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CARBOHYDRATE INTAKE, MUSCLE METABOLISM, AND ENDURANCE RUNNING PERFORMANCE IN MAN

by

KONSTANTINOS CHRYSSANTHOPoulos

A Doctoral Thesis

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

July 1995

This work is dedicated to the memory of my father
Ev oide oti, oudein oide
Sokrates, 5os aiwonas p.χ

The only thing I know is that I know nothing
Socrates, 5th century B.C
ABSTRACT

The purpose of this thesis was to study the effects of a pre-exercise carbohydrate meal on metabolism, endurance capacity and performance during prolonged running when carbohydrate was, or was not consumed during exercise.

The first study (Chapter 4) examined the effects on endurance running capacity of ingesting a carbohydrate-electrolyte solution during treadmill exercise to fatigue at 70% VO2 max after subjects (10 males) had undergone an overnight fast (P+C), or when fed with a 2.5 g.kg⁻¹ BW carbohydrate meal 3 hours before exercise (M+C). Exercise time to exhaustion was longer in the M+C (147.4 ± 9.6 min) and P+C (125.1 ± 7.0 min) trials compared with the control condition (P+P: 115.1 ± 17.6 min) (p< 0.01 and p< 0.05 respectively). Also, exercise time was longer in the M+C compared with the P+C trial (p< 0.01). The improvement in endurance capacity in the M+C trial occurred despite a higher carbohydrate oxidation rate during the first hour of exercise.

The second study (Chapter 5) examined whether a pre-exercise carbohydrate meal (M+W) can improve endurance capacity, and further examined if the combination of a pre-exercise meal together with the ingestion of a carbohydrate-electrolyte solution during exercise (M+C) would be superior to the carbohydrate meal (M+W) alone. Ten males volunteered in this study. Although the consumption of the meal increased carbohydrate oxidation during the first hour of exercise, exercise time to fatigue at 70% VO2 max was longer in the M+C (125.1 ± 5.3 min) and M+W (111.9 ± 5.6 min) trials compared with the control trial (P+W : 102.9 ± 7.9 min) (p< 0.01 and p< 0.05 respectively). Also, exercise time was longer (p< 0.05) in the M+C compared with the M+W trial.

The third study (Chapter 6) investigated whether the high carbohydrate meal can influence muscle glycogen levels. Eight male subjects participated in the study. Three hours after the ingestion of the 2.5 g.kg⁻¹ BW carbohydrate meal, muscle glycogen concentration was 10.6% higher (p< 0.05) in the vastus lateralis muscle (347.3 ± 31.3 mmol.kg dw⁻¹) compared with the muscle glycogen concentration before feeding (314 ± 33.9 mmol.kg dw⁻¹).

The fourth study (Chapter 7) examined the influence of ingesting a carbohydrate-electrolyte drink (M+C) on the muscle glycogen utilisation during 60 min running at 70% VO2 max in subjects (8 males) who had consumed a carbohydrate meal 3 hours before exercise (M+W). Muscle glycogen concentrations were not different before (M+C : 321.9 ± 27.2 vs M+W : 338.8 ± 32.8 mmol.kg dw⁻¹), as well as after exercise (M+C : 225.8 ± 26.7 vs M+W: 261 ± 40.5 mmol.kg dw⁻¹) between the two experimental trials. Neither was there any difference in the rate of muscle glycogen utilisation (M+C : 96.1 ± 22.1 vs M+W : 77.9 ± 11.7 mmol.kg dw⁻¹.hr⁻¹).

The aim of the last study (Chapter 8) was to investigate whether, after an overnight fast, the ingestion of a carbohydrate-electrolyte solution during a 30
Self-paced treadmill run (C) would be as effective as the consumption of a carbohydrate meal (M) (2.0 g.kg\(^{-1}\) BW carbohydrate) 4 hours before exercise. Ten males volunteered for this study. The overall performance times in the M and C trials were identical (M: 121.8 ± 3.6 min vs C: 121.7 ± 4.1 min). No differences were found between the two trials in running speeds over each successive 5 km, or even when running speed was analysed every kilometre. Also, no reduction in the self-selected speeds of subjects was observed towards the end of the 30 km run in both conditions.

The ingestion of a carbohydrate meal, providing 2.5 g.kg\(^{-1}\) BW carbohydrate, 3 hours before exercise increases muscle glycogen concentration and improves endurance running capacity, despite an elevated carbohydrate oxidation rate during the first hour of exercise. It seems that the amount of carbohydrate given before exercise compensates for the greater carbohydrate used. Furthermore, the combination of both a pre-exercise carbohydrate meal and a carbohydrate-electrolyte solution ingested during exercise further improves endurance capacity.
Unless otherwise indicated the work contained in this thesis is that of the author and has not previously submitted for another degree in this or any other University.

Part of the work contained in this thesis has been published as follows:

**Published Communications**


**Published Paper**

ACKNOWLEDGEMENTS

This work was partially supported by SmithKline Beecham and a studentship from Loughborough University of Technology.

I am particularly indebted to my supervisor, Professor Clyde Williams not only for giving me the opportunity to undertake this research and for making financially possible for me to complete my studies, but for his continuous encouragement, support and wise guidance throughout the course of my studies. Professor Clyde Williams was always next to me inside and outside the laboratory not only in the good times, but also in some very difficult times especially during the final stage of this work.

This thesis would not have been possible without the support, encouragement, and understanding of my parents Georgia and Ioannis. I would especially like to thank my wife Angeliki for encouraging me to undertake this research and for her patience, and understanding all these years.

The valuable time and expertise given by Dr. Adrea Nowitz who performed the muscle biopsies and catheterisation required for this thesis is gratefully acknowledged. My thanks also go to Dr. Leslie Boobis for performing some of the biopsies in the study presented in Chapter 7.

I wish to thank my colleagues Lucy Asher, Lynda Hearne, Christina Kotsiopoulou, Veronica Vleck, and Maria Nute for their invaluable assistance during the experimental days. Special thanks also go to Dr. Wendy Wilson for her assistance in the first study of this thesis, and to Miss Lisa Piearce for analysing the dietary information obtained from the subjects.

I am grateful to Dr. Gregory Bogdanis for his help in the analysis of muscle samples in Chapter 6, and to Dr. Kostas Tsintzas and Miss Natassa Tsetsonis for their assistance during the 'busy' biopsy studies and for their friendship and support.

I would like to thank Dr. Henryk Lakomy for rescuing me with his technical expertise on the occasions, invariably at the most inconvenient times, when
the treadmill decided to break down.

The assistance of Dr. Alan Nevill in the statistical analysis of the data and the encouragement of my director of research Professor Harry Thomason are gratefully acknowledged.

I would like to acknowledge Dr. Paul Greenhaff (Medical School, Nottingham University) for giving me access to his laboratory for the weighing of the muscle tissue.

Many thanks to all my colleagues in the Sports Science Research Group for their support and friendship throughout my studies in Loughborough.

Finally, I want to express the debt I owe to all the individuals who gave their time and considerable efforts during their participation in these studies. Without their commitment this work would never have been completed.
# LIST OF CONTENTS

1. **INTRODUCTION**  

2. **REVIEW OF LITERATURE**  
   2.1 Introduction  
   2.2 Carbohydrate metabolism and prolonged exercise  
      2.2.1 Regulation of carbohydrate metabolism  
      2.2.2 Exogenous carbohydrates during exercise and carbohydrate metabolism  
   2.3 Fatigue in prolonged exercise  
      2.3.1 Central fatigue  
      2.3.2 Peripheral fatigue  
   2.4 The effects of carbohydrate ingestion before exercise on exercise metabolism, endurance capacity and performance  
      2.4.1 Postprandial metabolic and respiratory responses of a carbohydrate load  
      2.4.2 Pre-exercise carbohydrate feedings and exercise metabolism  
      2.4.3 The influence of pre-exercise carbohydrate feedings on endurance capacity and performance  
   2.5 The influence of carbohydrate ingestion during exercise on endurance capacity and performance  
      2.5.1 Cycling studies  
      2.5.2 Running studies  
      2.5.3 Possible mechanisms by which carbohydrate ingestion during exercise improves endurance capacity and performance  
   2.6 Influence of fluid loss and fluid ingestion during exercise on temperature regulation  
   2.7 Gastric emptying rate and intestinal absorption of carbohydrate feedings  
      2.7.1 Gastric emptying rate of liquids  
      2.7.2 Intestinal absorption of liquids  
      2.7.3 Gastric emptying and absorption of solids  
   2.8 Summary
3. GENERAL METHODS
3.1 Introduction 47
3.2 Preliminary measurements 47
   3.2.1 Familiarisation 48
   3.2.2 Body weight, height, heart rate, and rectal temperature measurements 48
   3.2.3 Collection and analysis of expired air samples 48
   3.2.4 Speed-Oxygen uptake test 49
   3.2.5 Maximal oxygen uptake test (V\text{O}_2 \text{ max}) 49
   3.2.6 Speed-Lactate test 49
   3.2.7 Diet and training control 50
3.3 Experimental protocol 50
   3.3.1 Endurance capacity protocol 51
   3.3.2 Endurance performance protocol 51
   3.3.3 Carbohydrate feedings 51
3.4 Collection, treatment, and analysis of blood samples 53
   3.4.1 Blood sample collection 53
   3.4.2 Treatment and analysis of blood samples 54
3.5 Collection, treatment, and analysis of muscle samples 57
   3.5.1 Collection of muscle samples 57
   3.5.2 Treatment and analysis of muscle samples 57

4. INFLUENCE OF A PRE-EXERCISE MEAL AND A CARBOHYDRATE-ELECTROLYTE SOLUTION ON ENDURANCE RUNNING CAPACITY: COMPARISON WITH A CARBOHYDRATE-ELECTROLYTE SOLUTION 60
4.1 Introduction 60
4.2 Methods 61
4.3 Results 65
4.4 Discussion 75

5. INFLUENCE OF A PRE-EXERCISE MEAL AND A CARBOHYDRATE-ELECTROLYTE SOLUTION ON ENDURANCE RUNNING CAPACITY: COMPARISON WITH A PRE-EXERCISE MEAL 80
5.1 Introduction 80
5.2 Methods 80
5.3 Results 85
6. THE EFFECTS OF A HIGH CARBOHYDRATE MEAL ON SKELETAL MUSCLE GLYCOGEN CONCENTRATION
   6.1 Introduction
   6.2 Methods
   6.3 Results
   6.4 Discussion

7. CARBOHYDRATE INGESTION DURING RUNNING AND SKELETAL MUSCLE GLYCOGEN UTILISATION IN FED HUMANS
   7.1 Introduction
   7.2 Methods
   7.3 Results
   7.4 Discussion

8. COMPARISON BETWEEN CARBOHYDRATE FEEDINGS BEFORE AND DURING EXERCISE ON ENDURANCE RUNNING PERFORMANCE
   8.1 Introduction
   8.2 Methods
   8.3 Results
   8.4 Discussion

9. GENERAL DISCUSSION

REFERENCES

APPENDICIES
LIST OF FIGURES

4.1 Schematic illustration of the experimental protocol 64
4.2 Respiratory exchange ratio (RER) during the M+C, P+C, and P+P trials (mean ± SE; n=9) 71
4.3 Carbohydrate oxidation rate (g.min⁻¹) during the M+C, P+C, and P+P trials (mean + SE; n=9) 71
4.4 Blood glucose concentration (mmol.l⁻¹) in M+C, P+C, and P+P trials (mean ± SE; n=9) 72
4.5 Blood lactate concentration (mmol.l⁻¹) in M+C, P+C, and P+P trials (mean ± SE; n=9) 72
4.6 Serum insulin concentration (mU.l⁻¹) in M+C, P+C, and P+P trials (mean ± SE) 73
4.7 Plasma FFA concentration (mmol.l⁻¹) in M+C, P+C, and P+P trials (mean ± SE) 73
4.8 Plasma glycerol concentration (mmol.l⁻¹) in M+C, P+C, and P+P trials (mean ± SE) 74
5.1 Schematic illustration of the experimental protocol 84
5.2 Blood glucose concentration (mmol.l⁻¹) during the postprandial period in the M+C, M+W, and P+W trials (mean ± SE) 93
5.3 Serum insulin concentration (µU.l⁻¹) during the postprandial period in the M+C, M+W, and P+W trials (mean ± SE; n=9) 93
5.4 Plasma FFA concentration (mmol.l⁻¹) during the postprandial period in the M+C, M+W, and P+W trials (mean ± SE; n=9) 94
5.5 Plasma glycerol concentration (mmol.l⁻¹) during the postprandial period in the M+C, M+W, and P+W trials (mean ± SE; n=9) 94
5.6 Respiratory exchange ratio (RER) in the M+C, M+W, and P+W trials (mean ± SE; n=8) 95
5.7 Carbohydrate oxidation rate (g.min⁻¹) in M+C, M+W, and P+W trials (mean ± SE; n=8) 95
5.8 Blood glucose concentration (mmol.l⁻¹) during exercise in the M+C, M+W, and P+W trials (mean ± SE; n=9) 96
5.9 Serum insulin concentration (µU.l⁻¹) during exercise in the M+C, M+W, and P+W trials (mean ± SE; n=8) 96
5.10 Plasma FFA concentration (mmol.l⁻¹) during exercise in the M+C, M+W, and P+W trials (mean ± SE; n=8) 97
5.11 Plasma glycerol concentration (mmol.l⁻¹) during exercise in the M+C, M+W, and P+W trials (mean ± SE; n=8) 97
6.1 Schematic illustration of the experimental protocol of experiment I 107
6.2 Blood glucose concentration (mmol.l⁻¹; mean ± SE) 112
6.3 Serum insulin concentration (mU.l⁻¹; mean ± SE) 112
6.4 Plasma FFA concentration (mmol.l⁻¹; mean ± SE) 113
6.5 Blood lactate concentration (mmol.l⁻¹; mean ± SE) 113
7.1 Schematic illustration of the experimental protocol 121
7.2 Blood glucose concentration (mmol.l⁻¹) during exercise in the M+C and M+W trials (mean ± SE) 128
7.3 Serum insulin concentration (mU.l⁻¹) during exercise in the M+C and M+W trials (mean ± SE) 128
7.4 Plasma FFA concentration (mmol.l⁻¹) during exercise in the M+C and M+W trials (mean ± SE) 129
7.5 Plasma glycerol concentration (mmol.l⁻¹) during exercise in the M+C and M+W trials (mean ± SE) 129
8.1 Schematic illustration of the experimental protocol 138
8.2 Speed (m.s⁻¹) per kilometer during the M and C trials (mean ± SE) 145
8.3 Speed (m.s⁻¹) per 5 km during the M and C trials (mean ± SE) 145
8.4 Blood glucose concentration (mmol.l⁻¹) during the postprandial period (mean ± SE) 146
8.5 Blood lactate concentration (mmol.l⁻¹) during the postprandial period (mean ± SE) 146
8.6 Blood glucose concentration (mmol.l⁻¹) during exercise in the M and C trials (mean ± SE) 147
8.7 Blood lactate concentration (mmol.l⁻¹) during exercise in the M and C trials (mean ± SE) 147
8.8 Plasma FFA concentration (mmol.l⁻¹) during exercise in the M and C trials (mean ± SE) 148
LIST OF TABLES

2.1 Studies that have examined the influence of carbohydrate ingestion or infusion during exercise on skeletal muscle glycogen utilisation 14

2.2 Studies that have examined the influence of pre-exercise carbohydrate feedings on skeletal muscle glycogen utilisation during exercise 27

2.3 Studies that have examined the influence of pre-exercise carbohydrate feedings on endurance capacity 28

2.4 Studies that have examined the influence of pre-exercise carbohydrate feedings on endurance performance 29

2.5 Studies that have examined the effects of carbohydrate ingestion during cycling on endurance capacity 35

2.6 Studies that have examined the effects of carbohydrate ingestion during cycling on endurance performance 36

2.7 Studies that have examined the effects of carbohydrate ingestion during running on endurance capacity 37

2.8 Studies that have examined the effects of carbohydrate ingestion during running on endurance performance 38

3.1 Coefficient of variation (C.V %) of blood, plasma, and serum metabolite assays (n =15) 56

3.2 Coefficient of variation (C.V %) of muscle metabolite assays (n =15) 59

4.1 Average daily energy intake, carbohydrate (CHO), fat, and protein consumed during the 2 days prior to each trial (mean ± SE) 67

4.2 Oxygen uptake (V̇O₂), heart rate (HR), and perceived rate of exertion (PRE) during the M+C, P+C, and P+P trials (mean ± SE; n=9) 68

4.3 Gut fullness (GFS), and abdominal discomfort (ADS) during the M+C, P+C, and P+P trials (mean ± SE; n=9) 69

4.4 Rectal temperature (°C) during the M+C, P+C, and P+P trials (mean ± SD; n=4) 69

4.5 Serum sodium and potassium concentrations (mmol.l⁻¹) in the M+C, P+C, and P+P trials (mean ± SE) 70

5.1 Average daily energy intake, carbohydrate (CHO), fat, and protein consumed during the 2 days prior to each trial (mean ± SE) 88

5.2 Gut fullness (GFS), and abdominal discomfort (ADS) during the
postprandial period (mean ± SE) 88

5.3 Gut fullness (GFS), and abdominal discomfort (ADS) during exercise (mean ± SE; n=9) 89

5.4 Oxygen uptake (\(\dot{V}O_2\)), heart rate (HR), and perceived rate of exertion (PRE) during the M+C, M+W, and P+W trials (mean ± SE; n=9) 90

5.5 Oxygen uptake (\(\dot{V}O_2\)), heart rate (HR), and perceived rate of exertion (PRE) during the M+C, M+W, and P+W trials (mean ± SE; n=9) 91

5.6 Blood lactate, plasma urea, serum sodium, and serum potassium concentrations (mmol.l\(^{-1}\)) during exercise (mean ± SE; n=8) 91

5.7 Plasma adrenaline and plasma noradrenaline concentrations (nmol.l\(^{-1}\)) in the M+C, M+W, and P+W trials (mean ± SE) 92

5.8 Adrenaline to insulin concentration (A/I) and noradrenaline to insulin concentration (NA/I), ratios in the M+C, M+W, and P+W trials (mean ± SE; n=8) 92

6.1 Muscle glycogen, glucose, G-6-P, Lactate, ATP, PCr, and Cr concentrations (mmol.kg dry weight\(^{-1}\)) in vastus lateralis muscle before and 3 hours after the ingestion of the meal (mean ± SEM, n=6) 110

6.2 Oxygen consumption (\(\dot{V}O_2\)), respiratory exchange ratio (RER), carbohydrate oxidation rate (CHO Oxid. Rate), and fat oxidation rate (Fat Oxid. Rate) in the F and M trials (mean ± SE) 111

7.1 Average daily energy intake, carbohydrate (CHO), fat, and protein consumed during the 3 days prior to each trial (mean ± SE) 123

7.2 Oxygen uptake (\(\dot{V}O_2\)), respiratory exchange ratio (RER), carbohydrate oxidation rate (CHO Oxid. Rate), heart rate (HR), perceived rate of exertion (PRE), gut fullness (GFS), and abdominal discomfort (ADS), during the M+C and M+W trials (mean ± SE) 124

7.3 Blood lactate, serum sodium, and serum potassium concentrations (mmol.l\(^{-1}\)) in the M+C, and M+W trials (mean ± SE) 125

7.4 Muscle metabolites (mmol.kg dm\(^{-1}\)) before (Pre) and after (Post) the 60-min treadmill run in the M+C and M+W trials (mean ± SE) 126

7.5 Treadmill running at 70 % \(\dot{V}O_2\) max for one hour. Comparison of physiological characteristics of subjects (mean ± SE) 127

7.6 Pooled muscle glycogen data (mmol.Kg dm\(^{-1}\)) from control trials from two studies (mean ± SE) 127
8.1 Average daily energy intake, carbohydrate (CHO), fat, and protein consumed during the 2 days prior to each trial (mean ± SE) 141

8.2 Oxygen uptake (\(\dot{V}O_2\)), respiratory exchange ratio (RER), carbohydrate oxidation rate (CHO Oxid. Rate), gut fullness (GFS), and abdominal discomfort (ADS) during the postprandial period (mean ± SE) 142

8.3 Oxygen uptake (\(\dot{V}O_2\)), respiratory exchange ratio (RER), carbohydrate oxidation rate (CHO Oxid. Rate), heart rate (HR), perceived rate of exertion (PRE), gut fullness (GFS), and abdominal discomfort (ADS) during exercise (mean ± SE) 143

8.4 Serum insulin, plasma glycerol, plasma ammonia, plasma urea, serum sodium and serum potassium concentrations before feeding and before and immediately after exercise in the M and C trials (mean ± SE) 144
CHAPTER 1

INTRODUCTION

More than half a century ago Christensen and Hansen (1939) demonstrated the importance of a high carbohydrate diet in performing prolonged exercise. The re-introduction of the Duchene needle biopsy technique by Bergstrom (1962) gave the opportunity to scientists to directly investigate metabolic alterations in the muscle cell during various types of activities. It was then realised that the onset of fatigue during prolonged heavy exercise (i.e: > 65% \( \dot{V} \text{O}_2 \text{max} \)) was associated with a marked reduction in the glycogen content in exercising muscle (Ahlborg et al. 1967; Hermansen et al. 1967). Furthermore, it was observed that with a combination of diet and exercise muscle glycogen stores were supercompensated (Bergstrom and Hultman 1966), a factor which could lead to enhanced endurance performance (Karlsson and Saltin, 1971).

Since then, various methods of enhancing and maintaining body carbohydrate stores have been employed in an attempt to delay the onset of fatigue, improve endurance performance, and speed-up the recovery from prolonged heavy exercise. The supercompensation diet nowadays is a standard recommendation for the endurance athlete (Costill 1988; Sherman 1983). Carbohydrate feedings during exercise have also become a very popular practice among athletes. There is cumulative evidence that carbohydrate feedings during exercise delay the onset of fatigue and improve performance during endurance activities (Coggan and Coyle 1991; Maughan 1991).

However, fatigue during prolonged exercise performed in a hot (i.e: > 30\( ^\circ \) C) environment may develop as a result of disturbances in thermoregulation and fluid balance rather than a reduced availability of body carbohydrate stores. Carbohydrate feedings during exercise, in the form of carbohydrate-electrolyte solutions, seem to help in reducing the environmental and exercise-induced disturbances in thermoregulation and fluid homeostasis (Coyle and Montain 1993; Lamb and Brodowicz 1986).

Therefore, the nutritional practices of consuming a high carbohydrate diet the days before an important endurance activity, and the ingestion of
carbohydrate during this activity are well accepted as nutritional aids to the endurance athlete (Williams and Devlin 1992). However, there is still some hesitation regarding the consumption of carbohydrate the hour before an endurance event. Early studies on this area concluded that ingesting carbohydrate 30 min before exercise is detrimental to endurance capacity (Costill et al. 1977; Foster et al. 1979). On the other hand, recent studies have shown that pre-exercise carbohydrate feedings in a liquid form may, or may not improve endurance capacity and performance, but do not exert any detrimental effect upon them (Sherman 1991).

Nevertheless, for social and practical reasons, athletes need to consume food rather than single nutrients in liquid form. To the best of the author's knowledge two studies have examined the influence of pre-exercise carbohydrate food on endurance capacity (Thomas et al. 1991), and performance (Sparks et al. 1994) during cycling. There is a lack of information regarding the effects of pre-exercise carbohydrate food on exercise metabolism, endurance capacity and performance during running exercise.

Therefore, the primary purpose of this thesis was to study the effects of a pre-exercise carbohydrate meal on metabolism, endurance capacity and performance during prolonged running.

This thesis is presented in eight main chapters. The review of literature (Chapter 2) provides a brief summary of the available literature regarding the effects of carbohydrate feedings before and during exercise on metabolism, temperature regulation, endurance capacity and performance during prolonged exercise. The possible causes of fatigue as well as the factors influencing the availability of the ingested carbohydrate are also examined. The general methodology employed in this thesis is outlined in Chapter 3.

The main aim of the first two studies (Chapters 4 and 5) were (a): to examine if a pre-exercise carbohydrate meal can influence endurance running capacity when a carbohydrate-electrolyte solution is ingested during exercise, (b): to examine if a pre-exercise carbohydrate meal can improve endurance running capacity compared to an overnight (15 h) fast, (c): to examine if endurance capacity is further improved when a pre-exercise meal is combined with a carbohydrate-electrolyte solution ingested during exercise, and (d): to study
some of the physiological and metabolic responses during exercise when carbohydrate feedings are ingested before and/or during exercise.

The main purposes of the next two studies (Chapters 6 and 7) were (a): to examine the influence of a high carbohydrate meal on skeletal muscle glycogen concentration, and (b): to examine the influence of a carbohydrate-electrolyte solution on skeletal muscle glycogen use when a carbohydrate meal is ingested before exercise.

The aim of the last study (Chapter 8) was to investigate whether, after an overnight fast, the ingestion of a carbohydrate-electrolyte solution during a 30 km self-paced race would be as effective as the consumption of a carbohydrate meal before running.

Finally, the last chapter of this thesis (Chapter 9) summarises the main findings of all the studies conducted and provides some ideas about further research.
2.1 Introduction

The aim of this chapter is to provide a brief review of the available literature regarding the effects of carbohydrate feedings before and during exercise on metabolism, endurance capacity, and performance during prolonged exercise.

This chapter has been divided into six main sections. The first part (section 2.2) outlines the regulation of carbohydrate metabolism. The influence of exogenous carbohydrate on carbohydrate metabolism is also presented. Section 2.3 gives a brief description about the possible causes of fatigue in prolonged exercise, whereas the next two parts deal with the influence of carbohydrate ingestion before (section 2.4) and during exercise (section 2.5) on metabolism, endurance capacity and performance. Section 2.6 examines the influence of fluid loss and fluid ingestion during exercise on temperature regulation and fluid homeostasis. Finally, section 2.7 reviews the factors that influence gastric emptying rate and intestinal absorption of liquids and solids.

2.2 Carbohydrate Metabolism and Prolonged Exercise

Any movement performed by the human body is the result of muscular contraction produced by movement between the myofilaments. This muscular contraction is powered by chemical energy released from hydrolysis of ATP. However, the amount of ATP present in the muscle at any instant is so small that it must be continuously replenished from other energy sources if muscular contraction is to be maintained.

The main energy sources for ATP resynthesis during prolonged exercise are fats and carbohydrates (Gollnick 1985), although there is evidence that proteins have a small contribution (Dohm 1986; Romijn and Wolfe 1992) which may be as high as 10% of the overall body energy requirements.
(Butterfield 1991). The relative contribution of fats and carbohydrates depends on factors such as the intensity and duration of exercise, the diet and carbohydrate status of the body, the state of physical training (Gollnick 1985), the environmental conditions (Young 1990), or even the gender especially among less trained individuals (Ruby and Robergs 1994). During prolonged exercise, requiring an oxygen uptake above 60% VO$_2$ max, carbohydrates and in particular muscle glycogen seem to be the most important fuels for energy provision (Saltin and Karlsson 1971).

2.2.1 Regulation of carbohydrate metabolism

The provision of energy from carbohydrates to the working muscle can be summarised within three main metabolic pathways: (a) breakdown of muscle glycogen to glucose-6-phosphate (G-6-P) (muscle glycogenolysis), (b) the glucose uptake by the muscle and its phosphorylation to G-6-P (glucolysis), and (c) the breakdown of G-6-P to pyruvate (glycolysis). The pyruvate formed, is oxidised through the Kreb's cycle depending on oxygen availability.

**Muscle glycogenolysis**

Muscle glycogen is broken down to glucose-1-phosphate (G-1-P) under the influence of the enzyme phosphorylase. Glycogenolysis is a regulated process since phosphorylase, at time of low metabolic demand such as rest, exists in its inactive form phosphorylase b. The activation of glycogenolysis is linked to the contractile activity of the muscle fibers. At the onset of contraction, a rapid elevation in free calcium and alkalisation occurs in muscle. These events activate the enzyme phosphorylase b kinase, which converts phosphorylase b to its active form phosphorylase a (Gollnick 1988). Phosphorylase activity produces G-1-P, which in turn is converted to G-6-P by the enzyme phosphoglucomutase. Since phosphorylase catalyses the flux-generating step for muscle breakdown, factors influencing its activity may influence the overall flux of the metabolic pathway.

Except calcium, an elevated concentration of AMP and IMP in contracting muscle may influence phosphorylase activity, especially in fast-twitch fibers.
(Richter et al. 1986). Also, inorganic phosphate (Pi) has been suggested as one of the main factors determining phosphorylase activity (Chasiotis et al. 1982). However, Ren and Hultman (1989) observed a high Pi content and extensive transformation of phosphorylase b to the a form but low glycogenolytic activity. Also, as exercise progresses there is a decline in phosphorylase a despite the fact that glycogenolysis continues (Cartier and Gollnick 1985; Conlee et al. 1979). These observations seem to suggest that factors other than the transformation of phosphorylase b to a may be involved in the regulation of glycogenolysis.

There is evidence that adrenaline acts as a stimulator of muscle glycogenolysis in both animals (Richter et al. 1981; 1982) as well as in humans (Chasiotis et al. 1983; Greenhaff et al. 1991; Jansson et al. 1986; Spriet et al. 1988). Furthermore, the rate of muscle glycogen utilisation seems to be affected by the initial muscle glycogen concentration (Gollnick et al. 1972; Richter and Galbo 1986; van Hall et al. 1994; Hargreaves et al. 1995).

However, when considering the net muscle glycogen breakdown, one should take into account factors that may influence glycogen synthesis. It has been found in rats that glycogen synthesis during exercise is a dynamic process which is not necessarily associated with extensive glycogen depletion (Hutber and Bonen 1989). Insulin is a powerful stimulator of muscle glycogen synthesis and a high insulin concentration decreases the breakdown of glycogen during muscle contractions in vitro (Berger et al. 1976 cited by Richter et al. 1986). However, the extent to which the decrease in plasma insulin concentration, that occurs during exercise, can influence muscle glycogen breakdown has not been directly studied (Richter et al. 1986).

In addition, as exercise continues there is a reduction in the rate of muscle glycogen breakdown (Gollnick et al. 1973) and an increase FFA contribution to energy metabolism (Ahlborg et al. 1974). Elevated plasma FFA levels may decrease muscle glycogen utilisation (Costill et al. 1977; Hickson et al. 1977), although this is not always the case (Hargreaves et al. 1991). Therefore, when considering whole body metabolism during prolonged exercise, there may be shifts in the way in which glycogenolysis is regulated (Gollnick 1988).

Finally, there is a different pattern of glycogen breakdown in the different muscle fiber types. The slow-twitch muscle fibers (Type I) seem to be
recruited more during the initial periods of moderate intensity exercise. As exercise continues and Type I muscle fibers become depleted, there is an increased glycogen breakdown in the fast-twitch muscle fibers (Type II) (Essen 1978; Gollnick et al. 1973; Vollestad et al. 1984; Tsintzas 1993).

Hepatic glucose production, uptake and phosphorylation

Another source of G-6-P formation in the muscle is hepatic blood glucose. Liver is considered as the main reservoir for glucose storage, mainly in the form of glycogen, which releases glucose into the bloodstream for use by various tissues. In liver, glycogen is broken down to G-6-P which is converted to glucose. This glucose is transported into the bloodstream, taken by the muscle and phosphorylated to G-6-P.

- Hepatic glucose production
Liver glycogenolysis appears to have a curvilinear relationship with running speed in rats (Winder 1985). In humans little direct information is available regarding liver glycogenolytic rate during exercise. In a group of subjects who exercised one hour on a cycle ergometer liver glycogen was found to be about 145 mmol/kg wet liver tissue lower than in people who did not exercise (Hultman and Nilsson 1971). Assuming linearity, the liver glycogenolytic rate for the exercised subjects was 2.4 mmol/kg/min, whereas in the resting state was about 0.3 mmol/kg/min (Hultman and Nilsson 1971).

Hepatic glucose production is related to intensity and duration of exercise. The reported values of glucose production during exercise above resting levels vary greatly depending on the sampling intervals and the method used. At 60-80% \( \dot{V}O_2 \) max glucose production has been reported to be from 116% up to 360% above resting values (Romijn and Wolfe 1992). Also, hepatic glucose production is probably suppressed by a low carbohydrate diet (Hultman and Nilsson 1973; Bjorkman and Eriksson 1983).

Several mechanisms have been suggested for the regulation of glycogenolysis in the liver: glucagon or adrenaline induced activation of adenylate cyclase, adrenaline or noradrenaline induced increase in cytoplasmic free calcium via alpha-adrenergic receptors with consequent allosteric activation of phosphorylase kinase, and insulin inhibition of the action of catecholamines
and glucagon on hepatic glycogenolysis (Winder 1985). These mechanisms, however, have been identified in vitro studies. In vivo, it has been suggested that liver glucose output is increased via a 'feedback' mechanism that prevents changes in blood glucose concentration that would otherwise occur as a result of increased glucose uptake by the muscles (Felig and Wahren 1979; Newsholme and Leech 1983; Jenkins et al. 1985). On the other hand, blood glucose concentration may increase early in exercise before any substantial glucose uptake by the working muscle takes place and lowers its concentration. Therefore, a different mechanism has been proposed which states that hepatic glucose production at the onset of exercise is 'feedforward' regulated and controlled by mechanisms intimately related to activity in the central nervous system (Richter et al. 1986; Sonne and Galbo 1985; Sonne 1989).

In general, however, external factors such as glucagon, catecholamines, increased muscle glucose uptake, high glucagon to insulin and catecholamine to insulin ratios stimulate liver glucose production, whereas insulin seems to suppresses it (Coggan 1991; Farrell 1992; Kjaer 1992; Richter et al. 1986; Romijn and Wolfe 1992; Vranic and Lickley 1990; Winder 1985).

- Glucose uptake and phosphorylation
Blood glucose uptake depends on the duration and intensity of exercise (Ahlborg et al. 1974; Ahlborg and Felig 1982; Katz et al. 1986; Wahren et al. 1971). During incremental exercise, as it happens with hepatic glucose production in rats (Winder 1985), there is a curvilinear relationship between glucose uptake and exercise intensity in man, which seems to be due to the arteriovenous glucose difference (Coggan 1991).

The working muscle can take up and oxidise blood glucose at rates of about 0.5 g.min⁻¹ (Broberg and Sahlin 1989), or as high as 1.2 g.min⁻¹ (Coggan and Coyle 1987). It has been suggested that when muscle glycogen is low blood glucose is the major fuel for oxidation (Coggan and Coyle 1987; Coyle et al. 1986).

In addition to exercise intensity and duration, glucose uptake and oxidation may be influenced by diet (Galbo et al. 1979), the muscle mass involved (Richter et al. 1988), or training status (Coggan et al. 1990). Muscle glycogen concentration, however, influences glucose uptake in the rat (Richter and

The transport of glucose into the muscle cell is facilitated by specific glucose transporters. At least five structurally related transporters have been identified (Carruthers 1990). However, for the muscle tissue glucose transport-1 (GLUT-1) and especially glucose transport-4 (GLUT-4) seem to be responsible for facilitating glucose transport across the cell membrane, and therefore, have attracted more attention (Brozinick et al. 1992; Han et al. 1995; Henriksen et al. 1990; Douen et al. 1989; 1990).

Insulin and muscle contraction are the main factors which seem to regulate glucose uptake by the muscle. Muscle contraction has an insulin-like effect (Ivy 1987) and increases the number of glucose transporters in the plasma membrane (Douen et al. 1989). Insulin, however, is not required for the stimulation of glucose uptake by muscle during contraction (Nesher et al. 1985; Ploug et al. 1984). The effects of these two factors may be synergistic (DeFronzo et al. 1981), or additive (Ivy 1987; Nesher et al. 1985; Bonen et al. 1989), acting via independent mechanisms (Nesher et al. 1985; Bonen et al. 1989).

After the translocation through the muscle membrane, glucose is phosphorylated to G-6-P under the action of the enzyme hexokinase. A high concentration of G-6-P has been shown to inhibit the action of hexokinase (Katz et al. 1991). Furthermore, glucose uptake is inversely related to G-6-P concentration of the cell and increases as the muscle glycogen level declines (Ivy 1987). Therefore, as exercise progresses, muscle glycogen concentration drops, and less G-6-P is formed from glycogenolysis, the rate-limiting step in glucose utilisation may shift from phosphorylation to glucose transport (Katz et al. 1991).

Glycolysis

The G-6-P formed from glycogen or blood glucose is converted to pyruvate through a series of chemical reactions. The glycolytic pathway is considered to be regulated by the key enzymes phosphofructokinase (PFK) and pyruvate kinase (PK) (Newsholme and Leech 1983). The activity of PFK may be
enhanced by several factors such as Pi, AMP, NH₄, fructose-1,6-biphosphate, and a low ATP/ADP ratio, whereas is inhibited by ATP, PCr, Mg, H⁺, and citrate (Gollnick 1988; Hultman et al. 1987; Newsholme and Leech 1983). Recently, however, it has been shown that citrate does not inhibit PFK activity to the extent it was believed earlier (Peters and Spriet 1995). The PK activity, on the other hand, is activated by ADP, but inhibited by ATP and PCr (Newsholme and Leech 1983).

Finally, the pyruvate formed, when considering aerobic metabolism, is oxidised and energy is generated through the Krebs cycle and oxidative phosphorylation. According to the allosteric theory (Monod et al 1963 cited by Newsholme and Start 1973) factors such as Ca, ADP, and a high NAD/NADH ratio stimulate the activity of key enzymes of the Krebs cycle and oxidative phosphorylation, whereas ATP and citrate inhibit these enzymes (Newsholme and Leech 1983). It has been suggested that intracellular oxygen pressure may also play a key role in regulating mitochondrial oxidative phosphorylation (Wilson 1994).

It is unclear, however, especially in vivo, how all these factors integrate and operate to regulate the pathways of carbohydrate metabolism in the muscle (Gollnick 1988).

2.2.2: Exogenous carbohydrates during exercise and carbohydrate metabolism

When exogenous carbohydrates are ingested or infused during exercise changes in carbohydrate metabolism take place. The extent of these changes depend on a number of factors such as exercise intensity and duration, type, amount, and ingestion pattern of the ingested carbohydrate, mode of exercise, initial carbohydrate status of the body, and possibly training status of the subjects involved, and methodology employed.

- Blood glucose and insulin
During low-intensity exercise (about 30% VO₂max) carbohydrate ingestion produces a larger increase in blood glucose and insulin concentrations than the corresponding increases produced when exercise intensity is above 60%
\( \text{VO}_2 \text{ max (Coggan and Coyle 1991). However, the common observation is that at moderate intensities (50\% - 75\% \text{VO}_2 \text{ max}) blood glucose concentration is also higher when carbohydrates are ingested compared to water or placebo ingestion. This is the case during cycling (Bjorkman et al. 1984; Coggan and Coyle 1988; Coyle et al. 1983; 1986; Fielding et al. 1985; Flynn et al. 1987; Hargreaves et al. 1984; Hargreaves and Briggs 1988; Ivy et al. 1979; Mitchell et al. 1989a; Murray et al. 1991; Wright et al. 1991), as well as during running (MacLaren and Otter 1988; Sasaki et al. 1987a; Tsintzas et al. 1993b; Wilber and Moffatt 1992).}

On the other hand, serum insulin concentrations are higher in some (Bjorkman et al. 1984; Coyle et al. 1983; Coggan and Coyle 1988; Murray et al. 1991; Nicholas et al. 1994; Tsintzas et al. 1993b), but not in all studies (Coyle et al. 1986; Hargreaves and Briggs 1988; Mitchell et al. 1989a) as a result of carbohydrate ingestion during exercise. This may be due to the fact that at higher exercise intensities the greater sympathetic nervous system activity inhibits insulin secretion (Coggan and Coyle 1991).

Agreement exists, nevertheless, regarding the effect of carbohydrate ingestion on plasma FFA and glycerol concentrations during exercise. Almost in all studies, where plasma FFA and glycerol concentrations were determined, the ingestion of carbohydrates during exercise blunted their responses. Since the respiratory exchange ratio values are not usually altered by carbohydrate feedings, at least during the first 100 min of exercise (Coyle et al. 1983; 1986; Coggan and Coyle 1988; Erickson et al. 1987; Flynn et al. 1987; Maughan et al. 1989; Mitchell et al. 1989a; Murray et al. 1991; Tsintzas et al. 1993b), seems to suggest that the substrate utilisation is probably not affected (Coggan and Coyle 1991). Unfortunately, no direct observations are available on peripheral or intramuscular FFA oxidation during exercise when fed carbohydrate.

- Fate of the exogenous carbohydrates

The fate of exogenous carbohydrates has been determined using radioactive \( ^{14} \text{C} \) or stable \( ^{13} \text{C} \) isotope techniques. Both techniques, however, seem to possess methodological problems.

