Gonzalez-Alonso et al. (1992) dehydrated subjects by means of cycling for 60 min at 60-80% \( \dot{V}O_2 \text{max} \) at 32°C (40% relative humidity); subjects then drank 2000 ml of fluid within 2 h. Whereas Costill and Sparks (1973) employed 4 to 5 treadmill runs followed by a 3 h rehydration period in which subjects ingested a 10% glucose electrolyte solution. Even though the approaches to the question of how best to rehydrate after exercise are different, there appears to be agreement that complete rehydration does not occur during recovery periods of only 3 to 6 h when the volume of fluid ingested is equivalent to the weight loss during exercise.
The incomplete rehydration found in the present study and other studies, was partly due to continuous fluid loss during recovery brought about by urine formation, insensible sweating and respiration (Costill and Sparks, 1973; Gonzalez-Alonso et al., 1992). In the present study, 37% of the total volume of fluid ingested during the AL trial was excreted as urine whilst 30% was excreted in the PI trial. This was despite the fact that the subjects were dehydrated and required fluid. The tendency for greater fluid retention in the PI trial may have been brought about by the more evenly distributed fluid intake pattern during the REC.

One of the notable findings of the present study was that there was no difference in the total volume ingested in the two trials. However, a greater volume of fluid was ingested in the fourth hour of the PI trial \( (P < 0.05) \). In the first 2 h period, the subjects ingested \( 983 \pm 52 \) and \( 1181 \pm 167 \) ml in the PI and AL trials respectively \( (NS) \). It was reported that the upper limits for fluid replacement during exercise-heat stress and recovery are set by the maximal gastric emptying rates, which approximate 1.0 - 1.5 l·h\(^{-1}\) for an average adult male (Sawka, 1992). Since our data for both trials are within this range, gastric emptying was probably not limiting during the REC. However, considering that the volume ingested is one of the main factors affecting gastric emptying, the larger volume ingested in the AL trial within the first 2 h period may, therefore, explain why the corresponding blood glucose values also tended to be higher and vice versa in the second 2 h period during the REC. The tendency for a greater percent rehydration during the PI trial may have been brought about by the more stable and balanced passage of fluid from the stomach into the gut over the rehydration period. In such a condition, fluid absorption is better maintained and urine formation decreased.

The ingestion of CHO during rapid rehydration would appear to be advantageous, as the rate of muscle glycogen resynthesis is three times faster if CHO is ingested immediately after exercise as opposed to delaying feeding by 2 h (Ivy et al., 1988a). Therefore, in an attempt to hasten recovery of muscle glycogen in the present study, subjects ingested 50 g of CHO immediately after T1 in the PI trial based on the recommendations of Coyle (1993). Although this 50 g of CHO was not prescribed in the AL trial, subjects still consumed this amount of CHO and exceeded the optimal rate of glucose ingestion advocated by Blom et al. (1987) in the first 2 h of the REC. This amount of CHO intake may have stimulated a near optimal rate of glycogen resynthesis within this period. However, in the 3 and 4 h period during the REC, the subjects ingested a significantly different amount of glucose in both trials which were both below 0.7 g·kg\(^{-1}\)·h\(^{-1}\). Therefore, this glucose ingestion may have induced different rates of glycogen resynthesis in the REC before T2 with the greater rate in
the PI trial. The observed enhanced endurance capacity during T2 in the PI trial could be attributed to this greater glucose provision during the REC, even though there were no differences in the total amount of glucose ingested and the percent rehydration between trials.

Furthermore, unlike the abrupt decrease of the volume of fluid ingested during the REC in the AL trial, subjects drank the CHO solution in equivolmetric measures hourly during the PI trial. It has been suggested that a more frequent CHO feeding schedule may be needed to hasten the muscle glycogen replenishment (Doyle et al., 1993). Thus, the prescribed fluid intake schedule in the present study would not only have promoted rehydration, but also produced a more consistent stimulus for glycogen resynthesis during the REC.

In conclusion, the results of this study suggest that drinking a prescribed volume of a 6.9% CHO-electrolyte solution after prolonged exercise, calculated to replace the body fluid losses, restores endurance capacity to a greater extent than ad libitum drinking over a 4 h recovery, even though the total volumes ingested were the same.
CHAPTER 5

INFLUENCE OF REHYDRATION WITH CARBOHYDRATE-ELECTROLYTE SOLUTION AND WATER DURING 4 HOURS RECOVERY FROM PROLONGED, SUBMAXIMAL RUNNING ON SUBSEQUENT EXERCISE CAPACITY

5.1 INTRODUCTION

Rapid restoration of muscle glycogen and rehydration after exercise are of importance for optimal substrate provision, cardiovascular function, and thermoregulation during subsequent bouts of exercise (Costill and Sparks, 1973; Montain and Coyle, 1992b; Heaps et al., 1994). It has also been suggested that at least 50 g of CHO should be ingested immediately after exercise in order to hasten the glycogen resynthesis process (Coyle, 1993). However, it is unclear whether consuming only 50 g of CHO immediately after exercise, as suggested, is sufficient for restoring endurance capacity for subsequent exercise. Theoretically, this is unlikely because providing a greater amount of CHO after prolonged exercise is believed to be more beneficial in replenishing the body's glycogen stores and, thus, further enhancing the subsequent endurance capacity. In the study reported in Chapter 4, it was found that ingesting a prescribed CHO-electrolyte solution, during a 4 h recovery period following prolonged running, restored endurance capacity to a greater extent than ad libitum drinking. Thus, it is reasonable to use the prescribed fluid intake pattern again in examining the effectiveness of different amounts of CHO during the REC on subsequent exercise capacity.

Therefore, the purpose of this study was to investigate the influence of ingesting 50 g of CHO immediately after exercise, either with subsequent serial CHO feeding or water ingestion during the REC from prolonged, submaximal running, on rehydration and subsequent exercise capacity.
5.2 METHODS

Subjects

Eight endurance-trained male subjects participated in this study. All were post-graduate physical education students at Loughborough University. The physical characteristics (mean ± SEM) of the subjects were: age, 31.4 ± 2.6 years; height, 177.0 ± 1.8 cm; weight, 74.4 ± 2.9 kg; maximum heart rate, 181 ± 4 beat·min⁻¹; and VO₂ max, 56.9 ± 1.1 ml·kg⁻¹·min⁻¹.

Protocol

Dietary Analysis
Subjects completed the weighed-food intake dietary record prior to the main trials as described previously (Section 3.1.4)

Preliminary Measurements
After subjects became familiar with treadmill running and the experimental procedures, they performed a series of preliminary tests as previously described (Section 3.1.5)

Experimental Design
The standardised "recovery" protocol (Section 3.2) was used again in this study. The runners were randomly assigned to each trial and no order effect was observed between trials.

The first treadmill run (T1) required the subjects to run for 90 min at 70% VO₂ max, or until volitional fatigue, whichever came first. Immediately after T1, the 4 h rehydration-recovery period (REC) started and fluid intake was confined to the REC. During the second treadmill run (T2), subjects were required to run at the same speed for as long as possible, such that their endurance capacity could be measured in terms of subsequent exercise time to fatigue (Fig. 5.1).
During the REC, the subjects ingested a prescribed volume of fluid equal to the body mass lost during T1 in both experimental conditions. In order to provide 50 g of CHO, the subjects drank 725 ml of a 6.9% CHO-electrolyte solution (Lucozade-Sport; Na⁺: 24 mmol·l⁻¹; K⁺: 2.6 mmol·l⁻¹; Osmolality: 300 mOsm·l⁻¹) 15 min after T1 on both occasions as their first prescribed fluid intake. This volume of fluid was subtracted from body weight losses during T1 in order to calculate the remaining hourly fluid intake. After this ingestion, the subjects drank either the same solution (CE) or water (W) at each hour after T1 during the REC. In an attempt to avoid GI distress during T2, no fluid was ingested during the last 50 min of the REC in both conditions. As in study 1 (Chapter 4), whole body rehydration was estimated according to Gonzalez-Alonso et al. (1992) and percent rehydration represented the amount of ingested fluid that was retained within the body after the REC. Urine voided during the REC was collected and measured for calculation of rehydration.

**Analyses**

After the subjects had maintained a relaxed standing position for 20 min, a 10 ml venous blood sample was obtained, whilst duplicate 20 μl capillary blood samples were taken for the determination of blood glucose and blood lactate. Similarly, venous and capillary blood samples were taken before, and at the cessation of, each trial, as well as at 1 h intervals during the REC. Further capillary blood samples were taken at 30 min intervals during each trial. Blood samples were treated, stored, and subsequently analysed as described previously (Section 3.3).

Expired air samples were collected for 2 min at 15 min intervals and during the last min prior to perceived exhaustion during T1 and T2. A 5-min sample was also obtained at each hour during the REC. Simultaneously, the subjective ratings of perceived exertion, perceived thirst and abdominal discomfort were recorded. The method of collection and analysis of expired air samples was the same as previously described (Section 3.1.3.3).

The performance times, total volume ingested, percent rehydration, and changes of body weight were compared using Student's t-tests for paired data. A two-way analysis of variance (ANOVA) for repeated measures on two factors (experimental treatment and time) was used to analyse overall differences between the physiological and blood biochemical responses in both trials. When a significant interaction was obtained, a Tukey post hoc test was used to identify differences between means. The accepted level of significance was set at P < 0.05. Data are reported as means ± SEM.
Fig. 5.1  Schematic representation of the experimental procedures
5.3 RESULTS

Endurance capacity

All subjects completed the 90 min run during T1 on both occasions. During T2, the exercise time to exhaustion was $54.2 \pm 9.2$ min in the CE trial (range: 17.8 - 109.4 min) and $52.2 \pm 6.2$ min in the W trial (range: 35.5 - 86.8 min), respectively (NS).

Body mass changes, fluid balance and urine volume

After exercising for 90 min in T1, the subjects lost $2.8 \pm 0.3\%$ (2.2 ± 0.2 kg) in the CE trial and $2.6 \pm 0.1\%$ (2.0 ± 0.1 kg) in the W trial of their pre-exercise body mass (BM), respectively. At the end of the REC in both conditions, the subjects had not restored their body mass to pre-exercise values (range, CE: 0.2 - 0.8 kg; W: 0.4 -1.6 kg below the BM) and the subjects were hypohydrated by $0.7 \pm 0.1\%$ of their BM in the CE trial and $1.0 \pm 0.2\%$ in the W trial. This was in spite of the fact that in both conditions, the fluid ingested was calculated to replace the total fluid loss during T1.

There was no difference in the total volume of fluid ingested during the REC between trials (CE: 2157 ± 255 ml; W: 1950 ± 90 ml). However, the percent of body mass loss that was regained at the end of the REC, i.e., the percent rehydration, during CE trial was 10.5% higher than that of the W trial (CE: 73.5 ± 4.2% vs. W: 63 ± 5.7%; $P < 0.05$) (Fig. 5.2). As expected, the total amount of glucose ingested during the CE trial was greater than that during the W trial (CE: 148.8 ± 17.7 g vs. W: 50 g; $P < 0.01$) (Table 5.1).

Although there was a trend for urine volume, during W trial, to be greater than that of CE trial (359 ± 120 ml vs. 288 ± 75 ml; range, W: 115 - 1110 ml; CE: 113 - 660 ml), no differences were found between experimental treatments. This volume of urine represented 14.6% and 18.6% of fluid ingested during the CE and W trials, respectively (NS).
Plasma volume and blood changes

Figure 5.3 shows the changes in plasma volume as a result of exercise-induced dehydration in T1 and during the REC and T2. All values are expressed as a percent change from the resting levels. The mean plasma volume decrease was similar between the two experimental treatments immediately after T1 (CE: 4.5 ± 1.4%; W: 4.1 ± 1.2%, NS). After the first hour of rehydration, plasma volume was restored in both conditions. In the CE trial, the rate of increase levelled off during the REC but still remained elevated (2.6 ± 1.4%) before T2, whereas in the W trial, it continued to increase up to 5.3 ± 3.3% at the third hour; however, it returned to the initial level before the start of T2. Unlike the plasma volume changes after T1, there were no changes in either trial after T2 and both values were found close to the initial level (CE: -0.2 ± 1.5%; W: -0.3 ± 3.0%). There were no differences between trials at any time due to large individual variability.

During T1, blood glucose concentrations were well maintained within the normal range in both conditions (Fig. 5.4). Glucose concentrations then increased to the highest level (CE: 6.5 ± 0.3 mmol·l⁻¹; W: 6.6 ± 0.1 mmol·l⁻¹) on both occasions during the first hour of the REC after the ingestion of the 50 g of CHO. When drinking water during the W trial, blood glucose began to drop after the first 60 min of REC (P < 0.01). Although continually ingesting a CHO-electrolyte beverage in the CE trial, glucose concentration returned to 5.4 ± 0.7 mmol·l⁻¹ (P < 0.01) at the third hour, but remained elevated throughout the REC. As expected, glucose concentration was higher (P < 0.01) in the CE trial during the REC. Notably, blood glucose dropped after 30 min during the CE trial in T2 and was lower than that of W trial (CE: 3.6 ± 0.2 mmol·l⁻¹ vs. W: 4.5 ± 0.3 mmol·l⁻¹, P < 0.01). However, no differences were found at the end of T2 between trials.

The nature of energy metabolism did not differ between the two trials during T1 (Table 5.2), though the respiratory exchange ratios were higher in the CE trial compared with the W trial during the T2 (P < 0.05). Greater CHO oxidation rates were also observed during T2 in the CE trial (P < 0.05) (Table 5.2). Carbohydrate oxidation contributed 72.5% of the total energy requirement of the CE trial during T1, compared with 77.3% during T2 (NS). Whilst 69.2% of the energy requirement was provided by CHO oxidation during T1 in the W trial, compared with 52.9% during T2 (P < 0.05).
The changes in serum insulin mirrored the changes of blood glucose concentration (Fig. 5.5). After the first hour during the REC, the continuing ingestion of the CHO beverage resulted in higher serum insulin levels ($P < 0.01$) as compared to those elicited by ingestion of water. However, no differences were observed at the end of T2 between trials. Figure 5.6 shows the change of blood lactate concentration and the responses in both conditions were similar during T1, REC and T2.

At the end of T1, plasma FFA level increased twofold ($P < 0.05$) in both trials and remained nearly fourfold higher ($P < 0.01$) during the REC and T2 in the W trial (Fig. 5.7). Similarly, plasma glycerol increased during T1 and T2 in both conditions following the onset of exercise (Fig. 5.7) and a higher value was found at the end of T2 between trials. During the REC, although plasma glycerol tended to be higher in the W trial, no differences were observed between trials.

Serum $\text{Na}^+$ concentrations increased during T1 in both trials ($P < 0.01$) and tended to decrease during the REC in the W trial (Table 5.3). However, serum $\text{Na}^+$ was greater ($P < 0.05$) at the end of the REC and during T2 when the CHO-electrolyte beverage was ingested during rehydration. Similarly, serum $\text{K}^+$ concentrations increased during exercise in T1 and T2 in both treatments ($P < 0.01$) (Table 5.3). During the REC, serum $\text{K}^+$ decreased in both trials ($P < 0.01$). Although the changes in the CE trial tended to be greater during the REC, serum $\text{K}^+$ was nearly back to the initial values and not different between trials after rehydration.