The most important factors influencing the exogenous carbohydrate oxidation rate seem to be the absolute exercise intensity as well as the total amount of carbohydrate ingested (Peronnet et al. 1992).
Because of ethical reasons the majority of studies have used $^{13}\text{C}$, which is a natural stable isotope. The studies which were conducted in 1970's and 1980's reported exogenous carbohydrate oxidation rates between about 0.2 and 0.8 g.min$^{-1}$ during exercise intensity ranging 45-64% $\dot{\text{VO}}_2$ max and exercise duration from 105 min up to 285 min (Jandrain et al. 1989; Krzentowski et al. 1984; Massicotte et al. 1986; Pallikarakis et al. 1986; Pirnay et al. 1977a; 1977b; 1981; 1982). These studies, however, probably overestimated the actual exogenous carbohydrate oxidation because it was assumed that the background oxidation of endogenous substrates rich in natural $^{13}\text{C}$ in the experimental trials was similar to the isotopic composition of expired CO$_2$ observed at rest or during exercise without carbohydrate ingestion (Peronnet et al. 1992). The main limitation of the $^{13}\text{C}$ tracer methods seems to be the isotopic composition of CO$_2$ arising from endogenous substrate oxidation which changes significantly during exercise without carbohydrate ingestion (Peronnet et al. 1993), and is influenced by the subject's diet (Wagenmakers et al. 1993). Taking these factors into account more recent studies have reported carbohydrate oxidation rates of about 0.23 g.min$^{-1}$ when 30 g of glucose were given during 90 min of cycling at 68% $\dot{\text{VO}}_2$ max (Peronnet et al. 1993), and 0.32 g.min$^{-1}$ when 50 g of glucose were ingested during two hours cycling at 60.7% $\dot{\text{VO}}_2$ max (Adopo et al. 1994).

Few studies have used the $^{14}\text{C}$ tracer, which has the advantage of negligible background in the endogenous substrate pools (Peronnet et al. 1992). The early studies conducted by Costill et al. (1973a) and Van Handel et al. (1980) reported a very low rate of exogenous carbohydrate oxidation (about 0.10 g.min$^{-1}$). These studies have been criticised for underestimating the exogenous carbohydrate oxidation rate mainly because the low $^{14}\text{C}$ label recovered in expired CO$_2$ was the result of the slow passage of the tracer through the body's bicarbonate pools and not a limited rate of blood glucose oxidation (Coggan and Coyle 1991). More recent observations, however, have reported exogenous blood glucose utilisation rates reaching as high as 1.8 g.min$^{-1}$ (Hawley et al. 1991). This study may have overestimated the exogenous carbohydrate oxidation because of the mixing of labelling an insoluble type of carbohydrate with a soluble tracer (Saris et al. 1993). Recently, it has been suggested that exogenous carbohydrate rates do not exceed about 1 g.min$^{-1}$ during prolonged exercise (Saris et al. 1993; Hawley et al. 1992).
Despite the methodological problems of the tracer methods, the above studies collectively seem to suggest that a considerable amount of the exogenous carbohydrate is utilised during exercise. This is confirmed by other studies where the euglycaemic clamp technique has been used (Coggan and Coyle 1987; Coyle et al. 1991).

- Muscle glycogenolysis

Except the controversy regarding the extent to which exogenous carbohydrates are oxidised during exercise, controversy also exists regarding the influence of exogenous carbohydrates on muscle glycogenolysis during exercise.

The studies which have examined the influence of exogenous carbohydrate on the rate of muscle glycogen utilisation during exercise are presented in Table 2.1. It should be noted that in all studies where running was the exercise mode a glycogen sparing effect has been shown (Tsintzas et al. 1993c; 1994; Nicholas et al. 1994). Also, when carbohydrate feeding produced an increase in insulin concentration during exercise, glycogen utilisation is reduced compared with control conditions during both cycling (Bjorkman et al. 1984; Yaspelkis III and Ivy 1991) and running (Tsintzas et al. 1993c; 1994; Nicholas et al. 1994). On the other hand, when carbohydrate feedings fail to increase insulin levels, no difference in muscle glycogen utilisation is found (Coyle et al. 1986; Hargreaves and Briggs 1988; Mitchell et al. 1989a). However, although insulin concentration was higher after 60 min of cycling at 73% \( \dot{V}O_2 \) max when glucose was infused, muscle glycogen utilisation was unaffected (Coyle et al. 1991).

As far as hepatic glucose production is concerned, recent data indicate that carbohydrate ingestion during prolonged exercise (69% \( \dot{V}O_2 \) max) results in suppression of hepatic glucose production during cycling exercise (McConell et al. 1994).
Table 2.1: Studies that have examined the influence of carbohydrate ingestion or infusion during exercise on skeletal muscle glycogen utilisation.

<table>
<thead>
<tr>
<th>STUDY</th>
<th>N</th>
<th>TYPE OF CHO USED</th>
<th>TYPE OF EXERCISE</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bjorkman et al. 1984</td>
<td>8</td>
<td>G, F</td>
<td>C at 68% ( \dot{VO}_2 ) max</td>
<td>Sparing</td>
</tr>
<tr>
<td>Bergstrom and Hultman (1967a)*</td>
<td>10</td>
<td>G</td>
<td>C at 700-900 kpm/min</td>
<td>Sparing</td>
</tr>
<tr>
<td>Hargreaves et al. 1984</td>
<td>10</td>
<td>SC</td>
<td>C at 50% ( \dot{VO}_2 ) max</td>
<td>Sparing</td>
</tr>
<tr>
<td>Fielding et al. 1985</td>
<td>9</td>
<td>SC</td>
<td>C at 50% ( \dot{VO}_2 ) max</td>
<td>No-Sparing</td>
</tr>
<tr>
<td>Coyle et al. 1986</td>
<td>7</td>
<td>GP</td>
<td>C at 71% ( \dot{VO}_2 ) max</td>
<td>No-Sparing</td>
</tr>
<tr>
<td>Erickson et al. 1987</td>
<td>5</td>
<td>G</td>
<td>C at 65-70% ( \dot{VO}_2 ) max</td>
<td>Sparing</td>
</tr>
<tr>
<td>Neufer et al. 1987</td>
<td>10</td>
<td>GP+F,SC</td>
<td>C at 77% ( \dot{VO}_2 ) max</td>
<td>No-Sparing</td>
</tr>
<tr>
<td>Hargreaves &amp; Briggs 1988</td>
<td>5</td>
<td>GP</td>
<td>C at 70% ( \dot{VO}_2 ) max</td>
<td>No-Sparing</td>
</tr>
<tr>
<td>Mitchell et al. 1989a</td>
<td>8</td>
<td>GP,F,S+GP</td>
<td>C at 70% ( \dot{VO}_2 ) max</td>
<td>No-Sparing</td>
</tr>
<tr>
<td>Coyle et al. 1991*</td>
<td>8</td>
<td>G</td>
<td>C at 70% ( \dot{VO}_2 ) max</td>
<td>No-Sparing</td>
</tr>
<tr>
<td>Yaspelkis &amp; Ivy 1991</td>
<td>12</td>
<td>GP</td>
<td>C at 50% ( \dot{VO}_2 ) max</td>
<td>Sparing</td>
</tr>
<tr>
<td>Tsintzas et al. 1993c</td>
<td>7</td>
<td>GP+G+F</td>
<td>R at 70% ( \dot{VO}_2 ) max</td>
<td>Sparing</td>
</tr>
<tr>
<td>Tsintzas et al. 1994</td>
<td>8</td>
<td>GP+G+F</td>
<td>R at 75% ( \dot{VO}_2 ) max</td>
<td>Sparing</td>
</tr>
<tr>
<td>Nicholas et al. 1994</td>
<td>6</td>
<td>GP+G+F</td>
<td>Intermittent Running</td>
<td>Sparing</td>
</tr>
</tbody>
</table>

G=glucose; F= fructose; S= sucrose; GP= glucose polymer; SC= solid carbohydrate
C= cycling; R= running; N= number of subjects tested
* = glucose infusion studies
2.3 Fatigue in Prolonged Exercise

Fatigue has been defined as "the failure to maintain the required or expected force and power output" (Edwards 1983). Since the command chain from voluntary muscular activity involves many steps, from the brain to the actin-myosin cross bridge formation within the muscle, fatigue may occur as a result of functional failure at any one link in this chain (Edwards 1981). From this structural approach fatigue has been classified as central or peripheral. Central fatigue is caused by an impaired neural drive involving the brain, spinal cord, and peripheral nerve, whereas peripheral fatigue is an impairment of force generation by the muscle involving regions from the peripheral nerve down to myofilament cross bridge formation (Gibson and Edwards 1985; MacClaren et al. 1989).

2.3.1 Central fatigue

Central fatigue may occur because of malfunction of nerve cells, or due to central inhibition activated by signals originated from the fatigued muscles (Asmussen 1979). Studies conducted in 1920's emphasised the role of hypoglycaemia or neuroglucopenia in the development of fatigue during prolonged exercise (Coggan and Coyle 1991). Christensen and Hansen (1939) suggested that hypoglycaemia could cause fatigue by affecting the central nervous system (CNS). However, recent studies have shown that although hypoglycaemia (i.e: a blood glucose concentration below 2.5 mmol.l⁻¹) may occur during prolonged exhaustive work (Felig et al. 1982), it is not always associated with the development of fatigue (Felig et al. 1982), and is not frequently observed especially during running exercise (Chryssanthopoulos et al. 1994; Tsintzas et al. 1993b; 1994).

Recently, it has been suggested that central fatigue during prolonged exercise may occur as a result of an increased in the plasma free tryptophan to branched chain amino acid (BCAA) ratio, which in turn will lead to an increased synthesis of the neurotransmitter serotonin (5-hydroxytryptamine) in the brain (Newsholme 1987; Blomstrand et al. 1988). Serotonin is responsible for causing a state of tiredness, sleepiness and lethargy in man and experimental animals (Young 1986, cited by Davies 1995), which in turn may result in a reduced mental and physical performance during prolonged
exhaustive exercise in animals (Bailey et al. 1993), or in humans (Blomstrand et al. 1991; Wilson and Maughan 1992).

2.3.2 Peripheral fatigue

The re-introduction of the Duchene biopsy technique by Bergstrom (1962) provided the opportunity of investigating directly skeletal muscle metabolism during exercise. Several studies found that during cycling at 65-80% $\dot{V}O_2\text{max}$ depletion of muscle glycogen stores limits the ability to perform prolonged work and coincides with the development of fatigue (Ahlborg et al. 1967; Saltin and Karlsson 1971; Hermanssen et al. 1967). However, in some studies a considerable amount of glycogen was still present in muscle after prolonged running (Madsen et al. 1990; Costill et al. 1973b), or cycling (Coyle et al. 1986; Coggan and Coyle 1987). Nevertheless, except the study conducted by Madsen and co-workers (1990), histochemical analysis of muscle fibers demonstrated a depletion of glycogen in Type I muscle fibers at the point of fatigue (Gollnick et al. 1973; Vollestad et al. 1984; Coggan and Coyle 1987), suggesting a limitation in assessing glycogen utilisation using muscle samples of mixed fiber populations (Costill et al. 1973b). Indeed, when subjects became fatigued during treadmill running at 75% $\dot{V}O_2\text{max}$ their muscle glycogen concentration was depleted in Type I muscle fibers, while considerable amount of glycogen was still present in the Type II fibers (Tsintzas et al. 1994).

However, glycogen depletion may not be the mechanism per se by which fatigue is developed. Sahlin and co-workers (1990) suggested that glycogen depletion may impair aerobic energy production by reducing the level of Krebs cycle intermediates. On the other hand, Wagenmakers et al. (1991) have argued that low level of glycogen availability may result in an elevated BCAA oxidation which in turn will drain the tricarboxylic acid cycle. Furthermore, glycogen depletion has been associated with a reduction in the total adenine nucleotide pool, especially AMP, an increased muscle ammonia concentration, and an elevated concentration of IMP availability in Type I muscle fibers (Broberg and Sahlin 1989; Norman et al. 1988). An increased IMP concentration may indicate a decreased ATP resynthesis, (Broberg and Sahlin 1989; Norman et al. 1988), although Hargreaves and his colleagues
(1994) failed to demonstrate it. However, in that study only mixed muscle tissue was analysed (Hargreaves et al. 1994).

As far as the elevated ammonia accumulation is concerned, it has been associated with muscle cramping (Brouns et al. 1990), as well as affecting critical regions of the CNS, and therefore, may contribute to central fatigue (Banister and Cameron 1990).

Another possible cause for the development of fatigue during prolonged exercise is the loss of intracellular potassium which will decrease the depolarisation of muscle membrane and lead to disturbances of the excitation-contraction process (Sjøgaard 1990). However, other authors believe that this potassium loss may have little consequences for the electrical properties of the muscle cell (Sejersted, 1992). Furthermore, during prolonged exercise structural damages to the sarcoplasmic reticulum may compromise the ability of the muscle cell to regulate calcium release and hence a decline in muscle force will occur (Allen et al. 1992; Fitts 1994). In addition, echocardiographic data showed reduced ventricular diastolic dimension and fractional shortening of the myocardium in 21 triathletes after an Ironman Triathlon, suggesting cardiac fatigue (Douglas et al. 1987).

In conclusion, fatigue is a complex phenomenon that may be central, and/or peripheral in origin. The central and peripheral regions may also be linked as the 'tryptophan-serotonin' and 'ammonia' theories suggest. However, over the last three decades accumulated evidence suggest that muscle glycogen depletion is highly correlated with and undoubtedly contributes to fatigue during endurance exercise (Fitts 1994).

2.4 The Effects of Carbohydrate Ingestion Before Exercise on Exercise Metabolism, Endurance Capacity and Performance

The consumption of carbohydrate before exercise produces various postprandial metabolic and cardiorespiratory responses which are associated mainly with the body's digestion and absorption processes. Depending on the degree of these postprandial responses as well as the time difference between
carbohydrate ingestion and initiation of exercise, exercise metabolism may or may not be affected as a result of such feedings. The aim of the section of this chapter is to provide a brief outline about the postprandial metabolic and respiratory responses of a carbohydrate load and to describe its influence on exercise metabolism, endurance capacity and performance.

2.4.1 Postprandial metabolic and respiratory responses of a carbohydrate load

During the postprandial period following a carbohydrate meal several cardiovascular, respiratory, hormonal, and metabolic responses take place in the body. However, postprandial blood glucose and insulin responses seem to play a key role on the subsequent influence of pre-exercise carbohydrate consumption on exercise metabolism.

- Blood glucose and serum insulin response

Factors such as the type (high or low glycaemic food), the form (liquid or solid), and physical properties of carbohydrate, its amylose and fibre content, as well as the method of food processing seem to influence the glucose and insulin responses of a carbohydrate load.

When different types of complex (Crapo et al. 1977), or simple (Bohannon et al. 1980) carbohydrate are ingested the postprandial plasma glucose and insulin responses are different. For instance, the consumption of 50 g of potato elicited greater glucose and insulin responses than 50 g of rice or corn (Crapo et al. 1977). On the other hand, simple carbohydrates such as sucrose and glucose produced higher glycaemic and insulinaemic responses than fructose (Bohannon et al. 1980). Furthermore the rise in plasma glucose and insulin levels after dextrose and potatoes were found to be similar (Crapo et al. 1977). Therefore, with respect to postprandial glucose and insulin responses, the classification of carbohydrates into complex and simple form is insufficient. In order to overcome this difficulty Jenkins and co-workers (1981) introduced the concept of Glycaemic index. Based on the relative rise in plasma glucose after the ingestion of a single carbohydrate load, as compared to the same amount of glucose or white bread, a glycaemic index (GI) for different foods was proposed, according to the formula:

\[
\text{GI} = \frac{\text{Plasma glucose rise with test food}}{\text{Plasma glucose rise with reference food}} \times 100
\]
GI = \frac{\text{Area under blood glucose response curve of food}}{\text{Area under blood glucose response curve of glucose}} \times 100

Foods such as white bread, rice, cornflakes, and banana are classified as having a high glycaemic index and, therefore, they produce a high postprandial insulin and glucose response. On the other hand, foods like apples, figs, lentils, and milk have a low glycaemic index and do not produce high postprandial insulin and glucose responses (Jenkins et al. 1981; 1984; 1988).

However, other factors such as food processing may well affect glycaemic responses. It has been observed that boiled rice had a higher glucose response than baked rice (Gatti et al. 1987). Crapo and colleagues (1976) reported that raw starch ingestion resulted in 44% lower glucose response and 35-65% lower insulin response than did glucose or sucrose ingestion. It seems that the more processed a food is, the higher the glycaemic response it will produce (Brand et al. 1985). When carbohydrate is given as a meal glucose response is lower than when the same carbohydrate is given as a drink (Crapo et al. 1976). Furthermore, a high amylose (Goddard et al. 1984; Behall et al. 1988) or fibre content seems to reduce postprandial glycaemic and insulinaemic responses. The above factors may influence the rate of gastric emptying which in turn may induce different glycaemic responses (Mourot et al. 1988; Torsdottir et al. 1984).

Insulin response may also be influenced by performing regularly aerobic exercise (Lohmann et al. 1978; Heath et al. 1983; Young et al. 1989), by obesity (Segal et al. 1991), or by an acute effect of physical activity performed before the consumption of the carbohydrate load (Heath et al. 1983; Young et al. 1989; Oshida et al. 1991). Recently, it has been suggested that initially physical training induces a decreased secretory capacity of the β-cells which is later accompanied by increased peripheral insulin sensitivity (Mikines 1992).

**Dietary-induced thermogenesis**

The ingestion of carbohydrate, or food in general, is associated with an increased metabolic rate (Poehlman 1989). Terms such as 'heat increment', 'specific dynamic action', 'thermic effect of food', or 'dietary-induced thermogenesis' have been used to describe this response (Ravussin et al. 1985). This elevation in metabolic rate is not only due to energy requirements
for digestion, absorption, conversion and storage of substrates. Therefore, dietary-induced thermogenesis has been divided into obligatory and facultative thermogenesis. The obligatory component is the energy cost associated with digestion, absorption and synthesis of protein, fat and carbohydrate, whereas the energy expenditure in excess of the obligatory component is considered to be the facultative thermogenesis (Poehlman 1989). This facultative component is supposed to be at least partially due to insulin-mediated activation of the sympathetic nervous system which in turn exerts its thermogenic effect via release of catecholamines stimulating $\beta_1$-adrenoreceptors (Acheson et al. 1983; 1984).

Furthermore, it has been shown that the dietary-induced thermogenesis is lower in obese than lean humans (Segal et al. 1990; 1991; 1985; Kaplan and Leveille 1976). However, there is still some controversy among studies as well as on the implication of reduced dietary-induced thermogenesis in the obese (Sims and Danforth 1987; Jansky 1995). Except in the obese a reduced dietary-induced thermogenesis has been observed in exercise-trained individuals (LeBlanc et al. 1984; Poehlman et al. 1988; Tremblay et al. 1983), which seems to be related to a reduced activity of the sympathetic nervous system (LeBlanc et al. 1984).

Disposal of a carbohydrate load
The human body contains the enzymes necessary for the conversion of carbohydrate to fat (Newsholme and Leech 1983). Lipogenesis from ingested carbohydrate can be assessed by measuring the postprandial non-protein respiratory exchange ratio (RER). A RER value above 1.0 suggests that the rate of fat resynthesis exceeds the rate of fat absorption (Brody 1994), indicating that lipogenesis takes place. However, after humans consumed a 500-g carbohydrate load the RER values remained below 1.0 for most of the 10-hour postprandial period (Acheson et al. 1982). Furthermore, direct measurements of muscle and liver glycogen content after infusion of fructose or glucose suggested that less than 10% of the retained fructose or glucose load could have been stored in fat tissue (Nilsson and Hultman 1974). Therefore, there is the view that even large amounts of dietary carbohydrate do not influence the body fat stores (Acheson et al. 1982; 1988; Bjorntorp and Sjostrom 1978). It has been suggested that glycogen storage capacity in man is about 15g/kg body weight and can accommodate a gain of about 500 g
before net lipid synthesis contributes to increasing body fat mass (Acheson et al. 1988).

The carbohydrate ingested will mainly be stored in the liver and muscle as glycogen. Glycogen can be formed directly in the liver, or through gluconeogenic precursors such as lactate, alanine and pyruvate (Radziuk 1989; Katz and McGarry 1984). Felig and co-workers (1975), using the hepatic vein catheterization technique, reported that only 15 g of an oral load of 100 g of glucose escaped splanchnic bed during the 3 hours after ingestion, from which it was concluded that about 85% of the load was retained by the liver. On the other hand, Katz and colleagues (1983) suggested that about 65 g from a total 92 g oral glucose load were taken up by body muscles which suggests that more than 2/3 of the oral glucose load escaped splanchnic removal. This indicates that peripheral tissues quantitatively play the dominant role in glucose disposal (Katz et al. 1983). However, direct measurements of liver glycogen content have shown that a considerable amount of carbohydrate load given orally (Nilsson and Hultman 1973), or intravenously (Nilsson and Hultman 1974) was deposited as liver glycogen.

2.4.2 Pre-exercise carbohydrate feedings and exercise metabolism

Possibly the first study that triggered the interest of exercise physiologists in pre-exercise carbohydrate consumption was the study conducted by Costill et al. (1977). In that study it was shown that 75 g of glucose ingested 45 min before running at 70% \( \dot{V}O_2\text{max} \) caused the blood glucose concentration during exercise to decline at lower levels compared with the control trial. This low blood glucose concentration was associated with elevated pre-exercise insulin concentration and low FFA levels during the exercise period (Costill et al. 1977). This initial decline in blood glucose concentration as a result of pre-exercise carbohydrate feeding has been observed in many studies (Koivisto et al. 1981; 1985; Hargreaves et al. 1987; Levine et al. 1983; Coyle et al. 1985; Foster et al. 1979; Peden et al. 1989; Sherman et al. 1989; Chryssanthopoulos et al. 1994; Thomas et al. 1991; Alberici et al. 1993), but not in all (Fielding et al. 1987; Gleeson et al. 1986; Devlin et al. 1986; Bonen et al. 1981; Wright et al. 1991; Calles-Escandon et al. 1991). It seems that the initial decline in blood glucose concentration is of a transient nature and blood glucose usually returns to placebo/control levels within 30-45 min after the initiation of

It has been suggested that the blood glucose response, when carbohydrates are fed before exercise, depends on factors such as the amount of carbohydrate ingested, time of ingestion before exercise, and fitness status of subjects (Sherman 1991). It has been shown that when a carbohydrate meal was given 4-6 hours before cycling at 70% VO_{2}max blood glucose decrease was less in trained than in untrained individuals (Montain et al. 1991).

The transient drop in blood glucose concentration observed during the first 30-45 min of exercise seems to be the result of an increased glucose uptake by the muscle due to muscle contraction and elevated pre-exercise insulin and/or the inability of liver glycogen to replace blood glucose at the same rate as muscle takes up this substrate (Sherman et al. 1991). However, blood glucose may drop, relative to control values, despite a similar insulin concentration between control and carbohydrate trials (Coyle et al. 1985). It has been argued that insulin has a persistent effect that affects glucose homeostasis during exercise despite the fact that insulin levels before or during exercise are not elevated (Coyle et al. 1985).

Pre-exercise carbohydrate feedings are usually (Coyle et al. 1985; Costill et al. 1977; Koivisto et al. 1985; Wright et al. 1991; Thomas et al. 1991; Ahlborg and Felig 1977), but not always (Calles-Escandon et al. 1991; Alberici et al. 1993) associated with a depression of FFA concentrations during exercise which is probably attributed to antilipolytic activity of insulin (Newsholme and Leech 1983). In order to avoid a marked increase in serum insulin before exercise, several studies have used fructose instead of glucose. Fructose ingestion produces a smaller increase in pre-exercise insulin concentration compared to glucose and similar blood glucose responses during exercise to the blood glucose responses observed in the control trials (Hargreaves et al. 1987; Koivisto et al. 1981; 1985; Levine et al. 1983; Fielding et al. 1987). However, fructose ingestion still reduces plasma FFA levels during exercise (Koivisto et al. 1981; 1985).

Another way by which one can have smaller elevations of insulin and glucose before exercise is to ingest foods that have a low glycaemic index.
Sparks and co-workers (1994) (Febbraio 1995, personal communication) reported that the ingestion of 1 g/kg BW of lentils 45 min before cycling at 70% \(\dot{V}O_2\text{max}\) produced a smaller fall in glucose and FFA concentrations during exercise and a smaller increase in insulin compared with an equivalent amount of mashed potato. However, blood glucose and FFA concentrations during exercise were not different in a similar study conducted by Thomas et al. (1991).

Another factor that might be influenced by pre-exercise carbohydrate feedings is the muscle glycogen utilisation. Costill et al. (1977) reported that the ingestion of a 75 g glucose 30 min before exercise produced a higher muscle glycogen use compared to control conditions. The authors attributed this finding to elevated pre-exercise insulin and the suppressed FFA levels during exercise observed in the glucose trial. Since then, several authors have examined the influence of carbohydrate ingestion before exercise on skeletal muscle glycogen utilisation (Table 2.2). Except the studies conducted by Costill et al. (1977), and by Hargreaves et al. (1985) which showed an accelerated muscle glycogenolysis as a result of pre-exercise glucose feeding, the majority of studies have reported that muscle glycogen use is not affected by pre-exercise carbohydrate ingestion. Levine et al. (1983) found a lower muscle glycogen use as a result of fructose ingestion compared to glucose or control. However, this finding was not confirmed by other studies where fructose was used against glucose and placebo (Hargreaves et al. 1985; 1987; Koivisto et al. 1985; Fielding et al 1987).

Furthermore, pre-exercise carbohydrate ingestion has been shown to produce a greater reliance on carbohydrate metabolism compared to fasting control conditions (Coyle et al. 1985; Sherman et al. 1989; Wright et al. 1991; Montain et al. 1991; Willcutts et al. 1988). This is probably a result of a reduced peripheral lipolysis caused by the action of insulin. It has been shown that there is a linear relationship between the degree of lipolysis during exercise, as reflected by the glycerol concentration, and the length of fast, whereas FFA concentrations are inversely related to carbohydrate oxidation (Montain et al. 1991). It seems that at least 6 hours of fasting are necessary after the ingestion of 140 g carbohydrate before exercise metabolism will be similar to exercise metabolism after an overnight (12-16 hour) fast (Montain et al. 1991).
2.4.3: The influence of pre-exercise carbohydrate feedings on endurance capacity and performance

Endurance capacity has been defined as the time taken for an individual to exercise to exhaustion at a constant pace or workload, whereas endurance performance refers to the time taken to perform a set task or cover a certain distance (Williams 1989).

Several studies have examined the influence of pre-exercise carbohydrate ingestion on endurance capacity (Table 2.3) and performance (Table 2.4). Most of the authors have used cycling as the exercise mode, whereas few studies have been conducted on running.

As far as cycling exercise is concerned, only two studies have shown detrimental effects on endurance capacity (Foster et al. 1979; Keller and Schwarzkopf 1984), whereas the rest of the investigators have shown either no effect (Koivisto et al. 1981; Devlin et al. 1986; Hargreaves et al. 1987; Calles-Escandon et al. 1991; Alberici et al. 1993; Chryssanthopoulos et al. 1994), or an improvement (Gleeson et al. 1986; Okano et al. 1988; Wright et al. 1991), as a result of pre-exercise carbohydrate feedings. In the study conducted by Keller and Schwarzkopf (1984) the subjects were exhausted after about 19 min and 25 min of intermittent cycle exercise during glucose and placebo trials respectively. These endurance times are short compared with those usually reported by other investigators. They were probably the consequence of the high exercise intensity (85% \( \dot{V}O_2 \max \)), and/or the endurance runners who were selected as subjects were not accustomed to cycling at such high exercise intensity. On the other hand, the study by Foster et al. (1979) has been criticised because the criterion of fatigue the authors used was a subjective unwillingness on the part of the subjects to continue cycling instead of a physical inability to turn the pedals (Sherman 1991).

The two studies which have examined the influence of pre-exercise carbohydrate ingestion on running capacity have shown that the ingestion of glucose or fructose 30-45 min before treadmill exercise at 70-85% \( \dot{V}O_2 \max \) does not influence exercise time to exhaustion (McMurray et al. 1983; Chryssanthopoulos et al. 1994).
It has been shown that ingesting a low glycaemic food such as lentils 60 min before cycling exercise improves endurance capacity compared to glucose, mashed potato (high glycaemic foods), or water (Thomas et al. 1991). However, when Sparks and colleagues (1994) (Febbraio 1995, personal communication) provided the same amount of carbohydrate in the form of lentils no improvement in performance was observed compared with placebo or mash potato feeding during a 15-min 'all-out' ride. It may be argued that the protocol used by Sparks et al. (1994) (50 min constant pace + 15 min performance ride) did not challenge the carbohydrate stores of the body and so no performance changes occurred with increased carbohydrate intake. When carbohydrate feedings, equivalent to about 1-5 g/kg BW, were ingested 60-240 min before cycling exercise, performance was improved during longer (135-200 min) exercise periods (Sherman et al. 1989; Peden et al. 1989; Wright et al. 1991).

As far as running performance is concerned, Millard-Stafford et al. (1994) observed an improvement in the time to complete the last 1.6 km of a 15 km run in the heat as a result of pre-exercise ingestion of a carbohydrate electrolyte solution. However, Braun and colleagues (1994) failed to demonstrate an improvement during a 10 km self-selected paced treadmill run which was performed 60 min after ingesting 54 g of carbohydrate in various forms. Also, Williams et al. (1995) reported that performance was unaffected when subjects ingested 75 g of glucose 30 min before a biathlon race (1.371 km swim and 21.5 km run).

Since pre-exercise carbohydrate ingestion does not usually produce a glycogen sparing effect (Table 2.2), it has been suggested that the enhanced glucose availability and oxidation can partially account for any improvement in endurance capacity and performance (Sherman 1991). However, another possibility might be a higher pre-exercise muscle glycogen concentration as a result of carbohydrate feeding. Coyle and his colleagues (1985) found a 42% increase in glycogen concentration in the vastus lateralis muscle as a result of pre-exercise ingestion of 140 g of carbohydrate. On the other hand, Neufer et al. (1987) reported a non-significant 15% increase in muscle glycogen, when 200 g of carbohydrate was ingested before exercise. As mentioned earlier, both studies measured muscle glycogen only 4 hours after the ingestion of the meals.
In conclusion, despite a transient drop of blood glucose during the first 30-45 min of exercise and a greater reliance on carbohydrate metabolism, pre-exercise carbohydrate feedings do not adversely affect endurance capacity and performance. Also, when a substantial amount of carbohydrate is provided, an improvement in endurance capacity and performance may occur through an enhanced glucose oxidation and/or a greater pre-exercise muscle glycogen concentration.
Table 2.2: Studies that have examined the influence of pre-exercise carbohydrate feedings on skeletal muscle glycogen utilisation during exercise.

<table>
<thead>
<tr>
<th>STUDY</th>
<th>N</th>
<th>AMOUNT OF CHO</th>
<th>TYPE OF CHO</th>
<th>TIME OF FEEDING BEFORE EXERCISE</th>
<th>EXERCISE INTENSITY (% VO2max)</th>
<th>GLYCOGEN USE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Costill et al. 1977*</td>
<td>7</td>
<td>75 g</td>
<td>G</td>
<td>30 min</td>
<td>70</td>
<td>Sparing in C</td>
</tr>
<tr>
<td>Levine et al. 1983</td>
<td>8</td>
<td>75 g</td>
<td>G,F</td>
<td>45 min</td>
<td>75</td>
<td>Sparing in F</td>
</tr>
<tr>
<td>Hargreaves et al. 1985</td>
<td>8</td>
<td>50 g</td>
<td>G,F</td>
<td>45 min</td>
<td>75</td>
<td>Sparing in C</td>
</tr>
<tr>
<td>Coyle et al. 1985</td>
<td>7</td>
<td>140 g</td>
<td>SC</td>
<td>120 min</td>
<td>70</td>
<td>No Difference</td>
</tr>
<tr>
<td>Koivisto et al. 1985</td>
<td>8</td>
<td>75 g</td>
<td>G,F</td>
<td>45 min</td>
<td>55</td>
<td>No Difference</td>
</tr>
<tr>
<td>Devlin et al. 1986</td>
<td>8</td>
<td>43 g</td>
<td>SC</td>
<td>30 min</td>
<td>70</td>
<td>No Difference</td>
</tr>
<tr>
<td>Hargreaves et al. 1987</td>
<td>6</td>
<td>75 g</td>
<td>G,F</td>
<td>45 min</td>
<td>75</td>
<td>No Difference</td>
</tr>
<tr>
<td>Fielding et al. 1987*</td>
<td>6</td>
<td>75 g</td>
<td>G,F</td>
<td>30 min</td>
<td>70</td>
<td>No Difference</td>
</tr>
<tr>
<td>Ahlborg &amp; Bjorkman (1987)</td>
<td>6</td>
<td>200 g</td>
<td>G</td>
<td>50 min</td>
<td>30</td>
<td>No Difference</td>
</tr>
</tbody>
</table>

G=glucose; F=fructose; SC=solid carbohydrate
N= number of subjects tested; C= control trial
*= studies conducted on running (the rest of the studies used cycle ergometry)
Table 2.3: Studies that have examined the influence of pre-exercise carbohydrate feedings on endurance capacity.

<table>
<thead>
<tr>
<th>STUDY</th>
<th>N</th>
<th>AMOUNT OF CHO</th>
<th>TYPE OF CHO</th>
<th>TIME OF FEEDING BEFORE EXERCISE</th>
<th>EXERCISE INTENSITY (% VO\textsubscript{2max})</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foster et al. 1979</td>
<td>16</td>
<td>75 g</td>
<td>G</td>
<td>30 min</td>
<td>84</td>
<td>D in G</td>
</tr>
<tr>
<td>McMurray et al. 1983*</td>
<td>6</td>
<td>100 g</td>
<td>G,F</td>
<td>45 min</td>
<td>85</td>
<td>No Difference</td>
</tr>
<tr>
<td>Keller &amp; Schwarzkopf (1984)</td>
<td>5</td>
<td>100 g</td>
<td>G</td>
<td>60 min</td>
<td>85</td>
<td>D in G</td>
</tr>
<tr>
<td>Koivisto et al. 1981</td>
<td>9</td>
<td>75 g</td>
<td>G,F</td>
<td>45 min</td>
<td>70</td>
<td>No Difference</td>
</tr>
<tr>
<td>Devlin et al. 1986</td>
<td>8</td>
<td>43 g</td>
<td>SC</td>
<td>30 min</td>
<td>70</td>
<td>No Difference</td>
</tr>
<tr>
<td>Gleeson et al. 1986</td>
<td>6</td>
<td>70 g</td>
<td>G</td>
<td>45 min</td>
<td>73</td>
<td>Improvement</td>
</tr>
<tr>
<td>Hargreaves et al. 1987</td>
<td>6</td>
<td>75 g</td>
<td>G,F</td>
<td>45 min</td>
<td>75</td>
<td>No Difference</td>
</tr>
<tr>
<td>Okano et al. 1988</td>
<td>12</td>
<td>60-85 g</td>
<td>F</td>
<td>60 min</td>
<td>62-81</td>
<td>Improvement</td>
</tr>
<tr>
<td>Calles-Escandon et al. 1991</td>
<td>9</td>
<td>43-65 g</td>
<td>F,SC</td>
<td>30 min</td>
<td>70</td>
<td>No Difference</td>
</tr>
<tr>
<td>Wright et al. 1991</td>
<td>9</td>
<td>333 g</td>
<td>GP+S</td>
<td>180 min</td>
<td>70</td>
<td>Improvement</td>
</tr>
<tr>
<td>Thomas et al. 1991</td>
<td>8</td>
<td>70 g</td>
<td>Lentils</td>
<td>60 min</td>
<td>65-70</td>
<td>Improvement</td>
</tr>
<tr>
<td>Alberici et al. 1993</td>
<td>8</td>
<td>46-92 g</td>
<td>SC</td>
<td>30 min</td>
<td>70</td>
<td>No Difference</td>
</tr>
<tr>
<td>Chryssanthopoulos et al. 1994*</td>
<td>9</td>
<td>75 g</td>
<td>G</td>
<td>30 min</td>
<td>70</td>
<td>No Difference</td>
</tr>
</tbody>
</table>

G=glucose; F=fructose; SC=solid carbohydrate; GP=glucose polymer
N= number of subjects tested; D in G= Decrease of endurance capacity in Glucose trial
*= studies conducted on running (the rest of the studies used cycle ergometry)
Table 2.4: Studies that have examined the influence of pre-exercise carbohydrate feedings on endurance performance.

<table>
<thead>
<tr>
<th>STUDY</th>
<th>N</th>
<th>AMOUNT OF CHO</th>
<th>TYPE OF CHO</th>
<th>TIME OF FEEDING BEFORE EXERCISE</th>
<th>TYPE OF EXERCISE</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Sherman et al. 1989</td>
<td>10</td>
<td>312 g</td>
<td>G+GP</td>
<td>240 min</td>
<td>45 min PR</td>
<td>Improvement</td>
</tr>
<tr>
<td>- Peden et al. 1989</td>
<td>9</td>
<td>1.1-2.2 g/kg BW</td>
<td>G+GP</td>
<td>60 min</td>
<td>45 min PR</td>
<td>Improvement</td>
</tr>
<tr>
<td>- Wright et al. 1991</td>
<td>9</td>
<td>333 g</td>
<td>S+GP</td>
<td>180 min</td>
<td>3 min PR per 45 min</td>
<td>Improvement</td>
</tr>
<tr>
<td>- Millard-Stafford et al. 1994</td>
<td>12</td>
<td>80 g</td>
<td>C-E</td>
<td>60 min</td>
<td>1.6 km Run</td>
<td>Improvement</td>
</tr>
<tr>
<td>- Braun et al. 1994</td>
<td>10</td>
<td>54 g</td>
<td>G,F,S+G,SC</td>
<td>60 min</td>
<td>10 km Run</td>
<td>No Difference</td>
</tr>
<tr>
<td>- Sparks et al. 1994</td>
<td>6</td>
<td>75 g</td>
<td>L,MP</td>
<td>45 min</td>
<td>15 min PR</td>
<td>No Difference</td>
</tr>
<tr>
<td>- Williams et al. 1995</td>
<td>7</td>
<td>75 g</td>
<td>G</td>
<td>30 min</td>
<td>1.37 km Swim and 21.5 km Run</td>
<td>No Difference</td>
</tr>
</tbody>
</table>

G=glucose; F=fructose; S=sucrose; SC=solid carbohydrate; GP=glucose polymer
C-E=Carbohydrate electrolyte solution; L=lentils; MP=mash potato
N=number of subjects tested; PR=performance ride
2.5 The Influence of Carbohydrate Ingestion During Exercise on Endurance Capacity and Performance

Since muscle glycogen and blood glucose oxidation are important for both endurance capacity (Coyle et al. 1986; Tsintzas et al. 1993b), as well as endurance performance (Karlsson and Saltin 1971), it is reasonable to attempt to provide additional carbohydrate during exercise in order to delay the onset of fatigue. Since the early 1980's many studies have been conducted to investigate the effect of carbohydrate ingestion during exercise on endurance capacity or performance. The majority of studies, however, have used cycling as the mode of exercise, whereas fewer studies have been conducted on running. Furthermore, it is interesting to note that with the exception of few studies (Murray et al. 1991; Mitchell et al. 1988; 1989a; Peters et al. 1995; Powers et al. 1990; Rehrer et al. 1994; Wright et al. 1991), the vast majority of experiments were conducted after an overnight fast lasting usually 10-16 hours.

2.5.1 Cycling studies

Christensen and Hansen (1939) were probably the first to show that the administration of glucose in humans late in exercise can delay fatigue when cycling at 60-65% \( \text{VO}_2\text{max} \). Since then many studies have been conducted where the effect of carbohydrate ingestion during exercise on cycle time to fatigue was investigated (see Table 2.5). In these studies various exercise intensities (60-85% \( \text{VO}_2\text{max} \)), amounts, and types of carbohydrate have been used. The majority of studies have shown an improvement in endurance capacity when carbohydrates were ingested compared with water, or placebo ingestion. Carbohydrates were usually administered in a form of a solution. The various solutions ingested had concentrations ranging from 1.8% up to 50%, whereas the rate of carbohydrate intake was 13-111 g per hour. Although a dose-response relationship between carbohydrate intake and improvement in endurance capacity does not seem to exist, ingesting a very low dose of carbohydrate (13 g.h\(^{-1}\)) does not improve endurance capacity (Burgess et al. 1991).

In an attempt to measure endurance performance several authors have employed different protocols where subjects are required to complete as
much work as possible in a certain time, or to produce a certain amount of work in the shortest possible time (Table 2.6). Again the majority of studies have shown an improvement in cycling performance as a result of ingesting carbohydrates during the exercise, or during the exercise period immediately before the performance task. These studies also suggest that no dose-response relationship between carbohydrate ingestion rate and performance exists (Fielding et al. 1985; Hargreaves et al. 1984; Murray et al. 1991; Mitchell et al. 1989a). However, there are studies where carbohydrate ingestion failed to improve cycling performance (Ivy et al. 1979; Flynn et al. 1987; Cole et al. 1993; Madsen and Christensen 1994). It seems that when the initial muscle glycogen levels are high, usually after a supercompensation diet, carbohydrate feedings during cycling fail to improve endurance performance (Flynn et al. 1987; Widrick et al. 1992).