**Mean skin temperature, rectal temperature and heart rate**

The $\text{Tsk}$ profiles were similar in both trials during T1 (Fig. 5.8). Although the $\text{Tsk}$ tended to be higher during T2 in the CE trial, the values were not different from that of the W trial. The $\text{Trec}$ was similarly regulated during the two trials (Fig. 5.9). During T1 in the CE trial, values ranged from $36.8 \pm 0.1^\circ\text{C}$ at the start of exercise to a maximum of $39.0 \pm 0.3^\circ\text{C}$, and from $36.9 \pm 0.1^\circ\text{C}$ to $39.0 \pm 0.3^\circ\text{C}$ in the W trial, whereas in T2, values ranged from $36.8 \pm 0.2^\circ\text{C}$ to $38.7 \pm 0.2^\circ\text{C}$ in the CE trial, and from $37.0 \pm 0.1^\circ\text{C}$ to $38.8 \pm 0.2^\circ\text{C}$ in the W trial. No differences were observed between trials. Although there was a trend for $\text{Trec}$, in the W trial, to be higher than that of CE trial during the REC, no differences were observed between experimental treatments.
Exercising HR were similar during T1 in both trials (Fig. 5.10). However, after different fluids were prescribed during the REC, heart rates during T2 were about 5 beat·min\(^{-1}\) higher in the W trial (P < 0.01).

Ratings of perceived exertion increased similarly during T1 and T2 in both trials and no differences were observed between trials (Fig. 5.11). No GI discomfort was reported during treadmill running nor during the REC; nevertheless, the ratings of abdominal discomfort during T2 were higher in the CE trial (P < 0.05) (Fig. 5.11).
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<td>0</td>
</tr>
<tr>
<td>Glucose (g)</td>
<td></td>
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<tr>
<td>SEM</td>
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<td>18</td>
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</table>

**Table 5.1** The amount of glucose ingested in the first and second to fourth hour periods of the 4 h recovery during the CE and W trials; values represent means ($\pm$ SEM).
<table>
<thead>
<tr>
<th></th>
<th>Run Time (min)</th>
<th></th>
<th></th>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>30</td>
<td>45</td>
<td>60</td>
<td>75</td>
<td>90</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>RER</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>0.93</td>
<td>0.93</td>
<td>0.92</td>
<td>0.91</td>
<td>0.90</td>
<td>0.93</td>
<td>0.96</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>(0.02)</td>
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<td>(0.02)</td>
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<td>(0.01)</td>
</tr>
<tr>
<td>W</td>
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<td>0.91</td>
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<td>0.91</td>
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<tr>
<td>CHO Oxidation</td>
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<td></td>
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<td></td>
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<td></td>
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<tr>
<td>Rate (g·min⁻¹)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CE</td>
<td>2.70</td>
<td>2.62</td>
<td>2.55</td>
<td>2.52</td>
<td>2.38</td>
<td>2.78</td>
<td>2.95</td>
<td>2.71</td>
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<tr>
<td></td>
<td>(0.24)</td>
<td>(0.24)</td>
<td>(0.28)</td>
<td>(0.23)</td>
<td>(0.30)</td>
<td>(0.21)</td>
<td>(0.17)</td>
<td>(0.15)</td>
</tr>
<tr>
<td>W</td>
<td>2.70</td>
<td>2.48</td>
<td>2.50</td>
<td>2.26</td>
<td>2.15</td>
<td>2.48</td>
<td>2.14</td>
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<tr>
<td></td>
<td>(0.20)</td>
<td>(0.20)</td>
<td>(0.22)</td>
<td>(0.19)</td>
<td>(0.29)</td>
<td>(0.29)</td>
<td>(0.12)</td>
<td>(0.07)</td>
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</tbody>
</table>

Table 5.2 The respiratory exchange ratio (RER) and CHO oxidation rate (g·min⁻¹) during T1 and T2 in the CE and W trials; values represent mean (±SEM)

a P < 0.05, CE vs. W
**Table 5.3** Serum sodium, potassium concentrations (mmol·L⁻¹) during T1, REC and T2 in the CE and W trials; values represent means (± SEM)

<table>
<thead>
<tr>
<th>Serum</th>
<th>T1</th>
<th>Recovery</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>1h</td>
</tr>
<tr>
<td>Sodium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>138 (3)</td>
<td>143 (2)b</td>
<td>142 (3)</td>
</tr>
<tr>
<td>W</td>
<td>137 (2)</td>
<td>142 (2)b</td>
<td>140 (2)</td>
</tr>
<tr>
<td>Potassium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>4.1 (0.1)</td>
<td>5.0 (0.1)b</td>
<td>4.5 (0.1)</td>
</tr>
<tr>
<td>W</td>
<td>4.2 (0.2)</td>
<td>5.0 (0.2)b</td>
<td>4.5 (0.2)</td>
</tr>
</tbody>
</table>

* P < 0.05, CE vs. W; b P < 0.01 from Pre T1; c P < 0.01 from Post T1; d P < 0.01 from 1 h; e P < 0.01 from 4 h (Pre T2)
Fig. 5.2 Fate of the ingested volume during the 4 h recovery period in the CE and W trials. The height of the graph represents the total amount of fluid consumed (ml). The stacked bars represent the fate of the ingested volume: The ingested fluid was either retained in the body or lost in the form of urine and other insensible losses. The percent of the body weight loss that was regained, percent rehydration, was used as indicative of the volume retained.

\(^a\) P < 0.05, CE vs. W
Fig. 5.3 Change of plasma volume (%) of the carbohydrate (CE) and water (W) trials during T1, recovery, and T2 (mean±SEM)

a $P < 0.05$ from Post T1
Fig. 5.4  Blood glucose (mmol·l⁻¹) concentration of the carbohydrate (CE) and water (W) trials during T1, recovery and T2 (mean±SEM)

a $P < 0.01$, CE vs. W

b $P < 0.01$ from 1 hr in Recovery

c $P < 0.01$ from 0 min in T1

d $P < 0.01$ from 4 h
Fig. 5.5 Serum insulin (mU·l⁻¹) concentration of the carbohydrate (CE) and water (W) trials during T1, recovery and T2 (mean±SEM)

- a P < 0.01, CE vs. W
- b P < 0.01 from Post T1
- c P < 0.01 from 1 hr
- d P < 0.01 from Pre T1
Fig. 5.6  Blood lactate (mmol·l⁻¹) concentration of the carbohydrate (CE) and water (W) trials during T1, recovery and T2 (mean±SEM)

a P < 0.01 from 0 min in T1
b P < 0.01 from 90 min in T1
c P < 0.05 from 4 hr (Pre T2)
Fig. 5.7 Plasma free fatty acid (mmol·l⁻¹) and plasma glycerol (mmol·l⁻¹) concentration of the carbohydrate (CE) and water (W) trials during T1, recovery and T2 (mean±SEM)

- a P < 0.01, CE vs. W
- b P < 0.05 from Pre T1
- c P < 0.01 from Post T1
- d P < 0.01 from 1 hr
Mean skin temperature (°C) of the carbohydrate (CE) and water (W) trials during T1 and T2 (mean±SEM)

Fig. 5.8

a $P < 0.01$ from 0 min in T2
b $P < 0.05$ from 10 min in T2
Fig. 5.9 Rectal temperature (°C) of the carbohydrate (CE) and water (W) trials during T1, recovery and T2 (mean±SEM)

a P < 0.01 from 4 hr (0 min in T2)
Fig. 5.10  Heart rate (beat·min⁻¹) of the carbohydrate (CE) and water (W) trials during T1 and T2 (mean±SEM)

a P < 0.01, CE vs. W
Fig. 5.11 Rating of perceived thirst and rate of abdominal discomfort of the carbohydrate (CE) and water (W) trials during T1 and T2 (mean±SEM)

\[ a \ P < 0.05, \text{CE vs. W} \]
The main finding of this study was that ingesting approximately 150 g of CHO in the
form of a 6.9% CHO-electrolyte solution over a 4 h recovery period following
prolonged running is more effective for rehydration compared to the same volume of
fluid containing only 50 g of CHO and water, but does not have a greater effect on
subsequent endurance capacity.

Although there is a greater variety of sports drinks currently available than has been the
case in the past, some athletes still prefer water to these beverages. Thus, the
contribution of this study is to suggest that ingesting 50 g of CHO immediately after
prolonged exercise followed by only water may be equally effective for the restoration
of energy capacity as compared with ingesting 150 g of CHO in the form of sports
drinks during such a 4 h recovery period. However, the results also demonstrate the
efficacy of drinking a CHO-electrolyte solution on rehydration during a short-term
recovery when compared to ingesting only water. Nevertheless, all subjects remained
hypohydrated at the end of the REC in both conditions, despite the fluid ingested being
equal to the weight loss during T1.

Some investigators (Sole et al., 1989; Houmard et al., 1991) have reported that
solutions containing more than 2.5% CHO tend to empty from the stomach more
slowly than water or dilute saline solutions. Increasing the CHO content of drinks will
increase the rate of CHO delivery to the small intestine, but will decrease the volume of
fluid emptied. In the present study, a 6.9% CHO-electrolyte solution was consumed in
the CE trial and a total amount of approximately 150 g of CHO was ingested during the
REC. Although this fluid was effectively retained and absorbed in the body, as
reflected by the higher level of rehydration, it would be possible for the gastric
emptying rate of this beverage to be slower than the water in the W trial. Thus, the
CHO ingested might not be available for complete and optimal use during the
subsequent exercise in T2. Judging from the blood responses during the REC in the CE
trial, it appears that the glucose and electrolyte content of the beverage did increase the
delivery of CHO to the site of absorption in the small intestine. However, before the
start of T2, some of the extra CHO ingested would probably still be retained within the
GI tract and not incorporated into muscle glycogen. Since no muscle biopsies were
performed in this study, this question remains unanswered.
A larger and more sustained insulin and glucose response was observed during the hours after glycogen depleting exercise when a large amount of CHO was consumed (Ivy et al., 1988a; Doyle et al., 1993). During the CE trial in the present study, although subjects ingested 50 g of CHO immediately after exercise and consumed a total amount of 82.7 g of CHO within the first 2 h of the REC, this amount of CHO did not provoke similar large, and sustained, insulin and glucose responses to those found in the other studies. In view of the periodic decline in insulin concentrations during the REC noted in this study, it is speculated that the insulin response might not have caused maximal glucose uptake and stimulated maximal glycogen synthase activity, thereby leading to a sub-optimal glycogen replenishment in the CE trial. It has been suggested that a smaller and more frequent CHO feeding schedule may be needed to hasten the muscle glycogen replenishment (Doyle et al., 1993).

The blood glucose responses in the CE trial after the start of T2 is worth noting because the sudden drop in blood glucose might have further complicated the substrate availability during T2. The continued feeding of a further 100 g of CHO during the REC resulted in a rapid decline in blood glucose (to 3.6 mmol·l⁻¹) and a larger CHO oxidation during T2. The increased reliance on CHO in the CE trial during T2 could be attributed to a threefold higher insulin concentration at the start of T2, as compared with the W trial.

Another striking observation during the REC and T2 was the suppression of FFA when the CHO-electrolyte solution was continually ingested during the REC. The depressed FFA concentrations during the REC and T2 in the CE trial are indicative of impaired adipose tissue lipolysis (Acheson et al., 1988). This effect is mediated by the pre-T2 insulinemia after the ingestion of CHO (Costill et al., 1977; Hargreaves et al., 1985). In contrast, the ingestion of placebo following the first 50 g of CHO did not induce an excessive CHO challenge to suppress FFA to a critical level. Perhaps due to this pre-T2 insulinemia in the CE trial, no benefits were gained in terms of substrate provision, and performance time during T2 was adversely affected. This could occur despite an enhanced CHO oxidation, observed through the estimation of the respiratory exchange ratios. It would appear that subsequent exercise capacity after a short-term recovery from submaximal running was probably dependent upon factors other than rehydration. Rapid muscle glycogen replenishment and the resultant optimal substrate availability may still be the major determinant of this recovery process.
During the REC, the subjects followed the same prescribed drinking schedule and the total volume of fluid ingested was the same due to identical fluid losses in T1. The greater amount of rehydration in the CE trial, therefore, could be attributable to the composition of the CHO-electrolyte drink. Recent studies have shown that net intestinal absorption of fluid from a dilute solution containing CHO and Na⁺ occurs at a faster rate than that from plain water (Gisolfi et al., 1991, 1992; Maughan et al., 1994; Leiper and Maughan, 1986). This phenomenon is due to the fact that glucose transport across the intestinal membrane is coupled to the active transport of Na⁺ (Schedl, 1990).

The Na⁺ and K⁺ content of the CE solution in the present study was 23 mmol·l⁻¹ and 4 mmol·l⁻¹, respectively. This amount of Na⁺ should be more effective in restoration of fluid balance than that of the water in the W trial, in which only water was consumed after the first bolus of 725 ml of the CHO beverage. Although the Na⁺ concentration of the current drink might be regarded as low, it was still within the proposed range for rehydration solution (Brouns et al., 1992). It has been suggested that ingestion of only water after dehydration results in a rapid fall in the plasma Na⁺ concentration (Nose et al., 1988b) and this change has the effect of stimulating urine output which, in turn, affects the rehydration process. Although the values did not reach statistical significance, urine volume in the CE trial during the REC was 4.2% lower when compared to the W trial. The findings of the present study are in agreement with the observation of Nose et al. (1988b), as at the end of the REC, serum Na⁺ concentrations were significantly lower (135 ± 3 mmol·l⁻¹) when subjects had drunk water from the second hour onwards during the REC. In the absence of any information of the hormonal changes during the present experimental treatments, the mechanisms responsible for the observed effects on rehydration remain the subject of speculation. The results do, however, suggest that a greater level of rehydration can be achieved when CHO and Na⁺ are included in the ingested fluids for the post-exercise rehydration process.

The concentration of K⁺ may also play a role in facilitating rehydration of the intracellular fluid volume (Nielsen et al., 1986). Using perfusion techniques to investigate the intestinal absorption during 1 h recovery in cyclists, Gisolfi et al. (1991) reported that fluid absorption occurred significantly faster from a CHO-electrolyte solution than from water. During recovery in their study, the plasma Na⁺ and K⁺ for the 6% CHO-electrolyte solution and water trials were 142.5 ± 1.7 and 140.4 ± 0.5 mEq·l⁻¹, 3.8 ± 0.3 and 4.1 ± 0.3 mEq·l⁻¹, respectively. In the present study, subjects also drank a CHO-electrolyte solution of a similar concentration to that used by these authors but over a longer recovery period. The changes of K⁺ concentration observed at
the end of the REC were similar to those reported by these investigators, whereas serum Na⁺ concentrations were maintained throughout the REC.

A decrease in plasma volume leads to a higher HR (Sawka et al., 1979), whereas an increased blood volume results in a reduction in submaximal HR (Kanstrup and Ekblom, 1982). The higher HR observed during T2 in the W trial probably reflected the cardiovascular disturbances induced by dehydration (Montain and Coyle, 1992b). However, no severe thermoregulatory problems were observed during T2 in the W trial and the rectal temperature was maintained within the normal range under such strenuous exercise. The lack of differences in rectal temperature may have been due to the relatively low (CE: 0.7 ± 0.1%, W: 1.0 ± 0.2%) levels of dehydration during T2. Recent studies have shown that dehydration of up to 4% is associated with increases in rectal temperature of about 1°C or less (Barr et al., 1991; Hamilton et al., 1991; Montain and Coyle, 1992b).

In conclusion, the results of this study suggest that ingesting ~150 g of CHO in a 6.9% CHO-electrolyte solution during a 4 h recovery period following prolonged running is more effective for rehydration compared to the same volume of fluid containing only 50 g of CHO and water, but does not have a greater effect on subsequent endurance capacity.
CHAPTER 6

EFFECT OF INGESTING A LARGE VOLUME OF CARBOHYDRATE-ELECTROLYTE SOLUTION ON REHYDRATION DURING RECOVERY AND SUBSEQUENT EXERCISE CAPACITY

6.1 INTRODUCTION

In the previous studies (Chapter 4 and 5), it has been demonstrated that although a CHO-electrolyte solution combined with a controlled drinking pattern were employed during the 4 h rehydration-recovery period, complete rehydration was still not achieved when the volume of fluid ingested was equal to the body mass loss. In order to restore fluid balance as rapidly as possible after exercise-induced dehydration, it has been suggested that the ingestion of a larger fluid volume may be necessary during a short rehydration period (Grandjean et al., 1992). A recent study confirmed this hypothesis that complete rehydration could occur during a short period after exercise-induced dehydration when the volume ingested was substantially greater than the fluid loss during exercise (Maughan and Shirreffs, 1994). In that study, the subjects drank a volume of fluid with a high Na⁺ concentration (61 mmol·l⁻¹) equal to 200% of their body mass loss and positive fluid balance (+360 ml) was reported at the end of the 6 h recovery period.

Although more effective rehydration could occur with a solution containing a higher Na⁺ content (Maughan and Leiper, 1995) and/or low concentration of CHO (Maughan et al., 1996), the effects of ingesting such a large volume of fluid on subsequent exercise performance remain unknown and no studies so far have been performed to clarify this issue. Furthermore, if ingestion of a large volume of fluid during a relatively short, prescribed rehydration period restores hydration status to the pre-exercise level, the question arises as to the consequences during subsequent exercise if CHO is included in the fluid. Therefore, the purpose of the present study was to compare the effects of rehydration per se and CHO ingestion, during 4 h recovery, on subsequent endurance running capacity.
6.2 METHODS

Subjects

Nine endurance-trained male runners participated in this study. Their mean (± SEM) age, height, weight, maximum heart rate, and $\dot{V}O_2$ max were 26.4 ± 1.7 yr, 178.5 ± 2.5 cm, 71.0 ± 2.7 kg, 189 ± 3 beat-min⁻¹, and 59.5 ± 1.5 ml·kg⁻¹·min⁻¹.

Protocol

Dietary Analysis
In order to avoid any unwitting modification of diets before the main trials, subjects were requested to complete a 3-day weighed food intake diary (Section 3.1.4). Analyses of the nutritional content of their recorded diets were made and subjects were then required to replicate their diet at the same time three days before the second experiment.

Preliminary Measurements
Following familiarisation with running on a motor-driven treadmill and the experimental procedures, the subjects undertook a series of preliminary tests before performing the two main trials (Section 3.1.5).

Experimental Design
The standardised "recovery" protocol (Section 3.2) continued to be used in this study. The order of these experiments was randomised and administered in a double-blind cross-over design. Each subject was required to complete two experiments in which two treadmill runs (T1 and T2) were performed in each experimental condition, separated by at least seven days (Fig. 6.1).

The first treadmill run (T1) required the subjects to run for 90 min at 70% $\dot{V}O_2$ max, or until volitional fatigue, whichever came first. Immediately after T1, the 4 h rehydration-recovery period (REC) started and fluid intake was confined to the REC. During the second treadmill run (T2), subjects were required to run at the same speed for as long as possible, such that their endurance capacity could be measured in terms of subsequent exercise time to fatigue.
After T1, subjects rested in a seated position for 30 min before their body weights were taken. Dehydration was calculated as the difference between the pre-exercise body mass and the body mass recorded at the end of this resting period.

During the REC, the subjects ingested a prescribed volume of fluid equivalent to 200% of the body mass lost (g=ml) during T1 in both experimental trials. Subjects drank either a 6.9% CHO-electrolyte (CE) solution (Lucozade-Sport; Na+: 24 mmol·l⁻¹; K⁺: 2.6 mmol·l⁻¹; Osmolality: 300 mOsm·l⁻¹) or a CHO-free sweetened placebo (PL). The test drinks ingested were of the same colour, texture and taste. Subjects drank an initial 725 ml of the fluids 30 min after T1 on both occasions which, in the CE trial, provided 50 g of CHO. The remaining volume of fluid was then computed by deducting the first administration (725 ml) from the total volume prescribed. The fluid was then ingested in equivolumetric measures at 30 min intervals up to the beginning of the 4 h of the REC. This drinking pattern was chosen to maximise gastric emptying (Rehrer et al., 1990b; Noakes et al., 1991) and avoid GI distress during T2. The fluid was maintained at a temperature of 10-15°C and the overall time required for each ingestion was 10 min. Urine volumes were also measured at 30 min intervals during the REC. Whole body rehydration was estimated from the percent gain in body weight over the REC relative to weight loss during T1. As such, percent rehydration represented the amount of ingested fluid that was retained within the body after the REC.

Dry bulb temperatures in the laboratory were 20.6 ± 0.2 and 20.8 ± 0.3°C during the CE and PL trial, respectively. Whereas relative humidity for the two occasions were 55.6 ± 0.5 and 56.1 ± 0.8%, respectively. While running on the treadmill, subjects were cooled by electric fans and wet sponges were also available for use ad libitum.

**Analyses**

Venous and capillary blood samples were collected, treated and analysed as previously described (Section 3.3) Expired air samples were collected over 2 min at 15 min intervals and the last min prior to perceived exhaustion during T1 and T2. A 5 min sample was also obtained at each hour during the REC. Simultaneously, the subjective ratings of perceived exertion, perceived thirst and abdominal discomfort were recorded. The method of collection and analysis of expired air samples was the same as previously described (Section 3.1.3.3).
The performance times, total volume ingested, percent rehydration, and changes of body weight were compared using Student's *t*-tests for paired data. A two-way analysis of variance (ANOVA) for repeated measures on two factors (experimental treatment and time) was used to analyse overall differences between the physiological and blood biochemical responses in both trials. When a significant interaction was obtained, a Tukey *post hoc* test was used to identify differences between means. Differences were considered significant at $P < 0.05$. Results are presented as means ± SEM.
Fig. 6.1 Schematic representation of the experimental procedures
6.3 RESULTS

Exercise times to exhaustion

All subjects completed the 90 min run during T1 on both trials. However, during T2 in the CE trial, the run time to exhaustion was 54% longer (P < 0.01) than during T2 in the PL trial (CE: 69.3 ± 5.5 min vs. PE: 45.0 ± 4.2 min). All subjects were able to produce a longer run time under the CE experimental treatment. Run times during T2 in the CE trial ranged from 44.5 min to 95.5 min, whereas the run times during T2 in the PL trial ranged from 26.3 min to 69.3 min.

Percentage VO$_2$ max, RER and CHO oxidation rate

The average % VO$_2$ max values sustained during T2 in the CE and PL trials were 69.8 ± 1.0 and 69.8 ± 1.2%, respectively (NS). During T1, there were no differences in the RER between trials. However, the RER values were greater from the second hour of the REC and remained elevated during T2 in the CE trial (P < 0.01) (Table 6.3). The CHO oxidation rates during T1, as calculated from the VO$_2$ and RER values, averaged 2.6 ± 0.2 and 2.5 ± 0.2 g·min$^{-1}$ for the CE and PL trials (NS). During REC and T2, a greater amount of CHO was oxidised in the CE trial (0.2 ± 0.2 g·min$^{-1}$ and 2.7 ± 0.2 g·min$^{-1}$) when compared with the values in the PL trial (0.1 ± 0.2 g·min$^{-1}$ and 1.6 ± 0.2 g·min$^{-1}$) (P < 0.01) (Table 6.3). Thus, CHO oxidation contributed 74.2% of the total energy requirement of the CE trial during T1, compared with 79.3% during T2. Whilst 74.3% of the total energy requirement was provided by CHO oxidation during T1 in PL trial, compared with only 49.3% during T2.

Body mass changes and fluid balance

After the completion of T1, the subjects lost ~3.0% of their pre-exercise body mass in both trials (Table 6.1) and no differences were observed in these body mass changes between trials. Accordingly, the total volume of fluid prescribed was 4307 ± 315 ml and 4600 ± 531 ml for the CE and PL trials. However, this volume of fluid was not completely consumed and the total volume of fluid ingested during the REC was only 170.8 ± 12.6% and 172.6 ± 13.8% of the body mass lost after T1 (NS) for the CE and PL trials. In the CE trial, this fluid ingestion resulted in the consumption of 247.2 ± 16.1 g of CHO.
Despite the subjects' inability to drink all the fluid prescribed in both trials during the REC, they had restored their body mass to pre-T1 values and were in positive fluid balance by 423 ± 215 ml in the CE trial and 446 ± 239 ml in the PL trial before T2 (NS). Similarly, there were no differences in the percent rehydration between experimental treatment (Table 6.1).

**Urine volume and changes in plasma volume**

Cumulative urine output was similar between treatment conditions at the end of the REC (Fig. 6.2). Although there was a trend for cumulative urine production, during PL trial, to be greater than that of the CE trial, no differences were observed at any time point during the REC between experimental treatments. By the second hour of the REC, cumulative urine output was still below 100 ml in both trials and ~80% of the total urine volume was produced within the last hour during the REC in both trials. The total volume of urine represented 17.8% and 18.2% of fluid ingested during the CE and PL trial, respectively (NS).

Figure 6.3 shows the changes in plasma volume as a result of exercise-induced dehydration in T1 and during the REC and T2. All values are expressed as a percent change from the resting levels. The mean plasma volume decrease was similar between two experimental conditions immediately after T1 (CE: 2.6 ± 1.2%; PL: 2.4 ± 1.5%). After the first hour of rehydration, plasma volume was restored with both drinks and it continued to increase and remained elevated before T2. No changes occurred on both treatments after T2 and both values were still found higher than the initial level. There were no differences between trials at any time point due to large individual variability.

**Blood changes**

During T1, blood glucose concentrations were equally well maintained within the normal range in both trials (Fig. 6.4). Glucose concentration then increased to the highest level (7.4 ± 0.3 mmol·l⁻¹) in the CE trial by the first hour during the REC (P < 0.01). Although continually ingesting a CHO-electrolyte beverage in the CE trial, glucose concentration declined over the REC and eventually returned to 4.9 ± 0.2 mmol·l⁻¹ before T2 which was not different from that of the PL trial. However, as expected, glucose concentration was higher (P < 0.01) during the REC in the CE trial and it remained unchanged when drinking placebo during the PL trial. Similar to the
blood glucose responses during T2 in the second study in this thesis (Chapter 5: Fig. 5.4), a sudden drop on blood glucose also occurred at 30 min of T2 in the CE trial in this study (P < 0.05). Meanwhile, no differences were found at the end of T2 between the test drinks.

Serum insulin decreased with the onset of T1 in both trials (Fig. 6.5). During the REC, the ingestion of the CHO beverage resulted in higher serum insulin levels (P < 0.01) as compared to those elicited by ingestion of placebo. Serum insulin concentrations increased progressively after the ingestion of the CHO drink and a peak was observed by the 3 h of the REC. Although returning to a lower level prior to the T2, serum insulin was still higher in the CE trial compared to the PL trial (CE: 19.0 ± 3.2 mU·l⁻¹ vs. PL: 3.0 ± 0.4 mU·l⁻¹, P < 0.01). After the start of T2, serum insulin decreased but no differences were observed at the end of T2 between trials.

Figure 6.6 shows the change of blood lactate concentration during T1, REC and T2. Although the values tended to be higher during the REC and T2 in the CE trial, there were no differences between treatments. At the end of T1, plasma FFA level increased nearly threefold (P < 0.01) from pre-T1 values in both trials and remained elevated (P < 0.01) during the REC and T2 when drinking the placebo (Fig. 6.7), whereas FFA concentrations were restored in the CE trial prior to T2. Following the onset of exercise in T2, the values increased again in the CE trial (P < 0.01).

Serum Na⁺ concentrations increased during T1 (P < 0.01) and decreased during the REC (P < 0.01) in both trials (Table 6.2). However, the values remained greater (P < 0.01) during the REC and T2 when the CHO-electrolyte beverage was ingested during rehydration. Similarly, serum K⁺ concentrations increased during exercise in T1 and T2 in both treatments (Table 6.2). Nevertheless, placebo ingestion resulted in higher serum K⁺ concentrations during REC and T2 (P < 0.01). Serum osmolality changed over time in both trials, but no differences were found between treatments at any time (Table 6.2). Although there was a tendency for the values to be higher during REC and T2 in the CE trial, this did not reach statistical significance.
Mean skin temperature and rectal temperature

The Tsk profiles were similar in both trials over T1 and T2 (Fig. 6.8), whereas the Tree was similarly regulated during the two trials (Fig. 6.9). During T1 in the CE trial, values ranged from 37.0 ± 0.1°C at the start of exercise to a maximum of 39.1 ± 0.3°C, and from 36.8 ± 0.1°C to 39.2 ± 0.2°C in the PL trial. Whereas in T2, values ranged from 37.0 ± 0.1°C to 38.9 ± 0.2°C in the CE trial, and from 37.1 ± 0.1°C to 38.9 ± 0.2°C in the PL trial. No differences were found between treatments. Although Tree tended to be higher at 2 h and 3 h during the REC in the PL trial, there were no significant differences between trials.