2.5.2 Running studies

The studies that have used running as the mode of exercise have produced conflicting results regarding the influence of carbohydrate ingestion on running capacity (Table 2.7), as well as on running performance (Table 2.8). Sasaki et al. (1987a) found an improvement in running time to exhaustion at 80% $\dot{V}O_2$ max as a result of ingesting a sucrose solution, whereas when exercise intensity varied between 60-90% $\dot{V}O_2$ max the administration of sucrose solution failed to improve endurance capacity (Sasaki et al. 1987b). Also, ingestion of carbohydrates during exercise at 80% $\dot{V}O_2$ max may (Wilber and Moffat 1992) or may not (Rehrer et al. 1994) improve running capacity. However, the result of recent studies showed that ingesting a 5.5% carbohydrate-electrolyte solution improved treadmill running time to exhaustion at 70-75% $\dot{V}O_2$ max (Tsintzas et al. 1993b; 1994).

As far as running performance is concerned, Williams and his co-workers have reported no overall difference in distance covered during a 2-hour run (Williams et al. 1987 cited by Williams 1989), or in time taken to cover 30-km on a treadmill (Williams et al. 1990) as a result of ingesting carbohydrate-electrolyte solutions during exercise. Nevertheless, in both studies higher running speeds were observed towards the last 30 min and 10 km of the trials, when carbohydrates were provided. Recently, another study has been conducted, combining cycling and running exercise, where the administration
of carbohydrates in liquid form produced an improvement in both cycling performance and running capacity (Peters et al. 1995).

2.5.3: Possible mechanisms by which carbohydrate ingestion during exercise improves endurance capacity and performance

In the early study conducted by Christensen and Hansen (1939) the authors suggested that the ingestion of glucose reversed hypoglycaemia and the subjects were able to continue cycling at 60-65% \( \dot{V}O_2 \text{max} \) for one hour more compared to control conditions. Felig et al. (1982), on the other hand, showed that exercise can continue in the presence of hypoglycaemia. In more recent studies hypoglycaemia is not frequently observed (Coggan and Coyle 1991). However, during cycling there is a gradual decrease in the blood glucose concentration and the rate of total carbohydrate oxidation when no carbohydrates are provided (Coyle et al. 1983; 1986; 1991; Coggan and Coyle 1988; 1989; Bjorkman et al. 1984; Burgess et al. 1991; Wright et al. 1991; Murdoch et al. 1993). Therefore, it has been suggested that the main mechanism by which carbohydrate feedings improve endurance capacity is by maintaining carbohydrate oxidation rate and blood glucose concentration late in exercise (Coyle et al. 1986; Coggan and Coyle 1991). Furthermore, it has been argued that only people who experience a decrease in blood glucose concentration during exercise in the fasted state can benefit from the ingestion of carbohydrates during exercise (Coyle et al. 1983; Coggan and Coyle 1991). However, in some cycling studies carbohydrate ingestion improved endurance capacity although blood glucose concentration and carbohydrate oxidation rate were maintained in the fasted trial (Maughan et al. 1989, Murray et al. 1991). Furthermore, it has been observed that carbohydrate administration does not influence glycogen utilisation (Coyle et al. 1986; 1991; Mitchell et al. 1989a; Hargreaves and Briggs 1988; Fielding et al. 1985; Flynn et al. 1987), or carbohydrate oxidation rates during the first 90-100 min of exercise (Coyle et al. 1986; Coggan and Coyle 1988; Maughan et al. 1989; Wright et al. 1991), despite the fact that considerable amounts of carbohydrates were consumed. Therefore, one may question the fate or influence of this carbohydrate load on the body. Unfortunately, there is little information about the hepatic glucose metabolism during exercise when carbohydrates are ingested. However, a recent study, using \(^3\)H-glucose tracers, has shown that when glucose was ingested during a 2-hour cycling
exercise at 69% \( \text{VO}_2\text{max} \), hepatic glucose production was 51% lower compared to placebo ingestion (McConell et al. 1994).

On the other hand, some cycling studies have observed a reduced rate of muscle glycogen utilisation as a result of carbohydrate ingestion, compared to control conditions (Erickson et al. 1987; Hargreaves et al. 1984; Bjorkman et al. 1984). However, it has been argued that the lower rate of muscle glycogen utilisation observed in these studies was the result of longer exercise time in the experimental trial (Bjorkman et al. 1984), or higher resting muscle glycogen levels in the control trial (Hargreaves et al. 1984), rather than an effect of exogenous carbohydrates on muscle glycogenolysis (Coggan and Coyle 1991). Although this argument might be valid, a closer examination of the studies that have examined the influence of carbohydrate ingestion on muscle glycogen utilisation will show that the different experimental treatments have produced different impacts on metabolism during exercise (i.e., altering or not altering insulin levels), or have examined only mixed muscle samples (see paragraphs 2.2.2 and 2.3.2). These factors may have contributed to the differences reported in the literature.

In prolonged running exercise in the fasted state blood glucose concentration does not decline with respect to time (Chryssanthopoulos et al. 1994; Fruth and Gisolfi 1983; Madsen et al. 1990; Macaraeg et al. 1983; Millard-Stafford et al. 1992; Rehrer et al. 1994; Sasaki et al. 1987a; Riley et al. 1988; Tsintzas et al. 1993a; 1993b; 1995; Wilber and Moffatt 1992). Also, carbohydrate ingestion has been shown to improve both running capacity (Sasaki et al. 1987a; Tsintzas et al. 1993b; Wilber and Moffatt 1992), and running performance (Millard-Stafford et al. 1992), despite the fact that carbohydrate oxidation rate during exercise does not decline with time in the control trial. Recently, it has been found that the ingestion of a carbohydrate-electrolyte solution during treadmill running at 70-75% \( \text{VO}_2\text{max} \) reduces the rate of muscle glycogen utilisation in Type I muscle fibers (Tsintzas et al. 1993c; 1994).

In conclusion, it seems that usually, but not always, carbohydrate ingestion during exercise improves endurance capacity and performance when muscle glycogen levels are not supercompensated prior to exercise. Nevertheless, no study so far has produced a detrimental effect on endurance performance or capacity as a result of carbohydrate administration during exercise. Carbohydrate ingestion exerts its ergogenic effects either by maintaining
blood glucose concentration and carbohydrate oxidation rate, or by reducing the rate of muscle glycogen utilisation, or even by reducing glucose output from the liver.
Table 2.5: Studies that have examined the effects of carbohydrate ingestion during cycling on endurance capacity.

<table>
<thead>
<tr>
<th>STUDY</th>
<th>N</th>
<th>TYPE OF CHO INGESTED</th>
<th>EXERCISE INTENSITY (% VO₂max)</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Felig et al. 1982</td>
<td>19</td>
<td>G</td>
<td>60-65</td>
<td>No Improvement</td>
</tr>
<tr>
<td>Coyle et al. 1983</td>
<td>10</td>
<td>GP</td>
<td>70-79</td>
<td>Improvement</td>
</tr>
<tr>
<td>Bjorkman et al. 1984</td>
<td>8</td>
<td>G</td>
<td>68</td>
<td>Improvement</td>
</tr>
<tr>
<td>Coyle et al. 1986</td>
<td>7</td>
<td>GP</td>
<td>71</td>
<td>Improvement</td>
</tr>
<tr>
<td>Coggan &amp; Coyle 1988</td>
<td>7</td>
<td>GP</td>
<td>60-85</td>
<td>Improvement</td>
</tr>
<tr>
<td>Coggan &amp; Coyle 1989</td>
<td>6</td>
<td>GP+S</td>
<td>70</td>
<td>Improvement</td>
</tr>
<tr>
<td>Maughan et al. 1989</td>
<td>6</td>
<td>G</td>
<td>70</td>
<td>Improvement</td>
</tr>
<tr>
<td>Powers et al. 1990</td>
<td>9</td>
<td>GP</td>
<td>85</td>
<td>No Improvement</td>
</tr>
<tr>
<td>Burgess et al. 1991</td>
<td>9</td>
<td>S</td>
<td>80</td>
<td>No Improvement</td>
</tr>
<tr>
<td>Wright et al. 1991</td>
<td>9</td>
<td>GP+F</td>
<td>70</td>
<td>Improvement</td>
</tr>
<tr>
<td>Murdoch et al. 1993</td>
<td>8</td>
<td>SC,S-S</td>
<td>70</td>
<td>Improvement</td>
</tr>
<tr>
<td>Hargreaves et al. 1994</td>
<td>6</td>
<td>CE</td>
<td>70</td>
<td>Improvement</td>
</tr>
</tbody>
</table>

G=glucose; GP=glucose polymer; S=sucrose; F=fructose; SC=solid carbohydrate
S-S=semi-solid carbohydrate
N=number of subjects studied
Table 2.6: Studies that have examined the effects of carbohydrate ingestion during cycling on endurance performance.

<table>
<thead>
<tr>
<th>STUDY</th>
<th>N</th>
<th>TYPE OF CHO INGESTED</th>
<th>TYPE OF EXERCISE</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ivy et al. 1979</td>
<td>9</td>
<td>GP</td>
<td>120 min PR</td>
<td>No Improvement</td>
</tr>
<tr>
<td>Hargreaves et al. 1984</td>
<td>10</td>
<td>SC</td>
<td>SR</td>
<td>Improvement</td>
</tr>
<tr>
<td>Fielding et al. 1985</td>
<td>9</td>
<td>SC</td>
<td>SR</td>
<td>Improvement</td>
</tr>
<tr>
<td>Flynn et al. 1987</td>
<td>8</td>
<td>GP+F</td>
<td>120 min PR</td>
<td>No Improvement</td>
</tr>
<tr>
<td>Mitchell et al. 1988</td>
<td>8</td>
<td>G,F,S</td>
<td>12 min PR</td>
<td>Improvement</td>
</tr>
<tr>
<td>Mitchell et al. 1989</td>
<td>10</td>
<td>GP+F</td>
<td>15 min PR</td>
<td>Improvement</td>
</tr>
<tr>
<td>Murray et al. 1991</td>
<td>7</td>
<td>G+GP</td>
<td>4.8 km PR</td>
<td>Improvement</td>
</tr>
<tr>
<td>Cole et al. 1993</td>
<td>10</td>
<td>F,G,GP</td>
<td>15 min PR</td>
<td>No Improvement</td>
</tr>
<tr>
<td>Anantaraman et al. 1994</td>
<td>5</td>
<td>GP</td>
<td>60 min PR</td>
<td>Improvement</td>
</tr>
<tr>
<td>Madsen &amp; Christensen 1994</td>
<td>9</td>
<td>G</td>
<td>100 km</td>
<td>No Improvement</td>
</tr>
<tr>
<td>Below et al. 1995</td>
<td>8</td>
<td>GP</td>
<td>10 min PR</td>
<td>Improvement</td>
</tr>
</tbody>
</table>

G=glucose; GP=glucose polymer; S=sucrose; F=fructose; SC=solid carbohydrate; SR=sprint ride; PR=performance ride; N=number of subjects studied.
Table 2.7: Studies that have examined the effects of carbohydrate ingestion during running on endurance capacity.

<table>
<thead>
<tr>
<th>STUDY</th>
<th>N</th>
<th>TYPE OF CHO INGESTED</th>
<th>EXERCISE INTENSITY (% VO2max)</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macaraeg et al. 1983</td>
<td>12</td>
<td>GP+F</td>
<td>85</td>
<td>Improvement</td>
</tr>
<tr>
<td>Fruth &amp; Gisolfi 1983</td>
<td>7</td>
<td>G,F</td>
<td>70</td>
<td>No Improvement</td>
</tr>
<tr>
<td>Sasaki et al. 1987a</td>
<td>5</td>
<td>S</td>
<td>80</td>
<td>Improvement</td>
</tr>
<tr>
<td>Sasaki et al. 1987b</td>
<td>7</td>
<td>S</td>
<td>60-90</td>
<td>No Improvement</td>
</tr>
<tr>
<td>MacLaren &amp; Otter 1988</td>
<td>7</td>
<td>G</td>
<td>75</td>
<td>Improvement</td>
</tr>
<tr>
<td>Riley et al. 1988</td>
<td>9</td>
<td>GP+F</td>
<td>70</td>
<td>No Improvement</td>
</tr>
<tr>
<td>Wilber &amp; Moffatt 1992</td>
<td>10</td>
<td>GP+S</td>
<td>80</td>
<td>Improvement</td>
</tr>
<tr>
<td>Tsintzas et al. 1993b</td>
<td>7</td>
<td>GP+F+G</td>
<td>70</td>
<td>Improvement</td>
</tr>
<tr>
<td>Rehrer et al. 1994</td>
<td>6</td>
<td>GP+F+G</td>
<td>80</td>
<td>No Improvement</td>
</tr>
<tr>
<td>Tsintzas et al. 1994</td>
<td>8</td>
<td>GP+F+G</td>
<td>75</td>
<td>Improvement</td>
</tr>
<tr>
<td>Nicholas et al. 1995</td>
<td>8</td>
<td>GP+F+G</td>
<td>Walking to Sprint</td>
<td>Improvement</td>
</tr>
</tbody>
</table>

G=glucose; GP= glucose polymer; S=sucrose; F=fructose
N= number of subjects studied
Table 2.8: Studies that have examined the effects of carbohydrate ingestion during running on endurance performance.

<table>
<thead>
<tr>
<th>STUDY</th>
<th>N</th>
<th>TYPE OF CHO INGESTED</th>
<th>TYPE OF EXERCISE</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Williams et al. 1987</td>
<td>15</td>
<td>GP+S+F</td>
<td>2 hour Run</td>
<td>No Improvement</td>
</tr>
<tr>
<td>Williams et al. 1990</td>
<td>12</td>
<td>GP+G,GP+F</td>
<td>30 km</td>
<td>No Improvement</td>
</tr>
<tr>
<td>Millard-Stafford et al. 1992</td>
<td>8</td>
<td>GP+F</td>
<td>40 km</td>
<td>Improvement</td>
</tr>
<tr>
<td>Tsintzas et al. 1993a</td>
<td>7</td>
<td>GP+F</td>
<td>30 km</td>
<td>Improvement</td>
</tr>
<tr>
<td>Millard-Stafford &amp; Sparling 1994</td>
<td>10</td>
<td>GP+F+G</td>
<td>5 km</td>
<td>Improvement</td>
</tr>
<tr>
<td>Tsintzas et al. 1995</td>
<td>7</td>
<td>GP+F+G</td>
<td>42.2 km</td>
<td>Improvement</td>
</tr>
</tbody>
</table>

G=glucose; GP= glucose polymer; S=sucrose; F=fructose
N= number of subjects studied
2.6: Influence of Fluid Loss and Fluid Ingestion During Exercise on Temperature Regulation

Humans are homeothermic animals and therefore their body temperature should be maintained fairly constant. However, during exercise a considerable increase in heat production occurs since most of the chemical energy is converted to heat and only about 25% of this chemical energy can be transformed into mechanical energy.

The main mechanism by which the body maintains a narrow range of temperatures during exercise is by evaporation of sweat. Evaporation of 1 litre of sweat from the skin will remove about 580 Kcal of heat from the body (Maughan 1991). The evaporative capacity of sweat, however, is related to the ambient water vapour pressure. Sweat rates vary between individuals even when they exercise at the same intensity, under the same environmental conditions, and having the same body temperatures (Maughan 1991).

Sweat loss will result in a considerable reduction in body weight, especially when exercise is performed in the heat. Body weight loss may be up to 8% during a marathon race (Costill and Miller 1980) which would represent a 13% reduction in body water content, since total body water constitutes approximately 60% of an average adult's body weight (Sawka and Pandolf 1990). This sweat comes from the extravascular bed, since plasma volume seems to be fairly stable during prolonged exercise, except for an initial drop that takes place within the first 10-15 min of exercise (Coyle and Hamilton, 1990). Intracellular and extracellular water losses, however, take place with exception of vital organs such as brain and liver where strong homeostatic mechanisms probably defend their water content (Sawka and Pandolf 1990). Additional reasons for the stable plasma volume during prolonged exercise may include water release from metabolism and also redistribution of water from inactive muscles (Sawka 1992).

Except for the fluid shifts from the extravascular to intravascular space the body responds with an increase in the heart rate, which preserves cardiac output, and an elevated splanchnic vascular resistance, in an attempt to maintain sufficient blood flow to the skin and the working muscles (Nadel et al. 1990). However, if fluid loss continues, the cardiovascular drift and reduced splanchnic blood flow may not be sufficient to preserve blood
volume to the muscles and the periphery. Under these conditions blood flow to the skin may be compromised, leading to a reduced sweat rate, which in turn will increase body temperature (Sawka 1992).

Furthermore, fluid loss will cause a higher plasma osmolality (Sawka 1992). This plasma hyperosmolality may further reduce the fluid in the intracellular space, but may also act as a stimulus for the reduction in the sweat rate and an increase in core temperature (Fortney et al. 1984; Sawka 1992). Unless the body's compensatory mechanisms against all these disturbances are assisted with fluid ingestion, not only physical performance will suffer but the person's health will be in danger (Maughan 1991; Lamb and Brodowicz 1986; Sawka 1992; Sawka and Pandolf 1990; Noakes 1993).

Fluid ingestion has been shown to reduce the increase in rectal temperature and heart rate, and to maintain cardiac output and stroke volume when environmental temperature is 33 °C (Montain and Coyle 1992a; 1992b; 1993), or even when it is about 22 °C (Hamilton et al. 1991). It seems that the degree of the beneficial effects of fluid ingestion during prolonged exercise are associated with the total amount of fluid consumed (Montain and Coyle 1992b) rather than the timing of the fluid intake during exercise (Montain and Coyle 1993). The authors have suggested that fluid ingestion reduces the degree of hyperthermia by limiting the fluid loss-induced serum hyperosmolality which is associated with a lower dehydration level and a high skin blood flow (Coyle and Montain 1993).

In terms of endurance capacity it has been shown that when subjects received plain water during treadmill running at 70% VO₂max they were able to exercise 25 min longer than when no water was provided (Fallowfield, 1994). Recently, it has been demonstrated that endurance cycling performance is also improved when subjects receive fluid or carbohydrate solutions during exercise in the heat. This improvement was produced independently by fluid and carbohydrate ingestion and their effects seemed to be additive (Below et al. 1995).

Although some general guidelines have been proposed by some authors about the optimal strategy for fluid replacement during prolonged exercise (Gisolfi and Duchman 1992; Coyle 1994), the variability among individuals regarding responses in sweat rate, fluid tolerance and race conditions makes
it very difficult for somebody to give specific instructions. Athletes are advised to find their own fluid ingestion strategy by practising fluid ingestion in training or race-simulated condition, after estimating their personal sweat loss from the change in body weight (Maughan and Noakes 1991).

2.7 : Gastric Emptying Rate and Intestinal Absorption of Carbohydrate Feedings

As it was reported earlier the ingestion of carbohydrate solutions before and especially during exercise helps to maintain fluid homeostasis and also provides fuel to the working muscles during prolonged exercise. The effectiveness, however, of these carbohydrate feedings depend on how quickly they pass through the stomach, and absorbed by the small intestine and enter the circulation.

2.7.1 Gastric emptying rate of liquids

There is considerable variation among individuals regarding the capacity for gastric emptying. There are people who show little or no emptying 20-30 min after feeding, whereas others may empty 90-95% of a feeding within this time period (Costill 1990). In general, however, gastric emptying seems to have an exponential pattern with an initial rapid phase which is probably due to the volume effect (Hunt and Spurrell, 1951).

It has been suggested that the regulation of gastric emptying seems to be under a 'dynamic closed loop feedback' system. The initial rapid emptying phase will allow the bolus to enter the duodenum and trigger a large number of receptors sensitive to a variety of factors. These receptors, in turn, will inhibit or activate gastric emptying (Brener et al. 1983 cited by Brouns et al. 1987).

Several factors have been considered as stimuli on the rate of gastric emptying, namely: volume, energy density, mode and intensity of exercise, osmolality, acidity, environmental temperature, electrolytes, drink temperature, hormones, dehydration, acclimation, time of day, phase of
menstrual cycle, stress, fear, and anxiety (Maughan 1991; Rehrer 1991; Murray 1987; Brouns et al. 1987; Costill 1990). However, the most important factors seem to be the volume, energy content, and osmolality of the ingested fluid (Noakes et al. 1991; Costill 1990).

There is considerable discrepancy in the literature regarding the influence of the above factors on the rate of gastric emptying. Except for differences in experimental protocols and subjects' responses, the employed methodology may also contribute to a certain degree to this discrepancy (Rehrer 1991; Maughan 1991). For instance, measuring the stomach contents only once over the observation period assumes that there is a linear rate of gastric emptying (Rehrer 1991; Maughan 1991). In the past 8 years a group of scientists modified the double sampling technique of George (Beckers et al. 1988) which enables multiple measurements of gastric residue over a certain period of time (Rehrer 1990; Vist and Maughan 1993).

Using this technique the exponential time course of gastric emptying was verified (Rehrer et al. 1989; 1990). This fact emphasises the importance of volume as a strong factor influencing gastric emptying rate (Noakes et al. 1991; Maughan 1991). Therefore, volumes of about 400-600 ml or equivalent to 8 ml/kg body weight have been administered at the initiation of exercise to facilitate gastric emptying (Rehrer et al. 1989). In order to maintain a large gastric volume, however, repeated feeding should take place. Several studies have shown that with repeated drinking of about 100-200 ml every 15-20 min during exercise carbohydrate solutions empty from the stomach as effectively as water (Mitchell et al. 1988; 1989b; Ryan et al. 1989; Houmard et al. 1991; Rehrer et al. 1990). Larger volumes (350-400 ml) have also been used during cycling exercise and have produced higher average gastric emptying rates (Mitchell and Voss 1991; Ryan et al. 1989). However it is questionable whether such high volumes of fluid will be tolerated during running.

Another factor which may influence gastric emptying rate is the osmolality of the solution. Hunt and Pathak (1960) reported that hypertonic electrolyte solutions slowed gastric emptying rate. Also, Coyle et al. (1978) observed an inverse relationship between gastric emptying rate and osmolality. However, Hunt (1960) found that isocaloric carbohydrate solutions emptied the stomach at similar rates despite the fact that they possessed different osmolalities. This should lead to the conclusion that it was the energy content rather than the
osmolality that determined the gastric emptying of these solutions (Costill 1990). This conclusion is supported by several studies which have shown that when carbohydrate concentration, and consequently energy content, increases above 2.5% gastric emptying of carbohydrate solutions are slower than water (Costill and Saltin 1974; Vist and Maughan 1993; Neufer et al. 1986). Furthermore, solutions with higher energy content empty from the stomach slower than iso-osmotic solutions with lower energy content (Hunt and Stubbs 1975; Vist and Maughan 1993). Therefore, it has been suggested that the energy content of a solution is a stronger regulator of gastric emptying than osmolality (Costill 1990; Vist and Maughan 1993).

As far as the effect of exercise is concerned, when exercise intensity does not exceed 70-75% \( \dot{V}O_2 \text{max} \) gastric emptying rate of carbohydrate and electrolyte solutions is similar to resting values (Costill and Saltin 1974; Fordtran and Saltin 1967). However, Neufer and co-workers (1986; 1989) reported higher gastric emptying rates for carbohydrate solutions and water during running (\( \leq 70\% \dot{V}O_2 \text{ max} \)) compared to resting conditions. Nevertheless, when exercise intensity exceeds 75% \( \dot{V}O_2 \text{max} \) gastric emptying seems to be delayed (Rehrer et al. 1989; Costill and Saltin 1974; Sole and Noakes 1989).

2.7.2: Intestinal absorption of liquids

Although some exchange of water takes place in the stomach, the main site for fluid and nutrient absorption is the small intestine. Absorption of fluid and nutrient occurs through the process of isotonicity of the solution in the small intestine. Osmotic equilibration is possible because the muscosal membranes of the duodenum and jejunum are permeable to water and electrolytes (Murray 1987). Net water absorption, which is a passive process, occurs in the direction that keeps luminal contents isoosmotic. Therefore, carbohydrate-electrolyte solutions that are hypotonic promote water absorption (Leiper and Maughan 1986a; 1986b). Water absorption is also promoted by sodium, glucose, and sucrose (Gisolfi and Duchman 1992; Murray 1987).

Glucose absorption is sodium dependent, whereas fructose is not. However, fructose is less rapidly absorbed than glucose, and when it is ingested in large quantities can cause gastrointestinal distress (Gisolfi and Duchman 1992; Maughan 1991). Sucrose, on the other hand, is quickly hydrolysed in
glucose and fructose and has been shown to promote glucose absorption (Maughan 1991; Gisolfi and Duchman 1992).

Due to practical difficulties involved, few studies have attempted to study the rate of intestinal absorption during exercise. The results in the literature appear to be conflicting. Maughan and his colleagues (1990) (cited by Maughan 1991) examined the effect of cycling exercise intensity (42-80% $\dot{V}O_{2}\text{max}$) on the rate of accumulation of deuterium oxide in the plasma, which is an index of both gastric emptying and absorption. Subjects cycled for 30 min after consuming 200 mls of a carbohydrate-electrolyte solution labelled with $^{2}$H$_2$O. The rate of accumulation of deuterium oxide was greater at rest than during exercise at 61 or 80% $\dot{V}O_{2}\text{max}$.

A segmental perfusion technique, utilising a triple-lumen tube, has also been employed to study directly intestinal absorption in vivo (Gisolfi et al. 1990). This method measures the net fluid and solute flux across a select segment of intestine. Barclay and Turnberg (1988) (cited by Schedl et al. 1994) found low electrolyte and fluid absorption rates in subjects who received a fluid-electrolyte solution during cycling at 103 beats/min. On the other hand, other studies have shown no influence of exercise on intestinal absorption of fluid or solute during cycling (Gisolfi et al. 1991), or running (Fordtran and Saltin 1967) at exercise intensities ranging 30-78% $\dot{V}O_{2}\text{max}$. In Gisolfi et al.'s (1991) study, however, when data were pooled a 6% carbohydrate-electrolyte solution was superior to plain water in terms of fluid absorption. Nevertheless, it should be pointed out that the perfusion technique has certain limitations. It does not measure transport of the solution being perfused, but measures the fluxes only across a small (about 40 cm) segment of the small intestine. Also, it may not reflect the absorption rate when a solution is ingested (Schedl et al. 1994).

2.7.3: Gastric emptying and absorption of solids

Emptying of solid meals can not be measured using the aspiration method. Also, there are problems in ensuring that tracers added to the meal empty at the same rate as its contents (Maughan and Rehrer 1993). However, some
attempts have been made to monitor gastric emptying and intestinal absorption of solid meals during rest or exercise.

Meyer et al. (1976) used $^{99m}$technetium sulfur colloid ($^{99m}$Tc) as a marker in chicken liver in order to label solid food (hamburger or beef stew). They observed that water emptied faster than solids. Also, in one subject who consumed two meals, different in size, the small meal emptied quicker than the bigger one, although the rate of gastric emptying was not affected. Moore et al. (1981) examined the gastric emptying of various sizes (300-1692 g) of meals with different energy contents (196-1945 Kcal). They observed that half times of liquids were faster than solids, while the larger weight and energy content were associated with longer emptying times for both solids and liquids.

Solids may also be under the influence of particle size. It has been shown that when liver was given to humans, in large (1 cm) liver cubes emptied slower than when given in smaller (0.3 cm) liver cubes (Holt et al. 1982). Similar results were reported by Hinder and Kelly (1977) who found that 50 g of liver given to the dog into 1 cm cubes had to be further reduced to smaller sizes, about 2 mm or even smaller, before emptied the stomach. It was also observed that homogenisation of the liver accelerated gastric emptying. Furthermore, indigestible spheres of 7 mm diameter given to the animals remained in the stomach (Hinder and Kelly 1977), suggesting that probably pylorus does not allow the passage of particles greater than 1-2 mm (Meyer et al. 1976). It has been suggested that solid food is 'ground down' and 'liquefied' by the gastric peristalsis action before enters the duodenum (Holt et al. 1982).

As far as exercise is concerned Cammack et al. (1982) reported that intermittent cycling at 117 beats/min for 6 hours significantly increased gastric emptying (half time) of a 630 kcal meal labelled with $^{99m}$Tc, but had no effect upon small bowel transit time. On the other hand, however, Feldman and Nixon (1982) found no exercise effect on gastric emptying of the meal marker or the gastric acid secretory response to a steak meal consumed 45 min before cycling at 50-70% $\dot{V}O_2$max. Assuming that serum triglyceride concentration would reflect absorption of dietary lipids, they suggested that exercise did not influence absorption of dietary fat due to the fact that 75 min
post-exercise serum triglycerides were similar in exercise and control conditions.

In conclusion, gastric emptying of liquids is mainly influenced by volume and energy content, whereas solids are influenced by particle size. Also, carbohydrate-electrolyte solutions seem to provide the muscle with fuel without compromising fluid absorption.

2.8 Summary

Carbohydrate, in the form of muscle glycogen or blood glucose, is the main source of energy during prolonged heavy (> 65% VO₂ max) exercise. Although fatigue may be central or peripheral in origin, fatigue during prolonged exercise has been associated with a marked reduction in muscle glycogen concentration in the working muscle, a low blood glucose concentration, and a reduced carbohydrate oxidation rate. However, when exercise is performed in a hot environment fatigue may occur due to disturbances in thermoregulation and fluid homeostasis before muscle glycogen becomes depleted. Carbohydrate ingestion before or during exercise becomes available to the working muscle, provides an extra source of energy, and usually, but not always, delays the onset of fatigue and improves endurance performance. Furthermore, carbohydrate solutions seem to help in reducing the exercise-induced disturbances in thermoregulation and fluid homeostasis. Carbohydrate feedings during exercise may improve endurance capacity and performance by maintaining carbohydrate oxidation and glucose homeostasis late in exercise, or by sparing the endogenous glycogen stores. On the other hand, pre-exercise carbohydrate ingestion may improve endurance capacity and performance not only through an enhanced glucose oxidation, but also by elevating pre-exercise muscle and/or liver glycogen concentration.
CHAPTER 3

GENERAL METHODS

3.1 Introduction

In the present thesis, two different experimental models were employed to study the effects of carbohydrate feedings before and during exercise on endurance capacity and endurance performance during treadmill running. Endurance running capacity was assessed by the time taken to reach exhaustion while running at a constant speed corresponding to approximately 70% $\text{VO}_2$ max (Chapters 4 and 5 respectively). Endurance running performance was assessed by the time taken to cover 30 km on the treadmill (Chapter 8). Furthermore, a fixed duration (60 min) bout of running exercise was selected in order to investigate the effects of carbohydrate feeding during exercise on muscle metabolism in fed individuals (Chapter 7). Finally, a non-exercise experimental approach was adopted to examine the effect of carbohydrate feeding on skeletal muscle glycogen concentration (Chapter 6).

This chapter has been divided into four sections. The first section describes the preliminary measurements performed before the main experimental tests, whereas the second section outlines the experimental protocols employed. The third and fourth sections deal with the procedures followed during the collection, treatment, and analysis of blood and muscle samples respectively.

3.2 Preliminary Measurements

Before every individual acted as a subject in these studies he was fully informed about all the procedures involved during each test and also completed a health history questionnaire, and signed a statement of informed consent. All the studies had the approval of the University's Ethical Advisory Committee.
3.2.1 Familiarisation
Before taking part in any major experimental test subjects were fully familiarised with experimental procedures and especially with collection of expired air samples and ingestion of fluid while running on the treadmill.

3.2.2 Body weight, height, heart rate, and rectal temperature measurements
In the preliminary as well as in the main tests body weight was measured using a balance accurate to two decimal places (Avery 3306 ABV), whereas heart rate was continuously recorded by short range telemetry (Polar Electro sports tester PE 3000). Body height of all subjects was obtained using a stadiometer (Holtain Ltd). In Chapter 4 rectal temperatures were recorded using a rectal probe connected to a thermometer (Edale Instruments Ltd, Model C).

3.2.3 Collection and analysis of expired air samples
The collection of expired air samples during the preliminary as well as the main tests was carried out using the Douglas bag method. The subjects breathed through a low resistance respiratory valve which was connected to a 150 litre Douglas bag by lightweight smooth bore tubing (Falconia Ltd). Oxygen (O₂) and carbon dioxide (CO₂) content were determined using a paramagnetic oxygen analyser (Sybron-Taylor, model 570A) and an infrared carbon dioxide analyser (Lira, model 303), respectively. Analysers were calibrated against atmospheric air, and known O₂ and CO₂ gas mixtures prior to each analysis. A dry gas meter (Harvard Ltd) and a thermometer (Edale Instruments Ltd, model C) were used to measure the volume and the temperature of each air sample, respectively. The dry gas meter was also calibrated using a 600 litre Tissot spirometer (Collins Ltd, USA). Barometric pressure was obtained from a barometer (Griffen and George Ltd). Using the Haldane transformation formula, all gas volumes were corrected to STPD conditions. In this way, oxygen uptake (\(\dot{V}O_2\)), carbon dioxide (\(\dot{V}CO_2\)), minute ventilation (\(\dot{V}E\)), and respiratory exchange ratio (RER) were calculated. Carbohydrate and fat oxidation rates were calculated from a non-protein RER (Appendix E).
3.2.4 Speed- Oxygen uptake test
This test was carried out in all the studies except the study presented in Chapter 6. This test was conducted in order to determine the relationship between oxygen uptake and running speed (running economy).

The test was performed on a motorised treadmill. Two different motorised treadmills were used in all exercise tests reported in this thesis (Woodway, model ELG 2 for studies presented in Chapters 4, 5, and 7, and Quinton Seattle, Washington USA for studies presented in Chapters 7 and 8). However, the same treadmill was used by a subject for the whole of any one study.

The test consisted of 16 minute continuous submaximal running. The initial speed was set between 2.5 and 3.5 m.s\(^{-1}\), depending on subject's training status, on a level treadmill. The speed was increased every four minutes by 0.4-0.5 m.s\(^{-1}\) again depending on subject's fitness. Expired air samples, were collected during the last minute of each four-minute period, while heart rate was monitored throughout the test. From this test a regression equation relating oxygen uptake to running speed was obtained, from which running speeds at 70% \(\dot{V}O_2\) max could be predicted.

3.2.5 Maximal oxygen uptake test (\(\dot{V}O_2\) max)
This test involved uphill treadmill running using a continuous incremental test to fatigue (Taylor et al. 1955). The speed was kept constant throughout the test, whereas the inclination of the treadmill increased by 2.5% every three minutes from an initial 3.5%. Expired air samples were collected between 1:45-2:45 min of every 3-min period. The final expired air sample was taken during the last minute of the test, after the subject indicated, by raising his hand, that he was able to continue for only one more minute. Verbal encouragement was given to subjects during this test. Heart rate was continuously monitored, and perceived rate of exertion (PRE) (Borg, 1973) was recorded every 3 minutes. The \(\dot{V}O_2\) value obtained during the last expired air sample was considered to be the \(\dot{V}O_2\) max value of the subject.

3.2.6 Speed - Lactate test
This test was carried out only in the study presented in Chapter 8. The subjects run on a level treadmill at speeds corresponding to 60%, 70%, 80%, and 90% of their \(\dot{V}O_2\) max. The speeds were calculated using the data from
and 90% of their \( \dot{V}O_2 \) max. The speeds were calculated using the data from the preliminary tests. During the test subjects ran continuously for 4 min at speeds equivalent to each of the above exercise intensities. Expired air samples were collected between 3-4 min of each 4-min period. Heart rate was also recorded throughout the test. Duplicate 20 \( \mu \)l capillary blood samples from the subject's thumb were collected between 3:30-4:00 min of each 4 min period, and analysed for blood lactate concentration. In addition, a resting pre-exercise capillary blood sample was taken from a pre-warmed hand. This test enabled the running speeds corresponding to blood lactate concentrations of 2 and 4 mmol.l\(^{-1} \) to be calculated.

After all preliminary tests were completed a treadmill run at 70% \( \dot{V}O_2 \) max for 30-60 min was also performed 7-10 days before the first experimental trial (except in Chapter 6). This was performed to ensure that subjects were fully familiarised with experimental procedures and also to check the accuracy of the predicted speed corresponding to 70% \( \dot{V}O_2 \) max.

3.2.7 Diet and training control
In all studies subjects reported to the laboratory after a 12-hour overnight fast. The subjects weighed and recorded their normal food intake for 2-3 days prior to the first trial, and tried to replicate the same diet for the same period of time before the next trials. The diets were analysed (Paul and Southgate, 1978) for energy, carbohydrate, protein, and fat content and were found to be no different between each study's experimental trials. Furthermore, subjects refrained from exercise the day prior to main trials, and followed the same training schedule three days before each trial.

3.3 Experimental Protocol

All experiments were conducted in the laboratory under environmental conditions which were frequently measured (temperature: 19-24 °C, and humidity: 50-65%). Both dry and wet bulb temperatures were measured and adjusted accordingly throughout the tests using a whirling hydrometer (Brannan). From these values relative humidity was calculated by using a conversion scale.
completion of the exercise test. The post-exercise body weight was measured after the removal of the sweat from the skin. Body weight changes were corrected for fluid consumption during exercise.

3.3.1 Endurance Capacity Protocol
In Chapters 4 and 5, endurance capacity was measured as the time taken to reach exhaustion during running on a level treadmill at a constant speed corresponding to 70% of each subject's \( \dot{V}O_2 \) max. Exhaustion was defined as the point in time where subjects were unable to maintain the speed of the treadmill. However, in order to ensure that subjects had reached the fatigue point a two minute reduction of the speed to 60% \( \dot{V}O_2 \) max was allowed when subjects felt very tired. Thereafter, the speed was increased to the prescribed level and the subjects encouraged to continue running for as long as possible.

3.3.2 Endurance Performance Protocol
In Chapter 8 endurance performance was measured as the time taken to complete a 30 km distance on a level treadmill. Subjects were required to cover the distance as quickly as possible. The treadmill was interfaced with a microcomputer (BBC Master Series), and the speed, time, and distance elapsed were displayed on the computer's screen. This information was also stored on the computer for later analysis. Subjects did not know the exercise time, but they received information regarding distance and running pace. Using this instrumentation subjects were able to control the speed of the treadmill using a hand-held microswitch, in an attempt to complete each of the two runs as fast as possible.

In both experimental protocols all subjects were highly motivated and were verbally encouraged throughout the exercise period.

3.3.3 Carbohydrate feedings
In this thesis carbohydrate was consumed before exercise in the form of a meal. During exercise carbohydrate was provided in the form of a carbohydrate-electrolyte drink.
Pre-Exercise Meal
In all studies a high carbohydrate meal was consumed after a 12-hour overnight fast. In Chapters 4, 5, and 7 the meal was eaten 3 hours before exercise commenced, whereas in Chapter 8 exercise begun 4 hours after the consumption of the meal. In Chapter 6 no exercise was performed but the postprandial physiological and metabolic responses were studied for 3 hours after the consumption of the meal.
In the studies reported in Chapters 4, 5, 6, and 7 the meal was designed to provide 2.5 g.kg\(^{-1}\) BW carbohydrate, whereas in the study presented in Chapter 8 the meal provided 2 g.kg\(^{-1}\) BW carbohydrate (Appendix C). Both meals consisted of white bread, jam, cornflakes, skimmed milk, and orange juice, which amounted to about 86% of energy intake from carbohydrates, 11% from protein, and less than 3% from fat. About 88% of the carbohydrate included in the meals was obtained from foods classified as having a High Glycemic Index, whereas the rest was obtained from foods with Moderate and Low Glycemic Index (Jenkins et al. 1984; 1988).

The subjects had to consume the meals within 10-20 min. When a meal was consumed twice in a study (Chapters 5 and 7), subjects kept the same time for food consumption and the same order by which they ate the various food ingredients.

Since solid food was used a double blind design was almost practically impossible. In order to make the design single blind subjects were told that the purpose of the experiments (Chapters 4, 5, and 8) was to compare liquid and solid carbohydrate food. Therefore, in studies where endurance performance and endurance capacity were measured, subjects ingested once (Chapters 5 and 8) or twice (Chapter 4) 10 ml.kg\(^{-1}\) BW of a liquid placebo (dilute sugar free lemon and lime juice; less than 1 kcal pre 100 ml undiluted).

Carbohydrate feeding during exercise
In all studies, except in Chapter 6, an isotonic lemon and lime flavoured carbohydrate-electrolyte solution was provided during exercise. As control in Chapter 4 a placebo (dilute sugar free lemon and lime juice) was ingested, whereas in Chapters 5, 7, and 8, water was used. The carbohydrate-electrolyte solution was a commercially available sports drink (Lucozade
Sport, Still; SmithKline Beecham), which contained 6.9% carbohydrates (dextrose, maltodextrin, and glucose syrup) and four electrolytes (24 mmol.l⁻¹ sodium, 2.5 mmol.l⁻¹ potassium, 1.2 mmol.l⁻¹ calcium, and 0.8 mmol.l⁻¹ magnesium). The drinking pattern employed in each study is described in the individual chapters. Plastic 30 ml volumetric syringes were used by the subjects for fluid ingestion to avoid any spillage and ensure that the right amount was consumed. In this way fluid ingestion did not interrupt running. The solution was kept chilled to improve palatability, although no effort was made to be administered at a constant temperature.

In order to monitor the subjects' abdominal discomfort (ADS) and their sensation of gut fullness (GFS) two scales were used during exercise (Chapters 4, 5, 7, and 8), as well as during the 3 hours postprandial period (Chapter 5) after the consumption of the meal or placebo (Appendix D).

3.4 Collection, Treatment, and Analysis of Blood Samples

3.4.1 Blood Sample Collection

In Chapters 5, 6, and 7, venous blood samples (10 ml) were obtained from an ante-cubital vein using an indwelling catheter (Venflon, 16 G), which was inserted under local anaesthesia (0.5 ml of 1% lignocaine). The catheter was inserted after the subjects rested for 15 min on an examination couch, and was kept patent by infusion of sterile normal saline. Venous blood samples (10 ml) were also collected using a hypodermic needle before and immediately after exercise in Chapters 4 and 8 and before the ingestion of the meal or placebo in Chapters 6 and 8.