Heart rate, perceived rate of exertion and thirst

Exercising HR rose progressively during T1 in both conditions (Table 6.4). However, after the ingestion of the test drinks, heart rates were higher at 15 min during T2 in the PL trial (P < 0.05). There were no differences in the rates of perceived exertion during T1 between trials (Table 6.4). However, subjects found it harder to run after the ingestion of the placebo (P < 0.05). With respect to the subjects' perceived thirst during the trials, a consistent increase in the sensation of thirst was experienced by all subjects throughout T1 and T2. However, no differences existed as a result of the experimental treatments. At the end of the experiments, all subjects reported tolerable gastric distress during T2 owing to the volume of fluid ingested under the two trials. Ratings of abdominal discomfort tended to higher during T2 in the CE trial, but there were no differences at any time point due to large individual variability (Fig. 6.10).
<table>
<thead>
<tr>
<th></th>
<th>CE</th>
<th>PL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass after T1 (%)</td>
<td>-3.0 (0.1)</td>
<td>-3.2 (0.3)</td>
</tr>
<tr>
<td>Fluid prescribed during REC (ml)</td>
<td>4307 (315)</td>
<td>4600 (531)</td>
</tr>
<tr>
<td>Fluid ingested during REC (ml)</td>
<td>3582 (234)</td>
<td>3712 (249)</td>
</tr>
<tr>
<td>Fluid ingested of volume prescribed (%)</td>
<td>170.8 (12.6)</td>
<td>172.6 (13.8)</td>
</tr>
<tr>
<td>Fluid balance after REC (ml)</td>
<td>+423 (215)</td>
<td>+446 (239)</td>
</tr>
<tr>
<td>% Rehydration after REC</td>
<td>123.7 (10.6)</td>
<td>125.3 (10.0)</td>
</tr>
</tbody>
</table>

Table 6.1  Body fluid balance after T1 and during REC in the CE and PL trials; values represent mean (± SEM)
<table>
<thead>
<tr>
<th>Serum</th>
<th>T1</th>
<th>Recovery</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>1h</td>
</tr>
<tr>
<td>Sodium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>140 (1)</td>
<td>144 (1)</td>
<td>142 (1)</td>
</tr>
<tr>
<td>PL</td>
<td>139 (1)</td>
<td>143 (1)</td>
<td>140 (1)</td>
</tr>
<tr>
<td>Potassium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>4.1 (0)</td>
<td>4.7 (0.1)</td>
<td>4.4 (0.1)(^{ab})</td>
</tr>
<tr>
<td>PL</td>
<td>4.0 (0.1)</td>
<td>4.7 (0.1)</td>
<td>4.5 (0.1)</td>
</tr>
<tr>
<td>Osmolality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>294 (1)</td>
<td>306 (1)</td>
<td>304 (1)</td>
</tr>
<tr>
<td>PL</td>
<td>294 (2)</td>
<td>306 (1)</td>
<td>300 (1)</td>
</tr>
</tbody>
</table>

Table 6.2  Serum sodium, potassium concentrations (mmol·l\(^{-1}\)) and osmolality (mOsm·l\(^{-1}\)) during T1, REC and T2 in the CE and PL trials; values represent mean (± SEM)

\(^{a}\) P < 0.01, CE vs. PL; \(^{b}\) P < 0.01 from Post T1; \(^{c}\) P < 0.05 from Pre T1
<table>
<thead>
<tr>
<th>Run Time (min)</th>
<th>T1</th>
<th>Recovery</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>RER</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>0.92</td>
<td>0.92</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.02)</td>
</tr>
<tr>
<td>PL</td>
<td>0.94</td>
<td>0.93</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.01)</td>
</tr>
<tr>
<td>CHO Oxidation Rate (g·min(^{-1}))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>2.62</td>
<td>2.52</td>
<td>2.66</td>
</tr>
<tr>
<td></td>
<td>(0.12)</td>
<td>(0.16)</td>
<td>(0.16)</td>
</tr>
<tr>
<td>PL</td>
<td>2.80</td>
<td>2.82</td>
<td>2.63</td>
</tr>
<tr>
<td></td>
<td>(0.16)</td>
<td>(0.17)</td>
<td>(0.18)</td>
</tr>
</tbody>
</table>

Table 6.3  Respiratory exchange ratio (RER) and CHO oxidation rate (g·min\(^{-1}\)) during T1, recovery, and T2 in the CE and PL trials; values represent mean (± SEM)

\(^a\) \(P < 0.01\), CE vs. PL; \(^b\) \(P < 0.05\), CE vs. PL
<table>
<thead>
<tr>
<th>Run Time (min)</th>
<th>T1</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>Heart Rate (beat-min⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>149 (2)</td>
<td>154 (3)</td>
</tr>
<tr>
<td>PL</td>
<td>150 (2)</td>
<td>156 (2)</td>
</tr>
<tr>
<td>Rate of Perceived Exertion (Borg Scale)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>11 (1)</td>
<td>11 (1)</td>
</tr>
<tr>
<td>PL</td>
<td>11 (1)</td>
<td>11 (1)</td>
</tr>
</tbody>
</table>

Table 6.4  Heart rate (beat-min⁻¹) and rate of perceived exertion (Borg Scale) during T1 and T2 in the CE and PL trials; values represent mean (± SEM)

a P < 0.05, CE vs. PL
Fig. 6.2  Cumulative urine volume (ml) during recovery in the carbohydrate (CE) and placebo (PL) trials (mean±SEM)

- $P < 0.01$ from all other values
- $P < 0.01$ from 3 h
Fig. 6.3  Change of plasma volume (%) during T1, recovery, and T2 in the carbohydrate (CE) and placebo (PL) trials (mean±SEM)

\[ a \text{ P} < 0.01, \text{ from post T1 in both trials} \]
\[ b \text{ P} < 0.01 \text{ from 1 h in both trials} \]
Fig. 6.4  Blood glucose (mmol·l⁻¹) concentration during T1, recovery, and T2 in the carbohydrate (CE) and placebo (PL) trials (mean±SEM)

a P < 0.01, CE vs. PL
b P < 0.01 from 90 min of T1
c P < 0.05 from 1 h
d P < 0.05 from 0 min of T2 in CE trial
Fig. 6.5  Serum insulin (mU·l⁻¹) concentration during T1, recovery, and T2 in the carbohydrate (CE) and placebo (PL) trials (mean±SEM)

a P < 0.01, CE vs. PL
b P < 0.01 from post T1
Fig. 6.6  Blood lactate (mmol·l⁻¹) concentration during T1, recovery, and T2 in the carbohydrate (CE) and placebo (PL) trials (mean±SEM)

a $P < 0.05$ from 0 min of T2 in both trials
Fig. 6.7  Plasma free fatty acid (mmol·l⁻¹) concentration during T1, recovery, and T2 in the carbohydrate (CE) and placebo (PL) trials (mean±SEM)

a  P < 0.01, CE vs. PL
b  P < 0.01 from post T1
c  P < 0.01 from pre T2
Fig. 6.8 Mean skin temperature (°C) of the carbohydrate (CE) and placebo (PL) trials during T1 and T2 (mean±SEM)
Fig. 6.9  Rectal temperature (°C) of the carbohydrate (CE) and placebo (PL) trials during T1, recovery, and T2 (mean±SEM)

\[ a \ P < 0.05 \text{ from } 1 \text{ h during recovery in both trials} \]
Fig. 6.10  Rating of abdominal discomfort during T1, recovery, and T2 in the carbohydrate (CE) and placebo (PL) trials (mean±SEM)

\(^a\) P < 0.05 from 1 h in both trials
The main finding of this study was that positive fluid balance after prolonged running can be achieved by ingesting a prescribed volume of either a 6.9% CHO-electrolyte solution or a CHO-free sweetened placebo during a 4 h recovery period, calculated to replace ~170% of the body fluid loss. However, ingesting a CHO-electrolyte solution is more effective in restoring endurance capacity compared to the same volume of placebo solution. Performance during prolonged exercise is limited by a reduction, if not depletion, of the limited glycogen stores in the working muscles (Ahlborg et al., 1967b; Costill et al., 1988a; Tsintzas et al., 1996), and this may be compounded by dehydration. In the present study, since subjects had a similar hydration status after the REC in both conditions, the differences in performance during T2 between the placebo and CE trials can not be attributed to any difference in the degree of hydration level. Thus, it is reasonable to conclude that it was the provision of additional CHO which enabled the subjects to run longer during T2.

This improvement in subsequent endurance capacity following the ingestion of a CHO-electrolyte solution during short-term recovery confirmed the findings of Fallowfield et al. (1995). In that study, subjects ingested either 1.0 g CHO·kg⁻¹ body weight immediately after a 90 min run and then 2 h later, or an equal volume of a placebo solution at the same time points over a 4 h recovery period. Although their subjects were still hypohydrated before the second run in both conditions, they ran 22 min longer in the CHO trial. In the present study, subjects were in positive fluid balance in both trials after consuming approximately 170% of their body mass loss in T1. Despite the similar hydration levels, all nine subjects were able to run longer during T2 in the CE trial and the run time to exhaustion was 24.3 ± 4.4 min longer when compared with the PL trial. The difference in the amount of CHO ingested between the previous study and the present one was about 109 g. However, there is only ~2 min difference in the average run time to exhaustion between these two studies. Thus, it seems that excess CHO ingestion may not further improve the subsequent endurance capacity 4 h later. This suggestion is supported by the results of another similar study in which no differences were observed in run time during the subsequent exercise between the ingestion of 1.0 and 3.0 g CHO·kg⁻¹ body weight·h⁻¹ (Fallowfield, 1994).
The mechanism responsible for improving subsequent endurance capacity has been linked with the maintenance of a high rate of CHO oxidation (Fallowfield et al., 1995), which is the ergogenic effect of ingesting CHO-electrolyte solutions during a short-term recovery. Based on the CHO oxidation rate in the present study, it was estimated from indirect calorimetry that 234.2 ± 12.6 and 231.9 ± 10.9 g of CHO were oxidised during T1 in the CE and PL trials, respectively. During the REC, the fluid ingested provided 247.2 ± 16.1 g of CHO in the CE trial. This amount of CHO, if completely absorbed, would be sufficient to replace approximately 106% of that estimated to have been metabolised during T1. However, in the PL trial, only a negligible amount of CHO was contained in the placebo solution and this would, therefore, have not replaced the substantial amount of the CHO oxidised during T1 and, subsequently, very little glycogen synthesis would occur (MacDougall et al., 1977; Ivy et al., 1988a). As a result, the CHO drink administrated in the CE trial provided an optimal CHO replenishment during the short-term recovery.

The provision of CHO during the REC in the CE trial was also evident in the higher blood glucose concentrations and the greater CHO oxidation rates observed throughout the REC and T2. During T2, the RER indicated that the contribution of CHO oxidation to energy production was reduced by 25% in the PL trial, but increased 5% when ingesting the CHO-electrolyte solution during the REC. This reduction in CHO oxidation when ingesting the placebo was associated with a compensatory increase in fat metabolism during T2. The high insulin concentrations during the REC and T2 in the CE trial facilitated the uptake of blood glucose, as reflected by a reduction in blood glucose concentrations during the onset of T2. Thus, the higher blood glucose uptake and greater CHO oxidation during T2 in the CE trial resulted in the restoration of the energy capacity and, thereby, improved the subsequent exercise capacity. Furthermore, as the activity of glycogen synthase has been shown to be greater after prolonged exercise (Bak and Pedersen, 1990; Mæhlum and Hermansen, 1978; Mæhlum et al., 1977), the elevated and sustained increase of insulin with CHO ingestion was likely to optimise the muscle glycogen resynthesis.

Endurance exercise not only stresses the body's energy system, but also disturbs the body's fluid balance. The body mass loss during T1 in both trials was greater than the 2.0% threshold beyond which athletic performance appears to be impaired (Armstrong et al., 1985a). The levels of rehydration found in this study are in agreement with previously reported values (Maughan et al., 1994). In that study, a high Na⁺ concentration (61 mmol·l⁻¹) drink was ingested in a volume equal to 200% of the subjects' body fluid loss. At the end of the 6 h rehydration period, their subjects were
in positive fluid balance by 360 ± 205 ml. Considering the differences of the Na\(^+\) concentration of the test drinks between that study and the present study, it is reasonable to speculate that lower positive fluid balance and greater cumulative urine output would have occurred in the present study if the 4 h recovery period was extended to 6 h.

The combined active transport of glucose and Na\(^+\) across the gut membrane is very rapid which stimulates water absorption due to the osmotic action of these solutes (Schedl and Clifton, 1963; Levinson and Schedl, 1965; Ferrannini et al., 1982; Schedl, 1990). It might, therefore, be expected that a more complete rehydration would occur in the CE trial. One of the notable findings of the present study was that a similar positive fluid balance was achieved between treatments and no differences in the total urine output were observed at the end of the REC. Whilst Na\(^+\) concentration (Maughan and Leiper, 1995) and CHO content (Gisolfi et al., 1995) of the ingested fluid are believed to be the major regulators for the post-exercise rehydration process, the reason for the similar levels of rehydration between the CE and PL solutions in this study is unclear.

In order to maximise the rate of gastric emptying during the REC, a serial feeding schedule was used in this study. However, our subjects were not able to consume all the fluids prescribed. Since all subjects complained about the GI discomfort during T2, it is reasonable to assume that the gastric emptying rate was not fast enough for ingestion of such a large volume of fluid within a 4 h recovery period. When a fluid volume of 200% of body weight loss was prescribed within this period, it is noteworthy that 4 out of 9 subjects could not manage this large volume of fluid intake. Compared with the mean value of the fluid ingested in both conditions, which is approximately 170% of the fluid loss, these 4 subjects could only drink 134.3 ± 12.1% and 138.2 ± 20.8% of the fluid loss in the CE and PL trial, respectively. However, they still reported GI discomfort during T2 to a level which was similar to the responses of those subjects who had consumed all the fluid prescribed.

A large individual variability existed in the drinking capability of the subjects in this study, an observation which has been rarely reported in the scientific literature. However, this variability does make it very difficult, sometimes, to compare or interpret the results of other studies of this kind. For instance, one subject in this study only ingested 2260 ml of the 4000 ml prescribed fluid in one trial and his cumulative urine output was 380 ml which is 16.8% of the volume consumed. Another subject drank 5000 ml during the REC without any GI problems and, more surprisingly, his total urine output prior to the T2 was only 75 ml. The percent rehydration of this
particular subject was 191.3% of the fluid loss and he still completed T2 without severe GI discomfort. It appears that post-exercise rehydration with continued fluid and CHO ingestion needs to be carefully monitored in terms of GI responses if the recovery period between the bouts of exercise is relatively short. In the present study, although subjects achieved euhydration with both drinks after the REC, the reported GI discomfort during subsequent exercise indicates the practical constraints of ingesting such a large volume of fluid within a short period.

In conclusion, positive fluid balance can be achieved by ingesting a prescribed volume of either a 6.9% CHO-electrolyte solution or a CHO-free sweetened placebo, during a 4 h recovery period following prolonged running, calculated to replace ~170% of the body fluid loss. Despite a similar hydration status after the REC in both conditions, ingesting a CHO-electrolyte solution is more effective in restoring endurance capacity compared to the same volume of placebo solution.
CHAPTER 7

EFFECT OF INGESTING DIFFERENT AMOUNTS OF CARBOHYDRATE ON REHYDRATION DURING 4 HOURS RECOVERY AND SUBSEQUENT EXERCISE CAPACITY

7.1 INTRODUCTION

In the study reported in Chapter 5, it was found that ingesting a solution containing ~150 g of CHO, during a 4 h period following prolonged running, was more effective in terms of rehydration compared to the same volume of fluid containing only 50 g of CHO and water. Yet, this advantage did not have a greater effect on subsequent endurance capacity. These findings were unexpected because providing a greater amount of CHO after prolonged exercise was expected to be beneficial in replenishing the body's glycogen stores rapidly; thus the subsequent endurance capacity should have been enhanced. However, this was not the case. In the Chapter 5 study, subjects were not euhydrated in both trials prior to T2 and this dehydration may have confounded the benefits gained from the additional CHO ingested. Furthermore, subjects knew one of the solutions ingested was water which may have influenced their subsequent performance psychologically.

The results reported in Chapter 6 demonstrated that positive fluid balance could be achieved, and subsequent exercise capacity improved, when the volume of the CHO-electrolyte solution ingested was substantially greater (~170%) than the fluid loss. Nevertheless, GI discomfort during subsequent exercise indicated the practical constraints of ingesting a large volume of fluid within such a short period. For achieving rapid rehydration following prolonged, submaximal running, a fluid volume between 100% and 170% of the exercise-induced body weight loss would appear best. However, the volume consumed will be dependent upon the possible GI tolerances of individuals.
Therefore, in this study, it was intended to further investigate whether the ingestion of 50 g of CHO in the form of a CHO-electrolyte solution, calculated to replace 150% of fluid loss in order to achieve euhydration during the REC, would provide sufficient substrate for subsequent endurance exercise.

### 7.2 METHODS

#### Subjects

Nine endurance-trained male runners participated in this study. Their mean (± SEM) age, height, weight, maximum heart rate, and $\bar{V}O_2$ max were 34.3 ± 2.4 yr, 180.0 ± 1.5 cm, 70.4 ± 2.7 kg, 185 ± 3 beat·min⁻¹, and 58.9 ± 2.3 ml·kg⁻¹·min⁻¹.

#### Protocol

**Dietary Analysis**

A dietary record was obtained and analysed as previously described (Section 3.1.4)

**Preliminary Measurements**

After subjects became familiar with treadmill running and the experimental procedures, they performed a series of preliminary tests as previously described (Section 3.1.5)

**Experimental Design**

The standardised "recovery" protocol (Section 3.2) was used again in this study. The trials were conducted in a double-blind cross-over design and the runners were randomly assigned to each trial.

The first treadmill run (T1) required the subjects to run for 90 min at 70% $\bar{V}O_2$ max, or until volitional fatigue, whichever came first. Immediately after T1, the 4 h rehydration-recovery period (REC) started and fluid intake was confined to the REC. During the second treadmill run (T2), subjects were required to run at the same speed for as long as possible, such that their endurance capacity could be measured in terms of subsequent exercise time to fatigue (Fig. 7.1).
During the REC, a fixed volume of fluid equivalent to 150% of the body weight loss (g=ml) during T1 was consumed on both occasions. In order to provide 50 g of CHO, the subjects ingested 770 ml of a 6.5% CHO-electrolyte solution (Lucozade-Sport; Na+: 24 mmol·l⁻¹; K+: 2.6 mmol·l⁻¹; Osmolality: 300 mOsm·l⁻¹) 30 min after T1 in both experimental conditions as their first prescribed fluid intake. This volume of fluid was subtracted from body weight losses during T1 in order to calculate the remaining fluid intake. After this ingestion, the subjects drank either the same solution (CE) or a CHO-free sweetened placebo (PL) in equivolumetric measures at 30 min intervals up to the beginning of the 4 h of the REC. The test drinks ingested were of the same colour, texture and taste. As in the study reported in Chapter 6, this particular drinking schedule was designed to maximise gastric emptying (Rehrer et al., 1990; Noakes et al., 1991) and avoid GI discomfort during T2. The fluid was maintained at a temperature of 10-15°C and the overall time allowed for each ingestion was 10 min. Urine volumes were also measured at 30 min intervals during the REC. Whole body rehydration was estimated from the percent gain in body weight over the REC relative to weight loss during T1.

Dry bulb temperatures in the laboratory were 18.5 ± 0.4 and 18.4 ± 0.4°C during the CE and PL trial, respectively. Whereas relative humidity for the two occasions were 54.2 ± 1.6 and 55.5 ± 2.4%, respectively. While running on the treadmill, subjects were cooled by electric fans and wet sponges were also available for use ad libitum.

**Analyses**

Venous and capillary blood samples were collected, treated and analysed as previously described (Section 3.3). Expired air samples were collected for 1 min at 15 min intervals and during the last min prior to perceived exhaustion during T1 and T2. A 5-min sample was also obtained at each hour during the REC. Simultaneously, the subjective ratings of perceived exertion, perceived thirst and abdominal discomfort were recorded. The method of collection and analysis of expired air samples was the same as previously described (Section 3.1.3.3).

The performance times, total volume ingested, percent rehydration, and changes of body weight were compared using Student's *t*-tests for paired data. A two-way analysis of variance (ANOVA) for repeated measures on two factors (experimental treatment and time) was used to analyse overall differences between the physiological and blood biochemical responses in both trials. When a significant interaction was obtained, a Tukey post hoc test was used to identify differences between means. The accepted level of significance was set at *P* < 0.05. Data are reported as means ± SEM.
Fig. 7.1 Schematic representation of the experimental procedures
7.3 RESULTS

Exercise times to exhaustion

All subjects completed the 90 min run during T1 in both conditions. During T2, the run time to exhaustion was 56.9 ± 8.1 min in the CE trial (range: 29.5 - 113.5 min) and 65.4 ± 7.8 min in the PL trial (range: 27.9 - 105.6 min), respectively (NS).

Percentage $\dot{V}O_2\text{max}$, RER and CHO oxidation rate

The mean relative exercise intensities during T2 in the CE and PL trials were 69.7 ± 1.0 and 69.8 ± 1.1%, respectively (NS). There were no differences in the RER between trials during T1 (Table 7.3). Nevertheless, the RER values were higher from the 3-h sample point onwards during the REC and remained greater during T2 in the CE trial (P < 0.01), indicating a decrease in fat oxidation and enhanced CHO oxidation. The CHO oxidation rates during T1, as calculated from the $\dot{V}O_2$ and RER values, averaged 1.8 ± 0.2 and 1.7 ± 0.2 g·min⁻¹ for the CE and PL trials (NS). A greater amount of CHO was oxidised in the CE trial (0.2 ± 0.0 g·min⁻¹ and 2.2 ± 0.2 g·min⁻¹) during REC and T2 when compared with that in the PL trial (0.1 ± 0.0 g·min⁻¹ and 1.3 ± 0.1 g·min⁻¹) (P < 0.01) (Table 7.3). Thus, CHO oxidation contributed 55.8% and 54.6% of the total energy requirement of the CE and PL trials during T1, respectively (NS). However, during T2, a greater proportion of the total energy requirement was provided by CHO oxidation in the CE trial (75.8%), compared with 50.2% in the PL trial (P < 0.01).

Body mass changes, fluid balance and urine volume

As described in the Standard Experimental Procedures (Section 3.2), care was taken to ensure that subjects arrived at the laboratory in an euhydrated state on both occasions; the constancy of the pre-exercise body mass suggests that this was the case. After exercising for 90 min in T1, the subjects lost 2.5 ± 0.2% of their pre-exercise body mass in both trials (Table 7.1). Thus, the total volume of fluid prescribed was 2575 ± 198 ml and 2570 ± 148 ml for the CE and PL trials, respectively. All the subjects were able to ingest this volume of fluid over the REC in both conditions. In the CE trial, this fluid ingestion resulted in the consumption of 167.4 ± 12.9 g of CHO, compared with only 50 g of CHO in the PL trial (P < 0.01).
By the end of the REC, subjects were approximately euhydrated in both trials (CE: 0 ± 184 ml; PL: -27 ± 120 ml) (NS). Similarly, there were no differences in the percent rehydration between experimental treatments (Table 7.1) or in the fraction of the drink which was retained [CE: 64.2 ± 7.1% (range: 34.4 - 88.9%); PL: 64.3 ± 4.9% (range: 42.9 - 84.7%)] (Fig. 7.2).

Cumulative urine output was similar between trials at any time point during the REC (Fig. 7.3). The total volume of urine during the REC in the CE trial ranged from 140 to 1080 ml, whereas total volume of urine during the REC in the PL trial ranged from 190 to 1140 ml. No urine output was recorded during the first 1.5 h in both conditions and ~50% of the total urine volume was produced within the last 30 min during the REC in both trials. The total volume of urine represented 22.0 ± 6.1% and 24.4 ± 5.1% of fluid ingested during the CE and PL trial, respectively (NS).

**Plasma volume and blood changes**

Plasma volume decreased by 0.6 ± 1.8% and 0.7 ± 1.0% after T1 in CE and PL trials (NS) (Fig. 7.4). During the fluid ingestion period, plasma volume was restored in both conditions and remained elevated even at the end of T2. No significant treatment effects on plasma volume were observed at any time point in either trial.

Blood glucose was equally well maintained over T1 in both trials, increasing from 4.7 ± 0.1 to 5.2 ± 0.2 mmol·l⁻¹ in the CE trial and from 4.6 ± 0.2 to 5.4 ± 0.2 mmol·l⁻¹ in the PL trial (Fig. 7.5), whereas serum insulin decreased by ~29% in both conditions (Fig. 7.6). During the REC, blood glucose peaked 30 min after the feeding of the 50 g of CHO on both occasions (CE: 7.3 ± 0.5 mmol·l⁻¹; PL: 7.5 ± 0.4 mmol·l⁻¹) (NS). When continually ingesting the CHO-electrolyte solution in the CE trial, blood glucose remained higher over the REC (P < 0.01). However, concentrations did not differ prior to T2, but were higher at 30 min during T2 in the PL trial. No significant differences were found at the end of T2 in the two trials.
The pattern of changes on serum insulin were essentially similar to the blood glucose (Fig. 7.6). After a peak occurred at 1 h during the REC (CE: 39.3 ± 5.0 mU·l⁻¹; PL: 36.4 ± 4.5 mU·l⁻¹) (NS), significant differences in serum insulin were observed from 2 h over the REC (P < 0.01). Serum insulin was still higher in the CE trial prior to T2 (CE: 22.8 ± 3.7 mU·l⁻¹ vs. PL: 8.5 ± 2.0 mU·l⁻¹; P < 0.01) but there were no differences at the end of T2.

Blood lactate responses did not differ between the trials during T1 (Fig. 7.5). Although the values tended to be higher from 3 h during the REC in the CE trial, there were no differences between treatments. However, a greater lactate response was observed at 30 min in the CE trial during T2 (P < 0.01).

Plasma FFA doubled over T1 in both trials (P < 0.05), whereas plasma glycerol increased by fivefold (P < 0.05) (Fig. 7.7). During the REC, decreases in plasma FFA (P < 0.05) were greater in the CE trial, whilst FFA and glycerol concentrations remained elevated from the 3-h sample point onwards during the REC and T2 in the PL trial (P < 0.05). Following the onset of T2, plasma glycerol increased in the PL trial (P < 0.01). Plasma FFA remained elevated in the PL trial during T2, whereas concentrations increased in the CE trial (P < 0.01).

Serum Na⁺ increased during T1 (P < 0.05) but returned to initial levels after the REC in both trials (Table 7.2). There were no treatment effects at any time point between the trials. Similarly, serum K⁺ increased during exercise in T1 in both treatments (Table 7.2). Ingestion of the CHO-electrolyte solution resulted in a greater decrease on serum K⁺ concentrations during the REC (P < 0.05); however, no significant differences were observed at any time point between the two conditions. Serum osmolality was not different between trials during T1, REC and T2 (Table 7.2).

**Thermoregulatory responses**

Rectal temperature followed similar profiles during T1 and T2 (Fig. 7.8). During T1 in the CE trial, values ranged from 36.7 ± 0.1°C at the start of exercise to a maximum of 38.8 ± 0.3°C, and from 36.5 ± 0.1°C to 38.7 ± 0.2°C in the PL trial, whereas in T2, values ranged from 36.9 ± 0.1°C to 38.6 ± 0.2°C in the CE trial, and from 36.9 ± 0.1°C to 38.8 ± 0.2°C in the PL trial. No significant differences were observed between treatments. Although Trec tended to be higher at 3 h during the REC in the CE trial, there were no significant differences between trials.
Heart rate, perceived rate of exertion, thirst and abdominal discomfort

The pattern of change in exercising HR was similar during T1 in both trials (Table 7.4). However, at the end of the REC, HR were still higher (P < 0.05) and did not return to pre-T1 values in both conditions.

The ratings of perceived exertion (Table 7.5) and thirst did not differ between the two trials during T1, REC and T2.

Although a volume of 150% of fluid loss was prescribed on both occasions, subjects reported no GI discomfort during the REC. However, the ratings of abdominal discomfort increased following the onset of T2 in the two trials (P < 0.05), although no differences existed during T2 between the experimental treatments (Table 7.4).
<table>
<thead>
<tr>
<th></th>
<th>CE</th>
<th>PL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight after T1 (%)</td>
<td>- 2.5 (0.2)</td>
<td>- 2.5 (0.2)</td>
</tr>
<tr>
<td>Fluid ingested during REC (ml)</td>
<td>2575 (198)</td>
<td>2570 (148)</td>
</tr>
<tr>
<td>% Rehydration after REC</td>
<td>96.2 (10.7)</td>
<td>96.5 (7.3)</td>
</tr>
<tr>
<td>Cumulative urine during REC (ml)</td>
<td>567 (130)</td>
<td>627 (114) (NS)</td>
</tr>
<tr>
<td>Run Time in T2 (min)</td>
<td>56.9 (8.1)</td>
<td>65.4 (7.8) (NS)</td>
</tr>
</tbody>
</table>

Table 7.1  Body weight change, volume of fluid ingested, and fluid balance in the CE and PL trials; values represent mean (± SEM)
<table>
<thead>
<tr>
<th>Serum</th>
<th>T1</th>
<th>Recovery</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>1h</td>
</tr>
<tr>
<td>Sodium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>141 (1)</td>
<td>145 (2)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>142 (1)</td>
</tr>
<tr>
<td>PL</td>
<td>140 (1)</td>
<td>144 (1)</td>
<td>141 (1)</td>
</tr>
<tr>
<td>Potassium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>4.2 (0.1)</td>
<td>4.9 (0.1)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.3 (0.1)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PL</td>
<td>4.2 (0.1)</td>
<td>4.9 (0.1)</td>
<td>4.2 (0.1)</td>
</tr>
<tr>
<td>Osmolality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>293 (1)</td>
<td>304 (2)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>298 (2)</td>
</tr>
<tr>
<td>PL</td>
<td>294 (3)</td>
<td>305 (2)</td>
<td>301 (1)</td>
</tr>
</tbody>
</table>

Table 7.2 Serum sodium, potassium concentrations (mmol·l<sup>-1</sup>) and osmolality (mOsm·l<sup>-1</sup>) during T1, REC and T2 in the CE and PL trials; values represent mean (± SEM)

<sup>a</sup> P < 0.05 from Pre T1 in both trials; <sup>b</sup> P < 0.05 from Post T1 in both trials
<table>
<thead>
<tr>
<th>Run Time (min)</th>
<th>T1</th>
<th>Recovery</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>RER</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>0.91</td>
<td>0.88</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>(0.01)</td>
<td>(0.02)</td>
<td>(0.02)</td>
</tr>
<tr>
<td>PL</td>
<td>0.91</td>
<td>0.87</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>(0.01)</td>
<td>(0.02)</td>
<td>(0.02)</td>
</tr>
<tr>
<td>CHO Oxidation Rate (g·min⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>2.35</td>
<td>1.99</td>
<td>1.84</td>
</tr>
<tr>
<td></td>
<td>(0.18)</td>
<td>(0.22)</td>
<td>(0.22)</td>
</tr>
<tr>
<td>PL</td>
<td>2.28</td>
<td>1.88</td>
<td>1.72</td>
</tr>
<tr>
<td></td>
<td>(0.25)</td>
<td>(0.28)</td>
<td>(0.26)</td>
</tr>
</tbody>
</table>

Table 7.3  Respiratory exchange ratio (RER) and CHO oxidation rate (g·min⁻¹) during T1, recovery, and T2 in the CE and PL trials; values represent mean (± SEM)

a P < 0.01, CE vs. PL
<table>
<thead>
<tr>
<th>Run Time (min)</th>
<th>T1</th>
<th>Recovery</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>15</td>
<td>30</td>
<td>45</td>
</tr>
<tr>
<td>Heart Rate (beats·min⁻¹)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>49 (4)</td>
<td>149 (4)</td>
<td>153 (4)</td>
</tr>
<tr>
<td>PL</td>
<td>50 (3)</td>
<td>149 (3)</td>
<td>153 (3)</td>
</tr>
<tr>
<td>Rate of Abdominal Discomfort</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>-</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>PL</td>
<td>-</td>
<td>1 (1)</td>
<td>2 (1)</td>
</tr>
</tbody>
</table>