Duplicate 20 (Chapter 8) or 25 (Chapter 4) µl capillary blood samples were collected from the subjects' thumb before the ingestion of the meal or placebo (Chapters 4 and 8), during the post prandial period (Chapter 8) and during exercise (Chapters 4 and 8). All resting capillary blood samples were collected from the thumb of a prewarmed hand.

Before and after exercise venous samples were used to estimate changes in plasma volume using the changes in haemoglobin concentrations and haematocrit values according to the method by Dill and Costill (1974). Since
the relative differences between conditions were the main objective, and bearing in mind that posture may well affect plasma volume, it was important to standardise body position during the collection of pre and post exercise venous samples. Therefore, although posture was different from study to study (i.e: standing for 20 min in Chapters 5 and 8, sitting for 1 min in Chapter 4, and lying for 10 min in Chapter 6), in all the trials within a study the same body position was used. However, because in Chapter 7 the pre-exercise venous sample was taken while subjects were lying on a couch, the baseline for plasma volume was chosen to be the 10th min into exercise.

3.4.2: Treatment and analysis of blood samples

Venous blood samples
In all studies venous samples were collected into 5 ml lithium heparin and serum tubes. In Chapter 8 about 1 ml of blood was also immediately placed into calcium heparin plastic tubes (50 U.ml⁻¹) and centrifuged (Eppendorf, Model 5414) for 3 min at 1,200 rpm. The plasma obtained was stored at -70 °C and analysed within 48 hrs for ammonia (da Fonseca-Wollheim, 1973) using a spectrophotometer (Cecil, CE 393).

The 5 ml venous blood samples collected into serum tubes were left to clot for 60 min at room temperature. Following this, samples were centrifuged in a refrigerated (3 °C) centrifuge (Burkard) for 15 min at 6000 rpm. The serum obtained was stored at -70 °C and later analysed for insulin (¹²⁵I radioimmunoassay; Coat-A-Count Insulin, DPC kit) using a gamma counter (Packard, Cobra 5000), and for sodium and potassium concentrations by flame photometry (Corning 435 equipped with Dilutor 800).

From the 5 ml venous blood samples collected into lithium heparin tubes duplicate 20 µl aliquots of whole blood were taken and deproteinised in 200 µl of perchloric acid (2.5 %), centrifuged for 5 min at 13000 rpm and stored at -20 °C (Chapters 5, 6, and 7). These samples were analysed at a later date for blood lactate concentrations (Maughan, 1982) using a flurometer (Locarate, Model 8-9), and for blood glucose (Boehringer Mannheim Glucose test combination, GOD/Perid method in Chapters 5, and 6; Roche Gluc GDH kit in Chapter 7) using a spectrophotometer (Chapters 5, and 6), or an automatic photometric analyser (Cobas Bio, Chapter 7). Another duplicate 20 µl of whole venous blood were used for the determination of haemoglobin
concentration (Boehringer Mannheim GmbH test combination). Triplicate 20 µl aliquots of blood were also analysed for haematocrit values using a microcentrifuge (Hawksley Ltd). As mentioned previously, haemoglobin and haematocrit values were used to estimate percentage changes in plasma volume.

The remained of the whole venous blood was centrifuged in a cool (3 °C) centrifuge for 15 min at 6000 rpm, and the plasma obtained was stored at -20 °C before being analysed at a later date for FFA (Wako kit) using an automatic photometric analyser, glycerol (Laurell and Tibbling, 1966) using a fluorometer, and urea (Boehringer Mannheim kit, Chapters 5 and 8) using a spectrophotometer. Also, in Chapter 5 about 600 µl of plasma were treated with 200 µl of an antioxidising agent which was made up of 100 mmol.1⁻¹ EGTA (ethylenglycerol tetra-acetic acid) and 100 mmol.1⁻¹ GSH (reduced glutathione). The treated plasma was stored at -70 °C and later analysed for plasma adrenaline and plasma noradrenaline concentrations (Forster et al. 1991), using high performance liquid chromatography (Gilson, Model 302).

**Capillary blood samples**

The 20 µl capillary blood samples (Chapter 8) were treated as described before for the 20 µl venous deproteinised samples. The 25 µl capillary blood samples (Chapter 4) were deproteinised in 250 µl of perchloric acid (2.5 %) and treated in the same way as the 20 µl venous deproteinised samples. The capillary blood samples were analysed for blood lactate (as described above), and blood glucose concentrations (spectrophotometrically using Boehringer Mannheim Glucose test combination GOD/Perid in Chapter 8, and Roche Gluc GDH kit using an automatic photometric analyser in Chapter 4).

With the exception of the assays where the analysis was automated (FFA and Glucose using an automatic photometric analyser, and Sodium and Potassium using Flame Photometry with automated sample dilution) an automatic dispenser was used for aspirating and dispensing various volumes during the assay analyses (Hamilton MicroLab 1000). However, serum insulin was analysed manually. Also, the pH of the various buffers was measured using a pH meter (Corning, Model 240) which was calibrated with appropriate buffer solutions (Fisons).
using a pH meter (Corning, Model 240) which was calibrated with appropriate buffer solutions (Fisons).

The coefficient of variation \([\text{S.D/mean} \times 100]\) of the blood, plasma, and serum metabolite assays is shown in Table 3.1. The coefficient of variation \((n=15)\) for haemoglobin and haematocrit were 0.9% and 0.6% respectively.

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

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Concentration</th>
<th>C.V (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood lactate</td>
<td>2 mmol.l(^{-1})</td>
<td>1.8</td>
</tr>
<tr>
<td>Blood glucose (spectrophotometrically)</td>
<td>6 mmol.l(^{-1})</td>
<td>1.2</td>
</tr>
<tr>
<td>Blood glucose (automated photometric analysis)</td>
<td>6 mmol.l(^{-1})</td>
<td>1.0</td>
</tr>
<tr>
<td>Plasma FFA</td>
<td>0.30 mmol.l(^{-1})</td>
<td>1.5</td>
</tr>
<tr>
<td>Plasma ammonia</td>
<td>75 µmol.l(^{-1})</td>
<td>3.5</td>
</tr>
<tr>
<td>Plasma glycerol</td>
<td>0.20 mmol.l(^{-1})</td>
<td>2.6</td>
</tr>
<tr>
<td>Plasma urea</td>
<td>5 mmol.l(^{-1})</td>
<td>0.8</td>
</tr>
<tr>
<td>Serum insulin</td>
<td>35 mU.l(^{-1})</td>
<td>5.8</td>
</tr>
<tr>
<td>Serum sodium</td>
<td>142 mmol.l(^{-1})</td>
<td>0.3</td>
</tr>
<tr>
<td>Serum potassium</td>
<td>4.2 mmol.l(^{-1})</td>
<td>0.3</td>
</tr>
<tr>
<td>Plasma adrenaline</td>
<td>0.35 nmol.l(^{-1})</td>
<td>10.0</td>
</tr>
<tr>
<td>Plasma noradrenaline</td>
<td>3.00 nmol.l(^{-1})</td>
<td>3.0</td>
</tr>
</tbody>
</table>
3.5 Collection Treatment, and Analysis of Muscle Samples

3.5.1 Collection of muscle samples

Muscle biopsy samples were obtained from vastus lateralis by a precutaneous needle biopsy technique (Bergstrom, 1962) with suction being applied (Evans et al., 1982). All samples were taken under local anaesthesia (2 ml of 1% lignocaine), and their size ranged between 30 and 100 mg wet weight. The vastus lateralis muscle was selected because, apart from being easily accessible, it has been shown to be heavily involved during treadmill running (Tsintzas et al. 1994).

3.5.2: Treatment and analysis of muscle samples

Sample treatment
All muscle samples were immersed in liquid nitrogen within 2-3 sec of sampling. Sampling in Chapter 7 took place 15-60 sec after subjects had stopped running on the treadmill. The samples were quickly removed from the needle, placed in screw-top plastic tubes (Eppendorf), and kept in liquid nitrogen until they were freeze-dried. After samples were freeze-dried they were stored at -70 °C in plastic tubes. At a later date, samples were removed from the freezer, left to thaw at room temperature, and were washed twice with 1 ml petroleum ether (30-40 °C) to remove the fat. Each time the samples were mixed before removing the ether. Finally, the tubes were left open in a fume cupboard for the evaporation of the petroleum ether until the sample was completely dry. Following this, samples were dissected free of visible blood and connective tissue, powdered using an agate pestle and mortar, placed in clean plastic tubes and weighed using an electrical balance accurate to five decimal places. The samples then were stored at -70 °C for analysis at a later date.

Sample analysis
On the day of the analysis muscle metabolites were extracted by adding 1 ml of 5 mmol.l⁻¹ perchloric acid (HClO₄), containing 1 mmol.l⁻¹ EDTA.Na₂ (ethylenediamine tetra-acetic sodium salt), for every 10 mg of muscle
powder which was kept in liquid nitrogen so that the extracting solution froze on introduction into the tube (Harris et al., 1974). Each sample was then placed at -20 °C for the extracting solution to thaw. The samples were then placed on ice and agitated (Stuart flask shaker) for 30 min, and then centrifuged (3 °C) at 6000 rpm for 10 min. The supernatant was then removed using a volumetric pipette, carefully measured, placed into clean cool plastic tubes, and neutralised by adding 1/4 volume of 2.1 mol.l⁻¹ potassium bicarbonate (KHCO₃). The remaining muscle pellet was kept at 3 °C for later analysis. The extracts of the samples were then mixed and left in the fridge at 3 °C with loose caps to let the CO₂ formed escape, before being centrifuged for 5 min at 3 °C. Following this, the supernatant was removed, placed into clean plastic tubes and kept on ice. The pH of the extract was 7.0, and 1 ml of it was equivalent to 8 mg of muscle powder (Harris et al., 1974).

All muscle assays were performed within two days. The analysis of ATP, PCr, Cr, and acid insoluble glycogen was performed on the first day immediately following the extraction procedure. The remaining extract was stored at -70 °C and analysed the following day for free glucose, G-6-P, lactate, and acid soluble glycogen. The assay methods were modified methods described by Harris et al. (1974), and Lowry and Passonneau (1972).

Glycogen was assayed by hydrolysis in 1 mol.l⁻¹ hydrochloric acid (HCl) both on the neutralised extract (acid-soluble glycogen) and on the precipitated muscle pellet (acid insoluble glycogen). The total muscle glycogen concentration was calculated by adding the acid soluble and acid insoluble glycogen concentrations.

All the muscle metabolites, except acid insoluble glycogen, were assayed fluorometrically. The analysis was based on enzyme catalysed reactions, the coenzymes NAD⁺ and NADP⁺ being simultaneously reduced to NADH and NADPH, respectively. The acid insoluble glycogen was assayed spectrophotometrically for glucose using a commercially available glucose kit (Boehringer Mannheim Glucose test combination, GOD/Perid method). All assays were carried out manually using pre-calibrated volumetric pipettes. Assay procedures are presented in detail in Appendix A.

All chemicals (Grade I) were obtained as standard commercial items from Boehringer, Fisons and Sigma. All reagents were made up with double
distilled water the day prior to analysis. The molarity of standards was checked on the same day spectrophotometrically using the coefficient of extinction of NADH (Appendix B). The standard-fluorescence curves were always linear \( (r = 0.999-1.000) \). All muscle metabolite concentrations were determined in dry weight to avoid changes in concentration due to water shift during exercise. Where necessary, the concentrations of the metabolites were adjusted to the true molarity of the standards, and to the highest content of total creatine \((\text{PCr}+\text{Cr})\) in each subject series (Harris et al., 1976). The correction for total creatine was performed in order to compensate for any admixture of elements such as connective tissue, fat droplets, or blood contamination of muscle samples. However, muscle glucose and lactate were not adjusted for total creatine content since these two metabolites occur in significant amounts in both muscle and blood and the amount present of the contaminating blood was not possible to determine. The coefficient of variation for all the muscle assays is shown in Table 3.2.

Table 3.2: Coefficient of variation (C. V %) of muscle metabolite assays \((n = 15)\).  

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Concentration (mmol.Kg dw(^{-1}))</th>
<th>C.V (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine triphosphate (ATP)</td>
<td>26.4</td>
<td>3.5</td>
</tr>
<tr>
<td>Phosphocreatine (PCr)</td>
<td>85.8</td>
<td>2.1</td>
</tr>
<tr>
<td>Creatine (Cr)</td>
<td>36.5</td>
<td>3.1</td>
</tr>
<tr>
<td>Acid insoluble glycogen</td>
<td>240</td>
<td>1.1</td>
</tr>
<tr>
<td>Acid soluble glycogen</td>
<td>53.0</td>
<td>3.3</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.8</td>
<td>3.2</td>
</tr>
<tr>
<td>Glucose-6-Phosphate (G-6-P)</td>
<td>1.4</td>
<td>3.4</td>
</tr>
<tr>
<td>Lactate</td>
<td>3.8</td>
<td>2.2</td>
</tr>
</tbody>
</table>
CHAPTER 4

INFLUENCE OF A PRE-EXERCISE MEAL AND A CARBOHYDRATE-ELECTROLYTE SOLUTION ON ENDURANCE RUNNING CAPACITY: COMPARISON WITH A CARBOHYDRATE-ELECTROLYTE SOLUTION

4.1 Introduction

Fatigue during prolonged submaximal (60-80% \( VO_2 \) max) exercise is associated with the depletion of the limited glycogen stores in active skeletal muscles (Hermansen et al. 1967). Consumption of high carbohydrate diets maximises body carbohydrate stores and improves endurance running performance (Karlsson and Saltin, 1971). Also, the consumption of carbohydrate solutions during exercise helps to minimise disturbances in fluid balance and thermoregulation (Coyle and Montain 1992; Maughan 1991), and seems to delay the onset of fatigue during both endurance cycling (Bjorkman et al. 1984; Coggan and Coyle 1988; 1989; Coyle et al. 1983; 1986; Mitchell et al. 1988; 1989a; Murray et al. 1991) as well as endurance running (Sasaki et al. 1987a; Tsintzas 1993b; Wilber and Moffatt 1992).

However, all the studies mentioned above, with the exception of those by Mitchell et al. (1988; 1989a) and by Murray et al. (1991), were conducted after subjects had undergone an 8-16 hour fast. Prolonged fasting, however, reduces liver glycogen stores (Nilsson and Hultman, 1973) and is associated with detrimental endurance performance in humans (Aragon-Vargas 1993). Therefore, athletes are advised to avoid prolonged fasting and ingest a meal high in carbohydrate and low in fat, protein and fibre about 4 hours before exercise (Coyle, 1992). Only a few studies have examined the influence of carbohydrate ingestion during exercise on endurance cycling performance after a short (4 hours) postabsorptive period (Neufer et al. 1987; Peters et al. 1995; Wright et al. 1991).
Therefore, the aim of the present study was to examine the effects on endurance running capacity of consuming a carbohydrate-electrolyte solution during exercise after subjects were either in the fed or fasted state.

4.2 Methods

Subjects
Ten male recreational/club level runners volunteered to take part in this study. Their age, height, body weight, VO₂ max, and maximum heart rate were 34.9 ± 2.5 years, 174.5 ± 2.9 cm, 72.4 ± 3.6 kg, 58.6 ± 1.9 ml.kg⁻¹.min⁻¹, and 186 ± 4 b.min⁻¹ respectively (mean ± SE). Nine subjects completed all three trials, and one completed only two trials.

Preliminary measurements
After subjects became familiar with treadmill running and experimental procedures, they performed two preliminary tests (see Chapter 3) : (a) a speed-VO₂ test, and (b) a maximum oxygen uptake test (VO₂ max). They also undertook a 60 min treadmill run, at 70% VO₂ max, about one week before the first experimental trial to fully familiarise them with the drinking pattern and the measurements used during the main trials.

Diet control
Subjects weighed and recorded their normal food intake for two days before the first main trial, and replicated the same diet for the same period of time before the following trials. The dietary information obtained was then analyzed as previously described (see Chapter 3). Furthermore, subjects followed the same training schedule three days before each trial.

Experimental Design
Each subject was required to run to exhaustion at 70% VO₂ max on a motorised level treadmill on three different occasions separated by one week (Fig. 4.1). On the first occasion a high carbohydrate meal (2.5 g of CHO.kg⁻¹ BW; Appendix C) was consumed 3 hours before exercise; and during exercise a 6.9% carbohydrate-electrolyte solution (see Chapter 3) was ingested (M+C). On the second and third occasions subjects ingested 10 ml.kg⁻¹ BW of a placebo solution 3 hours before exercise, and
during exercise they drank a placebo (P+P), or the 6.9% carbohydrate-electrolyte solution (P+C). The order of the three trials was random and the experiment was conducted in a single-blind manner (see Chapter 3).

After a 12 hour overnight fast subjects arrived at the laboratory at 8:00 a.m. After sitting quietly for 15 min duplicate 20 µl capillary blood samples were taken from the thumb of a pre-warmed hand. Following this, subjects ingested either 10 ml.kg⁻¹ BW of a placebo solution (P+C and P+P), or the high carbohydrate meal (M+C). During the 3-hour postprandial period subjects remained in the laboratory, or were involved with low physical activities (e.g., attending lectures, doing office work etc.) outside the laboratory. These activities were very similar in all experimental trials.

Three hours after the ingestion of the placebo or the meal a venous blood sample as well as a duplicate 20 µl capillary blood sample were taken while subjects were sitting. After that, subjects ingested 5 ml.kg⁻¹ BW of the 6.9% carbohydrate-electrolyte solution (M+C, and P+C) or equivalent amount of placebo (P+P). During exercise 2 ml.kg⁻¹ BW of the assigned fluid was ingested every 20 min. In the M+C trial subjects consumed 259 ± 13.8 g of carbohydrate (181 ± 9 g as meal and 78 ± 6 g during exercise), whereas in the P+C trial they ingested 70 ± 4 g of carbohydrate.

After the consumption of the 5 ml.kg⁻¹ BW placebo or carbohydrate-electrolyte drink subjects had a 5 min warm-up on the treadmill at 60% \( \dot{V}O_2 \) max. Following this, the speed of the treadmill was increased to 70% \( \dot{V}O_2 \) max and the subjects continued running till exhaustion. One minute expired air samples and duplicate 20 µl capillary blood samples were collected at 10 and 20 min into exercise and every 20 min thereafter. Expired air and capillary blood samples were also collected at the last minute of the run. Perceived rate of exertion (PRE), abdominal discomfort (ADS), and gut fullness (GFS) were also recorded at the same times. Immediately after subjects stopped exercise a further venous blood sample was taken while they were seated. Heart rate and rectal temperatures were continuously monitored throughout the trials. Each subject's pre-exercise and post-exercise nude body weights were also measured. Wet sponges were available to the subjects to be used ad libitum throughout the runs.
All trials were conducted under similar laboratory temperature (M+C: 19.8 ± 0.8 °C, P+C: 20.3 ± 0.6 °C, and P+P: 20.1 ± 0.8 °C) and relative humidity (M+C: 56.9 ± 2.8%, P+C: 53.3 ± 2.4%, and P+P: 51.9 ± 2.3%) (mean ± SE; n.s).

Analyses
Blood and air samples were collected and analysed as previously described (Chapter 3). Capillary blood samples were analysed for glucose and lactate concentrations, whereas venous blood samples were analysed for haematocrit values, haemoglobin, plasma FFA, plasma glycerol, serum potassium, serum sodium, and serum insulin concentrations.

A two-way analysis of variance (ANOVA) for repeated measures on two factors (treatment by time) was used to compare cardiovascular changes and blood lactate and glucose responses to all trials of the study. The remaining responses were examined using a two-tailed Student's t test for dependent samples. When significant differences were revealed, using the ANOVA, then a Tukey post hoc test was performed. The accepted level of significance was set at p < 0.05. Data are reported as mean ± SE.
Running at 70% VO2 max

Fig. 4.1: Schematic illustration of the experimental protocol
4.3 Results

Exercise time was longer in the M+C (147.4 ± 9.6 min; range: 110.9-198.7 min; n=9) and P+C (125.3 ± 7.0 min; range: 94.7-158.2 min; n=10) trials compared with the P+P (115.1 ± 7.6 min; range: 86.8-135.4 min; n=9) trial (p< 0.01 and p< 0.05 respectively). Also, exercise time was longer in the M+C compared with the P+C trial (p< 0.01). No difference was found, however, when exercise time to exhaustion was analysed by order (T1: 126.3 ± 10.8 min vs T2: 129.8 ± 9.3 min vs T3: 130.0 ± 6.5 min; n.s).

There were no differences between the three trials in the average daily energy intake, carbohydrates, fat, or protein consumed during the 2 days prior to each main trial (Table 4.1).

Oxygen uptake (\(\dot{V}_O_2\)), heart rate (HR) (Table 4.2), gut fullness (GFS), and abdominal discomfort (ADS) (Table 4.3) responses were not different between trials. The average % \(\dot{V}_O_2\) max values sustained during the M+C, P+C and P+P trials were 69.8 ± 0.6%, 70.5 ± 0.6%, and 71.0 ± 0.8% respectively (n.s). The perceived rate of exertion (PRE) was higher in the P+P trial at 80 min of exercise (Table 4.2) compared with the M+C (p< 0.01) and P+C (p< 0.05) trials. As far as rectal temperatures are concerned complete data were obtained for only 4 subjects (Table 4.4).

Respiratory exchange ratio (RER), was higher (p< 0.01) in the M+C trial compared with the P+C, and P+P trials during the first hour of exercise (Fig. 4.2). As a result of that, carbohydrate oxidation rate (Fig. 4.3) was higher (p< 0.01) in the M+C condition compared with the P+C and P+P conditions during the first hour as well as at 80 min of exercise between M+C and P+P conditions (p< 0.01).

The volume of fluid consumed during exercise was 1137 ± 90 ml, 1015 ± 60 ml, and 968 ± 70 ml in the M+C, P+C, and P+P trials respectively. These values were significantly different only between the M+C and P+P trials (p< 0.05; n=9). The average decrease in body mass as a result of exercise was 3.31 ± 0.27 kg, 2.82 ± 0.25 kg, and 2.60 ± 0.25 kg in M+C, P+C, and P+P trials respectively (p< 0.01). These decreases represented a body weight changes of 4.7 ± 0.4%, 3.9 ± 0.3%, and 3.6 ± 0.4% respectively. The decrease in body
weight as expressed both in kg and percent change (%) of body weight, was higher in the M+C trial compared with the P+C (p< 0.05, n=9), and P+P (p< 0.01, n=9) trials. However, no difference was observed between the three trials in the mean change of plasma volume (M+C: - 8.3 ± 1.9 % vs P+C: - 7.5 ± 1.1% vs P+P: -9.3 ± 1.8%).

Blood glucose concentration (Fig. 4.4) was higher at exhaustion in the M+C trial compared with the P+P trial (p< 0.05). However, in the P+P trial blood glucose concentration at exhaustion (4.2 ± 0.2 mmol.l⁻¹) was not different from the blood glucose concentration at the start of exercise (0 min: 4.6 ± 0.1 mmol.l⁻¹). Also, blood glucose concentration tended to be higher (p= 0.05) in the P+C trial at 10 min, and 60 min of exercise compared with the P+P and M+C, and P+P trials respectively.

Blood lactate concentrations (Fig. 4.5) were similar between trials throughout exercise and averaged 3.2 ± 0.3 mmol.l⁻¹, 3.4 ± 0.5 mmol.l⁻¹, and 3.1 ± 0.5 mmol.l⁻¹ in the M+C, P+C, and P+P trials respectively.

At the start of exercise serum insulin concentration (Fig. 4.6) in the M+C trial was about 3.2 fold and 4.4 fold higher compared with the P+C and P+P trials respectively (p< 0.01). Plasma FFA concentrations (Fig. 4.7) were lower (p< 0.01) at the beginning of exercise in the M+C trial compared with the P+C and P+P trials. Also, postexercise plasma FFA concentrations were lower (p< 0.05) when carbohydrates were consumed during exercise (M+C, and P+C) compared with placebo consumption (P+P). Plasma glycerol concentrations (Fig. 4.8) were also lower in the M+C condition compared with the P+C (p< 0.05) and P+P (p< 0.01) conditions.

Finally, preexercise serum sodium concentration was found to be higher (p< 0.05) 3 hours after the consumption of the meal (M+C), whereas serum potassium concentration was not different between the three conditions (Table 4.5).
Table 4.1: Average daily energy intake, carbohydrate (CHO), fat, and protein consumed during the 2 days prior to each trial (mean ± SE).

<table>
<thead>
<tr>
<th></th>
<th>Energy Intake (Kcal)</th>
<th>CHO (g)</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Kj)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>2665 ± 165</td>
<td>361 ± 26</td>
<td>106 ± 7</td>
<td>94 ± 8</td>
</tr>
<tr>
<td></td>
<td>± 690</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P+C</td>
<td>2847 ± 258</td>
<td>384 ± 37</td>
<td>110 ± 7</td>
<td>103 ± 11</td>
</tr>
<tr>
<td></td>
<td>± 1078</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P+P</td>
<td>2724 ± 203</td>
<td>368 ± 28</td>
<td>103 ± 6</td>
<td>98 ± 9</td>
</tr>
<tr>
<td></td>
<td>± 849</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4.2: Oxygen uptake (\(\dot{V}O_2\)), heart rate (HR), and perceived rate of exertion (PRE) during the M+C, P+C, and P+P trials (mean ± SE; n=9).

<table>
<thead>
<tr>
<th>Variable</th>
<th>10 min</th>
<th>20 min</th>
<th>40 min</th>
<th>60 min</th>
<th>80 min</th>
<th>Exhaustion</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\dot{V}O_2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ml.kg(^{-1}).min(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>40.1(^{a}) ± 1.7</td>
<td>40.2(^{a}) ± 1.6</td>
<td>40.1(^{a}) ± 1.8</td>
<td>40.8(^{a}) ± 1.5</td>
<td>40.8(^{a}) ± 1.4</td>
<td>44.4 ± 1.6</td>
</tr>
<tr>
<td>P+C</td>
<td>40.8(^{a}) ± 1.3</td>
<td>40.2(^{a,b}) ± 1.4</td>
<td>41.6 ± 1.2</td>
<td>41.3(^{a}) ± 1.2</td>
<td>42.0 ± 1.1</td>
<td>42.9 ± 1.2</td>
</tr>
<tr>
<td>P+P</td>
<td>40.7(^{a}) ± 1.6</td>
<td>41.5(^{a}) ± 1.7</td>
<td>41.0(^{a}) ± 1.6</td>
<td>41.5(^{a}) ± 1.7</td>
<td>42.1(^{c}) ± 1.6</td>
<td>43.8 ± 1.7</td>
</tr>
<tr>
<td>(\dot{V}O_2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(l.min(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>2.9(^{a}) ± 0.2</td>
<td>2.9(^{a}) ± 0.2</td>
<td>2.9(^{a}) ± 0.2</td>
<td>2.9(^{a}) ± 0.2</td>
<td>2.9(^{a}) ± 0.2</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>P+C</td>
<td>2.9(^{a}) ± 0.2</td>
<td>2.8(^{a}) ± 0.2</td>
<td>2.9 ± 0.2</td>
<td>2.9 ± 0.2</td>
<td>3.0 ± 0.2</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td>P+P</td>
<td>2.9(^{a}) ± 0.2</td>
<td>2.9(^{a}) ± 0.2</td>
<td>2.9(^{a}) ± 0.2</td>
<td>2.9(^{a}) ± 0.2</td>
<td>3.0(^{c}) ± 0.2</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>HR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b.min(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>147(^{a}) ± 5</td>
<td>149(^{a,e}) ± 5</td>
<td>154(^{a,d,b}) ± 5</td>
<td>156(^{a,d}) ± 5</td>
<td>158(^{a,d}) ± 5</td>
<td>168 ± 5</td>
</tr>
<tr>
<td>P+C</td>
<td>150(^{a}) ± 5</td>
<td>152(^{a,e}) ± 5</td>
<td>157(^{a,d,b}) ± 5</td>
<td>158(^{a,d}) ± 5</td>
<td>161(^{a,d}) ± 5</td>
<td>170 ± 4</td>
</tr>
<tr>
<td>P+P</td>
<td>149(^{a}) ± 4</td>
<td>152(^{a,e}) ± 4</td>
<td>157(^{a,d,b}) ± 5</td>
<td>158(^{a,d}) ± 5</td>
<td>161(^{a,d}) ± 5</td>
<td>168 ± 4</td>
</tr>
<tr>
<td>PRE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.7(^{a}) ± 0.4</td>
<td>10.9(^{a,h}) ± 0.5</td>
<td>11.4(^{a,b}) ± 0.4</td>
<td>11.9(^{a}) ± 0.6</td>
<td>12.7(^{a,d,f}) ± 0.6</td>
<td>17.7 ± 0.4</td>
</tr>
<tr>
<td>P+C</td>
<td>10.6(^{a}) ± 0.4</td>
<td>10.9(^{a,h}) ± 0.4</td>
<td>11.3(^{a,h}) ± 0.4</td>
<td>12.1(^{a,d}) ± 0.6</td>
<td>13.1(^{a,d,g}) ± 0.7</td>
<td>17.8 ± 0.4</td>
</tr>
<tr>
<td>P+P</td>
<td>10.7(^{a}) ± 0.4</td>
<td>11.2(^{a}) ± 0.4</td>
<td>11.6(^{a,h}) ± 0.4</td>
<td>12.7(^{a,d,h}) ± 0.6</td>
<td>14.3(^{a,d,i}) ± 0.7</td>
<td>17.7 ± 0.4</td>
</tr>
</tbody>
</table>

a: p< 0.01 from Exhaustion; b: p< 0.05 from 80 min; c: p< 0.05 from Exhaustion; d: p< 0.01 from 10 min; e: p< 0.01 from 40, 60, 80 min; f: p< 0.01 from P+P; g: p< 0.05 from P+P; h: p< 0.01 from 80 min; i: p< 0.01 from 20 min
Table 4.3: Gut fullness (GFS), and abdominal discomfort (ADS) during the M+C, P+C, and P+P trials (mean ± SE; n=9).

<table>
<thead>
<tr>
<th>Variable</th>
<th>M+C</th>
<th>P+C</th>
<th>P+P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 min</td>
<td>20 min</td>
<td>40 min</td>
</tr>
<tr>
<td>GFS</td>
<td>1.9 ± 0.5</td>
<td>1.7 ± 0.4</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>ADS</td>
<td>1.6 ± 0.4</td>
<td>1.8 ± 0.4</td>
<td>1.6 ± 0.3</td>
</tr>
</tbody>
</table>

\[a: \ p < 0.01 \text{ from } 10 \text{ min, } 20 \text{ min, } 40 \text{ min}\]

Table 4.4: Rectal temperature (°C) during the M+C, P+C, and P+P trials (mean ± SD; n=4).

<table>
<thead>
<tr>
<th></th>
<th>0 min</th>
<th>10 min</th>
<th>20 min</th>
<th>40 min</th>
<th>60 min</th>
<th>80 min</th>
<th>Exhaustion</th>
</tr>
</thead>
<tbody>
<tr>
<td>M+C</td>
<td>36.8± .1</td>
<td>37.8± .2</td>
<td>38.1± .2</td>
<td>38.4± .1</td>
<td>38.4± .1</td>
<td>38.6± .1</td>
<td>38.8± .2</td>
</tr>
<tr>
<td>P+W</td>
<td>36.6± .2</td>
<td>37.7± .1</td>
<td>38.1± .1</td>
<td>38.4± .1</td>
<td>38.5± .1</td>
<td>38.6± .1</td>
<td>38.9± .1</td>
</tr>
<tr>
<td>P+P</td>
<td>36.7± .1</td>
<td>37.8± .1</td>
<td>38.1± .2</td>
<td>38.5± .1</td>
<td>38.6± .2</td>
<td>38.7± .2</td>
<td>39.0± .3</td>
</tr>
</tbody>
</table>
Table 4.5: Serum sodium and potassium concentrations (mmol.l\(^{-1}\)) in the M+C, P+C, and P+P trials (mean ± SE)

<table>
<thead>
<tr>
<th></th>
<th>PRE-EXERCISE</th>
<th>POST-EXERCISE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum Sodium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>142 ± 0.5(^a)</td>
<td>144 ± 0.6(^b)</td>
</tr>
<tr>
<td>P+C</td>
<td>140 ± 0.4</td>
<td>143 ± 0.6(^c)</td>
</tr>
<tr>
<td>P+P</td>
<td>140 ± 0.4</td>
<td>142 ± 0.6(^c)</td>
</tr>
<tr>
<td><strong>Serum Potassium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>4.2 ± 0.1</td>
<td>4.8 ± 0.1(^b)</td>
</tr>
<tr>
<td>P+C</td>
<td>4.2 ± 0.1</td>
<td>4.8 ± 0.1(^b)</td>
</tr>
<tr>
<td>P+P</td>
<td>4.3 ± 0.1</td>
<td>4.7 ± 0.1(^b)</td>
</tr>
</tbody>
</table>

a: p< 0.05 from P+C (n=9) and P+P(n=8); b: p< 0.05 from pre-exercise
c: p< 0.01 from pre-exercise
Fig. 4.2: Respiratory exchange ratio (RER) during the M+C, P+C, and P+P trials (mean ± SE; n=9)
* p< 0.01 from P+C and P+P
a: p< 0.05 from M+C
b: p< 0.01 from 10-80 min

Fig. 4.3: Carbohydrate oxidation rate (g.min⁻¹) during the M+C, P+C, and P+P trials (mean ± SE; n=9)
* p< 0.01 from P+C and P+P
a: p< 0.01 from P+P
b: p< 0.01 from 10-80 min
Fig. 4.4: Blood glucose concentration (mmol.l⁻¹) in M+C, P+C, and P+P (mean ± SE; n=9)
- a: p = 0.05 from M+C, P+P
- b: p = 0.05 from P+P
- c: p < 0.05 from P+P

Fig. 4.5: Blood lactate concentration (mmol.l⁻¹) in M+C, P+C, and P+P (mean ± SE; n=9)
- a: p < 0.01 from 10 min to exhaustion
- b: p < 0.05 from 10 min, 20 min
- c: p < 0.01 from -180 min, 0 min
Fig. 4.6: Serum insulin concentration (mU.l⁻¹) in M+C, P+C, and P+P (mean ± SE)

- a: p < 0.01 from P+C (n=9) and P+P (n=8)
- b: p < 0.01 from post-exercise

Fig. 4.7: Plasma FFA concentration (mmol.l⁻¹) in M+C, P+C, and P+P (mean ± SE)

- a: p < 0.01 from P+C (n=9) and P+P (n=8)
- b: p < 0.05 from P+P (n=8)
- c: p < 0.05 from P+P (n=9)
- d: p < 0.01 from pre-exercise
Fig. 4.8: Plasma glycerol concentration (mmol.1-1) in M+C, P+C, and P+P (mean ± SE)
* p< 0.05 from P+C (n=9) and P+P (n=8)
a: p<0.01 from P+P (n=8); b: p<0.05 from P+C (n=9)
c: p<0.01 from pre-exercise
4.4 Discussion

The main finding of this study was an 18% improvement in endurance capacity as a result of combining the carbohydrate meal together with the carbohydrate-electrolyte solution (M+C) compared with the ingestion of the carbohydrate-electrolyte solution (P+C) alone. Furthermore, the 6.9% carbohydrate-electrolyte drink (P+C) produced a smaller (9%) but significant improvement in endurance capacity compared to control conditions (P+P). However, the biggest improvement (28%) compared to control (P+P), was achieved when the pre-exercise meal was combined with the ingestion of the carbohydrate-electrolyte solution (M+C) during exercise.

The improvement in endurance capacity after the high carbohydrate meal found in the present study is in agreement with Neufer and co-workers' (1987) study. In that study an increase in cycling performance was found after the ingestion of a 200 g CHO meal 4 hour before exercise compared with solid or liquid carbohydrate feedings ingested 5 min before exercise. However, in one study no additional improvement in cycling performance was found after a liquid carbohydrate meal was consumed 3 hours before exercise when carbohydrate solutions were ingested during exercise (Wright et al. 1991). One possible reason for this conflicting result might be the much larger size of meal provided in Wright et al. (1991) study (333 g carbohydrate) compared to Neufer et al. (1989) and to the present study (200 g and 181 g of carbohydrate respectively). Indeed, it has been suggested by the same authors that a very high dosage of carbohydrate ingested 3 hours before exercise may continue to provide an increased glucose availability to the working muscle during exercise (Wright et al. 1991). This could have masked, to some extent, the ergogenic effect of the carbohydrate solution ingested during exercise.

The difference in endurance capacity between the M+C and P+C trials was probably due to higher liver (Nilsson and Hultman 1973), and/or muscle carbohydrate stores (Coyle et al. 1985) before the start of exercise. However, the possibility that some portion of the meal was still in the stomach or small intestine when exercise begun, providing in this way some substrate to the working muscles during exercise, can not be totally excluded.

Various studies have shown an improved endurance capacity during cycling (Bjorkman et al. 1984; Coggan and Coyle 1988; 1989; Coyle et al. 1983; 1986;
Hargreaves et al. 1994; Maughan et al. 1989; Murdoch et al. 1993; Wright et al. 1991), or running (Macaraeg 1983; MacLaren and Otter 1988; Sasaki et al. 1987a; Tsintzas et al. 1993b; Wilber and Moffatt 1992), as a result of carbohydrate ingestion during exercise. However, there are some studies which have shown no difference in running time to exhaustion when carbohydrate solutions were ingested during submaximal (70% $\dot{V}O_2$ max) exercise compared to control (Fruth and Gisolfi 1983; Riley et al. 1988). In the study conducted by Fruth and Gisolfi (1983) no difference in running time to exhaustion was observed when glucose, fructose, or placebo solutions were ingested during exercise. The small number of subjects ($n=5$) involved in that study might be a reason for the lack of any reported beneficial effect of carbohydrate on running time to exhaustion. Furthermore, Riley et al. (1988) also failed to demonstrate an improvement in exercise time to exhaustion in subjects who exercised after a 21-hour fast when a carbohydrate solution was ingested during exercise. In that study the administration of the carbohydrate solution was probably not sufficient to offset the deleterious effects of prolonged fasting on endurance running capacity (Nieman et al. 1987; Aragon-Vargas 1993). Nevertheless, it should be mentioned here that the 6.9% carbohydrate-electrolyte solution used in the present study failed to improve endurance running capacity at 70% $\dot{V}O_2$ max in a previous study (Tsintzas et al. 1993b), despite the fact that there was a tendency for shorter exercise time in the control condition. One possible reason for this conflicting result might be the fact that in the study conducted by Tsintzas et al. (1993b) the carbohydrate-electrolyte drink was administered only for the first 60 min of exercise. In a different experiment, studying intermittent shuttle running (Nicholas et al. 1995), where this particular carbohydrate drink was ingested throughout exercise, an improvement in endurance capacity was found. Another possible explanation might be the gastrointestinal discomfort reported by the majority of runners in the study by Tsintzas et al. (1993b) (see also Tsintzas 1993) in contrast to the absence of such symptoms in the present study (Table 4.3). Indeed, gastrointestinal discomfort during running is an important factor since it has been interpreted as being a result of maldigestion and malabsorption (Brouns and Beckers, 1993).

Despite the fact that most studies have shown an improvement in endurance capacity or performance when carbohydrates are ingested during exercise, there is controversy over the mechanism by which ingestion of exogenous carbohydrate improves performance. Some investigators have concluded that
the main contribution of exogenous carbohydrate is to maintain blood glucose concentration and a high rate of carbohydrate oxidation by the working muscle during endurance cycling exercise (Coyle et al. 1986; Hargreaves and Briggs 1988; Mitchell et al. 1989a). This is because during cycling, fatigue in the fasted state has been associated with a decline in blood glucose concentration, and the rate of carbohydrate oxidation (Bjorkman et al. 1984; Coggan and Coyle 1987; 1988; 1989; Coyle et al. 1983; 1986; Wright et al. 1991). In the present study the respiratory exchange ratio (Fig. 4.2) and the rate of carbohydrate oxidation (Fig. 4.3) were not significantly reduced with time in the control trial (P+P). Neither was a significant drop in blood glucose concentration at exhaustion compared with the start of exercise (Fig. 4.4). It seems that when running is the mode of exercise blood glucose concentration and carbohydrate oxidation rate do not decrease with time to the same extent as during cycling (Nieman et al. 1987; Riley et al. 1988; Sasaki et al. 1987a; Tsintzas et al. 1993b; Wilber and Moffatt 1992). Therefore, it seems reasonable to suggest that the maintenance of carbohydrate oxidation rate and blood glucose concentration during exercise were not the mechanisms per se by which the carbohydrate-electrolyte drink (P+C) improved endurance capacity. Other investigators, however, have suggested that carbohydrate intake during exercise may reduce the rate of muscle glycogen utilisation and spare the limited muscle glycogen stores (Bjorkman et al. 1984; Erickson et al. 1987; Hargreaves et al. 1984). When considering the muscle glycogen utilisation during exercise one should take into account the different fiber composition of the muscle involved. Bearing in mind that at moderate exercise intensity (60-75 % $\dot{VO}_2$ max) Type I muscle fibers may be the first to become glycogen depleted (Gollnick et al. 1973; 1974), one can expect that these muscle fibers may benefit to a greater extent by the administration of exogenous carbohydrate. Indeed, recent studies, using the single fiber technique, have shown a reduced rate of glycogen breakdown in Type I muscle fibers when carbohydrates are ingested during exercise as opposed to water or placebo (Tsintzas et al. 1994; 1993c).

The higher respiratory exchange ratio and carbohydrate oxidation rate found in the M+C trial compared with the P+C and P+P trials seem to suggest that the consumption of the meal 3 hours before exercise altered substrate utilization during exercise. This alteration lasted mainly the first hour of exercise (Fig. 4.2 and 4.3). This finding is in agreement with other studies.
where a greater reliance on carbohydrate metabolism during cycling (Coyle et al. 1985; Montain et al. 1991; Neufer et al. 1987; Sherman et al. 1989; Wright et al. 1991), or running (Willcuts et al. 1988) was observed after carbohydrate meals were consumed before exercise as opposed to fasting conditions. In these studies the size of meals varied from about 2 g CHO.kg⁻¹ BW up to 5 g CHO.kg⁻¹ BW and the pre-exercise ingestion time from 30 min up to 12 hours. The alteration in substrate use during exercise is probably attributed to the higher pre-exercise insulin concentration found in the M+C trial (Fig. 4.6) compared to the P+C and P+P trials (Coyle et al. 1985). It is well known that insulin has an antilypolytic action which inhibits the activation of triacylglycerol lipase, an enzyme responsible for the fatty acid mobilisation from adipose tissue (Newsholme and Leech 1983).

The post-exercise FFA concentrations were lower when the carbohydrate drink was ingested during exercise (P+C) compared with the ingestion of placebo (P+P). This is a common observation which shows a depression of FFA mobilisation when carbohydrates are ingested during exercise (Coggan and Coyle 1991). However, the respiratory exchange ratio and carbohydrate oxidation rate were similar between the P+C and P+P trials indicating that an alternative source, such as intramuscular triglycerides, was probably used (Romijn et al. 1993; Essen et al. 1977).

Despite the elevated pre-exercise insulin concentration, and the higher carbohydrate oxidation rate observed in the M+C trial subjects improved their endurance capacity by 18% and 28% compared with the P+C and P+P trials respectively. It seems that the higher pre-exercise body carbohydrate stores in the M+C trial offset any possible detrimental effects the elevated pre-exercise insulin concentration and the accelerated rate of carbohydrate use could have on endurance capacity (Costill et al. 1977).

In summary, the result of this study confirmed the observation of many investigators that a carbohydrate-electrolyte solution ingested during exercise can improve endurance running capacity. Furthermore, the combination of both a pre-exercise carbohydrate meal and a carbohydrate-electrolyte solution (M+C) further improves endurance running capacity, despite an elevated carbohydrate oxidation rate during the first hour of exercise. However, this improvement could not be attributed to the meal per se, since the effect of the
meal ingestion on endurance capacity was not examined as a separate variable in this study. A possible combined effect (i.e: meal + carbohydrate drink) could have been responsible for, to a certain degree, the difference in endurance capacity between the M+C and P+P trials.
CHAPTER 5

INFLUENCE OF A PRE-EXERCISE MEAL AND A CARBOHYDRATE-ELECTROLYTE SOLUTION ON ENDURANCE RUNNING CAPACITY: COMPARISON WITH A PRE-EXERCISE MEAL

5.1 Introduction

In the previous study (Chapter 4), a carbohydrate meal improved endurance capacity when combined with a carbohydrate-electrolyte solution (M+C) to a greater extent than when compared with a carbohydrate-electrolyte solution alone (P+C). However, the influence of the meal on endurance capacity was not studied as a separate variable. As was mentioned earlier, a possible combined effect (i.e: meal + carbohydrate drink) could have been responsible for, to a certain degree, the difference in endurance capacity between the M+C and P+P trials. Therefore, to answer the question raised in the previous study, the present study was undertaken.

Thus, the aims of this study were: (a) to examine whether a pre-exercise carbohydrate meal can influence endurance running capacity, and (b) to examine if the combination of a pre-exercise meal together with the ingestion of a carbohydrate-electrolyte solution during exercise would be superior, in terms of running capacity, to a pre-exercise meal alone.

5.2 Methods

Subjects

Ten male recreational/club level runners or triathletes participated in this study. Their age, height, body weight, VO₂ max, and maximum heart rate were 30.1 ± 3.4 years, 176.9 ± 2.6 cm, 73.1 ± 4.0 kg, 63.5 ± 2.3 ml.kg⁻¹.min⁻¹,
and 186 ± 4 b.min⁻¹ respectively (mean ± SE). Nine subjects completed three trials and all ten completed two trials.

Preliminary measurements
After subjects became familiar with treadmill running and experimental procedures, they performed two preliminary tests (see Chapter 3): (a) a speed-\(\dot{V}O_2\) test, and (b) a maximum oxygen uptake test (\(\dot{V}O_2\) max). They also undertook a 60 min treadmill run at 70% \(\dot{V}O_2\) max, about one week before the first experimental trial to fully familiarise them with the drinking pattern and the measurements used during the main trials.

Diet control
Subjects weighed and recorded their normal food intake for two days before the first main trial, and replicated the same diet for the same period of time before the following trials. The dietary information obtained was then analyzed as previously described (see Chapter 3). Furthermore, subjects followed the same training schedule three days before each trial.

Experimental Design
Each subject was required to run to exhaustion at 70% \(\dot{V}O_2\) max on a motorised level treadmill on three different occasions separated by one week (Fig. 5.1). In one occasion a high carbohydrate meal (2.5 g CHO.kg⁻¹ BW; see Appendix C) was eaten 3 hours before exercise, and during exercise a 6.9% carbohydrate-electrolyte solution (see Chapter 3) was ingested (M+C). The high carbohydrate meal was also eaten 3 hours before exercise in another occasion (M+W), whereas in a different occasion subjects ingested 10 ml.kg⁻¹ BW of a placebo solution (P+W). During exercise only water was provided in the M+W, and P+W trials. The order of the three trials was random and the experiment was conducted in a single-blind manner (see Chapter 3).

After a 12 hour overnight fast subjects arrived at the laboratory between 7:00-8:00 a.m. While the subject was lying on an examination couch an indwelling catheter was inserted in an antecubital vein (see Chapter 3). After collecting a blood sample subjects consumed either the high carbohydrate meal (M+C, and M+W), or the 10 ml.kg⁻¹ BW placebo solution (P+W). Thirty minutes after the consumption of the placebo or the meal as well as every hour during the 3-hour postprandial period venous blood samples were obtained. The pre-
exercise blood sample (i.e: the last sample during the postprandial period) was taken after subjects had been standing for 15 min. Immediately after that, subjects ingested either 5 ml.kg⁻¹ BW of the 6.9% carbohydrate-electrolyte solution (M+C), or equivalent amount of water (M+W, and P+W). During exercise 2 ml.kg⁻¹ BW of the assigned fluid was consumed every 20 min. In the M+C trial subjects consumed a total of 252 ± 10.4 g of carbohydrate (181 ± 10.1 g as meal and 71 ± 4 g during exercise), whereas in the M+W trial they ingested 181 ± 10.1 g of carbohydrate.

After the consumption of the 5 ml.kg⁻¹ BW placebo or carbohydrate-electrolyte drink subjects had a 5-min warm-up on the treadmill at 60% VO₂ max. Following this, the speed of the treadmill was increased to 70% VO₂ max and the subjects continued running until exhausted.

Venous blood and one minute expired air samples were collected every 20 min intervals as well as at the last minute of the run. Perceived rate of exertion (PRE), abdominal discomfort (ADS), and gut fullness (GFS) were also recorded at the same time intervals. Heart rate was continuously monitored throughout the trials. Each subject's pre-exercise and post-exercise nude body weights were also measured. Wet sponges were available to the subjects to be used ad libitum throughout the runs.

All trials were conducted under similar laboratory conditions: temperatures: M+C: 20.3 ± 0.4 °C, M+W: 21.1 ± 0.6 °C, and P+W: 20.2 ± 0.3 °C; relative humidity: M+C: 61.6 ± 3.3 %, M+W: 62.2 ± 2.2 %, and P+W: 61.6 ± 3.3 % (mean ± SE; n.s).

Analyses
Venous blood and air samples were collected and analysed as previously described (Chapter 3). Venous blood samples were analysed for haematocrit and haemoglobin values, and for blood glucose, blood lactate, plasma FFA, plasma glycerol, plasma urea, plasma adrenaline, plasma noradrenaline, serum potassium, serum sodium, and serum insulin concentrations.

A two-way analysis of variance (ANOVA) for repeated measures on two factors (treatment by time) was used to compare cardiovascular changes and metabolic responses to all trials of the study. The postprandial metabolic responses were assessed separately from the metabolic responses during exercise. The remaining responses were examined using a two tailed
Student's t test for dependent samples. When significant differences were revealed, using the ANOVA, then a Tukey post hoc test was performed. The accepted level of significance was set at $p < 0.05$. Data are reported as mean ± SE.
Fig. 5.1: Schematic illustration of the experimental protocol
5.3 Results

Endurance Capacity
Exercise time to exhaustion was longer in the M+C (125.1 ± 5.3 min; range: 100-143.5 min; n=10) and M+W (111.9 ± 5.6 min; range: 92.2-138.9 min; n=10) trials compared with the P+W (102.9 ± 7.9 min; range: 64.9-134.5 min; n=9) trial (p< 0.01 and p< 0.05 respectively). Also, exercise time was longer in the M+C compared with the M+W trial (p< 0.05). No difference was found, however, when exercise time to exhaustion was analysed by order (T1: 117.5 ± 6.6 min vs T2: 111.7 ± 6.7 min vs T3: 112.1 ± 7.4 min; n.s).

Diet analysis
There were no differences between the three trials in the average daily energy intake, carbohydrates, fat, or protein consumed during the 2 days prior to each main trial (Table 5.1).

Postprandial Responses
No peak was found in blood glucose concentrations in either M+C or the M+W trials throughout the entire 3-hour postprandial period (Fig. 5.2). The blood glucose concentration fluctuated between 3.5 ± 0.2 and 4.1 ± 0.3 mmol.l⁻¹ and 4.0 ± 0.3 and 4.7 ± 0.3 mmol.l⁻¹ for the M+C and M+W respectively.

The consumption of the high carbohydrate meals resulted in an increase in blood lactate concentration, and therefore, the values obtained were higher (p< 0.01) than the corresponding ones in the P+W trial at 30 min, and 60 min postprandially (Table 5.5). Also, blood lactate concentration was higher in the M+C compared with the P+W trial at 120 min and 180 min postprandially (p<0.05). However, no difference was observed between the two meal trials during the postprandial period.

In contrast to blood glucose concentration, serum insulin concentration peaked at 30 min postprandially in both M+C and M+W trials (Fig. 5.3). Serum insulin concentration was higher (p< 0.01) in both M+C and M+W trials compared with the P+W during the first two hours postprandially. Three hours after the consumption of the carbohydrate meal serum insulin concentration was still 2.6-fold and 3.6-fold higher than prefeeding levels in the M+C and M+W trials respectively. Also, three hours after the
consumption of the meals serum insulin concentration was 3.7-fold (M+W) and 2.8-fold (M+C) higher than in the P+W trial. However, this difference did not reach significance due to high postprandial insulin concentration values.

Plasma FFA concentrations were higher (p< 0.01) in the P+W trial compared with both carbohydrate meal trials throughout the postprandial period (Fig. 5.4). Plasma glycerol concentrations were also higher (p< 0.01) in the P+W trial compared with both meal trials two and three hours postprandially (Fig. 5.5). However, plasma urea concentration (Table 5.5) was not different between conditions.

Sensation of gut fullness was higher (p< 0.01) in both meal trials compared with the P+W trial, whereas abdominal discomfort was not different between conditions (Table 5.2).

Responses During Exercise
Oxygen uptake (Table 5.4) was not different between trials and averaged 3.2 ± 0.1 l.min⁻¹ in all three exhaustion runs. The mean relative exercise intensities were 70.5 ± 1.1 % VO₂ max, 70.7 ± 1.1 % VO₂ max, and 70.7 ± 1.0 % VO₂ max in the M+C, M+W, and P+W trials (n.s). Heart rate (Table 5.4) was similar between conditions and averaged 164 ± 2 b.min⁻¹, 165 ± 2 b.min⁻¹, and 168 ± 2 b.min⁻¹ in M+C, M+W and P+W respectively. Perceived rate of exertion (Table 5.4) was higher (p< 0.05) in the P+W trial compared with the M+C trial at 60 min, whereas gut fullness (Table 5.3) was higher (p< 0.01) in the M+C compared with the P+W trial at 20 min of exercise. However, abdominal discomfort (Table 5.3) was similar between the three trials.

Respiratory exchange ratio was higher (p< 0.01) in two meal trials compared with the control (P+W) during the first hour of exercise (Fig. 5.6). Similarly, carbohydrate oxidation rate (Fig. 5.7) was higher (p< 0.01) during the first hour in the M+C and M+W trials compared with the P+W trial.

The volume of fluid consumed during exercise was 1022 ± 77 ml, 932 ± 69 ml, and 836 ± 49 ml in the M+C, M+W, and P+W trials respectively. These values were significantly different only between M+C and P+W trials (p< 0.05; n=9). There was an average decrease in body mass of 2.6 ± 0.2 kg, 2.6 ± 0.1 kg and 2.3 ± 0.1 kg during the M+C, M+W and P+W trials respectively (n.s). This decrease represented a body weight change of 3.7 ± 0.3 %, 3.6 ± 0.2 %, and 3.2
± 0.3 % respectively which was significantly different only between the P+W and M+C trials (p< 0.05; n=9). The mean change in plasma volume was 0.7 ± 3.0 %, 1.9 ± 2.0 % and 1.2 ± 3.8 % for the M+C, M+W, and P+P respectively (n.s).

Blood glucose concentration was higher (p< 0.01) at 20 min of exercise in the P+W compared with the M+W trial (Fig. 5.8). However, blood glucose concentration was not different between conditions at any other sampling point during exercise. In the P+W trial blood glucose concentration was lower (p< 0.01) at exhaustion (3.4 ± 0.3 mmol.l⁻¹) compared with the blood glucose concentration at 20 min, and 40 min of exercise (4.7 ± 0.1 mmol.l⁻¹) but was not different from the blood glucose concentration at the start of exercise (0 min: 4.1 ± 0.1 mmol.l⁻¹).

Blood lactate concentrations (Table 5.6) were similar throughout exercise in all treatments and averaged 2.1 ± 0.2 mmol.l⁻¹, 2.1 ± 0.1 mmol.l⁻¹ and 2.0 ± 0.3 mmol.l⁻¹ in the P+W, M+W and M+C respectively.

Serum insulin concentration (Fig. 5.9) was 3.7-fold (M+W) and 2.8-fold (M+C) higher in the meal trials compared with the serum insulin concentration in the P+W trial at the start of exercise (p< 0.01). No difference was found, however, between conditions during exercise. Nevertheless, at 20 min of exercise serum insulin concentration was 104% (M+C) and 60% (M+W) higher than the control trial but this difference did not reach significance (p> 0.05).

Plasma FFA (Fig. 5.10) and glycerol (Fig. 5.11) concentrations were lower in the M+C and M+W trials compared with the P+W trial throughout exercise (p< 0.01). However, plasma urea, serum sodium, and serum potassium concentrations (Table 5.6), as well as plasma adrenaline and plasma noradrenaline (Table 5.7) concentrations were not different between the three trials. Nevertheless, higher (p<0.05) adrenaline to insulin and noradrenaline to insulin ratios were found in the P+W trial compared with the M+C and M+W trials at the beginning (0 min) as well as at 20 min of exercise (Table 5.8).
Table 5.1: Average daily energy intake, carbohydrate (CHO), fat, and protein consumed during the 2 days prior to each trial (mean ± SE).

<table>
<thead>
<tr>
<th></th>
<th>Energy (kcal)</th>
<th>Intake (kJ)</th>
<th>CHO (g)</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M+C</td>
<td>3080 ± 164</td>
<td>12874 ± 686</td>
<td>479 ± 28</td>
<td>115 ± 8</td>
<td>92 ± 13</td>
</tr>
<tr>
<td>M+W</td>
<td>3179 ± 151</td>
<td>13288 ± 631</td>
<td>472 ± 33</td>
<td>120 ± 6</td>
<td>100 ± 12</td>
</tr>
<tr>
<td>P+W</td>
<td>3261 ± 173</td>
<td>13631 ± 723</td>
<td>473 ± 39</td>
<td>126 ± 7</td>
<td>104 ± 12</td>
</tr>
</tbody>
</table>

Table 5.2: Gut fullness (GFS), and abdominal discomfort (ADS) during the postprandial period (mean ± SE).

<table>
<thead>
<tr>
<th>Variable</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>0.5 ± 0.2</td>
<td>2.7 ± 0.5b</td>
<td>2.4 ± 0.5b</td>
<td>1.7 ± 0.5b</td>
<td>1.8 ± 0.4b</td>
</tr>
<tr>
<td>M+W</td>
<td>0.2 ± 0.1</td>
<td>3.0 ± 0.5b,c</td>
<td>2.3 ± 0.5b</td>
<td>1.5 ± 0.4b</td>
<td>1.4 ± 0.4b</td>
</tr>
<tr>
<td>P+W</td>
<td>0.3 ± 0.1a</td>
<td>1.1 ± 0.3a</td>
<td>0.8 ± 0.2a</td>
<td>0.3 ± 0.1a</td>
<td>0.3 ± 0.2a</td>
</tr>
<tr>
<td>ADS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>0.5 ± 0.2</td>
<td>2.1 ± 0.5b</td>
<td>1.8 ± 0.4b</td>
<td>1.3 ± 0.3</td>
<td>1.3 ± 0.5</td>
</tr>
<tr>
<td>M+W</td>
<td>0.4 ± 0.6</td>
<td>2.0 ± 0.6b</td>
<td>1.8 ± 0.6b</td>
<td>1.1 ± 0.4</td>
<td>0.9 ± 0.4</td>
</tr>
<tr>
<td>P+W</td>
<td>0.3 ± 0.5</td>
<td>1.1 ± 0.5</td>
<td>0.8 ± 0.3</td>
<td>0.9 ± 0.4</td>
<td>0.5 ± 0.3</td>
</tr>
</tbody>
</table>

a: p< 0.01 from M+C and M+W; b: p<0.01 from 0 min
c: p< 0.01 from 120 min, and 180 min
Table 5.3: Gut fullness (GFS), and abdominal discomfort (ADS) during exercise (mean ± SE; n=9).

<table>
<thead>
<tr>
<th>Variable</th>
<th>0 min</th>
<th>20 min</th>
<th>40 min</th>
<th>60 min</th>
<th>80 min*</th>
<th>Exhaustion</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>1.8 ± 0.5</td>
<td>3.0 ± 0.7</td>
<td>3.3 ± 0.6</td>
<td>3.8 ± 0.6b</td>
<td>3.5 ± 0.5c</td>
<td>3.8 ± 0.9b</td>
</tr>
<tr>
<td>M+W</td>
<td>1.4 ± 0.4</td>
<td>1.7 ± 0.4</td>
<td>1.9 ± 0.4</td>
<td>2.4 ± 0.6</td>
<td>2.4 ± 0.7</td>
<td>3.1 ± 0.6c</td>
</tr>
<tr>
<td>P+W</td>
<td>0.2 ± 0.2a</td>
<td>1.0 ± 0.3</td>
<td>2.0 ± 0.5b</td>
<td>2.4 ± 0.8b</td>
<td>3.5 ± 0.7b,d</td>
<td>2.4 ± 0.7b</td>
</tr>
<tr>
<td>ADS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>1.2 ± 0.5</td>
<td>3.3 ± 0.6b</td>
<td>3.4 ± 0.6b</td>
<td>3.4 ± 0.7b</td>
<td>3.0 ± 0.7</td>
<td>3.2 ± 0.9c</td>
</tr>
<tr>
<td>M+W</td>
<td>0.8 ± 0.5</td>
<td>2.8 ± 0.5b</td>
<td>2.4 ± 0.4</td>
<td>2.2 ± 0.5</td>
<td>3.0 ± 0.9b</td>
<td>3.3 ± 0.8b</td>
</tr>
<tr>
<td>P+W</td>
<td>0.2 ± 0.2</td>
<td>2.3 ± 0.6b</td>
<td>3.2 ± 0.6b</td>
<td>3.4 ± 0.7b</td>
<td>4.2 ± 0.6b,e</td>
<td>4.8 ± 0.5b,d</td>
</tr>
</tbody>
</table>

a: p< 0.05 from M+C; b: p< 0.01 from 0 min; c: p< 0.05 from 0 min
d: p< 0.01 from 20 min; e: p< 0.05 from 20 min
*N = 8
Table 5.4: Oxygen uptake (\(\dot{V}O_2\)) heart rate (HR), and perceived rate of exertion (PRE) during the M+C, M+W, and P+W trials (mean ± SE; n=9).

<table>
<thead>
<tr>
<th>Variable</th>
<th>20 min</th>
<th>40 min</th>
<th>60 min</th>
<th>80 min</th>
<th>Exhaustion</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\dot{V}O_2) (\text{ml.kg}^{-1}.\text{min}^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>42.7(^a,c)</td>
<td>43.4(^a,c)</td>
<td>43.9(^a,d)</td>
<td>46.3</td>
<td>47.7 (\pm 1.7)</td>
</tr>
<tr>
<td>M+W</td>
<td>42.6(^a,c)</td>
<td>43.4(^a)</td>
<td>43.6(^a)</td>
<td>45.6(^b)</td>
<td>47.8 (\pm 2.2)</td>
</tr>
<tr>
<td>P+W</td>
<td>43.7(^a)</td>
<td>44.0</td>
<td>44.0(^a)</td>
<td>46.3</td>
<td>46.2 (\pm 1.9)</td>
</tr>
<tr>
<td>(\dot{V}O_2) (\text{l.min}^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>3.1(^a,c)</td>
<td>3.1(^a,d)</td>
<td>3.2(^a)</td>
<td>3.3(^a)</td>
<td>3.5 (\pm 0.2)</td>
</tr>
<tr>
<td>M+W</td>
<td>3.1(^a)</td>
<td>3.2(^a)</td>
<td>3.1(^a)</td>
<td>3.2(^a)</td>
<td>3.4 (\pm 0.2)</td>
</tr>
<tr>
<td>P+W</td>
<td>3.2(^a)</td>
<td>3.2(^b)</td>
<td>3.2(^b)</td>
<td>3.3</td>
<td>3.4 (\pm 0.2)</td>
</tr>
<tr>
<td>HR (n=6) (\text{b.min}^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>158(^a)</td>
<td>161(^a)</td>
<td>164(^a)</td>
<td>163(^a)</td>
<td>171 (\pm 5)</td>
</tr>
<tr>
<td>M+W</td>
<td>158(^a)</td>
<td>159(^a)</td>
<td>161(^a)</td>
<td>164(^a)</td>
<td>174 (\pm 7)</td>
</tr>
<tr>
<td>P+W</td>
<td>161(^a)</td>
<td>164(^a)</td>
<td>167(^a)</td>
<td>170(^a)</td>
<td>173 (\pm 7)</td>
</tr>
<tr>
<td>PRE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>11.7(^a,c)</td>
<td>12.3(^a)</td>
<td>12.3(^a)</td>
<td>13.4(^a)</td>
<td>17.2 (\pm 0.6)</td>
</tr>
<tr>
<td>M+W</td>
<td>11.2(^a,c)</td>
<td>12.2(^a,c)</td>
<td>12.9(^a,f)</td>
<td>13.9(^a)</td>
<td>17.9 (\pm 0.6)</td>
</tr>
<tr>
<td>P+W</td>
<td>11.1(^a,c)</td>
<td>12.3(^a,c,g)</td>
<td>13.6(^a,e,f)</td>
<td>14.2(^a)</td>
<td>17.6 (\pm 0.6)</td>
</tr>
</tbody>
</table>

\(^a\): p< 0.01 from Exhaustion; \(^b\): p< 0.05 from Exhaustion; \(^c\): p< 0.01 from 80 min
\(^d\): p< 0.05 from 80 min; \(^e\): p< 0.05 from M+C; \(^f\): p< 0.01 from 20 min
\(^g\): p< 0.05 from 60 min

\(*\) N=8
Table 5.5: Blood lactate and plasma urea concentrations (mmol.1⁻¹) during the postprandial period (mean ± SE)

<table>
<thead>
<tr>
<th>Variable</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Lactate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>1.0±0.1</td>
<td>1.9±0.1</td>
<td>1.6±0.1</td>
<td>1.3±0.1</td>
<td>1.3±0.3</td>
</tr>
<tr>
<td>M+W</td>
<td>0.9±0.1</td>
<td>1.6±0.1</td>
<td>1.7±0.1</td>
<td>1.2±0.1</td>
<td>1.0±0.3</td>
</tr>
<tr>
<td>P+W</td>
<td>0.8±0.1</td>
<td>0.9±0.1</td>
<td>1.0±0.1</td>
<td>0.9±0.1</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>Plasma Urea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>5.9±0.2</td>
<td>5.8±0.3</td>
<td>5.7±0.3</td>
<td>5.6±0.3</td>
<td>5.6±0.3</td>
</tr>
<tr>
<td>M+W</td>
<td>6.0±0.2</td>
<td>5.9±0.2</td>
<td>5.8±0.2</td>
<td>5.8±0.2</td>
<td>5.8±0.2</td>
</tr>
<tr>
<td>P+W</td>
<td>6.3±0.3</td>
<td>6.1±0.3</td>
<td>5.9±0.3</td>
<td>5.7±0.3</td>
<td>5.6±0.3</td>
</tr>
</tbody>
</table>

a: p<0.01 from 0 min; b: p<0.01 from P+W; c: p<0.05 from P+W

Table 5.6: Blood lactate, plasma urea, serum sodium, and serum potassium concentrations (mmol.1⁻¹) during exercise (mean ± SE; n=8)

<table>
<thead>
<tr>
<th>Variable</th>
<th>0 min</th>
<th>20 min</th>
<th>40 min</th>
<th>60 min</th>
<th>Exhaustion</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Lactate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>1.2±0.1</td>
<td>1.7±0.1</td>
<td>1.6±0.1</td>
<td>1.6±0.1</td>
<td>2.9±0.2</td>
</tr>
<tr>
<td>M+W</td>
<td>1.0±0.1</td>
<td>2.0±0.3</td>
<td>2.1±0.3</td>
<td>1.9±0.2</td>
<td>2.5±0.3</td>
</tr>
<tr>
<td>P+W</td>
<td>0.9±0.03</td>
<td>1.8±0.2</td>
<td>1.9±0.2</td>
<td>1.9±0.2</td>
<td>2.6±0.3</td>
</tr>
<tr>
<td>Plasma Urea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>5.6±0.3</td>
<td>5.8±0.3</td>
<td>5.9±0.3</td>
<td>6.1±0.3</td>
<td>6.7±0.4</td>
</tr>
<tr>
<td>M+W</td>
<td>5.8±0.3</td>
<td>6.0±0.3</td>
<td>6.1±0.2</td>
<td>6.3±0.2</td>
<td>6.8±0.2</td>
</tr>
<tr>
<td>P+W</td>
<td>5.7±0.3</td>
<td>5.7±0.3</td>
<td>5.9±0.3</td>
<td>6.0±0.4</td>
<td>6.4±0.3</td>
</tr>
<tr>
<td>Serum Sodium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>141±0.5</td>
<td>142±0.5</td>
<td>142±0.3</td>
<td>142±0.3</td>
<td>144±0.4</td>
</tr>
<tr>
<td>M+W</td>
<td>141±0.6</td>
<td>142±0.5</td>
<td>142±0.5</td>
<td>142±0.5</td>
<td>144±0.6</td>
</tr>
<tr>
<td>P+W</td>
<td>140±0.6</td>
<td>141±0.4</td>
<td>141±0.4</td>
<td>141±0.3</td>
<td>142±0.7</td>
</tr>
<tr>
<td>Serum Potassium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>4.1±0.1</td>
<td>5.1±0.1</td>
<td>5.1±0.1</td>
<td>5.3±0.1</td>
<td>5.5±0.1</td>
</tr>
<tr>
<td>M+W</td>
<td>4.2±0.2</td>
<td>5.0±0.2</td>
<td>5.0±0.1</td>
<td>5.3±0.2</td>
<td>5.2±0.2</td>
</tr>
<tr>
<td>P+W</td>
<td>4.1±0.1</td>
<td>5.0±0.1</td>
<td>5.2±0.1</td>
<td>5.3±0.1</td>
<td>5.3±0.1</td>
</tr>
</tbody>
</table>

a: p<0.01 from Exhaustion; b: p<0.01 from 0 min; c: p<0.05 from Exhaustion
Table 5.7: Plasma adrenaline and plasma noradrenaline concentrations (nmol.l⁻¹) in the M+C, M+W, and P+W trials (mean ± SE)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Prefeeding</th>
<th>0 min</th>
<th>20 min</th>
<th>Exhaustion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma Adrenaline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n= 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>0.20± 0.03⁺</td>
<td>0.27± 0.06⁺</td>
<td>0.45± 0.10⁺</td>
<td>3.00± 0.90</td>
</tr>
<tr>
<td>M+W</td>
<td>0.34± 0.08⁺</td>
<td>0.21± 0.05⁺</td>
<td>0.45± 0.12⁺</td>
<td>4.62± 1.08</td>
</tr>
<tr>
<td>P+W</td>
<td>0.29± 0.05⁺</td>
<td>0.23± 0.06⁺</td>
<td>0.50± 0.12⁺</td>
<td>3.50± 1.10</td>
</tr>
<tr>
<td><strong>Plasma Noradrenaline</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n= 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>2.41± 0.76⁺, b</td>
<td>3.71± 0.83⁺, b</td>
<td>12.80± 1.17⁺</td>
<td>26.30± 4.40</td>
</tr>
<tr>
<td>M+W</td>
<td>2.52± 0.60⁺, b</td>
<td>3.61± 0.72⁺, b</td>
<td>12.60± 0.94⁺</td>
<td>25.06± 2.68</td>
</tr>
<tr>
<td>P+W</td>
<td>2.44± 0.60⁺, b</td>
<td>4.82± 1.19⁺, b</td>
<td>15.28± 0.94⁺</td>
<td>25.39± 2.34</td>
</tr>
</tbody>
</table>

a: p< 0.01 from Exhaustion; b: p< 0.01 from 20 min

Table 5.8: Adrenaline to insulin concentration (A/I) and noradrenaline to insulin concentration (NA/I) ratios in the M+C, M+W, and P+W trials (mean ± SE; n= 8)

<table>
<thead>
<tr>
<th></th>
<th>0 min</th>
<th>20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A/I</td>
<td>NA/I</td>
</tr>
<tr>
<td>M+C</td>
<td>0.02⁺</td>
<td>0.23⁺</td>
</tr>
<tr>
<td></td>
<td>± 0.01</td>
<td>± 0.06</td>
</tr>
<tr>
<td>M+W</td>
<td>0.01⁺</td>
<td>0.19⁺</td>
</tr>
<tr>
<td></td>
<td>± 0.004</td>
<td>± 0.05</td>
</tr>
<tr>
<td>P+W</td>
<td>0.05</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>± 0.02</td>
<td>± 0.35</td>
</tr>
</tbody>
</table>

a: p< 0.05 from P+W; b: p< 0.01 from 0 min
Fig. 52: Blood glucose concentration (mmol.l⁻¹) during the postprandial period in the M+C, M+W, and P+W trials (mean ± SE)

Fig. 53: Serum insulin concentration (µU.ml⁻¹) during the postprandial period in the M+C, M+W, and P+W trials (mean ± SE; n=9)

* p<0.01 from M+C, M+W
a: p<0.01 from 0 min
Fig. 5.4: Plasma FFA concentration (mmol.l⁻¹) during the postprandial period in the M+C, M+W, and P+W trials (mean ± SE; n=9)

* p< 0.01 from M+C, M+W
a: p< 0.01 from 0 min

Fig. 5.5: Plasma Glycerol concentration (mmol.l⁻¹) during the postprandial period in the M+C, M+W, and P+W trials (mean ± SE; n=9)

* p< 0.01 from M+C, M+W
a: p< 0.01 from 0 min
Fig. 5.6: Respiratory exchange ratio (RER) in the M+C, M+W, and P+W trials (mean ± SE; n=8)
* p< 0.01 from M+C, M+W
a: p< 0.01 from exhaustion in both M+C and M+W trials

Fig. 5.7: Carbohydrate oxidation rate (g.min⁻¹) in M+C, M+W, and P+W trials (mean ± SE; n=8)
* p< 0.01 from M+C, M+W
a: p< 0.01 from exhaustion in M+C
Fig. 5.8: Blood glucose concentration (mmol.L⁻¹) during exercise in the M+C, M+W, and P+W trials (mean ± SE; n=9)
*p < 0.01 from M+W; a: *p < 0.01 from 0 min in M+C
b: p < 0.01 from 20 min, 40 min

Fig. 5.9: Serum insulin concentration (µU.ml⁻¹) during exercise in the M+C, M+W, and P+W trials (mean ± SE; n=8)
*p < 0.01 from M+C, M+W
a: *p < 0.01 from 20 min, 40 min, 60 min, and exhaustion
Fig. 5.10: Plasma FFA concentration (mmol. l⁻¹) during exercise in the M+C, M+W, and P+W trials (mean ± SE; n=8)
* p<0.01 from M+C, M+W
a: p<0.01 from 0 min, 20 min, 40 min, and 60 min
b: p<0.05 from 0 min

Fig. 5.11: Plasma glycerol concentration (mmol. l⁻¹) during exercise in the M+C, M+W, and P+W trials (mean ± SE; n=8)
* p<0.01 from M+C, M+W; a: p<0.01 from 20 min, 40 min, 60 min, and exhaustion in P+W
b: p<0.01 from 0 min, 20 min, 40 min, and 60 min
5.4 Discussion

The main finding of the present study was that the consumption of the high carbohydrate meal 3 hours before exercise (M+W) improved endurance capacity (9%) compared with no meal (P+W). When the 6.9% carbohydrate-electrolyte solution was ingested during exercise (M+C) the endurance capacity was improved even further (22%). Furthermore, the combination of the meal and carbohydrate-electrolyte solution (M+C) was superior (12%) than the meal alone (M+W).

Improvements in cycling performance have been reported when liquid carbohydrate meals, providing 4.5-5.0 g CHO kg\(^{-1}\) BW, were consumed 3-4 hours before exercise (Sherman et al. 1989; Wright et al. 1991). However although performance was improved further when a pre-exercise meal was combined with carbohydrate feedings during exercise, this additional improvement was not found to be statistically significant (Flynn et al. 1989; Wright et al. 1991). In the study conducted by Flynn et al. (1989) a strong tendency (p=0.059) was found in favour of combining the pre-exercise meal with carbohydrate ingestion during exercise. Possibly, the protocol employed (15 min 'performance ride') was not sensitive enough to detect any differences in performance between treatments. Furthermore, as was argued earlier (Chapter 4), a possible reason for the discrepancy between the result of the present study and that of Wright and co-workers' (1991) is the fact that their subjects consumed almost double the amount of carbohydrate used in the present study. A very high dosage of carbohydrate ingested 3-4 hours before exercise may 'top-off' the glycogen stores in liver and muscle and may also continue to provide an increased glucose availability to the working muscles during exercise. This may reduce, to some extent, the ergogenic effect of a carbohydrate solution ingested during exercise. Indeed, when carbohydrate stores, and especially glycogen levels, are elevated carbohydrate ingestion during exercise does not result in an improvement in endurance performance (Flynn et al. 1987; Widrick et al. 1992).

Nevertheless, recently Rehrer et al. (1994) failed to demonstrate a difference in endurance running capacity in subjects who ingested a liquid carbohydrate meal 2 hours before exercise compared with the ingestion of both the liquid meal and carbohydrate during exercise, despite a tendency for longer endurance times in the second condition. A difference in the exercise protocol,
subjects variability, as well as the relatively small sample size (n=6) might be the reasons for the lack of any statistical difference between the two nutritional treatments.

Furthermore, Thomas and colleagues (1991) observed an improvement in cycling time to exhaustion after feeding subjects with a low glycaemic meal (lentils), but not when the meal had a high glycaemic index (mash potatoes). Probably, the amount of carbohydrates included in the high glycaemic meal (1 g per kg BW) was not enough to influence endurance cycling capacity. Indeed, when Sherman et al. (1989) compared the effects of feeding 45 g of carbohydrate versus 156 g of carbohydrate no difference was observed in cycling performance. However, when the amount of feeding increased to 312 g and was compared to fasting conditions an improvement was found during a 45 min performance ride.

It is possible that subjects had higher liver (Nilsson and Hultman 1973) and/or muscle glycogen stores (Coyle et al. 1985) before the initiation of exercise in the two meal trials. However, these two factors might not be the only possible mechanisms by which the pre-exercise carbohydrate meal improved endurance capacity. A portion of the meal might still have been in the gut when exercise begun providing in this way, a slow release of substrate for the working muscles during exercise. This suggestion is supported indirectly by the higher gut fullness rates observed in the two meal trials 3 hours after the ingestion of the meal (Table 5.2). Nevertheless, this possibility is speculative since no direct measurements on gastric emptying were performed in this study.

The pre-exercise carbohydrate meals (M+C, M+W) resulted in elevated respiratory exchange ratios (Fig. 5.6) and carbohydrate oxidation rates (Fig. 5.7) during the first hour of exercise and a suppression of plasma FFA (Fig. 5.10) and glycerol (Fig. 5.11) concentrations throughout exercise. This finding was consistent with the finding of the previous study (Chapter 4), and confirms the fact that pre-exercise carbohydrate feedings produce a greater reliance on carbohydrate metabolism during prolonged endurance exercise (Coyle et al. 1985; Montain et al. 1991; Neufer et al. 1987; Sherman et al. 1989; Willcutts et al. 1988; Wright et al. 1991). It seems that at least 6 hours of fasting are necessary before metabolism during exercise is similar to exercise metabolism after 8-12 hour fast (Montain et al. 1991).
Serum insulin concentrations were also higher in the M+C and M+W trials at the start of exercise (Fig. 5.9). This explains the difference in blood glucose concentration at 20 min of exercise between the M+W and P+W trials (Fig. 5.8). Indeed, that might be a consequence of an increased uptake of glucose by muscle in the presence of an increased plasma insulin concentration (in the M+W trial) and the inability of liver glycogen to replace blood glucose at the same rate as muscle takes up this substrate (Ahlborg and Felig 1976; Ahlborg and Bjorkman 1987; Sherman 1991).

This pre-exercise hyperinsulinemia can also explain the higher respiratory exchange ratio (RER) values and carbohydrate oxidation rates found during the first hour of exercise in the M+C and M+W trials, as well as the lower plasma FFA and glycerol concentrations observed throughout exercise (Coyle et al. 1985).

It has been suggested that elevated insulin concentrations at the start of exercise may cause a rapid decline in blood glucose concentration at the beginning of exercise, a reduced availability of FFA to the working muscles, and finally an elevated muscle glycogen utilisation (Costill et al. 1977), which eventually may lead to premature fatigue (Foster et al. 1979). However, despite the elevated carbohydrate metabolism observed in the present study in the two meal trials endurance capacity was improved compared to control conditions. Since the suggestion of Costill et al. (1977) and Foster et al. (1979) about the detrimental effects of pre-exercise carbohydrate feedings, many studies have examined the influence on endurance capacity and performance of ingesting carbohydrates before exercise (see Tables 2.3 and 2.4). With the exception of studies conducted by Foster et al. (1979), and Keller and Schwarzkopf (1984) all the studies have reported either no effect, or an improvement in endurance capacity or performance during cycling or running as a result of consuming carbohydrates in various forms and dosages before exercise (Table 2.3 and 2.4). Therefore, there is little evidence to support the idea that pre-exercise carbohydrate feedings may lead to premature fatigue.

Normally, there is a reduction in FFA mobilisation when carbohydrate is ingested during exercise compared to placebo or water (Coggan and Coyle 1991). However, in the present study no difference was observed in the
plasma FFA and glycerol concentrations between the M+C and M+W trials. Probably the depression of lipolysis caused by the pre-exercise meal in the M+W trial was too much to be further reduced by the administration of the carbohydrate-electrolyte solution in the M+C trial.

In agreement with the results reported in the previous study (Chapter 4), this study showed no significant drop in the carbohydrate oxidation rate, or blood glucose concentration at the point of fatigue, compared with the initiation of exercise, when subjects ingested no carbohydrates (P+W). Therefore, one might suggest that the possible mechanism by which the carbohydrate-electrolyte drink further improved running capacity in the M+C trial was a reduced glycogen utilisation in Type I muscle fibers (Tsintzas et al. 1993c; 1994). However, one subject had very low blood glucose concentration (2.1 mmol.l⁻¹) at exhaustion in the control trial. This subject had the largest improvement in endurance capacity with carbohydrate feedings (32 min and 63 min in the M+W and M+C trials respectively). It has been suggested that only people who experience a significant drop in blood glucose concentration at the point of fatigue in the fasted state benefit from the ingestion of carbohydrates during exercise (Coyle et al. 1983; Coggan and Coyle 1991). However, this particular subject was the only one, out of a total of 20 runners who participated in the first two studies of this thesis (Chapters 4 and 5), who became hypoglycaemic (i.e: blood glucose concentration < 2.5 mmol.l⁻¹) at exhaustion when no carbohydrate was provided.