Table 7.4 Heart rate (beat·min⁻¹) and rate of abdominal discomfort during T1, recovery, and T2 in the CE and PL trials; values represent mean (±SEM)

- a P < 0.05 from 1 h in both trials; b P < 0.05 from Pre T1 in both trials; c P < 0.05 from 4 h
<table>
<thead>
<tr>
<th>Run Time (min)</th>
<th>Rate of Perceived Exertion (Borg Scale)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PL</td>
</tr>
<tr>
<td></td>
<td>CE</td>
</tr>
<tr>
<td>15</td>
<td>10 (1)</td>
</tr>
<tr>
<td>30</td>
<td>10 (1)</td>
</tr>
<tr>
<td>45</td>
<td>11 (1)</td>
</tr>
<tr>
<td>60</td>
<td>11 (1)</td>
</tr>
<tr>
<td>75</td>
<td>12 (1)</td>
</tr>
<tr>
<td>90</td>
<td>12 (1)</td>
</tr>
<tr>
<td>Exh</td>
<td>13 (1)</td>
</tr>
<tr>
<td></td>
<td>14 (1)</td>
</tr>
<tr>
<td></td>
<td>14 (1)</td>
</tr>
<tr>
<td></td>
<td>14 (1)</td>
</tr>
<tr>
<td></td>
<td>15 (1)</td>
</tr>
<tr>
<td>T1</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7.5: Rate of perceived exertion (Borg Scale) during T1 and T2 in the CE and PL trials; values represent mean (± SEM)
Fig. 7.2  Fate of the ingested volume during the 4 h recovery period in the CE and PL trials. The height of the graph represents the total amount of fluid consumed (ml).
Fig. 7.3 Cumulative urine volume (ml) during recovery in the carbohydrate (CE) and placebo (PL) trials (mean±SEM)

a $P < 0.01$ from all other values
Fig. 7.4 Change of plasma volume (%) during T1, recovery, and T2 in the carbohydrate (CE) and placebo (PL) trials (mean±SEM)

\( a \ P < 0.01 \) from post T1 in both trials
Blood glucose (mmol·l⁻¹) and blood lactate (mmol·l⁻¹) concentration during T1, recovery, and T2 in the carbohydrate (CE) and placebo (PL) trials (mean±SEM)

Fig. 7.5

- P < 0.01, CE vs. PL
- b P < 0.01 from 1 h
- c P < 0.01 from 30 min of T1
- d P < 0.01 from 3 h in the CE trial
Fig. 7.6 Serum insulin (mU·I\(^{-1}\)) concentration during T1, recovery, and T2 in the carbohydrate (CE) and placebo (PL) trials (mean±SEM)

- a P < 0.01, CE vs. PL
- b P < 0.01 from 1 h
- c P < 0.01 from 1 h
- d P < 0.01 from post T1
Fig. 7.7 Plasma FFA (mmol·l⁻¹) and plasma glycerol (mmol·l⁻¹) concentration during T1, recovery, and T2 in the carbohydrate (CE) and placebo (PL) trials (mean±SEM)

a P < 0.05, CE vs. PL
b P < 0.01 from 2 h
c P < 0.05 from 1 h
d P < 0.05 from Pre T1
e P < 0.01 from Pre T2
**Fig. 7.8** Rectal temperature (°C) of the carbohydrate (CE) and placebo (PL) trials during T1, recovery, and T2 (mean±SEM)

- **a** $P < 0.05$ from 10 min in both trials
- **b** $P < 0.01$ from 4 h in both trials
7.4 DISCUSSION

The main finding of this study was that ingestion of a placebo solution containing 50 g of CHO during a 4 h period following prolonged running, calculated to replace 150% of body fluid loss, is equally effective in achieving approximate euhydration and restoring endurance capacity compared to the same volume of a 6.5% CHO-electrolyte solution containing ~167 g of CHO.

In a previous study (Chapter 5) reported in this thesis, when subjects ingested only water after a feeding of 50 g of CHO, there was no improvement in endurance capacity between this and ingestion of a 6.9% CHO-electrolyte solution. This performance result was, however, unexpected because existing research (Doyle et al., 1993) suggests that the provision of greater amounts of CHO during the post-exercise period would promote a more rapid replenishment of muscle glycogen stores. The resultant increase in muscle glycogen concentrations at the beginning of T2 would be expected to result in an enhanced endurance capacity. However, an improvement in endurance capacity was not found. If there had been a difference in performance times in the study reported in Chapter 5, it could have been argued that it was due to psychological factors because subjects knew one of the test drinks was water. In the present study, although subjects followed a similar but more frequent feeding schedule and ingested an identical volume of sweetened placebo during the REC, no improvement in subsequent exercise capacity was observed, thus confirming the results reported in Chapter 5. It seems reasonable to conclude from these two studies that ingesting 50 g of CHO during the 4 h recovery period is equally effective in providing substrate during the subsequent exercise, compared to the amounts of ~167 g of CHO ingested.

The effectiveness of post-exercise energy repletion with CHO solutions may be limited by gastric emptying, intestinal absorption, systemic transport, cellular restoration and utilisation (Blom et al., 1987; Ivy et al., 1988b). Although gastric emptying is influenced mainly by the volume of the drink ingested, it is also determined by the CHO content of the fluid consumed (Rehrer et al., 1989; Noakes et al., 1991). Unlike the feeding schedule reported in Chapter 5, in which the test drinks were given at hourly intervals, a 30-min feeding regimen was employed in this study in an attempt to increase the gastric emptying rate and, thereby, enhance the intestinal absorption. Despite the possible slower rate of gastric emptying for the CE trial due to the higher energy content of the CHO solution, greater CHO absorption would be expected from this rehydration regimen.
It has been estimated that the upper limit of glucose absorption from the intestine in normal subjects is equivalent to \(-1.0 \text{ g·min}^{-1}\) (Radzuik and Bondy, 1982). In the present study, 50 g of CHO was ingested in the first hour in both trials during the REC. Another \(-47 \text{ g of CHO was consumed in the CE trial during both 2 and 3 h, whereas the last ingestion in the 4 h was \(-24 \text{ g of CHO. Thus, theoretically, if completely emptied from the stomach, the amounts of glucose ingested in both trials in the present study would be completely absorbed over the REC.}

During T1, an estimated \(-160 \text{ g of CHO was metabolised in fueling exercise. The available CHO absorbed during the CE trial would cover \(-104\% of that oxidised during T1, whereas \(-31\% of that estimated to have been oxidised during T1 would be covered by the PL feeding regimen. Despite the ingestion of greater amounts of CHO (~46 g) during the first 2-h period in the CE trial compared to the PL trial, the rate of muscle glycogen resynthesis would probably have been similar because the amounts of CHO ingested during this period in both trials was within the optimal range for glycogen resynthesis, i.e., 0.7 - 1.5 g·kg\(^{-1}\)·2 h body weight of CHO. Without information on the changes in muscle glycogen, it is also uncertain whether the CHO ingested during the second 2-h REC period in the CE trial would result in greater muscle glycogen resynthesis because, as mentioned previously, some of the fluid ingested might still be retained in the GI tract without being fully emptied and absorbed prior to T2, even though the present feeding conditions were intended to enhance this process. In fact, the GI discomfort reported in the T2 could indirectly confirm this incomplete emptying.

Despite the fact that the rate of muscle glycogen resynthesis may have been increased during the REC in the CE trial, the indirect respiratory and blood biochemical results presented another aspect of the recovery process. These metabolic changes were thought to have offset the beneficial effects, if any, of the greater muscle glycogen resynthesis in the CE trial. The most striking observations during the REC and T2 were the increased rate of CHO oxidation and the suppression of FFA when CHO solution was continually ingested over the REC. Both the results reported in Chapter 5 and the present study suggest that the pre-exercise hyperinsulinemia and the suppression of FFA during T2 may explain why the endurance capacity during T2 was not greater in the CE trial (Acheson et al., 1988; Costill et al., 1977). In the present study, insulin reached \(-40 \text{ mU·l}^{-1}\) following the first ingestion of the 50 g of CHO in both trials. Insulin levels then remained elevated throughout the REC in the CE trial and the concentration was \(-25 \text{ mU·l}^{-1}\) prior to T2. The increased serum insulin concentrations promote muscle glucose uptake but suppresses FFA mobilisation (Hargreaves et al., 1985).
In contrast, the ingestion of placebo following the first 50 g of CHO did not result in similar drastic changes in plasma FFA. Blood glucose and serum insulin concentrations were well regulated during T2, whereas increased plasma FFA and glycerol during T2 indicated the enhanced lipid mobilisation and utilisation. Since this 50 g of CHO was ingested 30 min after T1, it should have been completely emptied from the stomach and absorbed into the body during the REC. Thus, it appears that ingestion of 50 g of CHO during the first 2 h of the REC ensures adequate substrate availability during subsequent exercise but, simultaneously, does not induce an excessive CHO challenge to suppress FFA to a critical level.

Using an identical experimental protocol to investigate the influence of increasing CHO intake from 1.0 to 3.0 g CHO·kg⁻¹ body weight·2 h⁻¹ during a 4 h recovery, Fallowfield (1994) reported no difference in subsequent endurance capacity with increased CHO intake. Enhanced CHO oxidation and suppressed fat oxidation during the REC and the second run to exhaustion were also observed in that study when subjects ingested a greater amount of CHO. It appears that in addition to incorporation into muscle and liver glycogen, enhanced CHO oxidation is a major path of glucose disposal during the short-term recovery and subsequent exercise in the present study. Ivy et al. (1988b) similarly observed enhanced post-exercise CHO oxidation with a high CHO intake. It has been suggested that glucose in excess of immediate requirements is either oxidised (Blom et al., 1987), or converted to lactate and released from the cell (Blom, 1989). These suggestions may explain why there were elevated lactate responses during the REC in the present study when CHO was continually ingested over the REC.

Reduced blood glucose levels during exercise may increase the reliance on muscle glycogen as the CHO source for muscle contraction. As in the previous studies (Chapter 5 and 6) reported in this thesis, a rapid drop in blood glucose with the onset of T2 was observed when CHO was consumed throughout the REC. Although it was transient, its physiological influence on performance could be detrimental because it happened at a time when muscle glycogen stores were at a critical level. The onset of T2 in the presence of hyperinsulinemia, as in the CE trial, resulted in this rapid fall in blood glucose and, possibly, an increase in muscle glycogen utilisation (Costill et al., 1977; Hargreaves et al., 1985). Furthermore, reduced FFA availability, which was also observed during T2 in the CE trial, has been shown to increase muscle glycogen utilisation during exercise (Bergstrom et al., 1969; Costill et al., 1977). However, this increase in muscle glycogenolysis is absent if the insulin level at the onset of exercise is lower (Hargreaves et al., 1985), as the case during T2 in the PL trial.
A substantially larger volume of fluid is needed for complete rehydration over a short period after exercise-induced dehydration (Maughan and Shirreffs, 1994). The effects of drinking such a large volume of fluid on subsequent exercise capacity have been investigated in Chapter 6. In that study, subjects could not consume all the fluid prescribed (200% of fluid loss) and GI discomfort was reported during T2 in both trials. For the present study, subjects were able to drink the prescribed volume of fluid over the REC which was 1.5 times their fluid loss. Nevertheless, similar GI discomfort still occurred during T2, even though it did not deter the subjects from running to exhaustion in either trial. The findings of Chapter 6 and the present study are, therefore, in agreement with that of the previous studies where complete, or approximate, euhydration can occur when the volume consumed is substantially greater than the loss. Furthermore, the results from these two studies also demonstrate that subsequent exercise can be completed by following such rehydration regimens, despite the presence of tolerable GI discomfort during subsequent exercise. However, there is a huge individual variability in GI responses when drinking a large volume of fluid within a short period. The occurrence of GI distress during exercise could be detrimental. Nevertheless, by practising the drinking regimens during and between training sessions, the drinking-induced GI problems could be alleviated, or even avoided.

In conclusion, the ingestion of a placebo drink containing only 50 g of CHO over a 4 h period following prolonged running, calculated to replace body fluid loss, is equally effective in achieving approximate euhydration and restoring endurance capacity compared to the same volume of a 6.5% CHO-electrolyte solution containing -167 g of CHO.
CHAPTER 8

GENERAL DISCUSSION AND CONCLUSIONS

The principal aim of this thesis was to examine the influence of rehydration with CHO-electrolyte solutions, during a short-term recovery period, on hydration status, physiological responses, and subsequent running capacity.

Despite the vast body of published data addressing the effectiveness of different rehydration fluids after exercise-induced dehydration and the rate of muscle glycogen resynthesis over various time periods, there are surprisingly few published studies which have investigated whether the restoration of exercise capacity parallels the replenishment of body fluid and CHO stores. Therefore, based on previous research findings, a standardised "recovery" protocol was used throughout the studies reported in this thesis in order to examine the influence of rehydration on subsequent exercise capacity. The protocol involved a 90 min constant pace run at 70% \( \dot{V}O_2 \) max (T1), followed by the 4 h rehydration-recovery period (REC), and a subsequent open-ended run to exhaustion at the same exercise intensity (T2).

In order to assess the effects of ingesting a CHO-electrolyte solution during the REC on rehydration and subsequent exercise capacity, it is essential to know the severity of the physiological disturbances resulting from T1. After running at 70% \( \dot{V}O_2 \) max for 90 min in the standardised protocol, subjects lost \( \geq 2.5\% \) of their pre-exercise body mass (Table 8.1). From the Review of Literature (Chapter 2), it is clear that even low levels of dehydration (< 2%) have a detrimental effect on physical performance (Sawka, 1985; Walsh, et al., 1994). In addition to the exercise-induced dehydration, a significant decrease in muscle glycogen stores would also have occurred during such prolonged, submaximal running. Using similar procedures to measure constant pace exhaustive running at 70% \( \dot{V}O_2 \) max, Tsintzas et al. (1993) reported a mean run time of 109 min. Extrapolating from this result, it could be cautiously speculated that the 90 min run (T1) in the present experimental protocol taxed \( \sim 85\% \) of the endurance capacity of subjects. By measuring samples of single muscle fibres, Tsintzas et al. (1996) also reported that such exhaustive running resulted in decreases in muscle glycogen concentration from 317 to 31.6 mmol·kg\(^{-1}\) dry weight in Type I fibres, and from 443 to 103 mmol·kg\(^{-1}\) dry weight in Type II fibres. Expressing these values as a mean for mixed fibre samples, muscle glycogen concentration decreased from 380 to 67.8 mmol·kg\(^{-1}\) dry weight. This reduction represents a decrease of muscle glycogen
concentration by \(-82\%\). Thus, extrapolating once again from these reported data, T1 in the present studies would reduce muscle glycogen concentration by \(-68\%\).