Hepatic glucose production during exercise is stimulated by glucagon and catecholamines and suppressed by insulin (Coggan 1991). Despite the fact that glucagon concentration was not determined in this study, the lower catecholamines to insulin ratio observed at the beginning of exercise in the meal trials (Table 5.8) supports the view that hepatic glycogenolysis might have been reduced when carbohydrate was provided (Coggan and Coyle 1991; McConell et al. 1994). Therefore, running capacity in the carbohydrate trials (M+C and M+W) could have been improved because of a reduced rate of hepatic glycogen utilisation. The decrease in the rate of hepatic glycogen degradation is in a sense a liver glycogen sparing effect which enables the liver to supply with fuel the working muscles late in exercise when the muscle glycogen reserves are low.
During the 3-hour postprandial period no peak was found in the blood glucose concentration in both M+C and M+W trials (Fig. 5.2). The reason for this finding is that glucose concentration may peak before the 30 min postprandial time as it was found in a subsequent study (Chapter 6).

The depression of plasma FFA (Fig. 5.4) and glycerol (Fig. 5.5) concentrations during the postprandial period in the meal trials was the result of the well known antilipolytic action of insulin (Newsholme and Leech 1983).

In summary, the results of this study show that ingestion of a high carbohydrate meal, providing 2.5 g CHO.kg⁻¹ BW, 3 hours before exercise at 70% VO₂ max improves endurance running capacity. Also, the combination of both the pre-exercise meal and a carbohydrate-electrolyte solution during exercise further improves endurance running capacity.
6.1 Introduction

The previous two chapters reported evidence showing an improvement in endurance running capacity as a result of consuming a pre-exercise high carbohydrate meal 3 hours before exercise. As mentioned earlier (Chapters 4 and 5) this improvement could have been due to an increase in pre-exercise muscle glycogen concentrations as a result of the pre-exercise feeding.

However, there is controversy regarding the effect of a high carbohydrate meal on skeletal muscle glycogen concentration. Coyle and his colleagues (1985) reported a 42% increase in muscle glycogen concentration 4 hours after subjects consumed about 140 g of carbohydrate. Neufer and his co-workers (1987), on the other hand, found a non-significant 15% increase in the muscle glycogen concentration 4 hours after subjects consumed 200 g of carbohydrate. In both studies muscle biopsies were performed only 4 hours after the ingestion of the meals and the muscle glycogen concentrations were compared with values observed in a separate experimental trial where subjects were fasted overnight. Despite the fact that diet and physical activity were controlled before the experimental trials, it is not certain whether subjects had similar fasting muscle glycogen concentrations in all experimental trials.

Therefore, the primary purpose of this study was to investigate whether a high carbohydrate meal alters the muscle glycogen concentration over a 3-hour post-prandial period (experiment I). In an subsequent study the respiratory responses to the meal and its thermic effect were also studied (experiment II).
6.2 Methods

Subjects
Eight male recreational/club level endurance runners volunteered for experiment I. Their age, weight, height, body mass index, maximal oxygen uptake, and maximum heart rate were 36.4 ± 5.2 years, 69.91 ± 3.9 kg, 175.9 ± 2.9 cm, 22.2 ± 0.6 kg.m⁻², 60.2 ± 3.5 ml.kg⁻¹.min⁻¹, and 179 ± 5 b.min⁻¹ respectively (mean ± SE).

Six subjects, of which 4 had participated in experiment I, volunteered for experiment II. Their age, weight, height, body mass index, maximal oxygen uptake, and maximum heart rate were 37.3 ± 5.6 years, 74.07 ± 4.1 kg, 175.6 ± 3.3 cm, 23.9 ± 0.5 kg.m⁻², 57.3 ± 3.0 ml.kg⁻¹.min⁻¹, and 182 ± 6 b.min⁻¹ respectively (mean ± SE).

Preliminary measurements
About one week before experiment I subjects performed a maximum oxygen uptake test (VO₂ max) (see Chapter 3). In experiment I subjects were instructed to record and weigh their normal food intake the three days before the experiment, and to refrain from any form of training the day before the experiment. The dietary information was then analysed and found that the average daily intake was 2648 ± 288 kcal (11069 ± 1204 kj) and its content was 51 ± 3.9% carbohydrate, 33.3 ± 2.9% fat, and 15.6 ± 2.2 % protein. In experiment II subjects recorded their normal food intake the day before the first trial and replicated the same diet the day before the second trial. Also, subjects in experiment II did not exercise the day before each trial, and followed the same training schedule two days before each trial.

Experimental Design

- Experiment I
After a 12-h overnight fast each subject arrived at the laboratory between 8:00-10:00 am, emptied his bladder and then his nude body weight was obtained. While the subject was lying on an examination couch a 10-ml venous blood was obtained from an antecubital vein via an indwelling catheter. After that a sample from the vastus lateralis muscle was obtained using the needle biopsy procedure (see Chapter 3). Thereafter, subjects
consumed a high carbohydrate meal designed to provide 2.5 g of carbohydrate per kg BW (see Appendix C). The average amount of carbohydrate consumed was 174.8±9.8 g. Three hours after the ingestion of the meal another muscle sample was obtained. Venous blood samples were also taken, while subjects were lying on an examination couch, at 15, 30, 60, 120, and 180 min after the ingestion of the meal (Fig. 6.1). During the 3-hour postprandial period subjects remained quietly in the laboratory, and were lying on the examination couch for at least 10 min before each venous sample.

During the experiment the laboratory temperature was 20.5 ± 0.1 °C, and the relative humidity was 56.4 ± 1.2 % (mean ± SE).

- Experiment II
After a 12-hour overnight fast each subject arrived at the laboratory between 8:00-9:00 am, emptied his bladder, and his nude body weight was obtained. After seating quietly for 15 min on a chair a 5-min expired air sample was collected, and then the subjects consumed either the same high carbohydrate meal (M) as in experiment I, or fasted for a further 3 hours (F). The F condition was conducted in order to account for fluctuations in energy expenditure due to circadian rhythms (Baily et al. 1973). In the M trial 185.2 ± 10.2 g of carbohydrates were consumed, whereas the total energy ingested was 862.2 ± 47.7 kcal. During the 3-hour postprandial (M) or postabsorptive period (F) 5-min expired air samples were collected every 30 min while subjects were sitting on a chair. During the 3-hour postabsorptive, or postprandial period subjects remained quietly in the laboratory. Also, subjects were sitting quietly on a chair at least 15 min before each expired air sample was collected. The expired air collections were taken at similar (about 15-30 min) corresponding times of the day in both the M and F trials. The two trials were separated by 3-7 days and the order of the two trials was random.

Both trials were conducted under similar laboratory temperature (M: 20.1 ± 0.4 °C, F: 20.8 ± 0.3 °C), and relative humidity (M: 55.9 ± 3.1 %, F: 52.8 ± 2.8 %) (mean ± SE).
Analyses

Muscle, blood, and expired air samples were collected and analysed as previously described (Chapter 3). Venous blood samples were analysed for glucose, lactate, FFA and insulin concentrations. Muscle samples were analysed for glycogen, glucose, G-6-P, ATP, PCr, Cr, and lactate concentrations.

A one-way analysis of variance (ANOVA) for repeated measures on one factor (i.e.: across time) was used to study the metabolic responses of the meal during the postprandial period (experiment I). A two tailed Student's t test for dependent samples was used to study the effect of the meal on the various muscle metabolites measured.

A two-way ANOVA for repeated measures on two factors (treatment by time) was used to compare respiratory responses between the two conditions in experiment II. The remaining responses were analysed using a two tailed Student's t test for dependent samples. When significant differences were revealed, using the ANOVA, then a Tukey post hoc test was performed. The accepted level of significance was set at p< 0.05. Data are reported as mean ± SE.
Fig. 6.1: Schematic illustration of the experimental protocol of experiment I
6.3 Results

- Experiment I
Blood glucose concentrations (Fig. 6.2) peaked 15 min after the ingestion of the meal, and afterwards gradually decreased with time. Three hours after the ingestion of the meal blood glucose was lower than the prefeeding levels by 0.7 mmol.l⁻¹, but this difference was not significant.

Serum insulin concentrations (Fig. 6.3) also peaked at 15 min postprandially, maintained elevated the first two hours, and decreased after the second hour. Three hours after the ingestion of the meal serum insulin concentration was still 3-fold higher than prefeeding levels (0 min: 6.7 ± 0.6 mU.l⁻¹ vs 180 min: 21.5 mU.l⁻¹). However, due to high postprandial insulin concentration values this difference did not reach significance.

After the ingestion of the carbohydrate meal plasma free fatty acids concentrations (Fig. 6.4) decreased with time and were 6-fold lower compared with prefeeding values at 180 min postprandialy.

Blood lactate concentrations (Fig. 6.5) were 75% higher at 30 min and 60 min postprandially compared with prefeeding concentration values. However, one hour after ingestion blood lactate concentrations were similar to pre-ingestion levels.

The muscle metabolite concentrations before and 3 hours after the carbohydrate meal are shown in table 6.1. Muscle glycogen concentration was 10.6% higher three hours after the ingestion of the meal. However, muscle glucose, G-6-P, ATP, PCr, Cr, and Lactate concentrations were not different three hours after the ingestion of the meal.

- Experiment II
Oxygen consumption was higher (p< 0.01) during the first two hours in the M condition compared with the F (Table 6.2). However, oxygen consumption was not different in the last hour between the postabsorptive and postprandial states.
Respiratory exchange ratios (Table 6.2) were higher (p< 0.01) only during the third hour of the postprandial state (M) compared with the postabsorptive state (F). However, carbohydrate oxidation rate (Table 6.2) was higher throughout the 3-hour experimental period in the M trial compared with the F trial. It should be mentioned here that during the M trial only one subject had RER above 1.0 and that happened only at 180 min. The RER value for this subject at that point was 1.01, whereas all other RER values during the postprandial period for this as well as for the rest of the subjects were below 1.0.

The energy expenditure over the 3-hour period during the M trial was higher than during the F trial (M: 237.2 ± 14.4 kcal vs F: 187.2 ± 14.7 kcal, p< 0.05). The thermic effect of the high carbohydrate meal (as calculated by subtracting the 3-hour energy expenditure during the postabsorptive trial from the 3-hour energy expenditure during the postprandial trial) was 50 ± 7.5 kcal.

Fat oxidation rate (Table 6.2) was lower in the third hour in the postprandial state compared with the postabsorptive state.

The total amount of carbohydrates oxidised (assuming a non-protein respiratory exchange ratio) during the 3-hour experimental period were more in the M trial compared with the F trial (M: 39.95 ± 2.57 g vs F: 18.55 ± 3.10 g, p< 0.01). However, the energy value (about 86 kcal) of the excess 21.4 ± 2.1 g of carbohydrates consumed during the M trial exceeds the 50 kcal which represented the thermic effect of the meal. That was due to the fact that the total fat oxidised in the F trial were more than the amount of fat oxidised in the postprandial state (F: 12.6 ± 2.0 g vs M: 8.6 ± 0.9 g). However, this difference did not reach significance (p= 0.07).
Table 6.1: Muscle glycogen, glucose, G-6-P, Lactate, ATP, PCr, and Cr concentrations (mmol.kg dry weight⁻¹) in vastus lateralis muscle before and 3 hours after the ingestion of the meal (mean ± SEM, n=6).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before Meal</th>
<th>After Meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen</td>
<td>314 ± 33.9</td>
<td>347.3 ± 31.3*</td>
</tr>
<tr>
<td>Glucose</td>
<td>2.5 ± 0.5</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>G-6-P</td>
<td>1.1 ± 0.3</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>Lactate</td>
<td>3.4 ± 0.3</td>
<td>3.5 ± 0.2</td>
</tr>
<tr>
<td>ATP</td>
<td>22.8 ± 1.5</td>
<td>23.7 ± 0.6</td>
</tr>
<tr>
<td>PCr</td>
<td>77.7 ± 3.9</td>
<td>80.7 ± 2.7</td>
</tr>
<tr>
<td>Cr</td>
<td>43.8 ± 5.7</td>
<td>40.7 ± 3.6</td>
</tr>
</tbody>
</table>

*: p< 0.05 from before meal
Table 6.2: Oxygen consumption (\(\dot{V}O_2\)), respiratory exchange ratio (RER), carbohydrate oxidation rate (CHO Oxid. Rate), and fat oxidation rate (Fat Oxid. Rate) in the F and M trials (mean ± SE).

<table>
<thead>
<tr>
<th>Variable</th>
<th>0 min</th>
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<th>90 min</th>
<th>120 min</th>
<th>150 min</th>
<th>180 min</th>
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<tr>
<td>(\dot{V}O_2) (ml.min(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>190</td>
<td>200</td>
<td>220</td>
<td>220</td>
<td>210</td>
<td>230</td>
<td>240(^a)</td>
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<td>±20</td>
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<td>±20</td>
<td>±20</td>
<td>±20</td>
<td>±20</td>
<td>±20</td>
</tr>
<tr>
<td>M</td>
<td>240(^b)</td>
<td>280(^c)</td>
<td>290(^a,c)</td>
<td>280(^c)</td>
<td>290(^a,c)</td>
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<td>±20</td>
<td>±20</td>
<td>±20</td>
<td>±30</td>
<td></td>
</tr>
<tr>
<td>RER</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>.84</td>
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<td>±.04</td>
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<td>.88</td>
<td>.89</td>
<td>.90(^c)</td>
<td>.93(^a,c)</td>
<td>.95(^a,c)</td>
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</tr>
<tr>
<td>CHO Oxid. Rate (g.min(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>±.03</td>
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<td>±.02</td>
<td>±.02</td>
<td>±.02</td>
<td>±.02</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>.11</td>
<td>.20(^a,b)</td>
<td>.20(^a,c)</td>
<td>.22(^a,c)</td>
<td>.23(^a,c)</td>
<td>.23(^a,c)</td>
<td>.25(^a,c)</td>
</tr>
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<td>±.02</td>
<td>±.02</td>
<td>±.02</td>
<td>±.02</td>
<td>±.01</td>
<td>±.02</td>
<td></td>
</tr>
<tr>
<td>Fat Oxid. Rate (g.min(^{-1}))</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>M</td>
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<td>.06</td>
<td>.06</td>
<td>.05</td>
<td>.05</td>
<td>.04(^a,c)</td>
<td>.03(^a,c)</td>
</tr>
<tr>
<td>±.01</td>
<td>±.01</td>
<td>±.01</td>
<td>±.003</td>
<td>±.004</td>
<td>±.01</td>
<td>±.01</td>
<td></td>
</tr>
</tbody>
</table>

a: p< 0.01 from 0 min; b: p< 0.05 from F; c: p<0.01 from F
Fig. 6.2: Blood glucose concentration (mmol.l⁻¹; mean ± SE)
  a: p< 0.01 from 15 min; b: p< 0.01 from 30 min

Fig. 6.3: Serum insulin concentration (mU.l⁻¹; mean ± SE)
  a: p< 0.01 from 0 min; b: p< 0.01 from 15 min
Fig. 6.4: Plasma FFA concentration (mmol.l⁻¹; mean ± SE)
a: p< 0.01 from 0 min; b: p<0.05 from 15 min
c: p< 0.01 from 15 min

Fig. 6.5: Blood lactate concentration (mmol.l⁻¹; mean ± SE)
a: p< 0.01 from 0 min; b: p< 0.05 from 180 min
c: p< 0.01 from 180 min
6.4 Discussion

The main finding of this study was that the ingestion of the carbohydrate meal (174.8 ± 9.8 g CHO) resulted in a 10.6% increase in the glycogen content of vastus lateralis muscle. This increase was similar to that reported by Neufer and his co-workers (1987), although the authors reported that increase (15%) as not statistically significant. Coyle and his colleagues (1985), however, have reported a more impressive increase (42%) in muscle glycogen concentration after ingestion of 140 g carbohydrates.

A factor which could account for the discrepancy between studies is the amount of physical activity subjects undertake the day before the experiment. It has been shown that carbohydrate administration produces a greater resynthesis of glycogen in previously exercised skeletal muscle (Bergstrom and Hultman, 1967b). In the present study subjects refrained from any physical activity the day before the experiment, whereas in the study of Neufer and co-workers (1987) subjects exercised for 60 min at 70% \( \dot{V}O_2 \) max the day before in order to lower the glycogen stores within the active muscles. However, the authors reported that muscle glycogen concentration was not influenced significantly, despite the fact that they consumed greater amount of carbohydrates (200 g) than it was consumed in the present study (175 g in experiment I), as well as in Coyle and colleagues' study (140 g) (Coyle et al. 1985). Possibly, as mentioned in the introduction to this Chapter, the main reason for the discrepancy among these studies is the methodology employed. In the present study biopsies were performed before and three hours after the consumption of the meal. This was not the case, however, in the two previous studies (Coyle et al. 1985; Neufer et al. 1987). Despite the fact that in both studies (Coyle et al. 1985; Neufer et al. 1987) training and diet were controlled the days before the experiments, muscle glycogen levels might have been different before carbohydrate ingestion compared to the fasting condition.

Three hours after the ingestion of the meal the carbohydrate load consumed could have the following theoretical fates: (a) taken up by skeletal muscle, (b) disposed as liver glycogen, (c) oxidised, (d) excreted in the urine, (e) converted to fat; (f) some of it remained in the free glucose pool, or (g) some of it remained in the stomach, and/or the small intestine.
The possibilities that some of the carbohydrate load was excreted in the urine or remained in the free glucose pool should be excluded. This is because in healthy subjects even much higher amounts of dietary carbohydrates ingested do not produce any glucosuria (Acheson et al. 1982). As far as the free glucose pool option is concerned, the blood glucose concentration 3 hours after the meal was even slightly lower than prefeeding levels (probably due to still elevated serum insulin concentrations).

The fact that the majority of the RER values obtained during the postprandial period were below 1.0 seems to suggest that the rate of fat resynthesis during the 3 hour observation period did not exceed the rate of fat oxidation (Brody 1994). This indicates that lipogenesis from the ingested carbohydrates was almost negligible. This is also supported by observations that even ingestion of large amounts of dietary carbohydrate do not influence the body fat stores (Acheson et al. 1982, 1988; Bjorntorp and Sjostrom 1978).

Therefore, if one assumes that the meal had been absorbed completely from the gastrointestinal tract within 3 hours, then the carbohydrate load was either taken up by the liver/or muscles, or was oxidised. Assuming that the excess carbohydrate oxidised in the M trial was derived from the ingested meal (Acheson et al. 1982), the excess 21.4 g of carbohydrates oxidised (experiment II) represented 11.6% of the ingested load (i.e.: \[21.4/185.2\] * 100). This would represent about 20 g of carbohydrates being oxidised in experiment I (i.e.: 0.116 * 175), which leaves about 155 g to be accounted for. Assuming a molecular weight of 165 for the stored glycogen expressed in glucosyl units (Nilsson and Hultman, 1974), a muscle mass of 40% of total body weight, a dry/wet weight ratio of muscle tissue of 4.3, and a similar degree of glycogen synthesis by the various muscles, the increase in muscle glycogen content found in experiment I accounts for about 36 g of carbohydrates ingested. This represents about 21% of the total amount of carbohydrate consumed. There are still about 120 g of carbohydrates, 68% of the total carbohydrate load, to be accounted for.

However, the possibility that some portion of the meal was still in the stomach or small intestine after the 3 hours (see gut fullness data in Table 5.2) should not be totally discounted. This possibility is also supported by the fact that serum insulin concentration had not returned to pre-ingestion levels 3
hours after ingestion of the meal in the present study, nor during the postprandial period, in previous studies (Chapters 4 and 5).

Nevertheless, the above assumptions may not be supported because accurate estimates concerning the disposal of the ingested carbohydrate load can not be made from the available data. It is reasonable to suggest, however, that a considerable amount of ingested carbohydrate was disposed as liver glycogen (Bjorntorp and Sjostrom 1978; Nilsson and Hultman 1973; 1974).

The elevated blood lactate concentration observed during the first hour postprandially (Fig. 6.5) is consistent with other studies where blood lactate concentration increase after the administration of high carbohydrate (Chapters 5 and 8), or mixed meals (Segal et al. 1990). This postprandial lactate may reflect a conversion of ingested carbohydrate to lactate formed by an indirect pathway, in which postprandial hepatic glycogen formation takes place partially through gluconeogenesis as glucose is converted to lactate, this lactate is released into the blood stream and goes to the liver to be converted to glycogen (Katz and McGarry 1984; Radziuk 1989).

In summary, the ingestion of a high carbohydrate meal, providing 2.5 g carbohydrate per kilogram BW, produced about an 11% increase in the glycogen concentration of the vastus lateralis muscle. However, 3 hours after ingestion a considerable amount (about 68%) of the carbohydrate ingested was either still in the gastrointestinal tract, and/or disposed as glycogen in the liver.
CHAPTER 7

CARBOHYDRATE INGESTION DURING RUNNING AND SKELETAL MUSCLE GLYCOGEN UTILISATION IN FED HUMANS

7.1 Introduction

In the study reported in Chapter 5 it was found that the combination of a pre-exercise meal together with a carbohydrate-electrolyte solution ingested during exercise (M+C) is superior, in terms of endurance capacity, than a pre-exercise meal alone (M+W). Many studies have shown that ingesting carbohydrates during cycling (see Table 2.5), or during running (see Table 2.7) improve endurance capacity. A possible mechanism by which carbohydrate ingestion improves endurance capacity is by reducing the rate of muscle glycogen utilisation (see Table 2.1 and paragraph 2.5.3). On the other hand, other investigators were unable to provide evidence to support this suggestion (see Table 2.1). Recently, however, in our laboratory it was observed that ingesting a carbohydrate-electrolyte solution during treadmill running at 70-75% \( \dot{V}O_2 \) max reduces the rate of muscle glycogen breakdown (Tsintzas et al. 1993c; 1994). However, these studies were conducted after subjects had undergone an overnight fast of 12 to 16 hours.

Therefore, the aim of this study was to examine whether the ingestion of a carbohydrate-electrolyte solution during running can influence the rate of muscle glycogen utilisation when a high carbohydrate meal is ingested 3 hours before exercise. In this way, the hypothesis that glycogen sparing was the reason for the improvement in endurance capacity in the M+C trials compared with the M+W trials in Chapter 5 was also examined.
7.2 Methods

Subjects
Eight male recreational/club level runners participated in this study. Their age, height, body weight, $\dot{V}O_2$ max, and maximum heart rate were 33.7±3.1 years, 172.8±2.0 cm, 73.3±2.2 kg, 58.7±2.1 ml.kg$^{-1}$.min$^{-1}$, and 193±3 b.min$^{-1}$ respectively (mean±SE).

Preliminary measurements
After subjects became familiar with treadmill running and experimental procedures, they performed two preliminary tests (see Chapter 3): (a) a speed-$\dot{V}O_2$ test, and (b) a maximum oxygen uptake test ($\dot{V}O_2$ max). Also, another 30 min treadmill run at 70% $\dot{V}O_2$, max was undertaken about one week before the first experimental trial to fully familiarise subjects with the drinking pattern and the measurements used during the experimental trials.

Diet control
Subjects weighed and recorded their normal food intake for three days before the first main trial, and replicated the same diet for the same period of time before the next trial. The dietary information obtained was then analyzed as previously described (see Chapter 3). Furthermore, subjects followed the same training schedule three days before each trial.

Experimental Design
Each subject was required to run for 60 min at 70% $\dot{V}O_2$ max on a motorised level treadmill on two different occasions separated by one week (Fig. 7.1). On both occasions a high carbohydrate meal (2.5 g CHO.kg$^{-1}$ BW; see Appendix C) was consumed 3 hours before exercise, whereas during exercise either water (M+W), or a 6.9% carbohydrate-electrolyte solution (see Chapter 3) was administered (M+C). The order of the two trials was random.

After a 12-hour overnight fast subjects arrived at the laboratory between 8:00-10 a.m. While the subject was sitting on a chair a venous sample was obtained from an antecubital vein using a hypodermic needle. Following
this, subjects consumed the high carbohydrate meal. During the three hours postprandial period no measurements were made, however, the subjects remained quietly in the laboratory.

About 30 min before the completion of the 3-hour postprandial period each subject emptied his bladder and his nude body weight was obtained. While the subject was lying on an examination couch an indwelling catheter was inserted in an antecubetal vein (see Chapter 3). Furthermore, two sites, 3 cm apart from each other, were prepared on the same leg under local anaesthetic (see Chapter 3) in order to obtain a tissue sample from the vastus lateralis muscle. When the 3-hour postprandial period was completed a venous blood as well as a muscle sample were obtained.

After that, subjects ingested either 5 ml.kg⁻¹ BW of the 6.9% carbohydrate-electrolyte solution (M+C), or equivalent amount of water (M+W). During exercise 2 ml.kg⁻¹ BW of the assigned fluid were ingested at 20 min and 40 min of exercise. In the M+C trial subjects consumed 228.5± 7.9 g of carbohydrate (183± 6.5 g as meal and 45.5± 1.4 g during exercise), whereas in the M+W trial they ingested 183± 6.5 g of carbohydrate. After the consumption of the 5 ml.kg⁻¹ BW water or carbohydrate-electrolyte drink subjects had a 5-min warm-up on the treadmill at 60% \( \dot{V}O_2 \) max. Following this, the speed of the treadmill was increased to 70% \( \dot{V}O_2 \) max and the subjects continued running for one hour.

Venous blood and one minute expired air samples were collected at 10, 20, 40, and 60 min of exercise. Perceived rate of exertion, abdominal discomfort, and gut fullness were also recorded at the same time intervals. Heart rate was continuously monitored throughout the trials.

When the 60-min run was completed a further muscle sample was taken within 15-60 sec after subjects stopped running. Each subject's post-exercise nude body weight was also measured. Wet sponges were available to subjects to be used ad libitum throughout the runs.

All trials were conducted under similar laboratory temperature (M+C: 24.2± 0.4 °C, M+W: 24.8± 0.4 °C) and relative humidity (M+C: 69.6± 1.8%, and M+W: 61.6 ± 3.4 %) (mean ± SE; n.s).
Analyses
Venous blood, muscle, and air samples were collected and analysed as previously described (Chapter 3). Venous blood samples were analysed for haematocrit and haemoglobin values, and for blood glucose, blood lactate, plasma FFA, plasma glycerol, serum insulin, serum sodium, and serum potassium concentrations. Muscle samples were analysed for glycogen, glucose, G-6-P, ATP, PCr, Cr, and lactate concentrations.

A two-way analysis of variance (ANOVA) for repeated measures on two factors (treatment by time) was used to compare cardiovascular changes, and metabolic responses to all trials of the study. The remaining responses were examined using a two tailed Student's t test for dependent samples. When significant differences were revealed, using the ANOVA, then a Tukey post hoc test was performed. The accepted level of significance was set at $p < 0.05$. Data are reported as mean ± SE.
Running at 70% VO2 max

Fig. 7.1: Schematic illustration of the experimental protocol
7.3 Results
There were no differences between the two trials in the average daily energy intake, carbohydrates, fat, or protein consumed during the 3 days prior to each main trial (Table 7.1).

Oxygen uptake (Table 7.2) was not different between trials and averaged 3.0± 0.2 l.min⁻¹, and 3.0± 0.1 l.min⁻¹ in the M+C, and M+W trials respectively (n.s). The corresponding mean relative exercise intensities were 70.9± 1.1 % $\dot{V}$O₂ max, and 70.1± 0.6 % $\dot{V}$O₂ max (n.s).

Heart rate (Table 7.2) was similar between conditions and averaged 163± 5 b.min⁻¹, and 162 ±5 b.min⁻¹ in the M+C and M+W respectively. The perceived rate of exertion, gut fullness, and abdominal discomfort responses were also similar between trials (Table 7.2).

No difference was found between trials in the respiratory exchange ratios which averaged 0.97± 0.01 and 0.98± 0.01 in the M+C and M+W trials respectively (Table 7.2). Similarly, carbohydrate oxidation rate (Table 7.2) was not different between the M+C (mean: 3.29± 0.25 g.min⁻¹) and M+W (mean: 3.34± 0.22 g.min⁻¹) trials throughout exercise.

The volume of fluid consumed during exercise was the same in the two conditions (660± 20 ml). The decrease in body mass (1.8± 0.9 kg) and the % of body weight change (2.5± 0.2%) were also the same in the two trials. Plasma volumes were almost unchanged in the M+C (-0.09± 1.5 %), and M+W (-0.03± 1.4) trials (n.s).

Blood glucose concentration (Fig. 7.2) was higher (p< 0.01) at 60 min of exercise in the M+C compared with the M+W trial. Blood glucose concentration was not different in any other sampling point between the two conditions. However, in the M+W trial blood glucose concentration was lower (p< 0.01) in the first 20 min of exercise compared with the initiation of exercise (0 min), a finding which was not observed in the M+C condition.
Blood lactate concentrations (Table 7.3) were similar between the two treatments and averaged 2.3± 0.4 mmol.l⁻¹ and 2.3± 0.2 mmol.l⁻¹ during exercise in the M+C and M+W trials respectively.

Serum insulin concentration (Fig. 7.4) was not different between the two conditions. However, serum insulin concentration was 3.6-fold and 3.4-fold higher (p< 0.01) at the start of exercise compared with fasting levels (-180 min) in the M+C and M+W trials respectively.

On the other hand, plasma FFA concentrations (Fig. 7.4) were 6.4-fold and 4.3-fold lower (p< 0.01) at the start of exercise compared with fasting levels in the M+C and M+W trials respectively. Nevertheless, no difference was found in the plasma FFA concentrations between the two conditions. Plasma glycerol (Fig. 7.5), serum sodium, and serum potassium (Table 7.3) concentrations were also similar between trials.

Muscle glycogen concentrations (Table 7.4) were not different before as well as after exercise between the two experimental trials. Neither was there any difference in the rate of muscle glycogen utilisation (M+C: 96.1± 22.1 mmol.kg.dm⁻¹.h⁻¹ vs M+W: 77.9± 11.7 mmol.kg.dm⁻¹.h⁻¹).

No difference was also found between the two conditions in the muscle glucose, G-6-P, ATP, PCR, Creatine, and lactate concentrations before or after the 60-min treadmill run (Table 7.4).

Table 7.1: Average daily energy intake, carbohydrate (CHO), fat, and protein consumed during the 3 days prior to each trial (mean ± SE).

<table>
<thead>
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<th>Energy Intake (kcal)</th>
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<th>Protein (g)</th>
<th>Fat (g)</th>
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<tr>
<td>M+C</td>
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<tr>
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<td>± 194</td>
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Table 7.2: Oxygen uptake (\(\dot{V}O_2\)), respiratory exchange ratio (RER), carbohydrate oxidation rate (CHO Oxid. Rate), heart rate (HR), perceived rate of exertion (PRE), gut fullness (GFS), and abdominal discomfort (ADS), during the M+C and M+W trials (mean ± SE).

<table>
<thead>
<tr>
<th>Variable</th>
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<th>40 min</th>
<th>60 min</th>
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<tr>
<td>M+C 42.2 ± 1.4</td>
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<tr>
<td>M+W 40.5 ± 1.6</td>
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<td>41.9 ± 1.7</td>
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<td>(\dot{V}O_2) (l.min(^{-1}))</td>
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</tr>
<tr>
<td>M+C 3.1 ± .2</td>
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<td>3.0 ± .2</td>
<td>3.0 ± .1</td>
<td></td>
</tr>
<tr>
<td>M+W 3.0 ± .1</td>
<td>3.0 ± .1</td>
<td>3.0 ± .2</td>
<td>3.0 ± .1</td>
<td></td>
</tr>
<tr>
<td>RER</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C 0.98 ± .01</td>
<td>0.97 ± .01</td>
<td>0.96 ± .01</td>
<td>0.97 ± .02</td>
<td></td>
</tr>
<tr>
<td>M+W 0.98 ± .01</td>
<td>0.97 ± .01</td>
<td>0.98 ± .01</td>
<td>0.97 ± .01</td>
<td></td>
</tr>
<tr>
<td>CHO Oxid. Rate (g.min(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C 3.47 ± .25</td>
<td>3.28 ± .21</td>
<td>3.18 ± .28</td>
<td>3.22 ± .27</td>
<td></td>
</tr>
<tr>
<td>M+W 3.36 ± .17</td>
<td>3.35 ± .18</td>
<td>3.36 ± .25</td>
<td>3.29 ± .26</td>
<td></td>
</tr>
<tr>
<td>HR (b.min(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C 155 ± 4</td>
<td>160 ± 5(^b)</td>
<td>167 ± 5(^a)</td>
<td>171 ± 5(^a)</td>
<td></td>
</tr>
<tr>
<td>M+W 155 ± 4</td>
<td>157 ± 4(^b)</td>
<td>165 ± 5(^a)</td>
<td>171 ± 5(^{a,c})</td>
<td></td>
</tr>
<tr>
<td>PRE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C 10.9 ± .2</td>
<td>11 ± .2(^b)</td>
<td>12 ± .3(^a)</td>
<td>12.6 ± .5(^a)</td>
<td></td>
</tr>
<tr>
<td>M+W 10.6 ± .2</td>
<td>11 ± .3(^b)</td>
<td>12.1 ± .5(^a)</td>
<td>12.6 ± .6(^a)</td>
<td></td>
</tr>
<tr>
<td>GFS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C 2.8 ± .4</td>
<td>2.3 ± .4</td>
<td>3.0 ± .4</td>
<td>3.3 ± .6</td>
<td></td>
</tr>
<tr>
<td>M+W 2.9 ± .5</td>
<td>2.3 ± .3</td>
<td>3.0 ± .7</td>
<td>2.5 ± .6</td>
<td></td>
</tr>
<tr>
<td>ADS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C 2.2 ± .5</td>
<td>2.0 ± .5</td>
<td>3.4 ± .6</td>
<td>3.1 ± .5</td>
<td></td>
</tr>
<tr>
<td>M+W 2.5 ± .4</td>
<td>2.6 ± .6</td>
<td>2.9 ± .7</td>
<td>2.5 ± .6</td>
<td></td>
</tr>
</tbody>
</table>

a: p< 0.01 from 10 min; b: p< 0.01 from 40 min, and 60 min

124
Table 7.3: Blood lactate, serum sodium, and serum potassium concentrations (mmol.L⁻¹) in the M+C, and M+W trials (mean ± SE).

<table>
<thead>
<tr>
<th>Variable</th>
<th>-180 min</th>
<th>0 min</th>
<th>10 min</th>
<th>20 min</th>
<th>40 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lactate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>0.8</td>
<td>1.1</td>
<td>2.5ᵃ</td>
<td>2.3ᵃ</td>
<td>2.0ᵃ</td>
<td>2.3ᵃ</td>
</tr>
<tr>
<td></td>
<td>±.08</td>
<td>±.2</td>
<td>±.4</td>
<td>±.3</td>
<td>±.3</td>
<td>±.4</td>
</tr>
<tr>
<td>M+W</td>
<td>0.8</td>
<td>1.2</td>
<td>2.4ᵃ</td>
<td>2.2ᵃ</td>
<td>2.2ᵃ</td>
<td>2.4ᵃ</td>
</tr>
<tr>
<td></td>
<td>±.09</td>
<td>±.2</td>
<td>±.2</td>
<td>±.2</td>
<td>±.2</td>
<td>±.3</td>
</tr>
<tr>
<td><strong>Serum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sodium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>140</td>
<td>140</td>
<td>142</td>
<td>141</td>
<td>142</td>
<td>142</td>
</tr>
<tr>
<td></td>
<td>±.6</td>
<td>±.5</td>
<td>±.8</td>
<td>±.7</td>
<td>±.7</td>
<td>±.8</td>
</tr>
<tr>
<td>M+W</td>
<td>141</td>
<td>141</td>
<td>142</td>
<td>142</td>
<td>142</td>
<td>142</td>
</tr>
<tr>
<td></td>
<td>±.6</td>
<td>±.5</td>
<td>±.6</td>
<td>±.6</td>
<td>±.6</td>
<td>±.5</td>
</tr>
<tr>
<td><strong>Serum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Potassium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M+C</td>
<td>4.3</td>
<td>4.0</td>
<td>5.1ᵃ</td>
<td>5.2ᵃ</td>
<td>5.3ᵃ</td>
<td>5.4ᵃ</td>
</tr>
<tr>
<td></td>
<td>±.09</td>
<td>±.08</td>
<td>±.1</td>
<td>±.1</td>
<td>±.1</td>
<td>±.1</td>
</tr>
<tr>
<td>M+W</td>
<td>4.2</td>
<td>4.0</td>
<td>5.1ᵃ</td>
<td>5.2ᵃ</td>
<td>5.3ᵃ</td>
<td>5.3ᵃ</td>
</tr>
<tr>
<td></td>
<td>±.08</td>
<td>±.07</td>
<td>±.09</td>
<td>±.1</td>
<td>±.1</td>
<td>±.1</td>
</tr>
</tbody>
</table>

ᵃ: p< 0.01 from -180 min, and 0 min
Table 7.4: Muscle metabolites (mmol.kg dm⁻¹) before (Pre) and after (Post) the 60-min treadmill run in the M+C and M+W trials (mean ± SE).

<table>
<thead>
<tr>
<th></th>
<th>M + C</th>
<th></th>
<th>M + W</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PRE</td>
<td>POST</td>
<td>PRE</td>
<td>POST</td>
</tr>
<tr>
<td>Glycogen</td>
<td>321.9 ± 27.2</td>
<td>225.8 ± 26.7*</td>
<td>338.8 ± 32.8</td>
<td>261 ± 40.5*</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.5 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>1.4 ± 0.2</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>G-6-P</td>
<td>1.3 ± 0.1</td>
<td>1.8 ± 0.3</td>
<td>1.1 ± 0.2</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>ATP</td>
<td>28.9 ± 1.5</td>
<td>27.6 ± 0.9</td>
<td>28.8 ± 1.0</td>
<td>28.0 ± 1.1</td>
</tr>
<tr>
<td>PCr</td>
<td>75.2 ± 3.0</td>
<td>65.5 ± 2.6*</td>
<td>75.2 ± 1.9</td>
<td>68.7 ± 4.0</td>
</tr>
<tr>
<td>Creatine</td>
<td>36.0 ± 1.2</td>
<td>45.7 ± 3.6</td>
<td>36.0 ± 3.1</td>
<td>42.6 ± 3.7</td>
</tr>
<tr>
<td>Lactate</td>
<td>4.6 ± 0.3</td>
<td>7.4 ± 1.1</td>
<td>5.3 ± 0.7</td>
<td>9.1 ± 1.0</td>
</tr>
</tbody>
</table>

* p < 0.01 from pre-exercise
Table 7.5: Treadmill running at 70% \( \dot{V}O_2 \) max for one hour. Comparison of physiological characteristics of subjects (mean ± SE)

<table>
<thead>
<tr>
<th></th>
<th>Tsintzas et al. 1993c</th>
<th>Present study</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAMPLE</td>
<td>7 Males</td>
<td>8 Males</td>
</tr>
<tr>
<td>AGE</td>
<td>29.2 ± 2.1 yrs</td>
<td>33.7 ± 3.1 yrs</td>
</tr>
<tr>
<td>B.W</td>
<td>75.2 ± 4.1 kg</td>
<td>73.3 ± 2.2 kg</td>
</tr>
<tr>
<td>HEIGHT</td>
<td>176.3 ± 1.8 cm</td>
<td>172.8 ± 2.0 cm</td>
</tr>
<tr>
<td>HR max</td>
<td>190 ± 2 b.min(^{-1})</td>
<td>193 ± 8 b.min(^{-1})</td>
</tr>
<tr>
<td>( VO_2 ) max</td>
<td>54.5 ± 2.0 ml.kg(^{-1}).min(^{-1})</td>
<td>58.7 ± 2.1 ml.kg(^{-1}).min(^{-1})</td>
</tr>
</tbody>
</table>

Table 7.6: Pooled muscle glycogen data (mmol.kg dm\(^{-1}\)) from control trials from two studies (mean ± SE)

<table>
<thead>
<tr>
<th></th>
<th>Tsintzas et al. (1993c)/FAST</th>
<th>Present Study/FED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-exercise</td>
<td>342.3 ± 21.0</td>
<td>338.8 ± 32.8</td>
</tr>
<tr>
<td>Post-exercise</td>
<td>191.4 ± 28.7</td>
<td>261.0 ± 40.5</td>
</tr>
<tr>
<td>Glycogen use</td>
<td>150.9 ± 19.9</td>
<td>77.9 ± 11.7*</td>
</tr>
</tbody>
</table>

* p< 0.05 from Tsintzas et al. (1993c) study (t-test for independent samples)
Fig. 7.2: Blood glucose concentration (mmol.l^{-1}) in the M+C and M+W trials (mean ± SE)  
* p<0.01 from the M+W  
a: p<0.01 from -180, 10, and 20 min; b: p<0.01 from 0 min  
c: p<0.05 from -180 min; d: p<0.05 from 10 min

Fig. 7.3: Serum insulin concentration (mU.l^{-1}) in the M+C and M+W trials (mean±SE)  
a: p<0.01 from -180, 10, 20, 40, and 60 min  
b: p<0.01 from -180, 0, and 60 min
Fig. 7.4: Plasma FFA concentration (mmol.l⁻¹) in the M+C and M+W trials (mean ± SE)
a: p < 0.01 from 0, 10, 20, 40, and 60 min

Fig. 7.5: Plasma glycerol concentration (mmol.l⁻¹) in the M+C and M+W trials (mean ± SE)
a: p < 0.01 from 40 and 60 min; b: p < 0.01 from 60 min
c: p < 0.05 from 60 min in the M+C trial; d: p < 0.05 from 60 min in the M+W trial
7.4 Discussion

The main finding of this study was that the ingestion of the 6.9% carbohydrate-electrolyte solution during a 60-min treadmill run at 70% \( \dot{V}O_2 \) max did not influence the skeletal muscle glycogen utilisation in fed runners.