The rate of muscle glycogen resynthesis is approximately \(5 - 7\) mmol\(\cdot\)kg\(^{-1}\)\(\cdot\)h\(^{-1}\) when \(\geq 50\) g of CHO is consumed every 2 h in the early stages of recovery (Ivy et al., 1988b; Blom et al., 1987). Thus, in the present studies, an estimated \(\geq 11\%\) of the muscle glycogen used during T1 was resynthesised during the REC when CHO-electrolyte solution was continually ingested. Furthermore, there is evidence that the rate of muscle glycogen resynthesis is 65% slower in the Type I fibres during the 0 - 90 min post-exercise period (Vollestad et al., 1989). The resultant deficit of muscle glycogen may, therefore, explain why fatigue occurred during subsequent exercise when blood glucose concentrations were still maintained the normal range (Fig. 8.1). Selective depletion of glycogen in Type I fibres, which are heavily recruited during prolonged exercise, is one of the main causes of fatigue during prolonged, submaximal running (Tsintzas et al., 1996). Thus, it appears that subsequent endurance running capacity, after 4 h recovery, is primarily determined by the replenishment of muscle glycogen, especially in Type I fibres, irrespective of the levels of rehydration reported in the present studies. However, gastric emptying and intestinal absorption may, in fact, have been limiting when a large amount of CHO was ingested within a short-term period, i.e. the CE trials in Chapter 6 (200% of fluid loss). It is possible that some of the solution ingested may still have been in the GI tract prior to T2, and was then slowly absorbed and used, as substrate for the working muscle during T2. This suggestion is supported indirectly by the higher blood glucose concentrations observed before and during T2 in this particular study.

One of the notable observations in the present studies was that running to exhaustion with or without CHO ingestion during the REC did not result in low blood glucose concentrations (Fig. 8.1). There was also no development of hypoglycaemic symptoms at the end of the exercise, a situation frequently observed at the end of exhaustive cycling. Total CHO oxidation rates also did not decline prior to fatigue, suggesting that CHO availability per se does not limit performance during prolonged, submaximal running (Tsintzas, 1993). Rather, as discussed previously, decreased glycogen availability within the Type I fibres appears to precipitate fatigue (Kirwan et al., 1988; Tsintzas, 1993, Tsintzas et al., 1995, 1996).
The provision of an adequate amount of CHO is not the only factor to be considered with respect to the recovery process. The ability to perform subsequent exercise is also influenced by fluid balance (Armstrong et al., 1985a). However, the phenomenon of "involuntary dehydration" has repeatedly been observed when individuals were allowed to drink *ad libitum* following exercise (Noakes et al., 1988; Greenleaf and Sargent, 1965). It was intriguing to find that the total volume of fluid ingested during the REC was similar when comparing the prescribed fluid intake with *ad libitum* drinking on rehydration (Chapter 4). The possible reason for this similar volume ingested may be the taste of the CHO-electrolyte solution used in this study. It has been shown that the palatability of fluid replacement beverages significantly contributed to rehydration because voluntary fluid intake increased with the perceived palatability of the drink (Boullé et al., 1983; Szlyk et al., 1987, 1989a). In addition, the results of this study also indicated that subsequent endurance capacity was enhanced when subjects followed the more balanced and frequent CHO feeding schedule, i.e. prescribed drinking. The prescribed fluid intake schedule was believed not only to promote rehydration, but also to produce a more consistent stimulus for glycogen resynthesis during the short-term recovery period. Based on the findings of this study, prescribed drinking was, therefore, adopted as a proven rehydration strategy for subsequent exercise in the other studies reported in this thesis.

When CHO was ingested at 1-h intervals, as in the CE trial in Chapter 5, the blood glucose and serum insulin (Fig. 8.1 and 8.2, 100%: CE vs. W) responses were transient, thus glycogen replenishment might not be optimal. It was then hypothesised that ingesting a larger amount of CHO in a more frequent feeding regimen (Fig. 8.2, 150%: CE vs. PL) could produce a more favourable condition for glycogen replenishment, thereby enhancing subsequent exercise capacity. This model was designed to maintain more stable elevations in blood glucose and insulin concentrations during the REC. However, as discussed previously, only limited muscle glycogen resynthesis could occur during this short-term period. It is also possible that the ~150 and ~167 g of CHO consumed over the REC in Chapter 5 and 7 may not have been completely emptied from the stomach and/or absorbed from the small intestine at the start of T2. This suggestion is supported by higher ratings of the abdominal discomfort during T2 (Fig. 5.11), as well as by the fact that the serum insulin concentration prior to T2 had not returned to pre-T1 levels. Thus, there may not be great differences in the repletion of muscle glycogen between these serial CHO feedings and the ingestion of only 50 g of CHO in the W and PL trials, as revealed in Chapter 5 and 7, respectively. Furthermore, the continued ingestion of the CHO solution over the REC resulted in increased rates of CHO oxidation during the REC and T2 (Table 8.1), and more importantly, an insulin-mediated suppression of plasma.
FFA during T2 (Fig 8.2) (Acheson et al., 1988). Both the pre-exercise hyperinsulinemia and the suppression of FFA during T2 may explain why the endurance capacity during T2 was not greater in the CE trials in these two studies.

In contrast, when subjects ingested only water (Chapter 5) or sweetened placebo (Chapter 7) following a feeding of 50 g of CHO at 30 min during the REC, blood glucose and serum insulin concentrations returned to normal levels prior to T2 and were well regulated during this test. Elevated plasma FFA and glycerol during T2 reflected enhanced mobilisation and utilisation (Fig. 8.2). An increase in plasma FFA concentration has been shown to decrease glucose uptake by the working muscles (Hargreaves et al., 1991). Thus, the greater oxidation of FFA may spare muscle glycogen stores, which were limited at that time, and hence contribute to maintain the endurance capacity. Furthermore, the ingestion of 50 g of CHO at 30 min after T1 allowed a further 3.5 h for gastric emptying and absorption of glucose to occur before the commencement of T2. Thus, it appears that ingesting 50 g of CHO at 30 min after T1 over a 4 h recovery period ensures substrate availability during subsequent exercise, but does not create the hyperglycaemic and hyperinsulinemic conditions which suppress FFA to a critical level during T2, after consumption of greater amounts of CHO.

Enhanced CHO oxidation and suppressed fat oxidation during a 4 h recovery and the subsequent run were also reported in a similar study when subjects ingested a larger amount of CHO (3.0 g CHO·kg⁻¹·body weight·2 h⁻¹) (Fallowfield, 1994). Similarly, there were no differences in run time during the subsequent exercise between the ingestion of 1.0 and 3.0 g CHO·kg⁻¹·body weight·2 h⁻¹ (Fallowfield, 1994). Ivy et al. (1988b) also observed enhanced post-exercise CHO oxidation with a high CHO intake. Thus, it appears that apart from incorporation into muscle and liver as glycogen, enhanced CHO oxidation is a major avenue of glucose disposal during the short-term recovery and subsequent exercise in the present studies.

Elevated blood lactate concentrations during the REC are possibly indicative of enhanced rates of "futile" CHO cycling (Bahr and Mæhlum, 1986). Blom et al. (1987) also suggest that glucose in excess of immediate requirements is either oxidised, or converted to lactate and released from the cell (Blom, 1989). This suggestion may explain why there were elevated lactate responses during the REC in the present studies when CHO was continually ingested over the REC.
Complete rehydration can occur during a short rehydration period after exercise-induced dehydration when the volume ingested was substantially greater than that loss (Maughan et al., 1994). However, the effects of ingesting such a large volume of fluid on subsequent exercise is unclear. The results reported in Chapter 6 clearly demonstrated that ingesting a volume of CHO-electrolyte solution equivalent to \( \sim 173\% \) of previous fluid loss during the REC improved subsequent endurance capacity compared to the same volume of placebo solution. A CHO-electrolyte solution achieved a similar level of rehydration over this period as was evident following the ingestion of a CHO-free placebo solution. Thus, differences in exercise capacity during T2 may be said to be due to the provision of CHO and electrolytes, as opposed to the body merely attaining complete rehydration. The suppression of FFA during T2 also occurred in this study when the CHO-electrolyte solution was ingested. However, the energy deficit induced by the decreased FFA oxidation was compensated by the large CHO provision during T2. Moreover, compared with the placebo solution in this particular study, greater muscle glycogen resynthesis should have occurred with the CHO-electrolyte solution during the REC. Therefore, there would have been considerable differences between these two experimental treatments in terms of substrate availability during T2.

The placebo trial in Chapter 6 is the only experimental condition in this thesis in which no CHO was provided during the REC. As expected, the run time during T2 of this trial was the shortest among all the studies reported (Table 8.1), even though subjects were euhydrated prior to T2 in this trial. These results, therefore, clearly demonstrate the importance of CHO as a substrate for energy metabolism during the short-term recovery and subsequent exercise, irrespective of the positive fluid balance achieved in both trials in this particular study.

It is of interest to note that there were no differences in percent rehydration and total urine volume between trials in Chapter 6 and 7 (Table 8.1). The Na\(^+\) concentration of the CHO-electrolyte solution was 24 mmol\(\cdot\)l\(^{-1}\) in these two studies. Thus, it was thought that more fluid would be retained in the body after the REC with the continued ingestion of the CHO-electrolyte solution compared to the placebo. Maughan and Leiper (1995) found a difference in fluid balance and urine output between two test drinks in which Na\(^+\) content was 2 and 26 mmol\(\cdot\)l\(^{-1}\). However, it was not the case in the present studies as similar hydration levels and urine volume output were observed between the CHO-electrolyte solution and placebo drinks. Although the Na\(^+\) content of the current CHO-electrolyte solution may be regarded as low, it was still within the range for rehydration solutions (Brouns et al., 1992). Furthermore, it seems that the addition of CHO in the fluid also made no difference in terms of
rehydration when compared to the placebo drinks, even though it was suggested that glucose was an important factor for enhancing intestinal water absorption (Gisolfi et al., 1995). The reason for these similar levels of fluid balance between the different drinks in these two studies is at present unclear. However, it may be related to the ingestion of such a large volume of fluid over a short period.

Because of the similar hydration levels, there were no differences in temperature and HR responses to exercise during T2 in the studies reported in Chapters 4, 6 and 7. However, in Chapter 5, higher HR were observed during T2 when rehydration was ~11% less after the REC when subjects drank only water following the ingestion of 50 g of CHO. Although the influence of dehydration per se was not reflected in the ingestion of 50 g of CHO. Although the influence of dehydration per se was not reflected in the subsequent run time in this particular study, this physiological perturbation did result in altered cardiovascular function during subsequent exercise. This finding is in agreement with the results of Montain and Coyle (1992b).

The current results reported in this thesis also indicate the importance of the timing of post-exercise CHO ingestion and the volume of fluid in optimising the rehydration process. Approximate (Chapter 7) or complete rehydration (Chapter 6) can be achieved by ingesting a substantially large volume of fluid which, as shown by the present studies, is ≥ 150% of fluid loss. The ingestion of such a large volume of fluid inevitably results in GI discomfort in some individuals. However, it is suggested that the occurrence of GI discomfort might be avoided by incorporating the rehydration strategies into an individual's training.

In conclusion, the studies reported in this thesis have provided evidence to support the suggestion that sufficient fluid replacement following exercise-induced dehydration maintains cardiovascular function and thermal balance during subsequent exercise. Thus, rapid restoration of the body's fluid balance plays an important role during post-exercise recovery. In order to achieve euhydration during recovery, a volume of fluid substantially larger (~150%) than that lost must be ingested. The provision of additional CHO (~150 to 170 g) would be expected to restore the body's CHO stores to a greater extent than a smaller amount of CHO (50 g) during the REC, and thereby, improve the subsequent endurance capacity. However, this was not the case. It appears that the ingestion of large amounts of CHO, during the REC, may have resulted in metabolic disturbances in the fat and CHO substrate provision which prevented an improvement in subsequent endurance capacity during T2. Further research is needed to elucidate how to limit the metabolic disturbances and hormonal changes induced by different rehydration regimens during the short-term recovery and subsequent exercise. The role of muscle glycogen during the subsequent prolonged


exercise in combination with different levels of rehydration also warrants further investigation.
<table>
<thead>
<tr>
<th>Variables</th>
<th>Chapter 4 (100% of fluid loss)</th>
<th>Chapter 5 (100% of fluid loss)</th>
<th>Chapter 6 (200% of fluid loss)</th>
<th>Chapter 7 (150% of fluid loss)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run Time in T2 (min)</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>PI: 69.9 ± 9.1</td>
<td>CE: 54.2 ± 9.2</td>
<td>CE: 69.3 ± 5.5</td>
<td>CE: 56.9 ± 8.1</td>
<td></td>
</tr>
<tr>
<td>AL: 60.2±10.2 (P &lt; 0.05)</td>
<td>W: 52.2±6.2 (NS)</td>
<td>PL: 45.0±4.2 (P &lt; 0.01)</td>
<td>PL: 65.4±7.8 (NS)</td>
<td></td>
</tr>
<tr>
<td>%ΔBM after T1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI: 2.6 ± 0.2</td>
<td>CE: 2.8</td>
<td>CE: 3.0 ± 0.1</td>
<td>CE: 2.5 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>AL: 2.5±0.3 (NS)</td>
<td>W: 2.6 (NS)</td>
<td>PL: 3.2±0.3 (NS)</td>
<td>PL: 2.5±0.2 (NS)</td>
<td></td>
</tr>
<tr>
<td>%ΔPV after T1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI: - 3.8</td>
<td>CE: - 4.5</td>
<td>CE: - 2.6</td>
<td>CE: - 0.6</td>
<td></td>
</tr>
<tr>
<td>AL: - 3.3 (NS)</td>
<td>W: - 4.1 (NS)</td>
<td>PL: - 1.2 (NS)</td>
<td>PL: - 0.7 (NS)</td>
<td></td>
</tr>
<tr>
<td>Volume Ingested (ml)</td>
<td></td>
<td></td>
<td>Volume Prescribed &amp; Ingested</td>
<td>Volume Prescribed &amp; Ingested</td>
</tr>
<tr>
<td>(6.9% CHO drink)</td>
<td>(6.9% CHO drink)</td>
<td>Volume Ingested (NS)</td>
<td>(6.5% CHO drink)</td>
<td></td>
</tr>
<tr>
<td>% Rehydration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI: 53.6 ± 7.6</td>
<td>CE: 73.5 ± 4.2</td>
<td>CE: 123.7 ± 10.6</td>
<td>CE: 96.2 ± 10.7</td>
<td></td>
</tr>
<tr>
<td>AL: 42.7±7.6 (NS)</td>
<td>W: 63.0±5.7 (P &lt; 0.05)</td>
<td>PL: 125.3±10.0 (NS)</td>
<td>PL: 96.5±7.3 (NS)</td>
<td></td>
</tr>
<tr>
<td>Total Urine Volume (ml)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of Fluid: PI: 30</td>
<td>% of Fluid: CE: 14.58</td>
<td>% of Fluid: CE: 17.8</td>
<td>% of Fluid: CE: 22.0</td>
<td></td>
</tr>
<tr>
<td>AL: 37 (NS)</td>
<td>W: 18.57 (NS)</td>
<td>PL: 18.2 (NS)</td>
<td>PL: 24.4 (NS)</td>
<td></td>
</tr>
<tr>
<td>CHO Ingested (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI: 103±10</td>
<td>CE: 148.8±17.7</td>
<td>CE: 247.2±16.1</td>
<td>CE: 167.4±12.9</td>
<td></td>
</tr>
<tr>
<td>AL: 97±18 (NS)</td>
<td>W: 50 (P &lt; 0.05)</td>
<td>PL: 0</td>
<td>PL: 50 (P &lt; 0.05)</td>
<td></td>
</tr>
<tr>
<td>CHO Oxidation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI: T1 - 60%</td>
<td>CE: T1 - 72.5%</td>
<td>CE: T1 - 74.2%</td>
<td>CE: T1 - 55.8%</td>
<td></td>
</tr>
<tr>
<td>T2 - 59%*</td>
<td>T2 - 77.3%*</td>
<td>T2 - 79.3%*</td>
<td>T2 - 75.8%*</td>
<td></td>
</tr>
<tr>
<td>AL: T1 - 60%</td>
<td>W: T1 - 69.2%</td>
<td>PL: T1 - 74.3%</td>
<td>PL: T1 - 51.6%</td>
<td></td>
</tr>
<tr>
<td>T2 - 61%</td>
<td>T2 - 52.9%</td>
<td>T2 - 49.3%</td>
<td>T2 - 50.2%</td>
<td></td>
</tr>
<tr>
<td>(NS from AL)</td>
<td>(P &lt; 0.05, CE vs. W)</td>
<td>(P &lt; 0.05, CE vs. PL)</td>
<td>(P &lt; 0.05, CE vs. PL)</td>
<td></td>
</tr>
</tbody>
</table>

Table 8.1 Summary of results of the chapters reported.
Mean blood glucose (mmol·l⁻¹) concentration during T1, recovery and T2 when different feeding schedules were employed, as described in the chapters reported.

Chapter 5: 100% of fluid loss, CE vs. W (50 g CHO and Water)
Chapter 6: 200% of fluid loss, CE vs. PL (CHO-free Placebo)
Chapter 7: 150% of fluid loss, CE vs. PL (50 g CHO and CHO-free Placebo)
Fig. 8.2  Mean serum insulin (mU·l⁻¹) and plasma FFA (mmol·l⁻¹) concentration during T1, recovery and T2 when different feeding schedules were employed, as described in the chapters reported.

Chapter 5: 100% of fluid loss, CE vs. W (50 g CHO and Water)
Chapter 6: 200% of fluid loss, CE vs. PL (CHO-free Placebo)
Chapter 7: 150% of fluid loss, CE vs. PL (50 g CHO and CHO-free Placebo)
REFERENCES


References


References


References


References


References


References


References


Appendix A: Statement of Informed Consent

LOUGHBOROUGH UNIVERSITY OF TECHNOLOGY
Department of Physical Education,
Sports Science and Recreation Management

Rehydration-Recovery Study
Statement of Informed Consent

I have read the above outline of procedure and requirements which are involved with this study and I understand what is required of me. I have had opportunity to ask for further information and for clarification of the demands of each of the procedures. I am aware that I have the right to withdraw from the study at any time with no obligation to give reasons for my decision.

I agree to take part in the study

Name ___________________________ Phone No. ___________________________

Age ___________________________ Date Of Birth ___________________________

Contact Address _______________________________________________________

Signed ___________________________ Witnessed by ___________________________

Date ___________________________
Appendix B: Medical History Questionnaire

NAME ........................................ SS/ ................................ AGE ......... DATE .........

ADDRESS ........................................ TELEPHONE (home) ................................

........................................ TELEPHONE (office) ................................

........................................ DATE OF BIRTH ................................

OCCUPATION ................................. PLACE OF EMPLOYMENT .............................

MARITAL STATUS: MARRIED ......... SINGLE .........

DOCTOR ........................................ ADDRESS ........................................

CHECK YES OR NO

PAST HISTORY
(Have you ever had?)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatic fever/heart murmur</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High blood pressure</td>
<td></td>
<td></td>
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<tr>
<td>Any heart trouble</td>
<td></td>
<td></td>
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<tr>
<td>Disease of arteries</td>
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<tr>
<td>Varicose veins</td>
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<tr>
<td>Lung disease</td>
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<tr>
<td>Asthma</td>
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<tr>
<td>Kidney disease</td>
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<tr>
<td>Gout</td>
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<tr>
<td>Diabetes</td>
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<tr>
<td>Epilepsy</td>
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<td></td>
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<tr>
<td>Thyroid disease</td>
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</table>

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

<table>
<thead>
<tr>
<th>Condition</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart attacks,</td>
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<td></td>
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<tr>
<td>who</td>
<td></td>
<td></td>
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<tr>
<td>age</td>
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<tr>
<td>High blood pressure</td>
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<td>High cholesterol</td>
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<tr>
<td>Stroke</td>
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<tr>
<td>Diabetes</td>
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<tr>
<td>Congenital heart disease</td>
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<tr>
<td>Heart operations</td>
<td></td>
<td></td>
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<tr>
<td>Early death</td>
<td></td>
<td></td>
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<tr>
<td>Other family illnesses</td>
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</tbody>
</table>

PRESENT SYMPTOMS REVIEW
(Have you recently had?)

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Yes</th>
<th>No</th>
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</thead>
<tbody>
<tr>
<td>Chest pain/discomfort</td>
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<td></td>
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<tr>
<td>Shortness of breath</td>
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<td>Heart palpitations</td>
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<tr>
<td>Skipped heart beats</td>
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<tr>
<td>Cough on exertion</td>
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<tr>
<td>Coughing of blood</td>
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<tr>
<td>Dizzy spells</td>
<td></td>
<td></td>
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<tr>
<td>Frequent headaches</td>
<td></td>
<td></td>
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<tr>
<td>Frequent colds</td>
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<td></td>
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<tr>
<td>Recurrent sore throat</td>
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<tr>
<td>Back pain</td>
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<tr>
<td>Arthritis/swollen, stiff, painful joints</td>
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<tr>
<td>Orthopaedic problems</td>
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<tr>
<td>Unexplained weight loss (&gt; 5 lb)</td>
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</table>

Are you presently taking any medications?     []  []

HOSPITALIZATIONS
Yes ...... No ......
Year Reason

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

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

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<thead>
<tr>
<th>Medication</th>
<th>Reason for Taking</th>
<th>How Long?</th>
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</thead>
<tbody>
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</tbody>
</table>
Appendices

Do you currently smoke? Yes .... No ....
If so, what? glasses How much? drinks

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 Beers Wine Wine glasses Hard Liquor drinks

How much caffeinated beverage do you consume per day?
What type? Coffee Coffee Tea Tea cups Soft Drinks drinks

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 ............... [ ] Poor ............... [ ]
Very good ................... [ ] Very poor ................... [ ]
Good ........................... [ ] Very, very poor .............. [ ]
Neither good nor poor ........ [ ]

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

<table>
<thead>
<tr>
<th></th>
<th>NEVER</th>
<th>Seldom</th>
<th>Sometimes</th>
<th>Frequently</th>
<th>Constantly</th>
</tr>
</thead>
<tbody>
<tr>
<td>I feel sad or depressed</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I am under considerable stress</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I feel tense and anxious</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I worry about things</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I have an intense desire to achieve</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I am a restless sleeper</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I am worried about my health</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I feel like I cannot cope with daily stress</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I feel like I need to get away</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>
Appendices

Appendix C: Composition of 6.5 and 6.9% CHO-Electrolyte Solution
(Lucozade Sport, SmithKline Beecham Plc)

<table>
<thead>
<tr>
<th></th>
<th>6.5%</th>
<th>6.9%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextrose (g)</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Fructose (g)</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Maltose (g)</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Higher Saccharides</td>
<td>3.3</td>
<td>3.7</td>
</tr>
<tr>
<td>Sodium (mg•100⁻¹ ml)</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>Potassium (mg•100⁻¹ ml)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Calcium (mg•100⁻¹ ml)</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Magnesium (mg•100⁻¹ ml)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Osmolality (mOsm•kg⁻¹)</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Energy Value (kJ•100⁻¹ ml)</td>
<td>120</td>
<td>120</td>
</tr>
</tbody>
</table>

Note: Non-carbonated
Appendix D: Food Record Diary

CONFIDENTIAL

NAME AND ADDRESS

.................................................
.................................................
.................................................
.................................................
.................................................
.................................................

SEX

M
F

AGE............ YRS

DATE OF START OF DIET........../........../......

FOOD RECORD DIARY

Please record everything you eat and drink during the next seven days. Instructions and an example are given inside.

Information about your diet will be treated in confidence and results will be returned to you as soon as possible.

Loughborough University
Department of Physical Education and Sports Science
Dietary Analysis Service
Loughborough
Leics. LE11 3TU
INSTRUCTION FOR USING THE FOOD DIARY

Everything that you eat and drink over the next seven days should be weighed and the weight and type of food or drink recorded.

For solid foods, the food should be placed on the scale on a plate or container. The plate or container must be weighed empty first and the scales can then be zeroed. Each item of food can then be added to the plate and weighed individually, returning the scales to zero between each item.

eg. Plate 150g, zero scale.
Roast Beef 100g, zero scale.
Potato 150g, zero scale.
Gravy 30g, zero scale.

For drinks, a cup or glass must first be weighed and then the scale can be returned to zero and the drink added. Please remember to record separately the weight of tea, milk and sugar put into a drink.

Do not forget to weigh and record second helpings and between meal snacks.

Any leftovers (eg apple cores) should also be weighed and recorded in the leftovers column.

Eating Out – Most people eat foods away from home each day, please do not forget to record these. Take your diary and scales with you where ever it is possible. If this is too inconvenient just record the type of food eaten with an estimated weight - but please say when a weight has been estimated.

Most snack foods will have the weight of the food on the packet so they do not need weighing if you eat the whole packet yourself.

Names and descriptions of foods should be as detailed as possible, including the brand name and any other information available.

eg. Cheese – is insufficient information.

Cheese, cheddar (Shape reduced fat) – is sufficient information.

Start a new page in your diary for each day, and record each item on a separate line. Record the time of day in the first column of each line.

eg. 10.30 am Mcvities Biscuits (2) 50g

Digestive

The space provided at the foot of each page for general comments is for you to give any further information about your diet and your training/activity for that day.

eg. Steady run, morning 1 hour.
Missed lunch due to stomach pains.

A full example sheet is given over page showing how to record a days food and how to fill in the comments section.

Please try to be as accurate as possible and try to choose a fairly typical week to record. For instance do not record a week when you are on holiday or when you are ill, if you feel that this would alter your normal diet or activities.
Please use a separate line for each item eaten; write in weight of plate; leave a line between different plate entries.

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>OFFICE USE ONLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 am</td>
<td>Kelloggs</td>
<td>Cornflakes</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Milk (whole)</td>
<td>120</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hot water</td>
<td>160</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Milk (whole)</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sugar white</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 am</td>
<td>Porridge</td>
<td>Coffee powder</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hot water</td>
<td>160</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Milk (whole)</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sugar white</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 pm</td>
<td>Hot sliced bread</td>
<td>4 slices</td>
<td>120</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Margarine</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Toothpaste</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cheese</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Apple (Ganny Smith)</td>
<td>140</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tea</td>
<td>180</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Milk (whole)</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sugar</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 pm</td>
<td>Scone (individual)</td>
<td>160</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chips (frozen)</td>
<td>150</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peas (boiled)</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Custard (toasted)</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mr Kipling Apple pie (individual)</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Birds Custard (toasted)</td>
<td>150</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GENERAL COMMENTS:

am Short run - 3 miles
pm Task session 10 x 400 m
Appendix E: Subjective Feelings Questionnaire

Name: ___________________________ Date: ____________

Trial: ____________________________

QUESTIONNAIRE

A. Did you suffer any of the following symptoms during the middle or towards the end of the race?

1) Nausea                      YES / NO    Comments:

2) Headache                    YES / NO    Comments:

3) Light headedness            YES / NO    Comments:

4) Loss of concentration       YES / NO    Comments:

5) None of the above           YES / NO

6) Other symptoms. Please specify:

B. Which of the following symptoms did your legs feel during the end of the race?

1) Heavy                      Yes / No

2) Weak at the knees           Yes / No

3) Painful                    Slightly: Yes / No Mildly: Yes / No Severely: Yes / No

4) Soreness                   Slightly: Yes / No Mildly: Yes / No Severely: Yes / No

5) Other symptoms. Please specify:

C. Did you experience any Gastrointestinal discomfort during the race?

Yes / No    Comments:

D. General comments about the taste, volume, etc. of the ingested fluid.

Comments:
Appendix F: Lactic Acid Assay

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

\[
\text{Lactate} + \text{NAD} \xrightarrow{\text{LDH}} \text{Pyruvate} & \text{NADH}
\]

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 μl LDH
   - for each ml of hydrazine (200 μl 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

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.
Appendices

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 µl of either the standard or supernatant was then transferred into a glass fluorimeter tube, whereupon 200 µl 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.
Appendix G: Glucose Assay

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

(i) Glucose + O2 + H2O → Gluconate + H2O

(ii) H2O + ABTS → Coloured Complex + H2O

Solutions *

(a) Perchloric acid: 25% w/v
(b) Phosphate buffer: 100.0 mmol·l⁻¹, pH 7.0
(c) P. Od: > 0.8 U·m⁻¹
(d) G. Od: < 10.0 U·m⁻¹
(e) ABTS: 1.0 mg·m⁻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

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

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 µl 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 (Mode 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:

\[ c = 5.55 \times \frac{A_{\text{sample}}}{A_{\text{standard}}} \ (\text{mmol}\cdot\text{l}^{-1}) \]
Appendix H: Haemoglobin Assay

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

\[
\text{Haemoglobin} + \text{Cyanide} + \text{Ferricyanide} \rightarrow \text{Cyanmethaemoglobin}
\]

Solutions*

Drabkin's 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 \(+15^\circ\)C to \(25^\circ\)C if stored in a brown glass bottle.

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

Procedure

1. 20 µl 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 1101 M) 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:

\[
c = (37.2\times A) + 0.06 \quad (g\cdot 100\, ml^{-1})
\]
Appendix I: Glycerol Assay

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

Solutions

(a) Zinc sulphate: 6.25 g ZnSO$_4$ - 7H$_2$O (mw 287.54) in 250 ml of DW
   0.087 M

(b) Barium hydroxide: 6.55 g Ba(OH)$_2$ - 8H$_2$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 1.0 M
   (Kept at 48°C) (wt·ml$^{-1}$ 1.03 g) in 250 ml DW (64% solution), with
   1.5 mM MgCl$_2$, i.e. 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
   -i.e. 0.4 g NaOH with 372.24 mg EDTA made up to 1000 ml with DW
Standards

1 Prepare approximately 4.0 mM solution, i.e. 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 ...

<table>
<thead>
<tr>
<th>0.4 mM (ml)</th>
<th>distilled water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% ... is ...</td>
<td>0.25</td>
</tr>
<tr>
<td>20%</td>
<td>0.5</td>
</tr>
<tr>
<td>40%</td>
<td>1.0</td>
</tr>
<tr>
<td>60%</td>
<td>1.5</td>
</tr>
<tr>
<td>80%</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Procedure

1 Pipette 0.25 ml zinc sulphate into small centrifuge tubes.

2 Add 50 µl 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 µl of supernatant into acid-washed fluorimetric tubes.

6 Add 100 µl 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.
Appendix J: Electrolytes (Na⁺, K⁺)

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

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 (i.e., 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 µl of sample or standard into bijou bottles.
2 Add 5 ml of 15 mmol·l⁻¹ lithium solution, mix and read.