As mentioned earlier, the available literature provides conflicting results regarding the influence of exogenous carbohydrates on the rate of muscle glycogen utilisation during prolonged (1-4 h) moderate intensity (50-75% \( \dot{V}O_2 \) max) exercise. Several studies have shown no effect in muscle glycogen use as a result of carbohydrate ingestion (Fielding et al. 1985; Coyle et al. 1986; Hargreaves and Briggs 1988; Mitchell et al. 1989), or infusion (Coyle et al. 1991) during cycling exercise when compared with control conditions. On the other hand, other researchers have reported a glycogen sparing effect when carbohydrates are ingested (Bjorkman et al. 1984; Hargreaves et al. 1984; Erickson et al. 1987; Yaspelkis III and Ivy 1991), or intravenously infused (Bergstrom and Hultman 1967a) during submaximal cycling exercise.

It is important to note that, with the exception of one study, all the above cycling studies were conducted after subjects had undergone an overnight fast of about 12-16 hours. Mitchell and his colleagues (1989), however, provided a small (48 g) liquid carbohydrate meal to their subjects 3 hours before exercise. They reported no difference in muscle glycogen use after 105 min of cycling at 70% \( \dot{V}O_2 \) max as a result of ingesting carbohydrate solutions compared with placebo. Recently, from our laboratory, three studies have been conducted using running as the exercise mode. Subjects in these studies had undergone an overnight (12-14 h) fast. The results clearly show a glycogen sparing effect after subjects ingested a carbohydrate-electrolyte solution during running compared with water or placebo (Tsintzas et al. 1993c; 1994; Nicholas et al. 1994).

In order to compare the results of the present study with the findings in other studies one should not only take into account the different exercise mode (i.e: cycling vs running) but certain other factors which
may well influence the glycogenolytic rate during prolonged moderate intensity exercise. The pre-exercise muscle glycogen levels, for instance, seem to play an important role in the glycogen breakdown (van Hall et al. 1994; Hargreaves et al. 1995). In the present study pre-exercise muscle glycogen concentrations were 320-340 mmol.kg dm⁻¹. These values are relatively small compared with the values reported in other studies where resting muscle glycogen concentrations were almost twice as high as in the present study (Coyle et al. 1986; Hargreaves and Briggs 1988; Yaspelkis III and Ivy 1991). Therefore, one reason for the conflicting results between the present study and those of other investigations might be the different resting muscle glycogen concentrations among subjects. However, some studies have reported that muscle glycogen concentration does not influence glucose uptake (van Hall et al. 1994; Hargreaves et al. 1995). On this basis, it seems that differences in pre-exercise muscle glycogen concentrations may not be so important to account for the discrepancies in the literature.

In the study conducted by Tsintzas et al. (1993c) pre-exercise muscle glycogen concentrations were very similar to the ones found in the present study (Table 7.6), yet the authors reported a glycogen sparing effect as a result of ingesting a carbohydrate-electrolyte solution during 60-min running at 70% VO₂ max compared with water ingestion. The similarity in the experimental procedures allows a direct comparison between the present study and that conducted by Tsintzas et al. (1993c). In table 7.5 the physiological characteristics of the subjects who participated in the two studies are presented (data obtained from Tsintzas 1993, and present study). A t-test for independent samples showed no statistical difference between the two groups. It is interesting to note, however, that the glycogen breakdown observed in the present study in the control trial (77.9 ± 11.7 mmol. kg dm⁻¹) was almost half of the glycogen used in the corresponding control trial in Tsintzas et al. (1993c) study (150.9 ± 19.9 mmol.kg dm⁻¹) where subjects had undergone an overnight fast of about 12-14 hours. A statistical comparison did not show a difference in post exercise muscle glycogen concentrations between the control trial of Tsintzas et al. (1993c) study and that of the present study (M+W), despite the fact that there was a tendency (p< 0.1) for higher muscle glycogen concentration after exercise in this study (Table 7.6). However, the muscle glycogen use
was lower \( (p< 0.05) \) after the ingestion of the meal in the fed-control trial of the present study compared with the fasting-control trial of Tsintzas et al. (1993c) study (Table 7.6). It may be argued, therefore, that the pre-exercise meal ingested in both trials of the present study might have produced a glycogen sparing effect which in turn masked any possible influence of the carbohydrate-electrolyte solution on the muscle glycogen breakdown. Nevertheless, the comparison of data from these two studies (Tsintzas et al. 1993c, and present study) are only indirect observations and do not provide any solid evidence for a reduced glycogen utilisation as a result of the consumption of the pre-exercise meal.

The serum insulin concentrations were higher at the beginning of exercise compared with fasting levels (Fig. 7.3). Also, serum insulin concentrations were similar between conditions which shows that the ingestion of the carbohydrate-electrolyte solution did not cause any change in serum insulin concentration during exercise. These findings are consistent with the findings in previous studies (Chapters 4 and 5). Also, the blood glucose concentration was similar between conditions during most of the exercise time, and reached higher values in the M+C trial only at the end of the run (Fig. 7.2). These findings, however, should not be considered as a result of a reduced gastric emptying or intestinal absorption of the carbohydrate-electrolyte drink. The amount of carbohydrate ingested at the beginning of exercise in the M+C trial (25 g) was probably not enough to further increase the already high (about 18 mU.l\(^{-1}\)) pre-exercise serum insulin concentrations (Fig. 7.3).

If one assumes that a possible glycogen sparing effect produced by carbohydrate feeding during exercise is insulin dependent, then the absence of any difference in glycogen breakdown may be due to the fact that the ingestion of the carbohydrate-electrolyte solution did not produce any rise in insulin concentration in the M+C trial. It is interesting to know that when carbohydrate feedings produce an increase in insulin concentration during exercise, glycogen utilisation is reduced compared with control conditions during both cycling (Bjorkman et al. 1984; Yaspelkiss III and Ivy 1991) and running (Tsintzas et al. 1993c; 1994; Nicholas et al. 1994), whereas when carbohydrate feedings fail to increase insulin levels no difference in
muscle glycogen utilisation is observed (Coyle et al. 1986; Hargreaves and Briggs 1988; Mitchell et al. 1989). However, Coyle and co-workers (1991) failed to show any change in muscle glycogen utilisation despite elevated insulin concentrations produced after glucose infusion during cycling at 73% VO₂ max.

The elevated pre-exercise serum insulin concentrations depressed dramatically the plasma FFA concentrations (Fig. 7.4). These elevated insulin concentrations together with the suppression of plasma FFA levels could favour an increased blood glucose uptake by the active muscles (Pernow and Saltin 1971). This can explain the initial drop of the blood glucose concentration during the first 20 min of exercise. This drop was more pronounced in the M+W trial due to the fact that no carbohydrates were provided.

In conclusion, the results of the present study demonstrate that the ingestion of a 6.9% carbohydrate-electrolyte solution during treadmill running at 70% VO₂ max does not influence the rate of muscle glycogen utilisation during the first hour of exercise when a carbohydrate meal providing 2.5 g.kg⁻¹ BW carbohydrate is ingested 3 hours before exercise.
CHAPTER 8

COMPARISON BETWEEN CARBOHYDRATE FEEDINGS BEFORE AND DURING EXERCISE ON ENDURANCE RUNNING PERFORMANCE

8.1 Introduction

The study presented in Chapter 5 showed that a pre-exercise carbohydrate rich meal can improve endurance capacity compared to an overnight fast. When considering endurance performance, however, many factors, other than carbohydrate availability, may influence the selection of racing speed. However, running speed is lower during endurance running, after an overnight fast, when runners ingest only water during exercise compared with the running speed achieved when runners drink a carbohydrate-electrolyte solution during exercise (Tsintzas et al. 1993a, 1995; Williams et al. 1990). On the other hand, many runners experience severe gastrointestinal discomfort during exercise when ingesting carbohydrate solutions (Brouns and Beckers 1993). Therefore, the question here is whether the endurance athlete who suffers from gastrointestinal discomfort when consuming carbohydrates during exercise can compensate by eating a carbohydrate rich meal before exercise. Similarly, do athletes who cannot tolerate eating a meal before competition compensate by drinking a carbohydrate solution during exercise?

To the best of the author’s knowledge no study has compared the effects of ingesting carbohydrates during exercise with the effects of consuming a preexercise meal on endurance running performance. Therefore, the purpose of this study was to examine whether, after an overnight fast, the ingestion of a carbohydrate-electrolyte solution during running would be as effective as the consumption of a carbohydrate meal 4 hours before running.
8.2 Methods

Subjects
Ten male subjects volunteered for this study. They were club-level athletes who competed regularly over half and full marathon distances or completed these distances in training. Their age, weight, height, maximal oxygen uptake, and maximum heart rate were 28.7 ± 2.6 years, 67.4 ± 2.1 kg, 175.4 ± 2.1 cm, 62.21 ± 1.7 ml.kg⁻¹.min⁻¹, and 189 ± 4 b.min⁻¹ respectively (mean ± SE).

Preliminary measurements
After subjects became familiar with treadmill running and experimental procedures, they performed three preliminary tests (see Chapter 3): (a) a speed-VO₂ test, (b) a maximum oxygen uptake test (VO₂ max), and (c) a speed-lactate test. Also, another 60 min treadmill run (the first 5 km at 70% VO₂ max and the rest of the time at each subject's own speed) was undertaken about one week before the first experimental trial in order to ensure that runners were completely familiar with the procedures and measurements used during the experimental trials.

Diet control
Subjects were required to record their training as well as to record and weigh their food intake during the two days prior to the first experimental trial and to replicate this in the second trial. The dietary information obtained was then analyzed as previously described (see Chapter 3). Furthermore, subjects followed the same training schedule two days before each trial.

Experimental Design
Each subject was required to complete two 30-km treadmill runs, trying to achieve a personal best time during each run. The two trials were separated by 2 weeks. Four hours before each run the subjects consumed either a liquid placebo solution (C) or a high-carbohydrate meal (M). On the day when runners consumed the liquid placebo, a 6.9% carbohydrate-electrolyte solution (see Chapter 3) was provided during the 30-km run (C), whereas when the meal was consumed only plain water was given during the 30-km run (M) (Fig. 8.1). The order of the two conditions was randomized.

After an overnight fast of approximately 10-12 h each subject arrived at the laboratory between 7:00 and 8:00 a.m and emptied his bladder, after which
his body weight was obtained. While the subject stood quietly a 3-min expired air sample and duplicate 20-µl samples of capillary blood were collected. A further 10-ml venous blood sample was also taken from an antecubital vein.

After resting blood and expired air samples were collected, subjects consumed either the placebo solution (C) or the high carbohydrate meal (M). During the 4-hr postprandial period 5 subjects remained in the laboratory, whereas the other 5 subjects were involved with low level physical activities (e.g. attending lectures, doing office work, etc.) outside the laboratory. These activities were very similar in both experimental trials. Every hour, during the 4-h postprandial period, capillary blood and expired air samples were obtained from the 5 subjects who remained in the laboratory.

Before the initiation of exercise, nude body weight and further expired air, capillary, and venous blood samples were obtained. Subjects had a 5-min warm-up on the treadmill at 60% $\tilde{V}O_2$ max, after which the running speed was increased to 70% $\tilde{V}O_2$ max and was maintained for the first 5 km of the 30-km trial. Thereafter, the subjects were free to control their own speed (see Chapter 3) in an attempt to complete each of the two runs as fast as possible. All subjects were highly motivated and were verbally encouraged throughout the 30-km trial. Subjects did not know the exercise time, but they received information regarding distance covered and running pace. Capillary blood and expired air samples were collected every 5 km. Perceived rate of exertion, abdominal discomfort, and gut fullness responses were recorded every 5 km as well. Heart rate was monitored throughout exercise. Abdominal discomfort, and gut fullness responses were also measured during the postprandial period. Immediately after the run was completed a venous blood sample was obtained; thereafter the subjects dried themselves, and postexercise nude body weight was recorded. Wet sponges were provided for the subjects to use ad libitum throughout the exercise period.

All trials were conducted under the same laboratory temperatures (21.6 ± 0.5 °C), and under similar relative humidity values (M: 52.0 ± 2.7%, and C: 48.5 ± 1.4 %) (mean ± SE; n.s).
Carbohydrate feedings
Four hours before each run subjects had to consume either 10 ml kg⁻¹ BW of a liquid placebo (C) (see Chapter 3), or a high-carbohydrate meal (M) designed to provide 2 g of carbohydrate per kilogram BW (see Appendix C). During exercise in the C trial a 6.9% carbohydrate-electrolyte solution was provided (see Chapter 3), whereas in the M trial only water was given. Immediately prior to the start of exercise subjects drank 8 ml kg⁻¹ BW of the carbohydrate solution or equivalent amount of water, and 2 ml kg⁻¹ BW of the assigned fluid every 5 km thereafter. The total amount of carbohydrate ingested in the M trial was 135 ± 4.3 g, whereas in the C trial was 83.8 ± 2.6 g.

Analyses
Blood and expired air samples were collected and analysed as previously described (Chapter 3). Capillary blood samples were analysed for glucose and lactate concentrations, whereas venous blood samples were analysed for haematocrit values, haemoglobin, plasma FFA, plasma glycerol, plasma ammonia, plasma urea, serum insulin, serum potassium, and serum sodium concentrations.

A two-way analysis of variance (ANOVA) for repeated measures on two factors (treatment by distance) was used to compare differences between running speeds, cardiorespiratory responses, and metabolic responses between trials. Also, the Wilcoxon signed-rank test was used to compare the results between the two treatments obtained during the postprandial period. The remaining responses were examined using a Student's t-test for dependent samples. When significant differences were revealed, using the ANOVA, then a Tukey post hoc test was performed. The accepted level of significance was set at p< 0.05. Data are reported as mean ± SE.
Fig. 8.1: Schematic illustration of the experimental protocol
8.3 Results

The overall performance times in the M and C trials were identical (M: 121.8 ± 3.6 min vs C: 121.7 ± 4.1 min) (range: M: 99.1-139.3 min vs C: 100.3-150.3 min). Neither was there an order effect between the first (T1) and second (T2) trials (T1: 122 ± 10.2 min vs T2: 121.5 ± 12.8 min ; n.s). The mean speed per 5 km ranged from 4.08 to 4.16 m.s⁻¹ in the M trial and 4.08 to 4.17 m.s⁻¹ in the C trial. No differences were found between the two trials in running speeds over each successive 5 km (Fig. 8.3), or even when running speed was analysed every kilometer (Fig. 8.2). Also, the last 5 km during both trials were covered within almost identical times (C: 20.4 ± 1.0 min vs M: 20.5 ± 1.1 min).

There were no differences between the two trials in the average daily energy intake, carbohydrates, fat, or protein consumed during the 2 days prior to each 30-km trial (Table 8.1).

The running speeds corresponding to blood lactate concentrations of 2 mmol.l⁻¹ and 4 mmol.l⁻¹ were 4.03 ± 0.15 m.s⁻¹ and 4.62 ± 0.09 m.s⁻¹, respectively. The running speed corresponding to 2 mmol.l⁻¹ was strongly correlated with performance time (r= -0.88, p< 0.01).

Postprandial Responses

The consumption of the high carbohydrate meal did not produce an increase in the oxygen consumption during the 4-hour postprandial period (Table 8.2). However, in the M trial the respiratory exchange ratios were higher during the 3 hours after the ingestion of the meal compared with the placebo ingestion (Table 8.2). The sensation of gut fullness was higher one hour after the ingestion of the meal, whereas the abdominal discomfort response was the same between the two conditions during the postprandial period (Table 8.2).

Blood glucose concentration was higher (p< 0.05) one hour after feeding in the M trial (Fig. 8.4). Also, blood lactate concentration was higher in the M trial during the first two hours postprandially (Fig. 8.5).

Responses During Exercise

Oxygen uptake (\(\dot{V}O_2\)) was similar between the two conditions, and averaged 45.2 ± 0.5 ml.kg⁻¹.min⁻¹ and 46.1 ± 0.6 ml.kg⁻¹.min⁻¹ in the M and C trials.
respectively (Table 8.2). However, \( \dot{V}O_2 \) was higher (p < 0.01) in both conditions at 30 km compared with the values obtained at the first 5 km. The relative exercise intensity at the first 5 km was 70.4 ± 1.6 % \( \dot{V}O_2 \) max and 69.3 ± 0.7 % \( \dot{V}O_2 \) max in the C and M, respectively. These values were lower (p < 0.05) than the corresponding ones at 30 km for both M (75.5 ± 2.9 % \( \dot{V}O_2 \) max) and C trials (76.4 ± 2.0 % \( \dot{V}O_2 \) max).

The respiratory exchange ratio was similar between the two trials throughout the exercise period (Table 8.3). Neither was there a difference in the carbohydrate oxidation rate between conditions (Table 8.3). The carbohydrate oxidation rate averaged 2.0 ± 0.3 g.min\(^{-1}\) and 2.2 ± 0.2 g.min\(^{-1}\) in the M and C trials respectively. In the M trial the oxidation rate for carbohydrates did not decrease with distance and was maintained above 1.8 g.min\(^{-1}\) throughout exercise.

Furthermore, perceived rate of exertion, heart rate, and abdominal discomfort were not different between trials, whereas gut fullness was higher (p < 0.01) at the end of the 30-km run in the C trial (Table 8.3).

There was an average decrease in body mass of 3.0 ± 0.2 kg and 2.9 ± 0.2 kg during the C and M, respectively. This decrease represented a change in body weight of 4.2 ± 0.2 % and 4.1 ± 0.2 % for C and M, respectively, which was not significantly different between the two conditions. The mean change in plasma volume for the C trial was 0.9 ± 1.7%, whereas for the M trial was -0.5 ± 2.0%. These changes in plasma volumes were not different.

Blood glucose concentration was higher (p < 0.01) in the C trial during the first 20 km (Fig. 8.6). However, mean blood glucose concentration did not decrease in the M trial during exercise and was maintained above 4.5 mmol.l\(^{-1}\).

Blood lactate concentrations were similar in both trials and averaged 4.1 ± 0.2 mmol.l\(^{-1}\) and 4.5 ± 0.2 mmol.l\(^{-1}\) in the C and M, respectively (Fig. 8.7). Plasma FFA concentration was higher (p < 0.01) before the initiation of exercise in the C trial, but was not different at the end of the run (Fig. 8.8). However, when the mean delta values were compared (i.e: prefeeding to preexercise and postexercise to preexercise) plasma FFA concentrations were lower in the M trial during the postprandial period (M: 0.07 ± 0.08 mmol.l\(^{-1}\) vs C: 0.33 ± 0.09...
mmol.l⁻¹; p< 0.05, n=9), whereas they were lower in the C trial during exercise (M: 0.40 ± 0.10 mmol.l⁻¹ vs C: -0.05 ± 0.12 mmol.l⁻¹; p< 0.05, n=9).

Finally, no differences were found between the two conditions in serum insulin, plasma glycerol, plasma ammonia, plasma urea, serum sodium, and serum potassium concentrations (Table 8.4).

Table 8.1: Average daily energy intake, carbohydrate (CHO), fat, and protein consumed during the 2 days prior to each trial (mean ± SE).

<table>
<thead>
<tr>
<th></th>
<th>Energy (kcal)</th>
<th>Intake (kJ)</th>
<th>CHO (g)</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>3504 ± 245</td>
<td>14647 ± 1024</td>
<td>514 ± 49</td>
<td>129 ± 11</td>
<td>109 ± 16</td>
</tr>
<tr>
<td>C</td>
<td>3325 ± 283</td>
<td>13899 ± 1183</td>
<td>501 ± 61</td>
<td>122 ± 7</td>
<td>95 ± 16</td>
</tr>
</tbody>
</table>
Table 8.2: Oxygen uptake ($\dot{V}O_2$), respiratory exchange ratio (RER), gut fullness (GFS), and abdominal discomfort (ADS) during the postprandial period (mean ± SE).

<table>
<thead>
<tr>
<th>Variable</th>
<th>0 min</th>
<th>60 min$^*$</th>
<th>120 min$^+$</th>
<th>180 min$^+$</th>
<th>240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\dot{V}O_2$ (ml.kg$^{-1}$.min$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>4.2</td>
<td>4.9</td>
<td>4.6</td>
<td>4.2</td>
<td>4.5</td>
</tr>
<tr>
<td>C</td>
<td>4.1</td>
<td>4.6</td>
<td>4.2</td>
<td>4.5</td>
<td>4.8</td>
</tr>
<tr>
<td>$\dot{V}O_2$ (1.min$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>0.29</td>
<td>0.34</td>
<td>0.31</td>
<td>0.28</td>
<td>0.31</td>
</tr>
<tr>
<td>C</td>
<td>0.28</td>
<td>0.32</td>
<td>0.29</td>
<td>0.31</td>
<td>0.33</td>
</tr>
<tr>
<td>RER</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>0.81</td>
<td>0.93$^{a,b}$</td>
<td>0.92$^{a,b}$</td>
<td>0.89$^a$</td>
<td>0.83$^c$</td>
</tr>
<tr>
<td>C</td>
<td>0.83</td>
<td>0.81</td>
<td>0.81</td>
<td>0.80</td>
<td>0.78</td>
</tr>
<tr>
<td>GFS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>0.7</td>
<td>2.0$^{a,b}$</td>
<td>1.5</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>C</td>
<td>0.4</td>
<td>0.8</td>
<td>0.3</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>ADS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>1.4</td>
<td>1.0</td>
<td>0.8</td>
<td>0.4</td>
<td>0.8</td>
</tr>
<tr>
<td>C</td>
<td>1.4</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
<td>0.9</td>
</tr>
</tbody>
</table>

$^{a}$: p< 0.05 from C trial; $^{b}$: p< 0.05 from 0 min; $^{c}$: p< 0.05 from 60 and 120 min
$^{*}$: n=6; $^{+}$: n=5
Table 8.3: Oxygen uptake ($\dot{V}O_2$), respiratory exchange ratio (RER), carbohydrate oxidation rate (CHO Oxid. Rate), heart rate (HR), perceived rate of exertion (PRE), gut fullness (GFS), and abdominal discomfort (ADS) during exercise (mean ± SE).

<table>
<thead>
<tr>
<th>Variable</th>
<th>5 km</th>
<th>10 km</th>
<th>15 km</th>
<th>20 km</th>
<th>25 km</th>
<th>30 km</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\dot{V}O_2$ (ml.kg$^{-1}$.min$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>43.1</td>
<td>45.3</td>
<td>44.8</td>
<td>45.6</td>
<td>45.8</td>
<td>46.9$^a$</td>
</tr>
<tr>
<td>± 1.1</td>
<td>± 1.3</td>
<td>± 1.5</td>
<td>± 1.6</td>
<td>± 1.4</td>
<td>± 2.1</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>43.7</td>
<td>46.1</td>
<td>46.5</td>
<td>46.1</td>
<td>46.7</td>
<td>47.7$^a$</td>
</tr>
<tr>
<td>± 0.8</td>
<td>± 1.3</td>
<td>± 1.4</td>
<td>± 1.5</td>
<td>± 1.7</td>
<td>± 2.2</td>
<td></td>
</tr>
<tr>
<td>$\dot{V}O_2$ (l.min$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>2.9</td>
<td>3.0</td>
<td>3.0</td>
<td>3.1</td>
<td>3.1</td>
<td>3.2$^a$</td>
</tr>
<tr>
<td>± .1</td>
<td>± .1</td>
<td>± .1</td>
<td>± .1</td>
<td>± .1</td>
<td>± .2</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2.9</td>
<td>3.1</td>
<td>3.1</td>
<td>3.1</td>
<td>3.1</td>
<td>3.2$^a$</td>
</tr>
<tr>
<td>± .1</td>
<td>± .1</td>
<td>± .1</td>
<td>± .1</td>
<td>± .1</td>
<td>± .1</td>
<td></td>
</tr>
<tr>
<td>RER</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>.88±.02</td>
<td>.87±.02</td>
<td>.87±.02</td>
<td>.86±.02</td>
<td>.85±.02</td>
<td>.87±.03</td>
</tr>
<tr>
<td>C</td>
<td>.86±.02</td>
<td>.88±.01</td>
<td>.87±.02</td>
<td>.88±.02</td>
<td>.89±.02</td>
<td>.91±.02</td>
</tr>
<tr>
<td>CHO Oxid. Rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(g.min$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>2.03±.23</td>
<td>1.97±.25</td>
<td>1.96±.25</td>
<td>1.96±.27</td>
<td>1.78±.29</td>
<td>2.14±.36</td>
</tr>
<tr>
<td>C</td>
<td>1.82±.22</td>
<td>2.20±.16</td>
<td>2.03±.23</td>
<td>2.19±.23</td>
<td>2.39±.28</td>
<td>2.65±.24$^a$</td>
</tr>
<tr>
<td>HR (b.min$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>157±5</td>
<td>164±5</td>
<td>165±6</td>
<td>166±6</td>
<td>169±5$^a$</td>
<td>173±4$^a$</td>
</tr>
<tr>
<td>C</td>
<td>163±4</td>
<td>170±4</td>
<td>170±4</td>
<td>170±5</td>
<td>173±5$^c$</td>
<td>176±4$^a$</td>
</tr>
<tr>
<td>PRE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>11.4±.4</td>
<td>12.9±.3$^a$</td>
<td>13.5±.3$^{a,e}$</td>
<td>14.2±.4$^{a,b,d}$</td>
<td>15±.5$^{a,d}$</td>
<td>16.5±.7$^{a,d}$</td>
</tr>
<tr>
<td>C</td>
<td>11.5±.5</td>
<td>13.2±.5$^{a,b}$</td>
<td>13.8±.6$^{a,e}$</td>
<td>14.8±.5$^{a,d,e}$</td>
<td>15.9±.5$^{a,b,d}$</td>
<td>17.2±.5$^a$</td>
</tr>
<tr>
<td>ADS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>1.5±.3</td>
<td>2.2±.5</td>
<td>3.4±.6$^a$</td>
<td>2.9±.5$^a$</td>
<td>3.9±.8$^a$</td>
<td>4.1±.8$^{a,f}$</td>
</tr>
<tr>
<td>C</td>
<td>2.0±.5</td>
<td>2.7±.5</td>
<td>3.2±.5$^b$</td>
<td>3.4±.5$^b$</td>
<td>4.6±.7$^{a,f}$</td>
<td>5.4±.8$^{a,f}$</td>
</tr>
<tr>
<td>GFS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>1.9±.6</td>
<td>1.8±.6</td>
<td>1.9±.5</td>
<td>1.6±.4</td>
<td>1.6±.6</td>
<td>1.6±.5</td>
</tr>
<tr>
<td>C</td>
<td>2.1±.5</td>
<td>2.2±.6</td>
<td>2.9±.7</td>
<td>2.6±.6</td>
<td>3.2±.8</td>
<td>3.6±1$^*$</td>
</tr>
</tbody>
</table>

*: p<0.01 from M; a: p<0.01 from 5 km; b: p<0.01 from 30 km; c: p<0.05 from 5 km; d: p<0.01 from 10 km; e: p<0.01 from 25 km and 30 km; f: p<0.05 from 10 km.
Table 8.4: Serum insulin, plasma glycerol, plasma ammonia, plasma urea, serum sodium and serum potassium concentrations before feeding and before and immediately after exercise in the M and C trials (mean ± SE).

<table>
<thead>
<tr>
<th>Variable</th>
<th>PRE-FEEDING</th>
<th>PRE-EXERCISE</th>
<th>POST-EXERCISE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (mU.l⁻¹)</td>
<td>M</td>
<td>7.9 ± 0.9b</td>
<td>8.7 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>7.6 ± 0.6a</td>
<td>6.3 ± 0.6e</td>
</tr>
<tr>
<td>Glycerol (mmol.l⁻¹)</td>
<td>M</td>
<td>.05 ± .005c</td>
<td>.05 ± .008b</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>.05 ± .009a</td>
<td>.07 ± .007a</td>
</tr>
<tr>
<td>Ammonia (µmol.l⁻¹)</td>
<td>M</td>
<td>39.2 ± 7c</td>
<td>33 ± 5.5b</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>42.8 ± 8a</td>
<td>39.0 ± 6.7a</td>
</tr>
<tr>
<td>Urea (mmol.l⁻¹)</td>
<td>M</td>
<td>5.9 ± 0.4</td>
<td>5.3 ± 0.3d,b</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>5.8 ± 0.3</td>
<td>5.2 ± 0.2a,e</td>
</tr>
<tr>
<td>Sodium (mmol.l⁻¹)</td>
<td>M</td>
<td>141.4±.2c</td>
<td>140.8±.5b</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>141.3±.5a</td>
<td>141.4±.5a</td>
</tr>
<tr>
<td>Potassium (mmol.l⁻¹)</td>
<td>M</td>
<td>4.8 ± 0.1c</td>
<td>4.7 ± 0.1b</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>4.7 ± 0.1a</td>
<td>4.6 ± 0.1a</td>
</tr>
</tbody>
</table>

a: p< 0.05 from postexercise (n=10) ; b: p< 0.05 from postexercise (n=9)  
c: p< 0.05 from post exercise (n=8) ; d: p< 0.05 from prefeeding (n=9)  
e: p< 0.05 from prefeeding (n=10)
Fig. 8.2: Speed (m.s⁻¹) per kilometer during the M and C trials (mean ± SE)

Fig. 8.3: Speed (m.s⁻¹) per 5 km during the M and C trials (mean ± SE)
Fig. 8.4: Blood glucose concentration (mmol/l⁻¹) during the postprandial period (mean ± SE)
*: p<0.05 from C trial (n=6); a: p<0.05 from 0 min (n=6)
+: n=5

Fig. 8.5: Blood lactate concentration (mmol/l⁻¹) during the postprandial period (mean ± SE)
*: p<0.05 from C trial (n=6); **: p<0.05 from C trial (n=5)
a: p<0.05 from 0 min (n=6); +: n=5
Fig. 8.6: Blood glucose concentration (mmol.l⁻¹) during exercise in the M and C trials (mean±SE)
*: p<0.01 from M; a: p<0.01 from 0 min

Fig. 8.7: Blood lactate concentration (mmol.l⁻¹) during exercise in the M and C trials (mean±SE)
*: p<0.01 from 5 km-30 km
Fig. 8.8: Plasma FFA concentration (mmol.l⁻¹) during the M and C trials (mean ± SE)

*: p< 0.01 from M (n=9); a: p< 0.01 from pre-exercise and post-exercise
b: p<0.01 from pre-feeding (n=8) and pre-exercise (n=9)
8.4 Discussion

The main finding of the present study was that the ingestion of the carbohydrate-electrolyte solution throughout exercise produced the same 30-km treadmill running performance time as the carbohydrate meal ingested 4 hours before exercise.

The consumption of the meal 4 hours before exercise enabled the runners to maintain their self-selected speeds for the whole of the trial despite the fact that only water was ingested during the M trial. In previous studies, subjects, after a 10-12 hour fast, could not maintain their self-selected speeds during the last 5 km of a 30-km run (Tsintzas et al. 1993a; Williams et al. 1990), a 42.2-km run (Tsintzas et al. 1995), or a 2 hour run (Williams et al. 1987 cited by Williams 1989) when only water or placebo was provided during exercise. Therefore, one can argue that the ingestion of the meal before the 30-km run enabled the runners to maintain their self-selected speeds towards the end of exercise, which may not had happen if subjects had undergone an overnight fast.

Improvements in cycling performance have been reported when liquid carbohydrate meals were ingested 3-4 hours before exercise, providing 4.5-5.0 g CHO.kg⁻¹ BW (Sherman et al. 1989; Wright et al. 1991). However, when subjects were fasted the ingestion of carbohydrate (175 g) during exercise was not found to be superior, in terms of endurance performance, to the consumption of a meal (333 g) 3 hours before exercise (Wright et al. 1991). In the present study the meal provided 2 g of carbohydrate per kilogram BW. The average consumption for the M trial was 135± 4.3 g, whereas for the C trial the subjects consumed 83.8 ± 2.6 g of carbohydrate. Interestingly, in both the present study as well as in Wright and colleagues's study (1991) performance was similar despite the fact that carbohydrate intake was lower during exercise. In the present study, carbohydrate was consumed during exercise at a mean rate of 42 g.hr⁻¹. It seems that the amount of carbohydrate ingested in the C trial exceeded the threshold response of the body above which no additional benefit was gained when more carbohydrate was provided. Another possibility could be that the difference between the carbohydrate feedings in the two trials (i.e: 135 g vs 84 g) was not big enough to produce any difference in performance (Sherman et al. 1989).
Coyle and his colleagues (1985) reported a 42% increase in the glycogen concentration of vastus lateralis muscle in subjects fed with 140 g of carbohydrate 4 hr prior to exercise compared with a condition in which subjects were fasted. However, in other studies a less impressive (10-15%) increase in muscle glycogen concentrations has been reported as a result of consuming 180-200 g of carbohydrate 3-4 hours before exercise (Chapter 6; Neufer et al. 1987). It is still not clear whether a meal providing only 2 g of carbohydrates per kilogram BW can increase muscle glycogen levels within 3-4 hours postprandially, or whether the carbohydrates consumed will be stored in the form of liver glycogen. Therefore, in the present study the meal's influence on performance could be attributed to elevated body carbohydrate stores, in the muscle and/or in the liver, before the 30-km run.

In the present study, blood glucose concentration was higher in the C trial during the first 20 km (Fig. 8.6). On the other hand, blood glucose in the M trial maintained above 4.5 mmol.l\(^{-1}\) throughout exercise. When subjects were fasted for about 12 hours before exercise in a similar study, blood glucose concentration decreased during the last 10 km of a 30-km treadmill run when only water was ingested (Williams et al. 1990). It seems that the ingestion of the meal 4 hours before the initiation of exercise maintained euglycemia and the substrate availability for the working muscles throughout the 30-km run and enabled the subjects to maintain their pace close to the pace selected in the C trial. Carbohydrate availability is also reflected by the maintenance of the RER values during exercise in the M trial (Table 8.2), and hence by the similar average rate of carbohydrate oxidation between the two conditions (M: 2.0 ± 0.3 g.min\(^{-1}\) vs. C: 2.2 ± 0.2 g.min\(^{-1}\)).

The prolonged fasting in the C trial elevated the preexercise plasma FFA concentrations (Fig. 8.8). This finding is consistent with the results of previous studies which reported that after prolong fasting plasma FFA concentrations were higher than the plasma FFA concentrations found after only 3 hours of fast (Chapters 4, and 5; Wright et al. 1991). During exercise the delta plasma FFA concentrations (i.e: postexercise to preexercise) were lower in the C trial. It seems that plasma FFA concentrations decrease when carbohydrate solutions are consumed during running exercise compared with water ingestion (Tsintzas et al. 1993a, 1995; Chapters 4 and 5), indicating a reduced fatty acid mobilization. However, the fact that oxidation of fat and carbohydrate was the same in both trials in the present study seems to
suggest either that the depression of FFA concentrations in the C trial was not strong enough to deny this substrate to the working muscle, or that an alternative source, such as intramuscular triglycerides, was used during exercise (Essen et al. 1977; Hurley et al. 1986; Romijn et al. 1993).

The blood lactate concentrations during the first two hours in the postprandial period were higher in the M trial compared with the C trial (Fig. 8.5). This finding is consistent with the results in other studies of this thesis (Chapters 5 and 6) and possibly reflects a conversion of ingested carbohydrate to lactate (Segal et al. 1990; Radziuk 1989).

Finally, as far as endurance performance is concerned, it is important to realize that the consumption of carbohydrate during running has been shown to improve endurance capacity (Chapter 4; Tsintzas et al. 1993b; Wilber and Moffatt 1992) to a greater extent (9-25%) than endurance performance (2%) (Tsintzas et al. 1993a, 1995). When endurance performance is considered, carbohydrate availability is not the only factor for success. Factors such as the utilisation of a large percentage VO2 max (Davies and Thompson 1979) as well as the highest exercise intensity an individual can sustain while still maintaining a metabolic steady state (Farrell et al. 1979; Sjodin and Svedenhag, 1985) seem to strongly influence performance in aerobic exercise. The latter is also confirmed by a strong correlation ($r= -0.88$, $p< 0.01$) between performance time and running speed equivalent to 2 mmol.l$^{-1}$ blood lactate concentration found in the present study. Furthermore, the selection of racing speed may be the result of the athlete's experience, the environmental conditions, the subjective well-being on the day, or the degree of difficulty of the course. Therefore one may argue that, since so many factors can influence endurance performance, carbohydrate availability may play a minimal role in the selection of racing speeds. Nevertheless, it has been shown that in laboratory (Tsintzas et al. 1995; Williams et al. 1990) as well as in field conditions (Tsintzas et al. 1993a) optimal running speeds cannot be maintained during endurance running, when carbohydrate availability is low, and hence poorer performance times are achieved (Tsintzas et al. 1993a; 1995).

In summary, the ingestion of the carbohydrate meal 4 hours before exercise proved to be as effective as the ingestion of the carbohydrate-electrolyte solution ingested during exercise in maintaining the self-selected running speeds of runners during the entire period of exercise.
CHAPTER 9

GENERAL DISCUSSION

The purpose of this thesis was to study the effects of a pre-exercise carbohydrate meal (M) on metabolism, endurance capacity and performance during prolonged running when carbohydrate was, or was not consumed during exercise.

From the first two studies (Chapters 4 and 5) it was found that the ingestion of a carbohydrate meal, which provided 2.5 g·kg⁻¹ BW carbohydrate, 3 hours before constant pace running, at 70% VO₂ max, improved endurance capacity. This improvement (9%) took place not only when no carbohydrate was given during exercise (M+W > P+W, Chapter 5), but more so (18%) when a carbohydrate-electrolyte solution was ingested during exercise (M+C > P+C, Chapter 4). It was also observed that when the meal was combined with the carbohydrate-electrolyte solution (M+C) the improvement in endurance capacity was greater than the improvement brought by the meal alone (M+W) (22% vs 9%), or by the carbohydrate-electrolyte drink (P+C) (28% vs 9%).

Possible mechanisms by which the pre-exercise meal improved endurance capacity

The data in this thesis do not suggest a clear mechanism by which the pre-exercise meal enhanced endurance capacity. However, a number of different factors can be suggested by which the carbohydrate meal exerted its ergogenic effect.

One possible factor might be an increased pre-exercise muscle glycogen concentration. The skeletal muscle glycogen concentrations were elevated by about 11% as the result of the carbohydrate meal (Chapter 6). This increase in muscle glycogen concentration was similar to the improvement in endurance capacity (9%) observed when the meal was given 3 hours before exercise compared with the fasted state (M+W vs P+W, Chapter 5), but was different (18%) when the meal was given before exercise during which the carbohydrate-electrolyte solution was ingested (M+C vs P+C, Chapter 4). This
difference might be, however, due to differences in subjects' responses and/or different nutritional interventions between the two studies.

The 181 g carbohydrate consumed 3 hours before exercise may not have been completely emptied from the stomach and/or absorbed from the small intestine at the start of exercise. This suggestion is supported by the subjective sensation of gut fullness reported by subjects 3 hours after the ingestion of the meal (see Table 5.2), as well as by the fact that the serum insulin concentration 3 hours postprandially had not returned to pre-feeding levels. If that was the case, a portion of the meal would simply act as a carbohydrate solution in the gut which would gradually provide substrate to the working muscles during exercise. Under these conditions, the meal might have produced a muscle glycogen sparing effect compared to the fasted state, as was suggested in Chapter 7. However, this is simply a suggestion from indirect observations and has to be determined directly before any conclusions can be made on this issue.

The 15-hour pre-exercise overnight fast in the control conditions (P+P and P+W in Chapters 4 and 5 respectively) would have markedly reduced liver glycogen (Nilsson and Hultman 1973). From the data presented in Chapter 6 it seems that a considerable amount of the carbohydrate meal was disposed as glycogen in the liver. Therefore, another factor which could have helped the subjects to exercise longer when ingested the meal might well have been a higher liver glycogen content. Furthermore, the pre-exercise carbohydrate meal may have reduced the rate of liver glycogenolysis at the beginning of exercise, as the catecholamine to insulin ratios indicated (Table 5.8), producing in this way a liver glycogen sparing effect.

The meal improved endurance capacity despite an elevated carbohydrate oxidation rate during the first hour of exercise, and a suppressed plasma FFA concentration. The total amount of carbohydrate oxidised during the first 60 min of exercise in the studies presented in Chapters 4 and 5 was about 195 g (193-197 g) for the M+C trials and 181 g for the M+W trial. On the other hand, in the control trials (P+P and P+W) about 149 g of carbohydrate (145-152 g) was oxidised during the first 60 min of exercise. Hence, the subjects utilised about 46 g of carbohydrate more in the M+C trials and 32 g more in the M+W trial than under fasting conditions. These amounts are equivalent to about 25% for the M+C and 18% for the M+W trial of the total carbohydrate oxidation.
consumed (181 g) in the pre-exercise meals. It seems, therefore, that the pre-exercise carbohydrate load more than compensated for the greater carbohydrate oxidation during the first hour of exercise.

The consumption of a meal providing 2.0 g.kg⁻¹ BW carbohydrates 4 hours before exercise seemed to help runners maintaining their self-selected running speeds during the entire period of a 30-km treadmill run (Chapter 8). However, the study provided no direct evidence about the influence of a pre-exercise carbohydrate meal on endurance running performance.

In summary, the pre-exercise carbohydrate meal improved endurance capacity either by increasing pre-exercise carbohydrate stores, or by reducing the rate of glycogen utilisation in the muscle or liver. The data in this thesis, however, directly support the elevation of pre-exercise carbohydrate stores as the possible reason for the improvement in endurance capacity. Nevertheless, it can be speculated that the mechanism by which a pre-exercise carbohydrate meal enhances endurance capacity can be altered by altering the size of the meal and/or the time of feeding before exercise commences. A large meal given close to the initiation of exercise may not have sufficient time to be absorbed, and therefore, act as a carbohydrate solution during exercise. On the other hand, a smaller meal given well before (5-6 h) exercise may elevate muscle and liver glycogen concentrations and improve, in this way, endurance capacity.

Possible mechanisms by which the carbohydrate-electrolyte solution improved endurance capacity

The study presented in Chapter 4 confirmed the observation of other investigations that a carbohydrate-electrolyte solution ingested during exercise improves endurance capacity (P+C > P+P). Furthermore, the ingestion of the carbohydrate-electrolyte drink improved endurance capacity even when the pre-exercise meal was ingested before exercise (M+C > M+W). Recent studies from our laboratory have demonstrated that, after an overnight fast, the ingestion of a carbohydrate-electrolyte drink during treadmill running at 70-75% VO₂ max results in a lower utilisation of glycogen in the Type I muscle fibers compared with water or placebo (Tsintzas et al. 1993c; 1994). Therefore, it is reasonable to suggest that the
difference in performance between the P+C and P+P trials was due to a reduction in muscle glycogen utilisation in the P+C trial.

However, the ingestion of the 6.9 % carbohydrate-electrolyte drink did not produce any glycogen sparing effect during the first hour of treadmill exercise when the 2.5 g.kg⁻¹ BW carbohydrate meal was ingested 3 hours before exercise (Chapter 7). It is possible that glycogen sparing took place later in exercise, beyond the first hour where muscle biopsies were performed (Chapter 7). On the other hand, one might argue that glycogen sparing might have occurred in Type I muscle fibers, and therefore, the mixed muscle samples analysed in Chapter 7 did not show any difference in the muscle glycogen breakdown. Although this might be a possibility, previous research has shown that glycogen sparing can be observed in both mixed muscle samples and single fibers when the vastus lateralis muscle is biopsied during running (Tsintzas 1993).

As mentioned earlier in the M+C and M+W trials liver glycogen was probably elevated before exercise as a result of the pre-exercise carbohydrate meal (Nilsson and Hultman 1973; 1974; Chapter 6). Furthermore, the carbohydrate-electrolyte solution ingested during the M+C trial may also have reduced the rate of liver glycogen utilisation during exercise (McConell et al. 1994). This would result in a higher liver glycogen availability late in exercise in the M+C trial compared to the M+W trial where no carbohydrate was available during exercise. That could have enabled the subjects to exercise longer in the M+C compared to the M+W trial.

It has been suggested that carbohydrate feedings during exercise improve endurance capacity by maintaining carbohydrate oxidation rate and blood glucose concentration (Coggan and Coyle 1991). Also, there is some evidence that only subjects who experience a marked reduction in blood glucose can benefit from carbohydrate ingestion during exercise (Coggan and Coyle 1991). However, in the present thesis the mean carbohydrate oxidation rate of 19 fasted subjects who exercised to fatigue was 2.5 ± 0.1 g.min⁻¹ in the first 20 min of exercise, whereas at exhaustion was still 2.4 ± 0.2 g.min⁻¹. Also, the resting blood glucose concentration was 4.4 ± 0.1 mmol.l⁻¹ and decreased to only 3.9 ± 0.2 mmol.l⁻¹ at the point of fatigue (n=18). Only a weak relationship was found (r= -0.15; n=18) between the degree of reduction in blood glucose concentration at the point of fatigue (i.e: resting blood glucose
concentration - blood glucose concentration at exhaustion) when fasted subjects exercised (P+P and P+W), and the degree of improvement in endurance capacity when carbohydrate was provided (M+C). However, the biggest drop in blood glucose concentration was about 43% experienced by two subjects in Chapter 5. Their blood glucose concentrations were 2.1 mmol.l⁻¹ and 2.6 mmol.l⁻¹ at the point of fatigue. Furthermore, their improvements in endurance capacity, as a result of carbohydrate feedings, was markedly different: 79% and 4% respectively. From these individual observations one may conclude that there are individuals who do become hypoglycaemic during prolonged running and they may benefit the most from the consumption of carbohydrate during exercise. On the other hand, however, a marked reduction of blood glucose during running is not always associated with a marked improvement in endurance capacity when carbohydrates are ingested during exercise.

In conclusion, the ingestion of a meal, providing 2.5 g.kg⁻¹ BW carbohydrate, 3 hours before exercise increases muscle glycogen concentration, and improves endurance running capacity despite an elevated carbohydrate oxidation rate during the first hour of exercise. It seems that the amount of carbohydrate given before exercise compensates for the greater carbohydrate used. Furthermore, the combination of both a pre-exercise carbohydrate meal and a carbohydrate-electrolyte solution ingested during exercise further improves endurance running capacity, but does not influence muscle glycogen utilisation during the first hour of exercise when a carbohydrate meal is ingested 3 hours before exercise.
Recomendations for further research

The following research projects may give a more complete picture about the influence of the pre-exercise meal on metabolism and endurance capacity.

- The possibility that the pre-exercise meal may influence muscle glycogen utilisation during running needs to be examined by employing the single fibre method.

- Gastric emptying and intestinal absorption measurements should be carried out 3 hours after the ingestion of the meal in order to obtain more information about the fate of the carbohydrate load over the 3-hour postprandial period.

- Although the carbohydrate load ingested before exercise compensates for the higher carbohydrate use during the first hour of exercise, giving the meal 5-6 hours before exercise, and therefore allowing the insulin concentration to return to pre-feeding levels, may be more advantageous, in terms of endurance capacity, than consuming the meal 3 hours before exercise.

- Finally, the study presented in Chapter 7 can be repeated by exercising the subjects for 90 min and by employing the single fiber method. This would clarify whether glycogen sparing occurs in the M+C trial beyond the first hour of exercise.
REFERENCES


159


172


APPENDIX A

MUSCLE METABOLITE ASSAYS

I) Adenosine triphosphate (ATP) and Phosphocreatine (PCr)

Principle:

PCr + ADP ======= Creatine Kinase =====> Creatine + ATP
ATP + Glucose ===== Hexokinase =====> ADP + G-6-P
G-6-P+NAPD+ ===== Glucose-6-P-DH =====> 6-P-Gluconolactone + NADPH + H+

Reagents:
Buffer: Tris-HCl 50 mmol.l⁻¹, pH 8.1 with 0.02% Bovine Serum Albumin (BSA)
Co-factor: NADP 5 mmol.l⁻¹
Enzymes: G6P-DH 14U.ml⁻¹; HK 28 U.ml⁻¹; CK 1260 U.ml⁻¹
Reagents: ADP 10 mmol.l⁻¹; Glucose 10 mmol.l⁻¹; Dithiothreitol (DTT) 50 mmol.l⁻¹; MgCl₂ 100 mmol.l⁻¹; Carbonate buffer 20 mmol.l⁻¹, pH 10.0; Tris-HCl 20 mmol.l⁻¹, pH 8.1 with 0.02% BSA (used for dilution of enzymes)
Standards: ATP 2 mmol.l⁻¹; PCr 2 mmol.l⁻¹ (Stock standards)

Working Standards (prepared daily):

<table>
<thead>
<tr>
<th>ATP 2 mmol.l⁻¹ (µl)</th>
<th>0</th>
<th>25</th>
<th>50</th>
<th>100</th>
<th>150</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double distilled water (µl)</td>
<td>2000</td>
<td>1975</td>
<td>1950</td>
<td>1900</td>
<td>1850</td>
<td>1800</td>
</tr>
<tr>
<td>ATP concentration (µmol.l⁻¹)</td>
<td>0</td>
<td>25</td>
<td>50</td>
<td>100</td>
<td>150</td>
<td>200</td>
</tr>
<tr>
<td>PCr concentration (µmol.l⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>200</td>
</tr>
</tbody>
</table>

Immediately prior to the analysis reaction mixture was prepared for three sets of duplicate samples and quadruplicate double distilled water blanks, and a set of quadruplicate standards.
Reaction Mixture - per ml of buffer (final concentrations):

- NADP 10 µl (0.046 mmol.l⁻¹)
- ADP 10 µl (0.091 mmol.l⁻¹)
- Glucose 10 µl (0.091 mmol.l⁻¹)
- DTT 10 µl (0.457 mmol.l⁻¹)
- MgCl₂ 50 µl (4.566 mmol.l⁻¹)
- G6P-DH 5 µl (0.064 mmol.l⁻¹)

Procedure:

1) Twenty µl of extract were pipetted into a fluorimeter tube and diluted with 100 µl of double distilled water. Ten µl aliquots of the diluted extract were pipetted into 3 sets of duplicate samples. Three sets of double distilled water blanks and one set of standards were also prepared using 10 µl volumes.

2) Two hundred µl of reaction mixture were added to one set of tubes and blanks (G-6-P determination; 1st step).

3) Five µl of HK (0.127 U.ml⁻¹) were added per ml of remaining reaction mixture. Two hundred µl of this reaction mixture were then pipetted to the second set of tubes, blanks and ATP standards (ATP + G-6-P determination; 2nd step).

4) Ten µl of CK (11.351 U.ml⁻¹) were added per ml of remaining reaction mixture, and 200 µl of this reaction mixture were then added to the third set of tubes and blanks (ATP + G-6-P + PCr determination; 3rd step).

Also, 1 ml of this reaction mixture was added to 50 µl of 200 µmol.l⁻¹ PCr standard, and the reaction was followed in the fluorimeter in order to confirm that the assay was running, and to ensure that the reaction had reached completion.

5) After 30-40 min incubation at room temperature 1 ml of carbonate buffer was added to each tube, and after thorough mixing fluorescence was measured.

For the determination of ATP the fluorescence of G-6-P was substracted from the fluorescence of ATP + G-6-P (i.e: Step 2- Step 1). The determination of PCr was achieved by substracting the fluorescence of ATP+ G-6-P+ PCr from the fluorescence of ATP+ G-6-P (i.e: Step 3 - Step 2). Concentrations were determined from the ATP standards. Values in mmol.Kg dry muscle⁻¹ were obtained after multiplying each value by 0.75 to account for the initial extraction dilution (factor 0.125) and the dilution of the extract (factor 6). Before any calculation was made the corresponding blanks were substracted from each sample and standard in every step.
II) Creatine (Cr)

Principle:

Creatine + ATP ======= Creatine Kinase ======> PCr + ADP
ADP + P-pyruvate======Pyruvate Kinase ======> ATP + Pyruvate
Pyruvate + NADH + H+ ==== Lactate Dehydrogenase ===> Lactate + NAD+

Reagents:
Buffer : Imidazole-HCl 50 mmol.1-1, pH 7.5
Co-factor : NADH 1 mmol.l-1
Enzymes : CK 1260 U. ml-1; PK 75 U.ml-1; LDH 240 U.ml-1
Reagents: ATP 10 mmol.l-1; MgCl₂ 100 mmol.l-1; KCl 3 mol.l-1; EDTA 100 mmol.l-1; Phosphoenol Pyruvate (PEP) 2 mmol.l-1; Carbonate buffer 20 mmol.l-1, pH 10.0; Tris-HCl 20 mmol.l-1, pH 8.1 with 0.02% BSA (used for dilution of enzymes)
Standard : Creatine 2 mmol.l-1 (stock solution)

Working Standards (prepared daily):

Creatine 2 mmol.l-1 (µl) 0 50 75 100 150
Double distilled water (µl) 2000 1950 1925 1900 1850
Creatine concentration (µmol.l-1) 0 50 75 100 150

Immediately prior to the analysis reaction mixture was prepared for two sets of duplicate samples and quadruplicate double distilled water blanks, and a set of quadruplicate standards.

Reaction mixture- per ml of buffer (final concentrations):

MgCl₂ 50 µl (4.413 mmol.l-1); KCl 10 µl (26.478 mmol.l-1); ATP 20 µl (0.177 mmol.l-1); NADH 15 µl (13 µmol.l-1); PEP 25 µl (44 µmol.l-1); EDTA 1 µl (88 µmol.l-1); LDH 2 µl (0.424 U.ml-1); PK 10 µl (0.662 U.ml-1)
Procedure:

1) Twenty µl of extract were pipetted into a fluorimeter tube and diluted with 100 µl of double distilled water. Ten µl aliquots of the diluted extract were pipetted into 2 sets of duplicate samples. Two sets of double distilled water blanks and 1 set of standards were also prepared using 10 µl volumes.

2) Two hundred µl of reaction mixture were added to one set of tubes and blanks (ADP + Pyruvate determination; 1st step).

3) Ten µl of CK (11.024 U.ml⁻¹) were added per ml of remaining reaction mixture. Two hundred µl of this reaction mixture were then pipetted to the second set of tubes, blanks and Creatine standards (Creatine + ADP + Pyruvate determination; 2nd step).

Also, 1 ml of this reaction mixture was added to 50 µl of 150 µmol.l⁻¹ Creatine standard, and the reaction was followed in the fluorimeter in order to confirm that the assay was running, and to ensure that the reaction had reached completion.

4) After 60 min incubation at room temperature 1 ml of carbonate buffer was added to each tube, and after thorough mixing fluorescence was measured. For the determination of Creatine the fluorescence of ADP + Pyruvate was subtracted from the fluorescence of Creatine + ADP + Pyruvate (i.e: Step 2 - Step 1). Concentrations were determined from the Creatine standards. Values in mmol.Kg dry muscle⁻¹ were obtained after multiplying each value by 0.75 to account for the initial extraction dilution (factor 0.125) and the dilution of the extract (factor 6). Before any calculation was made the corresponding blanks were subtracted from each sample and standard in every step.
III) Glycogen
Glycogen was measured on both the acid precipitated muscle pellet (acid insoluble glycogen) and the neutralised extract (acid soluble glycogen) by measuring the glucosyl units obtained after acid hydrolysis of glycogen (Jansson, 1981). The muscle glycogen values reported were the sum of soluble and insoluble glycogen, minus the free glucose and G-6-P present in the neutralised extract.

Acid Hydrolysis Procedure:

Reagents: HCl 1 mol.l⁻¹; NaOH 6 mol.l⁻¹

Procedure:
1) One hundred µl of 1 mol.l⁻¹ HCl per mg of muscle powder were added to the precipitated muscle pellet.
2) One hundred µl of 1 mol.l⁻¹ HCl were also added to 20 µl of undiluted neutralised extract from the same sample.
3) Both samples were gently mixed and incubated for 2 hours in tightly sealed screw-top eppendorf tubes in a boiling water bath. The acid hydrolysed extract was neutralised with 15 µl of 6 mol.l⁻¹ NaOH.

The acid insoluble glycogen was assayed spectrophotometrically using a Glucose Test Combination (GOD/Perid method). Acid soluble glycogen was assayed fluorimetrically for glucose.

IIIa) Acid insoluble glycogen (hydrolysed muscle pellet)

Principle:
Glucose + O₂ + H₂O =====> Glucose Oxidase ======> Gluconate + H₂O₂
H₂O₂ + ABTS ==== Horseradish Peroxidase ===> Coloured Complex + H₂O

where:
ABTS = di-ammonium 2,2'- azino-bis (3- ethylbenzothiazoline -6- sulphonate)
The intensity of the colour change is directly proportional to the concentration of glucose. Therefore, by using a standard of known concentration the glucose concentration of a sample can be calculated.

**Reagents:**
The GOD/Perid reagent contained the following:
Phosphate buffer 100 mmol.l⁻¹, pH 7.0; Horseradish peroxidase 0.8 U.ml⁻¹; Glucose oxidase 10 U.ml⁻¹; ABTS 1.0 mg.ml⁻¹. The reagent was stored in a dark bottle at 4 °C.

**Standard:**
Glucose 0.505 mmol.l⁻¹

**Procedure:**
1) Ten µl aliquots from the hydrolysed supernatant (i.e: the muscle pellet) were pipetted in triplicate into tubes. Five standards and five double distilled water blanks were also pipetted using 10 µl volumes.
2) Two ml of GOD/Perid reagent were added to each standard, sample, and blank.
3) After mixing samples were incubated for 30 min at room temperature. The absorbance (A) of samples and standards was measured against the distilled water blanks at 436 nm.

Acid insoluble glycogen concentration (mmol glucosyl units.kg dry muscle⁻¹) was calculated using the following formula:

\[ \text{[Glycogen]} = \left( \frac{A \text{ sample}}{A \text{ standard}} \right) \times 50.5 \]
III b) Acid soluble glycogen (hydrolysed muscle extract)

Principle:

\[
\text{ATP + Glucose} \rightleftharpoons \text{Hexokinase} \rightleftharpoons \text{ADP + Glucose-6-P} \\
\text{Glucose-6-P + NADP}^+ \rightleftharpoons \text{G6P-DH} \rightleftharpoons \text{6-P-gluconolactone + NADPH + H}^+ 
\]

Reagents:

- **Buffer**: Tris-HCl 100 mmol.l\(^{-1}\), pH 8.1 with 0.02% BSA
- **Co-factor**: NADP 5 mmol.l\(^{-1}\)
- **Enzymes**: G6P-DH 7 U.ml\(^{-1}\); HK 28 U.ml\(^{-1}\)
- **Reagents**: ATP 200 mmol.l\(^{-1}\); DTT 50 mmol.l\(^{-1}\); MgCl\(_2\) 100 mmol.l\(^{-1}\); EDTA 100 mmol.l\(^{-1}\); Carbonate buffer 20 mmol.l\(^{-1}\), pH 10.0; Tris-HCl 20 mmol.l\(^{-1}\), pH 8.1 with 0.02% BSA (used for dilution of enzymes)
- **Standard**: Glucose 0.505 mmol.l\(^{-1}\) (stock solution)

Working Standards (prepared daily):

<table>
<thead>
<tr>
<th>Glucose 0.505 (µl)</th>
<th>0</th>
<th>20</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double distilled water (µl)</td>
<td>505</td>
<td>485</td>
<td>455</td>
<td>405</td>
</tr>
<tr>
<td>Glucose concentration (µmol.l(^{-1}))</td>
<td>0</td>
<td>20</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

Immediately prior to the analysis reaction mixture was prepared for two sets of duplicate samples and quadruplicate double distilled water blanks, and a set of quadruplicate standards.

Reaction mixture - per ml of buffer (final concentrations):

- NADP 6 µl (29 µmol.l\(^{-1}\))
- ATP 1.5 µl (0.29 mmol.l\(^{-1}\))
- MgCl\(_2\) 10 µl (0.966 mmol.l\(^{-1}\))
- EDTA 5 µl (0.483 mmol.l\(^{-1}\))
- DTT 10 µl (0.483 mmol.l\(^{-1}\))
- G6P-DH 3 µl (0.02 U.ml\(^{-1}\))

197
Procedure:
1) Twenty µl of neutralised hydrolysed extract were pipetted into two sets of duplicate samples. Two sets of double distilled water blanks and one set of standards were also pipetted using 20 µl volumes.
2) Two hundred µl of the above reaction mixture were added to one set of samples and blanks (G-6-P determination; 1st step).
3) Five µl of HK (0.135 U.ml⁻¹) were added per ml of remaining reaction mixture and 200 µl of this reaction mixture were then added to the second set of samples, blanks and Glucose standards (Glucose + G-6-P determination; 2nd step). Also, 1 ml of this reaction mixture was added to 50 µl of 100 µmol.l⁻¹ Glucose standard, and the reaction was followed in the fluorimeter in order to confirm that the assay was running, and to ensure that the reaction had reached completion.
4) After 30 min incubation at room temperature 1 ml of carbonate buffer was added to each tube, and after thorough mixing fluorescence was measured. For the determination of acid soluble glycogen the fluorescence of G-6-P was substracted from the fluorescence of Glucose + G-6-P (i.e: Step 2- Step 1). Concentrations were determined from the Glucose standards. Values in mmol.kg dry muscle⁻¹ were obtained after multiplying each value by 0.125x6.75 to account for the initial extraction dilution and the dilution during acid hydrolysis. Before any calculation was made the corresponding blanks were substracted from each sample and standard in every step.

Muscle free glucose (obtained from another assay) was substracted from the result to give the true acid-soluble glycogen concentration.
IV) Free Glucose and G-6-P (Undiluted extract)

Principle:
ATP + Glucose ===> Hexokinase ===> ADP + Glucose- 6-P
Glucose-6-P + NADP+ ===> G6P-DH==> 6-P-gluconolactone + NADPH + H+

Reagents:
Buffer: Tris-HCl 100 mmol.l-1, pH 8.1 with 0.02% BSA
Co-factor: NADP 5 mmol.l-1
Enzymes: G6P-DH 7 U.ml-1; HK 28 U.ml-1
Reagents: ATP 200 mmol.l-1; DTT 50 mmol.l-1; MgCl2 100 mmol.l-1;
EDTA 100 mmol.l-1; Carbonate buffer 20 mmol.l-1, pH 10.0;
Tris-HCl 20 mmol.l-1, pH 8.1 with 0.02% BSA (used for
dilution of enzymes)
Standard: Glucose 0.505 mmol.l-1 (stock solution)

Working Standards (prepared daily):

<table>
<thead>
<tr>
<th>Glucose 0.505 (µl)</th>
<th>0</th>
<th>20</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double distilled water (µl)</td>
<td>505</td>
<td>485</td>
<td>455</td>
<td>405</td>
</tr>
<tr>
<td>Glucose concentration (µmol.l-1)</td>
<td>0</td>
<td>20</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

Immediately prior to the analysis reaction mixture was prepared for two sets
of duplicate samples and quadruplicate double distilled water blanks, and a
set of quadruplicate standards.

Reaction mixture - per ml of buffer (final concentrations):

NADP 6 µl (29 µmol.l-1); ATP 1.5 µl (0.29 mmol.l-1); MgCl2 10 µl (0.966
mmol.l-1); EDTA 5 µl (0.483 mmol.l-1); DTT 10 µl (0.483 mmol.l-1) G6P-DH 3 µl
(0.02 U.ml-1)
Procedure:

1) Twenty µl of undiluted extract were pipetted into two sets of duplicate samples. Two sets of double distilled water blanks and one set of standards were also pipetted using 20 µl volumes.

2) Two hundred µl of the above reaction mixture were added to one set of samples and blanks (G-6-P determination; 1st step).

3) Five µl of HK (0.135 U.ml⁻¹) were added per ml of remaining reaction mixture and 200 µl of this reaction mixture were then added to the second set of samples, blanks and Glucose standards (Glucose + G-6-P determination; 2nd step). Also, 1 ml of this reaction mixture was added to 50 µl of 100 µmol.l⁻¹ Glucose standard, and the reaction was followed in the fluorimeter in order to confirm that the assay was running, and to ensure that the reaction had reached completion.

4) After 30 min incubation at room temperature 1 ml of carbonate buffer was added to each tube, and after thorough mixing fluorescence was measured. For the determination of free Glucose the fluorescence of G-6-P was substracted from the fluorescence of Glucose + G-6-P (i.e: Step 2- Step 1). Also, G-6-P was determined from the first step of this assay. Concentrations were determined from the Glucose standards. Values in mmol.kg dry muscle⁻¹ were obtained after multiplying each value by 0.125 to account for the initial extraction dilution. Before any calculation was made the corresponding blanks were substracted from each sample and standard in every step.
V) Lactate

Principle:

\[
\text{Lactate} + \text{NAD}^+ \rightleftharpoons \text{Lactate Dehydrogenase} \rightleftharpoons \text{Pyruvate} + \text{NADH} + \text{H}^+
\]

Reagents:

Buffer: Hydrazine 1.1 mol.1⁻¹, pH 9.0 with 1 mmol.1⁻¹ EDTA
Co-factor: NAD 50 mmol.l⁻¹
Enzyme: Lactate dehydrogenase (LDH) 5500 U.ml⁻¹ (undiluted)
Standard: Lactate 2 mmol.l⁻¹ (stock solution)
Diluent: Carbonate buffer 20 mmol.l⁻¹, pH 10.0

Working Standards (prepared daily):

<table>
<thead>
<tr>
<th>Lactate 2 mmol.l⁻¹ (µl)</th>
<th>0</th>
<th>25</th>
<th>50</th>
<th>100</th>
<th>150</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double distilled water (µl)</td>
<td>2000</td>
<td>1975</td>
<td>1950</td>
<td>1900</td>
<td>1850</td>
</tr>
<tr>
<td>Lactate concentration (µl)</td>
<td>0</td>
<td>25</td>
<td>50</td>
<td>100</td>
<td>150</td>
</tr>
</tbody>
</table>

Immediately prior to the analysis reaction mixture was prepared for one set of duplicate samples and quadruplicate double distilled water blanks, and a set of quadruplicate standards.

Reaction mixture - per ml of buffer (final concentration):

NAD 10 µl (0.491 mmol.l⁻¹); LDH 8 µl (43.22 U.ml⁻¹)

Procedure:

1) Ten µl of undiluted extract were pipetted into a set of duplicate samples. A set of double distilled water blanks and 1 set of standards were also prepared using 10 µl volumes.
2) Two hundred µl of reaction mixture were then pipetted to each tube. Also, 1 ml of the reaction mixture was added to 50 µl of 150 µmol.l⁻¹ lactate, and the reaction was followed in the fluorimeter in order to confirm that the assay was running, and to ensure that the reaction had reached completion.

3) After 30-40 min incubation at room temperature 1 ml of carbonate buffer was added to each tube, and after thorough mixing fluorescence was measured. Concentration was determined from lactate standards after subtracting blanks from samples and standards. Values in mmol.kg dry muscle⁻¹ were obtained by multiplying each value by 0.125 to account for the initial extraction dilution.
APPENDIX B
SPECTROPHOTOMETRIC MUSCLE METABOLITE ASSAYS
FOR VALIDATION OF STANDARDS

GENERAL POINTS
All assays were carried out in a 1 ml semi-micro cuvette at 340 nm, reading against air, and were followed to completion on a chart recorder. The concentration of the standard was calculated using the following equation:

\[
[C] = \frac{V_c \times \Delta A}{6.22 \times V_s}
\]  

(1)

where: \([C]\) = the concentration of the standard (mmol.l\(^{-1}\))

- \(V_c\) = the final cuvette volume (ml)
- \(V_s\) = the volume of standard added (ml)
- \(\Delta A\) = change in absorbance = \(|AF - A_1| - |AE|\)
  - \(AF\) = final absorbance, \(A_1\) = initial absorbance,
  - \(AE\) = absorbance of enzyme

6.22 = the mmolar extinction coefficient for NADH/NADPH at 340 nm
I) Adenosine triphosphate (ATP)

Principle:
ATP + Glucose ======= Hexokinase =====> ADP + G-6-P
G-6-P+NAPD+ === Glucose-6-P-DH==> 6-P-Gluconolactone + NADPH + H+

Reagents:
Buffer: Tris-HCl 50 mmol.l⁻¹, pH 8.1 with 0.02% Bovine Serum Albumin (BSA)
Co-factor: NADP 50 mmol.l⁻¹
Enzymes: G6P-DH 7 U.ml⁻¹; HK 28 U.ml⁻¹
Reagents: Glucose 100 mmol.l⁻¹; Dithiothreitol (DTT) 50 mmol.l⁻¹;
MgCl₂ 100 mmol.l⁻¹; Tris-HCl 20 mmol.l⁻¹,pH 8.1 with
0.02% BSA (used for dilution of enzymes)
Standard: ATP 2 mmol.l⁻¹

Reaction Mixture - per ml of buffer (final concentrations):
NADP 10 µl (0.459 mmol.l⁻¹); Glucose 10 µl (0.917 mmol.l⁻¹); DTT 10 µl (0.459
mmol.l⁻¹); MgCl₂ 50 µl (4.587 mmol.l⁻¹); G6P-DH 10 µl (0.064 mmol.l⁻¹)

Procedure:
One ml of reaction mixture was added to 20 µl of 2 mmol.l⁻¹ ATP standard. A1 was then read. Ten µl of HK (0.272 U.ml⁻¹) were then added, the contents of the cuvette were mixed well and the reaction was followed to completion (5-6 min). Absorbance was then read (A2). Further 10 µl of HK were then added and absorbance was read again (AF) after the contents of the cuvette were mixed well. This was to determine the absorbance of the enzyme (AE= |A2- AF | ). The concentration of standard was calculated using the formula (I).
II) Phosphocreatine (PCr)

Principle:

PCr + ADP====== Creatine Kinase =====> Creatine + ATP
ATP + Glucose =====> Hexokinase ======> ADP + G-6-P
G-6-P+NAPD+ == Glucose-6-P-DH==> 6-P-Glucuronolactone + NADPH + H+

Reagents:
Buffer: Tris-HCl 50 mmol.1^{-1}, pH 8.1 with 0.02% Bovine Serum Albumin (BSA)
Co-factor: NADP 50 mmol.1^{-1}
Enzymes: G6P-DH 7 U.ml^{-1}; HK 28 U.ml^{-1}; CK 1290 U.ml^{-1}
Reagents: ADP 50 mmol.1^{-1}; Glucose 100 mmol.1^{-1}; Dithiothreitol (DTT) 50 mmol.1^{-1}; MgCl2 100 mmol.1^{-1}; Tris-HCl 20 mmol.1^{-1}, pH 8.1 with 0.02% BSA (used for dilution of enzymes)
Standard: PCr 2 mmol.1^{-1}

Reaction Mixture - per ml of buffer (final concentrations):

NADP 10 µl (0.450 mmol.1^{-1}); ADP 10 µl (0.450 mmol.1^{-1}); Glucose 10 µl (0.901 mmol.1^{-1}); DTT 10 µl (0.450 mmol.1^{-1}); MgCl2 50 µl (4.505 mmol.1^{-1}); G6P-DH 10 µl (0.063 mmol.1^{-1}); HK 10 µl (0.252 U.ml^{-1})

Procedure:
One ml of reaction mixture was added to 20 µl of 2 mmol.1^{-1} PCr standard. A1 was then read. Ten µl of CK (12.524 U.ml^{-1}) were then added, the contents of the cuvette were mixed well and the reaction was followed to completion (10-12 min). Absorbance was then read (A2). Further 10 µl of CK were then added and absorbance was read again (AF) after the contents of the cuvette were mixed well. This was to determine the absorbance of the enzyme (AE= |A2-AF | ). The concentration of standard was calculated using the formula (1).
III) Creatine (Cr)

Principle:

Creatine + ATP \rightarrow \text{Creatine Kinase} \rightarrow \text{PCr} + \text{ADP}

ADP + P-pyruvate \rightarrow \text{Pyruvate Kinase} \rightarrow \text{ATP} + \text{Pyruvate}

Pyruvate + NADH + H^+ \rightarrow \text{Lactate Dehydrogenase} \rightarrow \text{Lactate} + \text{NAD}^+

Reagents:
- Buffer: Imidazole-HCl 50 mmol.l^{-1}, pH 7.5
- Co-factor: NADH 1 mmol.l^{-1}
- Enzymes: CK 1260 U.ml^{-1}; PK 75 U.ml^{-1}; LDH 50 U.ml^{-1}
- Reagents: ATP 10 mmol.l^{-1}; MgCl_2 100 mmol.l^{-1}; KCl 3 mol.l^{-1}; Phosphoenol Pyruvate (PEP) 30 mmol.l^{-1}; Tris-HCl 20 mmol.l^{-1}, pH 8.1 with 0.02% BSA (used for dilution of enzymes)
- Standard: Creatine 2 mmol.l^{-1}

Reaction mixture- per ml of buffer (final concentrations):

- MgCl_2 50 µl (4.523 mmol.l^{-1}); KCl 10 µl (27.137 mmol.l^{-1}); ATP 100 µl (0.905 mmol.l^{-1}); NADH 75 µl (0.068 mmol.l^{-1}); PEP 6.5 µl (0.176 mmol.l^{-1}); LDH 4 µl (0.181 U.ml^{-1}); PK 10 µl (0.678 U.ml^{-1})

Procedure:
One ml of reaction mixture was added to 20 µl of 2 mmol.l^{-1} Cr standard. A1 was then read. Ten µl of CK (12.233 U.ml^{-1}) were then added, the contents of the cuvette were mixed well and the reaction was followed to completion (45-60 min). Absorbance was then read (A2). Further 10 µl of CK were then added and absorbance was read again (AF) after the contents of the cuvette were mixed well. This was to determine the absorbance of the enzyme (AE= | A2-AF | ). The concentration of standard was calculated using the formula (1).
IV) Lactate

Principle:

Lactate + NAD⁺ ⇌ Lactate Dehydrogenase ⇌ Pyruvate + NADH + H⁺

Reagents:
Buffer: Hydrazine 1.1 mol. l⁻¹, pH 9.0 with 1 mmol. l⁻¹ EDTA
Co-factor: NAD 50 mmol. l⁻¹
Enzyme: Lactate dehydrogenase (LDH) 5500 U. ml⁻¹ (undiluted)
Standard: Lactate 2 mmol. l⁻¹

Reaction mixture - per ml of buffer (final concentration):

NAD 40 µl (1.923 mmol. l⁻¹)

Procedure:
One ml of reaction mixture was added to 20 µl of 2 mmol. l⁻¹ lactate standard. A₁ was then read. Five µl of LDH (27.363 U. ml⁻¹) were then added, the contents of the cuvette were mixed well and the reaction was followed to completion (20-30 min). Absorbance was then read (A₂). Further 5 µl of LDH were then added and absorbance was read again (Aᶠ) after the contents of the cuvette were mixed well. This was to determine the absorbance of the enzyme (AE = |A₂ - Aᶠ|). The concentration of standard was calculated using the formula (1).
APPENDIX C

COMPOSITION OF MEALS

Composition of meal providing 2.5 g.kg⁻¹ BW carbohydrate*

<table>
<thead>
<tr>
<th>Type of food</th>
<th>Quantity</th>
<th>Energy (Kcal)</th>
<th>CHO</th>
<th>Fat</th>
<th>Protein</th>
<th>Fibre</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Bread</td>
<td>1.5 g</td>
<td>3.57</td>
<td>0.698</td>
<td>0.032</td>
<td>0.123</td>
<td>0.027</td>
</tr>
<tr>
<td>Jam</td>
<td>1.0 g</td>
<td>2.60</td>
<td>0.694</td>
<td>-</td>
<td>0.003</td>
<td>0.011</td>
</tr>
<tr>
<td>Corn Flakes</td>
<td>0.8 g</td>
<td>3.02</td>
<td>0.672</td>
<td>0.008</td>
<td>0.065</td>
<td>0.013</td>
</tr>
<tr>
<td>Skimmed Milk</td>
<td>4.0 ml</td>
<td>1.40</td>
<td>0.200</td>
<td>0.004</td>
<td>0.136</td>
<td>-</td>
</tr>
<tr>
<td>Orange Squash</td>
<td>2.1 ml</td>
<td>1.05</td>
<td>0.237</td>
<td>-</td>
<td>0.002</td>
<td>-</td>
</tr>
<tr>
<td>Water</td>
<td>4.5 ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Values are in grams per kg BW calculated from the food labels.

Typical values (g) for a 70 kg Man

<table>
<thead>
<tr>
<th>Type of food</th>
<th>Quantity</th>
<th>CHO</th>
<th>Fat</th>
<th>Protein</th>
<th>Fibre</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Bread</td>
<td>105 g</td>
<td>48.8</td>
<td>2.21</td>
<td>8.61</td>
<td>1.890</td>
</tr>
<tr>
<td>Jam</td>
<td>70 g</td>
<td>48.6</td>
<td>-</td>
<td>0.21</td>
<td>0.770</td>
</tr>
<tr>
<td>Corn Flakes</td>
<td>56 g</td>
<td>47.0</td>
<td>0.56</td>
<td>4.54</td>
<td>0.896</td>
</tr>
<tr>
<td>Skimmed Milk</td>
<td>280 ml</td>
<td>14.0</td>
<td>0.28</td>
<td>9.52</td>
<td>-</td>
</tr>
<tr>
<td>Orange Squash</td>
<td>147 ml</td>
<td>16.6</td>
<td>-</td>
<td>0.15</td>
<td>-</td>
</tr>
<tr>
<td>Water</td>
<td>315 ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Composition of meal providing 2.0 g kg\(^{-1}\) BW carbohydrate*

<table>
<thead>
<tr>
<th>Type of food</th>
<th>Quantity</th>
<th>Energy (Kcal)</th>
<th>CHO</th>
<th>Fat</th>
<th>Protein</th>
<th>Fibre</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Bread</td>
<td>1.2 g</td>
<td>3.20</td>
<td>0.594</td>
<td>0.017</td>
<td>0.086</td>
<td>0.032</td>
</tr>
<tr>
<td>Jam</td>
<td>0.6 g</td>
<td>1.56</td>
<td>0.416</td>
<td>-</td>
<td>0.002</td>
<td>0.007</td>
</tr>
<tr>
<td>Corn Flakes</td>
<td>0.7 g</td>
<td>2.71</td>
<td>0.601</td>
<td>0.006</td>
<td>0.064</td>
<td>0.009</td>
</tr>
<tr>
<td>White Sugar</td>
<td>0.045 g</td>
<td>0.18</td>
<td>0.045</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Skimmed Milk</td>
<td>3.1 ml</td>
<td>1.09</td>
<td>0.155</td>
<td>0.003</td>
<td>0.105</td>
<td>-</td>
</tr>
<tr>
<td>Orange Squash</td>
<td>0.8 ml</td>
<td>0.84</td>
<td>0.191</td>
<td>-</td>
<td>0.001</td>
<td>-</td>
</tr>
<tr>
<td>Water</td>
<td>4.5 ml</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Values are in grams per kg BW calculated from the food labels.

**Typical values (g) for a 70 kg Man**

<table>
<thead>
<tr>
<th>Type of food</th>
<th>Quantity</th>
<th>CHO</th>
<th>Fat</th>
<th>Protein</th>
<th>Fibre</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Bread</td>
<td>84 g</td>
<td>41.6</td>
<td>1.17</td>
<td>6.00</td>
<td>2.260</td>
</tr>
<tr>
<td>Jam</td>
<td>42 g</td>
<td>29.1</td>
<td>-</td>
<td>0.13</td>
<td>0.462</td>
</tr>
<tr>
<td>Corn Flakes</td>
<td>49 g</td>
<td>42.1</td>
<td>0.39</td>
<td>4.46</td>
<td>0.637</td>
</tr>
<tr>
<td>White Sugar</td>
<td>3.2 g</td>
<td>3.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Skimmed Milk</td>
<td>217 ml</td>
<td>10.9</td>
<td>0.22</td>
<td>7.38</td>
<td>-</td>
</tr>
<tr>
<td>Orange Squash</td>
<td>56 ml</td>
<td>16.0</td>
<td>-</td>
<td>0.06</td>
<td>-</td>
</tr>
<tr>
<td>Water</td>
<td>315 ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
APPENDIX D

Gut Fullness Scale

0 — Empty
1
2 — Slightly full
3
4 — Fairly full
5
6 — Moderately full
7
8 — Uncomfortably full
9
10 — Bloated
Abdominal Discomfort Scale

0 — Completely comfortable
1
2 — Fairly comfortable
3
4 — Slight discomfort
5
6 — Moderate discomfort
7
8 — Extreme discomfort
9
10 — Unbearable pain
APPENDIX E

Estimation of carbohydrate and fat oxidation using indirect calorimetry

The proportions of energy derived from carbohydrate and fat were estimated from the non-protein respiratory exchange ratio (RER) value. This assumes that the contribution of protein to energy metabolism is relatively small (Consolazio et al. 1963). The following method for calculating carbohydrate and fat oxidation by indirect calorimetry is adapted from McArdle et al. (1991):

- The oxidation of 1.0 g of carbohydrate uses 0.828 l of oxygen, and produces 0.828 l of carbon dioxide. The oxidation of 1.0 g of carbohydrate uses 1.989 l of oxygen, and produces 1.419 l of carbon dioxide. Whole body oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) is calculated from expired air analyses.

- Rates of carbohydrate and fat oxidation can be determined using simultaneous equations:

$$\dot{V}O_2 = 0.828x + 1.989y \quad (1)$$
$$\dot{V}CO_2 = 0.828x + 1.419y \quad (2)$$

By subtracting equation 2 from equation 1, gives rise to the following equations:

$$x = \frac{\dot{V}O_2 - (y \times 1.989)}{0.828} \quad y = \frac{\dot{V}O_2 - \dot{V}CO_2}{0.570}$$

where: $x =$ carbohydrate oxidised in g.min$^{-1}$, and $y =$ fat oxidised in g.min$^{-1}$

Reference: