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**INFLUENCE OF CARBOHYDRATE-ELECTROLYTE  
SOLUTIONS ON SELF-SELECTED ENDURANCE  
RUNNING PERFORMANCE**

by

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A Doctoral thesis

Submitted in partial fulfilment of the requirements  
for the award of Doctor of philosophy of the  
Loughborough Univeristy

September, 04-09-2009

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## ABSTRACT

The aim of this thesis was to advance the methods of assessing laboratory based running performance and study the influence of carbohydrate-electrolyte (CHO-E) solutions on self-selected running speeds.

The first study (Chapter 4) attempted to improve the methods used for treadmill time-trials by using an automated treadmill system that allows runners to rapidly change their running speed without engaging with the treadmill control panel. To this end, the study examined the repeatability of a 1 h running time-trial, in which the aim was to achieve the greatest distance in the set time. The coefficient of variation (CV), estimated using ANOVA with subject and trial as main effects, was 1.4%. Therefore, it was concluded that asking runners to cover as much distance as possible in 1 h, using an automated treadmill system is a reliable method of assessing endurance performance in endurance trained runners.

In the second study (Chapter 5) the automated treadmill system and test protocol was used to investigate the influence of CHO-E solution ingestion before and during exercise on 1 h running performance following a prolonged fast (12-15 h). The study used a double blind placebo (PLA) controlled design. On two occasions (P1, P2) runners consumed a placebo solution,  $8 \text{ ml} \cdot \text{kg BM}^{-1}$ , 30 min prior to and  $2 \text{ ml} \cdot \text{kg BM}^{-1}$  at 15 min intervals throughout the 1 h run. On a separate occasion runners consumed the same quantity of a 6.4% CHO-E solution (C). Total distance covered for P1, P2 and C trials was  $13685 \pm 1116 \text{ m}$ ,  $13715 \pm 1143 \text{ m}$  and  $14046 \pm 1104 \text{ m}$  respectively. Although there was no difference between the two PLA trials (P1, P2) ( $P > 0.05$ ), the distance covered during the C trial was significantly greater than both PLA trials ( $P < 0.05$ ). The ingestion of CHO resulted in a higher blood glucose concentration only at the onset of exercise ( $P < 0.05$ ), compared to the PLA trials. Blood lactate, RER and CHO oxidation were similar in all three trials. The ingestion of a 6.4% CHO-E solution before and during exercise was associated with improved running performance in runners compared to the ingestion of colour and taste matched placebo.

The third study (Chapter 6) investigated whether or not the improvements in endurance performance observed in Chapter 5, were still evident in fed runners, i.e. following the consumption of a pre-exercise meal 3 h prior to exercise. Endurance-trained male runners (n. =10) completed two trials that required them to run as far as possible in 1 h on the automated treadmill. Before each run, runners consumed a carbohydrate (CHO) meal (2.5 g CHO per kg body mass) 3 h before exercise. They then consumed either a 6.4 % CHO-E (C) or placebo (P) solution, 8 ml · kg BM<sup>-1</sup>, 30 min prior to and 2 ml · kg BM<sup>-1</sup> at 15 min intervals throughout the 1 h run. There was no difference in total distance covered i.e. 13680 ± 1525 m; and 13589 ± 1635 m for the P and C trials respectively ( $P > 0.05$ ). Blood glucose and lactate concentration, RER, and CHO oxidation during exercise were no different between trials ( $P > 0.05$ ). There were also no differences in perceived exertion (RPE), felt arousal (FAS) or pleasure-displeasure (FS) between trials ( $P > 0.05$ ). The results showed that the ingestion of a 6.4 % CHO-E solution did not improve 1 h running performance when a high CHO meal was consumed 3 h before exercise.

The aim of the fourth study (Chapter 7) was to investigate the mechanisms which may have been responsible for improved performance with CHO-E solution ingestion following a prolonged fast (13 h). Ten endurance-trained male runners completed two 1 h performance runs on the automated treadmill, whilst mouth-rinsing 25 ml of either a 6.4 % CHO-E (C) or placebo (P) solution immediately before and at 15-min intervals during the 1 h run. An additional ten healthy active males followed the same mouth-rinse procedure during a 1 h resting period. Finger prick blood samples were obtained for determination of blood glucose and plasma insulin concentrations. The runners covered 211 m (90 % CI = 42 m to 380 m,  $P = 0.048$ ) further during the C trial (14298 ± 685 m, mean ± SD) in comparison to the P trial (14086 ± 732 m). There was no change in blood glucose concentrations during the 1 h run (P; pre, 4.3 ± 0.2 mmol·l<sup>-1</sup>, post, 4.3 ± 0.3 mmol·l<sup>-1</sup>; C: pre, 4.3 ± 0.4 mmol·l<sup>-1</sup>, post, 4.3 ± 0.3 mmol·l<sup>-1</sup>). At rest there was no change in blood glucose (P; 4.3 ± 0.1 mmol·l<sup>-1</sup>, C: 4.3 ± 0.2 mmol·l<sup>-1</sup>) or plasma insulin (P; 6.2 ± 1.1 mU·L<sup>-1</sup>, C; 5.9 ± 1.0 mU·L<sup>-1</sup>) concentrations ( $P > 0.10$ ).

In the fifth study (Chapter 8) the observed beneficial effect of CHO-E solution mouth-rinse on the self-selection of speed towards an exercise task was explored further.

Endurance trained males (n. = 10) performed two trials, each involving a 10-min warm-up at 60%  $\dot{V}O_2$ peak followed by a 30-min run at a self-selected running speed. Participants were asked to run at speeds which equated to a RPE of “15”, mouth-rinsing either a CHO-E solution (C) or taste matched placebo (P) solution. The total distance covered was greater during the C than during the P trial ( $P < 0.05$ ). Faster speeds selected during the first 5-min of exercise corresponded with enhanced feelings of pleasure when mouth-rinsing the CHO-E solution.

The final study of the thesis (Chapter 9) investigated the effect of mouth-rinsing (Chapter 8) and ingesting (Chapter 5) a CHO-E solution on 1 h running performance. Following a prolonged fast (14-15 h), ten endurance-trained male runners completed three 1 h performance runs separated by 1 week. On two occasions runners ingested either a 6.4 % CHO-E solution (C) or placebo solution (P), 8 ml · kg BM<sup>-1</sup>, 30 min prior to and 2 ml · kg BM<sup>-1</sup> at 15 min intervals throughout the 1 h run. On a separate occasion, runners mouth-rinsed (R) a 6.5 % CHO-E solution without ingestion at the same time intervals as the ingestion trials. The total distances covered were 14515 ± 756 m; 14190 ± 800 m and 14283 ± 758 m for the C, P and R trials respectively ( $F_{(2,18)} = 9.3$ ,  $P = 0.002$ ). Runners covered 320 m (90% CI of difference = 140 m to 510 m,  $P = 0.01$ ) further during the C trial in comparison to the P trial and 230 m (90% CI of difference = 83 m to 380 m,  $P = 0.019$ ) in comparison to the R trial. The mean difference in distance covered between the R trial and P trial was 93 m (90% CI of difference = -13000 m to 13000 m,  $P = 1.0$ ). Thus the ingestion of a CHO-E solution was associated with increased distance covered during a 1 h performance run in comparison to mouth-rinsing a CHO-E solution and the ingestion of the same volume of placebo solution.

In conclusion it appears that the improved treadmill running performance following the ingestion of a CHO-E solution may not be entirely attributed to the provision of the exogenous substrate because there is an, as yet undermined, contribution from simply having carbohydrate in the oral cavity.

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

Rollo, I., Williams, C., & Nevill, A. (2008). Repeatability of scores on a novel test of endurance running performance. *Journal of Sports Sciences*, 26(13), 1-8.

Rollo, I., Williams, C., Gant, N., & Nute, M. (2008). The influence of carbohydrate mouth rinse on self-selected speeds during a 30-min treadmill run. *International Journal of Sports Nutrition and Exercise Metabolism* (18), 585-600.

Rollo, I., Williams, C. Influence of ingesting a carbohydrate-electrolyte solution before and during 1 hour running performance. *International Journal of Sports Nutrition and Exercise Metabolism*. (in press)

Rollo, I., Cole, M., Miller, R., Williams, C. The influence of mouth rinsing a carbohydrate solution on 1 hour running performance. *Medicine and Science in Sports and Exercise*. (in press, April 2010).

Rollo, I., Williams, C. Influence of ingesting a carbohydrate-electrolyte solution before and during 1 hour running performance in fed endurance trained runners. *Journal of Sports Sciences*. in press.

*This thesis is dedicated to my parents,  
John and Caroline*

## ACKNOWLEDGEMENTS

I would like to give special thanks to the following people for their help during my PhD:

My supervisor Professor Clyde Williams, for giving me the opportunity to complete these studies. His guidance, encouragement, friendship and enthusiasm are a constant inspiration for which I am eternally grateful.

My parents John and Caroline Rollo, without their support, love and understanding none of this would have been possible. My brother Dave for always looking out for me and my Nan for her unremitting confidence in me.

To Helen for her patience and understanding and also Helens family for their support.

Dr Mary Nevill, for accepting me as a student and for her encouragement and advice. Professor Alan Nevill for offering his time and invaluable experience in the statistical analysis of the data and my director of research Professor Mike Gleeson.

I would like to thank my colleagues Lewis James, for being a constant sounding board and great friend and Ruth Hobson, for her hospitality and bringing fun into everyday. Nicholas Gant and Maria Nute for their advice on testing and treadmill protocols. I would also like to thank all my other colleagues for their help and friendship which has made studying at Loughborough an enjoyable experience.

All the runners, whose participation made this thesis possible, made the collection of data enjoyable and taught me so much when talking about their running experiences.

The team at GlaxoSmithKline, in particular Gareth Wallis and Suzane Leser for their interest and support of my studies.

My family for their support Roger, Marujah and James Bristow, Laura and Kevin Brook.

My friends Dr Chris Shaw, Alex Young, Laurence Smith, Paul Breaden, Dominic Martin MSc, Helen Boyle, David Jones, Angie Cornelius. Dr Marwa Mattar, Diana Rockall, Olivia Saunders, Lindsey Marsh, Darren Turney, Steve and Claire Garret and family.

Angie, Ken, Lianne, Nickie, Mark and Ann Riley, Terry Doherty and Danny and Sam Anderson for always being there.



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# CHAPTER 1

## INTRODUCTION

Since the early recognition that fatigue during prolonged exercise is closely associated with the depletion of muscle glycogen stores (Bergstrom, Hermansen, Hultman & Saltin, 1967), nutritional studies have been directed at improving pre-exercise carbohydrate (CHO) concentrations as well as providing CHO during exercise.

Strategies to increase pre-exercise muscle glycogen stores include increasing daily dietary intake of CHO (Bergstrom et al., 1967; Brewer, Williams & Patton, 1988a; Pitsiladis, Duignan & Maughan, 1996; Sherman, Costill, Fink & Miller, 1981) and ingesting pre-exercise meals prior to performance. The consumption of easy to digest CHO meals 3 h before exercise has been shown to increase muscle glycogen concentrations by 11-15 % (Chryssanthopoulos, Williams, Nowitz & Bogdanis, 2004; Wee, Williams, Gray & Horabin, 1999). Ingesting a pre-exercise meal has been shown in several studies to improve endurance capacity of runners during treadmill running to fatigue at an intensity equivalent to approximately 70%  $\dot{V}O_2\text{max}$  (Chryssanthopoulos, Williams, Nowitz, Kotsiopolou & Vleck, 2002b; Wee et al., 1999; Wu & Williams, 2006).

The benefits of ingesting carbohydrate-electrolyte (CHO-E) solutions during prolonged exercise i.e. cycling and running, on endurance capacity have been established in well controlled laboratory studies. The ingestion of CHO during prolonged sub-maximal exercise has been shown to improve endurance capacity by maintaining euglycaemia late in exercise and under certain circumstances delaying the depletion of muscle glycogen stores (Coyle, Coggan, Hemmert & Ivy, 1986; Tsintzas, Williams, Boobis & Greenhaff, 1996a). When a pre-exercise meal rich in CHO is combined with the ingestion of a CHO-E solution during exercise then the improvements in endurance capacity during cycling (Wright, Sherman & Dernbach, 1991) and during running (Chryssanthopoulos, Williams, Nowitz, Kotsiopolou &

Vleck, 1994b) are reported to be greater than when either of these CHO interventions are adopted alone.

However, while there is a general consensus that providing CHO before and during prolonged sub-maximal exercise improves endurance capacity, there is less evidence to support the value of providing CHO immediately before and during high intensity ( $\geq 70\% \dot{V}O_2\text{max}$ ), shorter duration (approximately 1 h) exercise i.e. on time-trial performance. Time-trials assess endurance performance using tests that require the completion of a pre-set amount of external mechanical work (cycling) (Widrick, Costill, Fink, Hickey, McConell & Tanaka, 1993a) or distance (cycling or running) (Chryssanthopoulos, Williams, Wilson, Asher & Hearne, 1994c; Jeukendrup, Brouns, Wagenmakers & Saris, 1997b) in as fast a time as possible or by asking athletes to complete as much work as possible in a specified time (Schabort, Hopkins & Hawley, 1998). Endurance performance tests are of interest as they are more representative of “real life” races.

Many athletes avoid eating immediately before early morning training or competition. Although, given the choice, most athletes would prefer to have a meal a few hours before a race rather than to fast before exercise. Therefore, studies on the performance benefits of pre-exercise meals are worthwhile because they may give a better insight into the ‘real world’ responses of athletes to various nutritional strategies. However, there are relatively few studies investigating CHO ingestion during ‘real world’ road races. This is because of the difficulties of controlling variables that may influence the outcome of races, for example, the day-to-day differences in environmental conditions. Of those studies that have created road races to examine the efficacy of ingesting CHO on running performance only some (Tsintzas, Liu, Williams, Campbell & Gaitanos, 1993) but not all (Burke, Wood, Pyne, Telford & Saunders, 2005) have reported a performance benefit. Therefore, it is not surprising that most studies on nutrition and performance have been designed to try to create race conditions in well controlled laboratory environments.



Laboratory based studies typically use an exercise duration of 45 min to 1 h to assess endurance performance. During such exercise, endogenous glycogen stores are normally sufficient for the exercise task and are unlikely to limit performance (Hawley, Palmer & Noakes, 1997). McConell et al. (2000) found that only a small percentage (26 %) of total CHO consumed actually enters the peripheral circulation during high intensity endurance exercise. In addition the provision exogenous CHO contributes minimally to CHO oxidation in the muscle in comparison to the oxidation of endogenous muscle glycogen (Romijn, Coyle, Sidossis, Gastaldelli, Horowitz, Endert & Wolfe, 1993). Therefore, there does not appear to be a clear rationale for supplying additional CHO before a time-trial lasting an hour or less. Nevertheless, some studies (Ball, Headley, Vanderburgh & Smith, 1995; Below, Mora-Rodriguez, Gonzalez-Alonso & Coyle, 1995; Carter, Jeukendrup, Mundel & Jones, 2003; Neuffer, Costill, Flynn, Kirwan, Mitchell & Houmard, 1987) but not all (Anantaraman, Carmines, Gaesser & Weltman, 1995; Desbrow, Anderson, Barrett, Rao & Hargreaves, 2004; Widrick et al., 1993a) report that CHO ingestion improves endurance performance. Of particular interest, are studies which report improvements in performance without apparent influences on muscle metabolism (Jeukendrup et al., 1997b; Powers, Lawler, Dodd, Tulley, Landry & Wheeler, 1990). These studies are of interest because their results suggest a mechanism independent of glucose delivery to the circulation may be responsible for improved performance.

A key influence on performance appears to be the method by which CHO enters the circulation during exercise. Carter et al. (2004) reported that infusing glucose into cyclists ( $60 \text{ g} \cdot \text{h}^{-1}$ ) had no influence on their 1 h cycle time-trial performance compared with a non-CHO containing saline (Carter, Jeukendrup, Mann & Jones, 2004b). Surprisingly, mouth-rinsing a CHO solution without ingestion resulted in significant improvements in the performance of their cyclists (Carter, Jeukendrup & Jones, 2004a). These findings are also supported by Pottier et al. (2008) who reported that mouth-rinsing a CHO solution resulted in a greater improvement in 1 h cycle time-trial performance compared to ingesting the same solution (Pottier, Bouckaert, Gilis, Roels & Derave, 2008). These observations have led authors to speculate about possible links between CHO in the mouth and the stimulation of certain areas of the brain during exercise. These links have been recently explored by using functional magnetic resonance imaging techniques (Chambers, Bridge & Jones, 2009). The

concept that mouth-rinsing a CHO-E solution may exert a “central effect” is consistent with studies that have found runners experience more pleasure and report lower ratings of perceived exertion while ingesting CHO-E during prolonged exercise (Backhouse, Ali, Biddle & Williams, 2007; Backhouse, Bishop, Biddle & Williams, 2005).

The self-selection of running speed i.e. exercise intensity has been proposed to be determined by a centrally governed mechanism (Hampson, St Clair Gibson, Lambert & Noakes, 2001) which regulates energy expenditure to optimises the use of available energy reserves (Foster, De Koning, Hettinga, Lampen, La Clair, Dodge, Bobbert & Porcari, 2003). A study by Rauch et al. (2005) speculated that a chemoreceptor in muscle monitors glycogen concentration and its rate of decline. This speculation was based upon a study which reported cyclists’ finished time-trial performances with almost identical muscle glycogen concentrations despite starting exercise with significantly difference concentrations (high vs. low). Thus, the authors proposed that the central nervous system uses a “glycostat” to help set an optimum pacing strategy (Rauch, St Clair Gibson, Lambert & Noakes, 2005). If there is central recognition of the bodys CHO stores, it is reasonable to ask whether ingesting CHO during exercise alters the runners’ perception towards exercise and if so, whether this translates into faster self-selected running speeds. Whilst the administration of the RPE scale (Borg, 1982) provides information on the intensity of the perceived exertion it does not help describe ‘how the runners feel’ during exercise (Hardy & Rejeski, 1989). Therefore, the administration both a Feeling Scale (FS) and RPE scale, it is possible to assess not only “what” (RPE) but “how” (FS) a person feels (Hardy et al., 1989). In addition, whether runners’ feelings are ‘good or bad’ (pleasure-displeasure) or feel ‘energised’ (i.e. an activated state) during exercise are also relevant because it is likely that may influence their performance (Acevedo, Gill, Goldfarb & Boyer, 1996).

It is important to note that the majority of studies investigating CHO ingestion and time-trial performance have been conducted in cycling. In comparison, the number of studies investigating the influences of CHO solutions on running performance are few. The reason for the low number of studies investigating running performance under laboratory conditions is unclear. One possible explanation may be that the repeatability of endurance performance (~1 h duration) in treadmill running has

previously failed to produce coefficient of variations (CVs) that are comparable with the most reliable cycling tests (approximately 1 %) (Hickey, Costill, McConell, Widrick & Tanaka, 1992; Schabort et al., 1998). Schabort et al. (1998b) reported a CV of 2.7 % when runners were asked to run as far as possible in 60-min. A similar CV of 2 % was also reported by Whitham and McKinney (2007) when runners were asked to run as far as possible in 45 min after an initial run of 15 min at 65 %  $\dot{V}O_2$ max. Hopkins and Hewson (2001) reported that running tests need a CV of 2.5 % or less, to detect worthwhile differences in performance for half and full marathons and a CV of 1.5 % or less for races over shorter distances (Hopkins & Hewson, 2001).

Although there are clear advantages to conducting running studies in a well controlled laboratory environment, one of the limitations of using motorised treadmills is the runners' inability to spontaneously alter running speed (Laursen, Francis, Abbiss, Newton & Nosaka, 2007; Schabort et al., 1998; Whitham & McKinney, 2007). Instead, running speed is adjusted manually either by the runner or by the investigator. This approach does not allow runners to change their speed spontaneously according to how they 'feel' (Laursen et al., 2007; Whitham et al., 2007). Thus, treadmill tests which require conscious alterations of running speed, i.e. pressing appropriate buttons on the treadmill console, may not be optimal in detecting the influence of nutritional interventions on running performance. Although there are several studies that have used cycling time-trials lasting approximately 60 min or less there appears to be a lack of studies investigating the influence of CHO intake on endurance running performance of approximately 1 h in duration. Therefore, the aims of this thesis were to study the influences of ingesting and mouth-rinsing CHO on self-selected running speeds.

The results of this programme of research are presented in this thesis as ten chapters. The literature review (Chapter 2) provides a brief summary of the relevant literature on CHO nutrition and metabolism during running with specific reference to performance. The general methods and procedures used in this thesis are described in Chapter 3.

The first study (Chapter 4) attempted to improve the methods used for treadmill time-trials by using an automated treadmill system that allows runners to rapidly change their running speed with no manual input. To this end the study examined the repeatability of a 1 h time-trial.

The main aim of the second study (Chapter 5) was to use the new method of treadmill running (Chapter 4) to investigate the influence of CHO-E ingestion before and during exercise on 1 h running performance of recreational male runners.

The main aim of the third study (Chapter 6) was to investigate the influence of combining the ingestion of CHO-E solutions with the ingestion of a pre-exercise meal on 1 h running performance of recreational male runners.

The main aim of the fourth study (Chapter 7) was to investigate the influences of mouth-rinsing with a CHO-E solution on self-selected treadmill running speeds and distance covered in 1 h.

The main aim of the fifth study (Chapter 8) was to investigate the effect of mouth-rinsing a CHO-E solution on the self-selection of speed when runners are asked to select a pace that represented a rating of 15 (hard) on the Borg RPE scale (Borg, 1982). In addition, the study attempted to determine if runners 'felt better' while mouth-rinsing CHO-E solutions compared with mouth-rinsing a colour-matched placebo solution.

The last study (Chapter 9), investigated the influence of mouth-rinsing versus the ingestion of a CHO-E solution on 1 h running performance.

In the final Chapter 10, the main findings of the studies included in the thesis are discussed and some directions for future research are suggested.

## **CHAPTER 2**

### **LITERATURE REVIEW**

#### **2.1 Introduction**

This chapter is intended to provide information from the literature on topics related to carbohydrate (CHO) metabolism and exercise performance. By reviewing relevant information, this chapter will aim to provide a framework on which the present thesis is based and highlight how the studies in the thesis have contributed to understanding on this area of sports nutrition.

The literature review has been divided into eight main sections. The first section (2.2) outlines the evidence regarding the digestion, absorption and recognition of CHO along the gastrointestinal (G.I) tract. Section 2.3 provides a brief description on the effect of exercise on the G.I function. The next two sections address CHO metabolism (2.4) and its regulation (2.5). Section 2.6 reviews the current concepts on pacing strategies during running performance. Section 2.7 provides a brief description about the possible causes of fatigue during prolonged exercise and section 2.8 reviews the effect of ingesting CHO before and during exercise on metabolism. Finally section 2.9 reviews the studies which have investigated the effect of CHO ingestion on exercise performance.

## 2.2 Carbohydrate digestion and absorption

The endogenous stores of CHO are finite. Therefore in order to maintain the supply of energy for muscle and brain metabolism CHO must be replenished, either by dietary sources or intravenous infusion. Most interventions in sports nutrition involve the ingestion of dietary sources of CHO. Therefore, the effectiveness of nutritional interventions is dependent upon the regulation and absorption of CHO by the gastrointestinal (G.I) tract. In addition, it appears that the G.I tract has the ability to detect glucose in food. The ability of the body to recognise incoming energy and also potentially toxic substances is clearly evolutionary advantageous and may be an important factor when regulating energy expenditure during exercise.

The G.I tract is a continuous tube approximately 9 m long that extends from the mouth to the anus. The ingested bolus of food is moved along the G.I tract by strong peristaltic contractions. As the bolus is moved through the G.I tract it is mixed with extensive secretions of fluid containing various enzymes which aid in the digestion and absorption of nutrient. This section of the review will focus on the digestion, absorption and central recognition of CHO.

### *Mouth*

The digestion of CHO begins in the mouth. Food is broken down mechanically by mastication and mixed with saliva which is secreted by the salivary glands. The salivary amylase begins the breakdown of starch into smaller oligosaccharides. It is in the mouth where the tongue begins the analysis of food, determining whether it is nutritive (i.e. sugar) and should be ingested or is potentially harmful and therefore expectorated (Katz, Nicolelis & Simon, 2000). Sweet stimuli (glucose, sucrose, fructose, and artificial sweeteners) are detected by taste receptor cells (G-coupled receptor proteins; T1R2 and T1R3) on the tongue (Berthoud, 2003). These receptor cells, release a neurotransmitter ( $\alpha$ -gustducin) that is detected by primary afferent nerve fibre terminals, sending information to the brainstem. The central processing of sweet taste activates feeding circuits as well as brain reward systems that promote sweet appetite (Berthoud, 2003). The palatability of solutions is an important consideration when investigating CHO-E solutions. Several studies have reported that

flavouring or sweetening beverages can substantially increase the voluntary intake of fluid both during exercise and recovery from exercise (Passe, Horn & Murray, 2000; Wilmore, Morton, Gilbey & Wood, 1998).

The central response to ingesting CHO has been investigated by using functional magnetic resonance imaging (fMRI). In one study, participants ingested either 300 ml of water (control), a glucose solution, an aspartame (sweet taste) solution, or a maltodextrin (non-sweet CHO) solution. It was reported that both sweet taste and energy content are required for a hypothalamic response (Smeets, de Graaf, Stafleu, van Osch & van der Grond, 2005a). In addition, the activation of the hypothalamus was reported to be dose dependant to CHO i.e. the greater concentration of CHO the greater the response. Thus, a relationship was observed between the hypothalamic response and changes in the blood insulin concentration (Smeets, de Graaf, Stafleu, van Osch & van der Grond, 2005b).

The hypothalamus helps maintain the homeostasis of the body. It receives information about the state of the body and makes compensatory changes by releasing various hormones. The hypothalamus is considered the “control centre” of the body, regulating many physiological functions such as, motor functions, emotions, endocrine functions, energy intake and water intake.

In a recent study it was reported that both glucose (sweet) and maltodextrin (non-sweet) in the mouth activate regions in the brain associated with reward, such as the insula/frontal operculum, orbitofrontal cortex and striatum. These findings suggest that there may be a class of, so far unidentified, oral receptors that respond to CHO independently of sweetness (Chambers et al., 2009). Regions of the brain associated with reward are also believed to mediate behavioural responses to rewarding stimuli, such as taste (Rolls, 2007).

Receptors on the tongue also extract information about the texture and temperature of food. This processing prepares the G.I system for compounds in the mouth by causing the organism to salivate, masticate, swallow, or expel, as well as to release insulin and other peptides (Katz et al., 2000). In humans, simply tasting food in the oral cavity has been shown to stimulate the release of insulin from the pancreas, known as the

cephalic insulin release (CPIR). Under fasting conditions, both nutritive (sucrose) and non-nutritive sweetener (saccharin) solutions had an effect on the CPIR, when mouth-rinsed for 45 s and expectorated without ingestion (Just, Pau, Engel & Hummel, 2008). Food can be retained in the mouth for prolonged periods of time i.e. chewing gum. However when food is to be ingested, it is in the mouth for approximately less than 1 min before the pharynx and oesophagus aid in swallowing and transporting the food into the stomach.

A noteworthy study reported that the regular ingestion of CHO drinks during exercise caused significant tooth enamel decay in comparison to water and less acidic solutions (Venables, Shaw, Jeukendrup, Roedig-Penman, Finke, Newcombe, Parry & Smith, 2005). Therefore, when considering nutritional interventions involving the ingestion of CHO and acidic drinks participants should be advised to brush their teeth to maintain dental care.

### The stomach

In the stomach the food is subjected to vigorous movements that mix it with gastric juices, secreted from the stomach glands to form chyme. The stomach acts as a reservoir to receive food whilst intermittently delivering the chyme to the intestine. Ingested food stays in the stomach between 1 and 4 hours. The absorption of nutrients occurs almost entirely along the walls of the small intestine. Therefore the benefit of ingesting CHO and fluid at rest or during exercise is only obtained following the movement of chyme through the pyloric sphincter from the stomach into the duodenum (Gastric emptying). Numerous factors, such as temperature (Costill & Saltin, 1974), osmolality (Brouns, Senden, Beckers & Saris, 1995) and pH (Hunt & Knox, 1968) have been investigated as possible regulating factors of gastric emptying. However, none appear of greater importance than the volume or energy content of the ingested solution.

### *Energy density*

Water empties rapidly from the stomach. As CHO is added to the solution, typical of sports drinks, the energy content of the solution is increased and the rate of gastric



emptying is slowed. Studies investigating the ingestion of equivalent volumes of solutions, have reported that drinks containing a CHO content of 2.5% or less empty at approximately the same rate as water. Increasing the concentration of CHO in the solution to 4-5% has been shown to result in a small but significant slowing of gastric emptying (Costill et al., 1974). Drinks which have a CHO content of 6 % and above have been shown to slow gastric emptying. Houmard et al. (1991) reported that the modality of exercise had no impact on gastric emptying. The rate of gastric emptying was the same when participants ingested large volumes ( $10 \text{ ml} \cdot \text{kg BM} \cdot \text{h}^{-1}$ ) of a 7 % CHO solution, whilst running or cycling at 75 %  $\dot{V}O_2\text{max}$  (Houmard, Egan, Johns, Neuffer, Chenier & Israel, 1991).

In a recent study, it was suggested that gastric emptying can be increased by including a mixture of CHOs within the ingested solution. For example, adding fructose to a glucose solution has been shown to increase fluid delivery compared to a glucose only solution (Jeukendrup & Moseley, 2008). The mechanism by which gastric emptying is regulated by the energy content of the un-metabolised food is unknown (Maughan & Murray, 2001). However, that most sports drinks have a CHO concentration between 5 - 8% is reflective of the balance between maintaining a high rate of gastric emptying whilst delivering adequate concentrations of CHO to the intestine.

### *Gastric volume*

The stomach is highly distensible with the ability to change its capacity from approximately 50 ml when empty to 1000 ml when full, with only minimal changes in gastric pressure (Maughan et al., 2001). The gastric volume is an important determinant of the rate of gastric emptying (Noakes, Rehrer & Maughan, 1991). The ingestion of different volumes of a dilute glucose solution (200, 400, 600, 800 and 1000 ml) was shown to influence the rate of gastric emptying, recorded 15-min following ingestion (Costill et al., 1974). The ingestion of 600 ml resulted in a greater rate of emptying in comparison to 400 ml, which emptied faster than 200 ml. However, when volumes above 600 ml were ingested there was no further benefit to gastric emptying rate. This suggests that there is a limit at which additional fluid ingestion results in no further benefit to gastric emptying (Noakes et al., 1991).

Studies which have employed a repeated drinking design suggest that the gastric volume and therefore the pattern of drinking during exercise will have a significant influence on the rate of both CHO and water delivery from any solution (Noakes et al., 1991). Thereby, refilling the stomach at regular intervals, ensures the volume of the stomach is kept high and faster rates of gastric emptying can be maintained (Rehrer, Brouns, Beckers, ten Hoor & Saris, 1990).

### **Carbohydrate absorption**

After leaving the stomach, the chyme enters the duodenum which is the first part of the small intestine which links the stomach with the jejunum. The duodenum receives bile from the gall bladder and pancreatic secretions, important in facilitating the absorption of fats and the digestion of protein.

The jejunum (approximately 2.5 m in length) is a long convoluted tube, characterised by strong, rapid peristaltic contractions which sweep along its length. Most of the absorption of nutrients (CHO, electrolyte and water) occurs in the jejunum region. Carbohydrate entering the small intestine must first be hydrolysed to its constituent monosaccharide before its absorption and utilization (Holdsworth & Dawson, 1964). Water is absorbed passively as a result of an osmotic gradient created between the intestinal lumen and interstitial space by the movement of glucose, sodium ( $\text{Na}^+$ ) and other nutrients.

The terminal digestion of CHO occurs at or on the surface of the intestinal epithelium (Holmes, 1971). Disaccharidases are located on the brush border and microvillous membrane (Crane, 1968). The brush border contains several disaccharidases which hydrolyse the end products of pancreatic  $\alpha$ -amylase digestion. Thus, maltase acts on maltose liberating glucose, sucrase on sucrose releasing glucose and fructose, and lactase hydrolyses lactose to glucose and galactose (Holmes, 1971).

The transport mechanisms involved in the absorption of monosaccharides from the intestinal lumen are located in the brush border of the epithelial cell. The absorption of glucose occurs mainly in conjunction with the active transport of  $\text{Na}^+$ . Dietary monosaccharides are transported across the brush-border membrane of enterocytes

(intestinal absorptive cells) by the Na<sup>+</sup>/glucose cotransporter SGLT1 (Dyer, Vayro, King & Shirazi-Beechey, 2003). Glucose and galactose compete for the same transport. Fructose is absorbed by a separate transport system (Holdsworth et al., 1964). Fructose is transported by a different protein-carrier, GLUT-5 (Kristiansen, Darakhshan, Richter & Hundal, 1997) which is not Na<sup>+</sup> dependent and is less rapidly absorbed than glucose (Fordtran, 1975).

Located in the intestinal mucosa is the second line of glucose detection. The uptake of glucose by the sodium glucose transporter 1 (SGLT1) causes the release of a neurotransmitter, most likely 5-hydroxytryptamine (5-HT) (Raybould, 1998). The release of 5-HT, stimulates receptors on the vagal afferent terminals penetrating the lamina propria of the brain. Glucoreceptors located in the intestine are believed to provide information involved in regulating several G.I processes. Glucose detection in the intestine may stimulate insulin release and secretory functions via vago-vagal reflexes. These in turn contribute to satiation, informing the brain on the nutritive value of specific foods (Berthoud, 2003).

The hepatic portal system is a system of blood vessels consisting of hepatic portal veins which carry nutrients absorbed from the intestine to the liver for processing. Glucoreceptors are also found in the wall of the hepatic portal veins. These glucoreceptors are innervated by vagal afferent fibres and are important in the sympathoadrenal response to hypoglycaemia (Hevener, Bergman & Donovan, 2000). Information from peripheral glucose sensors along the G.I tract converges in the nucleus solitarius of the caudal medulla of the brain. The information is used by the medulla to generate appropriate oropharyngeal and autonomic motor responses, or it is relayed to the hypothalamus and taste/visceral cortex via the lateral parabrachial nucleus in the pons (Berthoud, 2003).

The final section of the small intestine is the ileum (approximately 3.6 m in length) which provides a reserve absorptive capacity. The ileum terminates at the ileocecal valve, where the large intestine begins. The large intestine (approximately 1.6 m in length) consists of the cecum, appendix, colon and anal canal. The colon is the main part consisting of three relatively straight sections; the ascending colon, the transverse colon and the descending colon – and sigmoid colon which leads to the rectum and in

turn the anal canal. The large intestine receives the liquid by-product of digestion. The absorption of most water and electrolytes along its tract forms the solid consistency of faeces. Peristalsis is slower and less propulsive in the large in comparison to the small intestine. Food can remain in the large intestine from approximately 10 h to several days.

### **2.3 Exercise and gastrointestinal function**

Ingesting sports drinks or solutions during exercise aims to improve performance via the prevention of dehydration and/or hypoglycaemia. However, during exercise the ingestion of sports drinks can cause G.I complaints which may adversely affect performance. The incidence of G.I complaints during exercise is greater during running in comparison to cycling (Brouns & Beckers, 1993). Gastrointestinal discomfort is therefore an important consideration when investigating CHO ingestion and its impact on running performance.

Runners participating in endurance events frequently suffer from G.I symptoms, which can be classified as upper G.I or lower G.I (Appendix A). Common symptoms of G.I discomfort include abdominal pain, urge to defecate, diarrhoea, heart burn, nausea and vomiting (Van Nieuwenhoven, Brummer & Brouns, 2000). In a survey of 707 marathon runners who ingested CHO-E drinks during exercise it was reported that urge to defecate, both during and immediately after running, occurred in approximately a third of runners. In the same study there was a higher incidence of lower rather than upper G.I symptoms (Keeffe, Lowe, Goss & Wayne, 1984).

The precise aetiology of G.I symptoms associated with exercise is yet to be determined. Possible causes of G.I symptoms could be a reduction in G.I blood flow or a reduced gastric emptying rate. Using a cycle ergometer, increasing exercise intensity from 40 % to 80 %  $\dot{V}O_2$ max was shown to reduce fluid delivery. Fluid absorption (measured by the appearance deuterium oxide in blood plasma) was reduced at 40 %  $\dot{V}O_2$ max compared to at rest, suggesting that even moderate exercise can influence the G.I tract. Whether the increased exercise intensity caused a

reduction in gastric emptying or impaired fluid absorption at the intestine could not be determined (Maughan, Leiper & McGaw, 1990).

Studies using constant paced treadmill running at 70%  $\dot{V}O_2$ max have shown that ingesting a 13 % glucose, 0.3 % Na<sup>+</sup> solution had no influence on the rate of gastric emptying compared to the rates of gastric emptying reported at rest. In addition, the action of running did not affect the absorption of CHO, water or electrolyte in the small intestine (Fordtran & Saltin, 1967).

Gastric emptying has also been investigated during intermittent high intensity running. Both a dilute CHO solution and flavoured water solution emptied at similar rates during intermittent high intensity running (Gant, Leiper & Williams, 2007). However, in comparison to walking, high intensity running was shown to slow gastric emptying (Leiper, Nicholas, Ali, Williams & Maughan, 2005). This suggests that gastric emptying is affected by running intensity. These trials were performed in a warm environment and symptoms of G.I discomfort were not reported.

A study investigating the ingestion of CHO sports drinks during an 18 km run in a cool environment found no performance benefit compared to the ingestion of water. In addition the ingestion of sports drinks led to a higher incidence of both upper and lower G.I complaint. Furthermore, including caffeine in a sports drink was reported to have no further effect on either running performance or G.I complaint (van Nieuwenhoven, Brouns & Kovacs, 2005).

Despite its complexity the rate of gastric emptying has been shown to be reproducible when conditions are kept constant and individuals ingest the same solution on different occasions (Beckers, Rehrer, Saris, Brouns, Ten Hoor & Kester, 1991). The incidence and severity of G.I symptoms will be influenced by the training status, exercise intensity, hydration status of the athlete prior to performance, as well as the type and timing of food ingested before and during exercise (Van Nieuwenhoven et al., 2000).

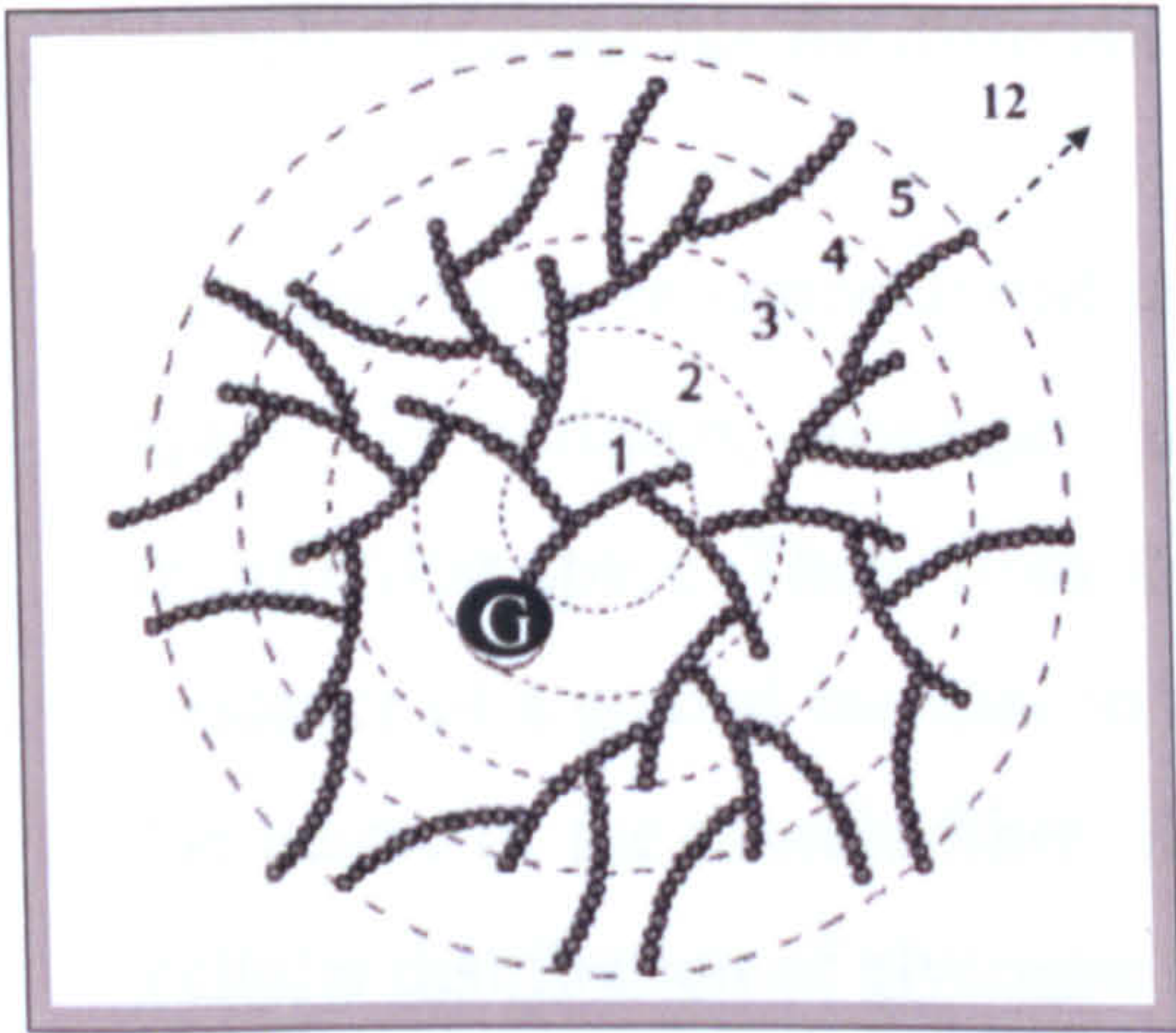
## 2.4 Carbohydrate metabolism

The completion of external mechanical work, i.e. muscular contraction, is a result of neural innervation of the working muscles by the central nervous system. Muscular contraction is powered by chemical energy released from the hydrolysis of adenosine triphosphate (ATP). However, the amount of ATP stored within the muscle cell is finite and is rapidly utilised at the onset of exercise. Thus, for muscular contraction to be maintained, an adequate supply of substrate is necessary for the continual resynthesis of ATP by the working muscles. The main energy sources for ATP resynthesis during aerobic exercise are glucose, glycogen and fatty acids (triglycerides) (Gollnick, 1985). The proportion of CHO and fat metabolism is dependent upon numerous factors such as exercise intensity (Romijn et al., 1993), substrate availability (Gollnick, Piehl, Saubert, Armstrong & Saltin, 1972), training status (Bergman, Butterfield, Wolfel, Casazza, Lopaschuk & Brooks, 1999; Gollnick, Armstrong, Saubert, Piehl & Saltin, 1972) and sex (Tarnopolsky, MacDougall, Atkinson, Tarnopolsky & Sutton, 1990). It is important to note, however, that glycogen is the most important fuel for exercise between 60 - 100% of an individual's maximal oxygen uptake (Romijn et al., 1993; Saltin, 1972).

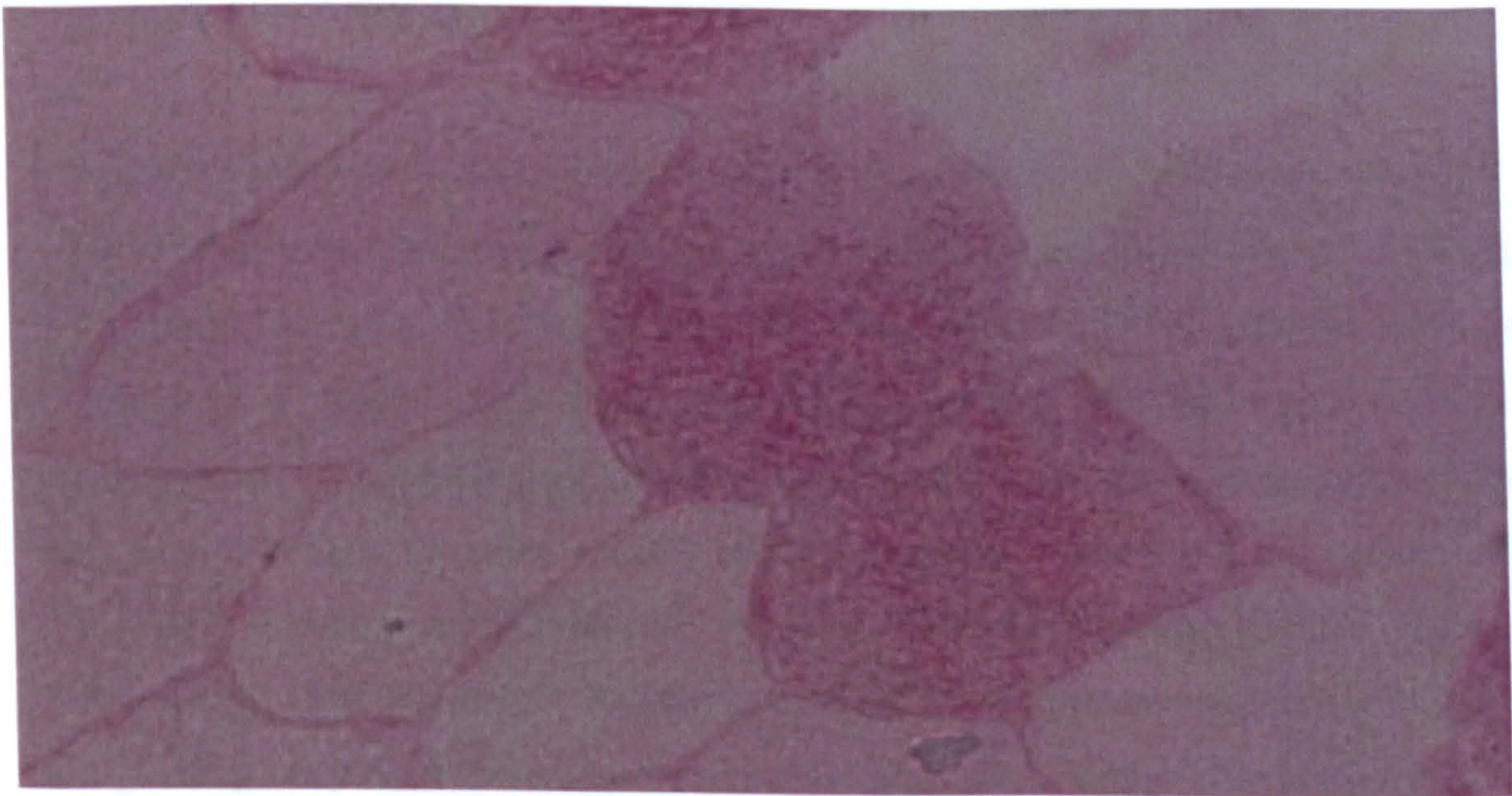
### Glycogen

Carbohydrate is available to the working muscles from blood glucose and muscle glycogen. Stores of glycogen are found in the liver (80 – 100 g) and muscles (250-500 g) and there is also a small energy reservoir of glycogen in the brain ( $3-4 \text{ mol} \cdot \text{g}^{-1}$ ) (Oz, Seaquist, Kumar, Criego, Benedict, Rao, Henry, Van De Moortele & Gruetter, 2007). Determined over thirty years ago, the structure of glycogen granule consists of type A chains and type B chains (Gunja-Smith, Marshall, Mercier, Smith & Whelan, 1971). Both chains are uniform in length with a mean value of thirteen glucose residues. The A chains are not branched (1-4 glycosidic bonds), whereas the B chains are branched, each with two branching points (1-6 glycosidic bonds) which create either A or B chains. There are 4 glucose residues between branches and a tail after the second branch in the B chains. Glycogen phosphorylase can only work on A chains, as the tail of the B chain is too short ( $\sim 4$  glucose residues, the limit of phosphorylase action). As a consequence of the degree of branching ( $r = 2$ ), the number of chains on any tier

is twice that of the previous one. Therefore, the glycogen molecule is organised into a spherical shape and arranged into concentric tiers, with every A chain in the most external tier (Figure 2.1). The arrangement of glucose into the glycogen polymer is a very efficient way of amassing large quantities of cytoplasmic glucose, without causing a significant change in cell osmolality (Melendez-Hevia, Waddell & Shelton, 1993). Glycogen can be formed directly in the liver by ingested glucose or through gluconeogenic pre-cursors such as lactate, alanine and pyruvate (Katz & McGarry, 1984).



**Figure 2.1.** Glycogen structure, adapted from Melendez (1993). The molecule has a spherical shape with 12 concentric tiers in the full molecule (only 5 are shown in the diagram). Glycogenin (G) the protein primer for glycogen synthesis is located at the core of the granule.



**Figure 2.2:** Muscle fibre type stained for glycogen. The “pinkest” cells are type 2 skeletal muscle fibres and the “whiter” cells are type 1 muscle fibres of the *vastus lateralis*. The fibres stained more pink, show a greater concentration of glycogen. Picture displayed with permission from Dr. C. Shaw, Birmingham University.

Recent investigations into glycogen metabolism have used descriptive transmission electron microscopy methods to quantify the distribution of skeletal muscle glycogen at a sub-cellular level (Marchand, Chorneyko, Tarnopolsky, Hamilton, Shearer, Potvin & Graham, 2002). Glycogen metabolism is believed to be regulated by intracellular compartmentalization (Prats, Helge, Nordby, Qvortrup, Ploug, Dela & Wojtaszewski, 2009). Glycogen storage has been reported in five topographically different locations (Friden, Seger & Ekblom, 1989), with each different glycogen pool probably responsible for different functions within the cell (Prats et al., 2009).

Glycogen is more concentrated in the sub-sarcolemmal space than in the myofibrillar space. In addition, glycogen particle volume was found to be greater in the myofibrillar space. Thus, from the transmission electron microscopy analysis there is evidence of a graded increase in single glycogen particle size from the sarcolemma to the centre of the muscle fibre. In addition, it is reported that differences in the sub-cellular distribution of glycogen between fibre types, with single particles of glycogen being bigger in type II fibres and the proportion of intra-myofibrillar glycogen greater in type I fibres. (Marchand et al., 2002).

Single glycogen particles exist in the muscle on a continuum, with sizes ranging from 10 to 44  $\mu\text{m}$  in diameter (Marchand et al., 2002). In the initial stages of glycogen formation, the granule is small with low CHO content. These granules have been described as proglycogen. When granules mature and gain more glucose residues (increase CHO content) they become larger and are termed macroglycogen. These molecules contain the same amount of protein but up to five times more glucose than the largest proglycogen molecule (Shearer, Wilson, Battram, Richter, Robinson, Bakovic & Graham, 2005). Shearer et al. (2005) propose glycogenin (GN-1) as the regulating enzyme responsible for glycogen granule formation. Shearer et al. (2005) found GN-1 was inactivated following a cycle to exhaustion at 70 %  $\dot{V}O_2$  peak, and five 30-s sprints to deplete muscle glycogen stores. However, post exercise, when the cyclists were fed  $75 \text{ g} \cdot \text{h}^{-1}$  CHO, GN-1 activity doubled after 30-min and GN-1 mRNA expression more than doubled 2 h into recovery. A 70% increase in GN-1 protein levels at 5-h post exercise suggests that a portion of the GM-1mRNA was translated into functional protein. It was concluded that the GN-1 mRNA levels likely



acted to replenish the GN-1 stores and subsequent glycogen regeneration. Having a free self-glycosylating protein present in skeletal muscle during exercise could be problematic, competing with glycolysis for glucose residues, which is why this enzyme may become inactivated during exercise (Shearer et al., 2005).

Post exercise, patterns of glycogen metabolism have been quantified by particle number, diameter and volume, and compared between sub-cellular locations using transmission electron microscopy. These changes were mapped for 48 h following a bout of glycogen depleting exercise at moderate exercise intensity. It was reported that each glycogen granule appears to contain the full complement of proteins required to regulate its metabolism. While individual granules are regulated based on their size (CHO content), there are also varying degrees of granule stimulation depending on sub-cellular location. It appears muscle glycogen concentration regulates its synthesis and that this operates at the level of sub-cellular location and, within this, at the level of individual granules (Marchand, Tarnopolsky, Adamo, Bourgeois, Chorneyko & Graham, 2007).

## **2.5 Regulation of carbohydrate metabolism**

The provision of energy from CHO to the working muscles can be categorised into three main metabolic pathways: (1) glycolysis, the uptake and phosphorylation of glucose to glucose-6-phosphate by the working muscles; (2) muscle glycogenolysis, the breakdown of muscle glycogen to glucose-6-phosphate and; (3) glycolysis, the breakdown of glucose-6-phosphate to pyruvate.

### **Glucolysis**

#### *Glucose uptake*

To date, there are six known glucose transport proteins responsible for the facilitated transport of glucose across plasma membranes in humans (Gaster, Handberg, Beck-Nielsen & Schroder, 2000). For a review of purification and recombinant DNA techniques to identify the proteins involved in the facilitated diffusion of glucose see (Carruthers, 1990). In skeletal muscle GLUT-1 and GLUT-4 appear to be the key

proteins responsible for the facilitation of glucose transport across the sarcolemma. However, the GLUT-1 protein is not abundantly expressed in adult human muscle fibre. Therefore, GLUT-4, is the key protein in facilitated glucose transport (Gaster et al., 2000). Studies investigating glucose uptake and the associated transport proteins have predominantly been conducted in vitro using rat hind limb muscle. It has been reported that both the contractile activity of muscle and the action of insulin promotes the translocation of GLUT-4 from an intracellular storage site, in the cytoplasmic pool to the plasma membrane (Douen, Ramlal, Rastogi, Bilan, Cartee, Vranic, Holloszy & Klip, 1990). The effects of muscle contraction and insulin are additive to glucose transport, but act via separate mechanisms (Nesher, Karl & Kipnis, 1985), as insulin is not required for contraction induced glucose uptake (Ploug, Galbo & Richter, 1984). In addition, it appears that the actions of muscle contraction and insulin recruit two different intracellular pools of GLUT-4 for translocation (Douen, Ramlal, Cartee & Klip, 1990; Douen, Ramlal, Klip, Young, Cartee & Holloszy, 1989). The glucose uptake capacity of the cell is also dependent upon muscle fibre type. Type I fibres have been found to have a greater content of GLUT-4 protein in comparison to type II fibres (Henriksen, Bourey, Rodnick, Koranyi, Permutt & Holloszy, 1990). Thus as a result, type I muscle fibres exhibit higher glucose uptake in comparison to type II muscle fibres (Bonen, McDermott & Hutber, 1989). In a study on rats it was found that the activation of muscle glucose transport and GLUT-4 translocation in response to a sub-maximal or maximal insulin stimulus can be modulated by the glycogen content in fast twitch but not slow-twitch skeletal muscle (Derave, Hansen, Lund, Kristiansen & Richter, 2000). The uptake of glucose and its phosphorylation to glucose-6-phosphate (G-6-P) by the working muscles are the main regulatory steps in exogenous glucose utilisation.

### *Glucose phosphorylation*

Once the glucose has entered the muscle cell, it is either phosphorylated to glucose-6-phosphate (G-6-P) (catalyzed by the enzyme hexokinase) or stored by being incorporated into glycogen. The enzyme hexokinase is closely associated with the glucose transporters at the plasma membrane. This results in 80 % of glucose entering the cell being phosphorylated to G-6-P before entry to the cells cytoplasmic pool (Carruthers, 1990). The phosphorylation of glucose prevents it from leaving the muscle cell. An accumulation G-6-P in the cytosol has been shown to inhibit the

action of hexokinase (Katz, Sahlin & Broberg, 1991). Glucose uptake is therefore reduced as the content of glucose-6-phosphate of the muscle cell increases and increased as glycogen is degraded throughout exercise (Ivy, 1987). Thus, during the latter stages of prolonged exercise, the limiting step of glucose utilisation shifts from the phosphorylation of glucose to the transport of glucose into the muscle cell (Katz et al., 1991). The hexokinase reaction requires 1 molecule of ATP per molecule of glucose. The conversion of glucose to glucose-6-phosphate also ensures a concentration gradient for glucose across the cell membrane, down which transport can occur (Maughan, Gleeson & Greenhaff, 1997). The net transport of glucose always occurs in the direction of high to low glucose concentration, thus, this passive mechanism does not require the involvement of transport proteins (Carruthers, 1990).

### *Muscle glycogenolysis*

The degradation of glucose from muscle glycogen is called glycogenolysis. Muscle glycogen breakdown is catalyzed by the enzyme glycogen phosphorylase, which exists in two enzymatically interconvertible forms, phosphorylase b (inactive) and phosphorylase a (active). The activation of phosphorylase from its inactive to active form is regulated by phosphorylase-b-kinase.

Calcium released from the sarcoplasmic reticulum at the onset of muscle contraction activates phosphorylase-b-kinase (Danforth, 1965), which converts phosphorylase b to its active form phosphorylase a. Phosphorylase a works on the A chain of the glycogen granule (section 2.4) to produce glucose-1-phosphate, which in turn is converted to glucose-6-phosphate by the enzyme phosphoglucomutase (Maughan et al., 1997).

During intense exercise the rate of ATP utilisation in skeletal muscle can exceed ATP resynthesis, resulting in an accumulation of ADP (adenosine diphosphate) and AMP (adenosine monophosphate). To avoid a large accumulation of AMP within the cell, AMP is deaminated to inosine monophosphate (IMP) and ammonia (NH<sub>3</sub>) via the enzyme AMP deaminase (Hellsten, Richter, Kiens & Bangsbo, 1999).

In addition to calcium, increases in intramuscular concentrations of AMP, IMP during prolonged exercise (Sahlin, Broberg & Ren, 1989) have been shown to exert allosteric

effects on phosphorylase activity (Aragon, Tornheim & Lowenstein, 1980; Chasiotis, Sahlin & Hultman, 1982). In addition, phosphocreatine breakdown has been shown to influence muscle glycogen utilisation, indicating that inorganic phosphate (Pi) also factors in phosphorylase activity (Chasiotis et al., 1982).

However, the regulation of glycogenolysis is far more intricate than the regulation of phosphorylase b to its more active form a. Studies show that glycogenolytic activity can be slow, despite high Pi and extensive transformation of phosphorylase b to phosphorylase a (Ren & Hultman, 1989). In addition glycogenolysis continues during exercise, despite significant reductions in phosphorylase a (Cartier & Gollnick, 1985; Stanley & Connett, 1991). These findings suggest that other metabolic systems have important roles in a complex regulation glycogenolysis during exercise (Ren et al., 1989).

#### *Hormonal control*

The rate of glycogen breakdown is also under hormonal control. The primary hormone promoting glycogenolysis is adrenaline. Adrenaline is secreted from the adrenal medulla and binds to specific receptors on muscle cell membranes. The infusion of adrenaline has been shown to increase glycogen breakdown in resting (Chasiotis, Sahlin & Hultman, 1983) and exercising skeletal muscle (Jansson, Hjemdahl & Kaijser, 1986). For example, at rest the infusion of adrenaline was shown to result in almost complete transformation of phosphorylase to its active form (a) (Chasiotis et al., 1983). Also, the infusion of adrenaline into a single leg during sub-maximal two-legged cycle ergometer exercise was shown to significantly increase muscle glycogen depletion (Jansson et al., 1986). Following intense short-term muscle stimulation, rapid muscle glycogenolysis appears to occur predominantly in type II muscle fibres, even though both type I and type II fibres are recruited. When adrenaline stimulated glycogenolysis occurs, it is more prominent in type I muscle fibres (Greenhaff, Ren, Soderlund & Hultman, 1991), thus suggesting that adrenaline stimulated glycogenolysis is more important in type I muscle fibres.

### *Glycolysis*

Glycolysis is the conversion of G-6-P to pyruvate via a series of chemical reactions. The reaction occurs in the cytoplasm of the cell, without the use of oxygen and is therefore considered an anaerobic process. The enzymes governing the rate of the glycolytic flux are believed to be phosphofructokinase (PFK) and pyruvate kinase (PK). Specifically, G-6-P is converted to fructose-6-phosphate (F-6-P) by the enzyme phosphoglucose isomerase. The F-6-P is then phosphorylated to 1,6-diphosphate (FDP), this reaction also requires a phosphate group to be donated by ATP and is catalysed by PFK. The PFK reaction is the first opportunity for regulation at a point which will affect the metabolism of both glucose and glycogen and is considered the rate-limiting reaction in glycolysis (Maughan et al., 1997). The activity of PFK is low in resting muscle and is inhibited by ATP, PCr,  $Mg^{2+}$ , hydrogen ions, cyclic AMP and  $K^+$ . Conversely, PFK is activated by numerous factors such as  $P_i$ , AMP, F6-P,  $NH_4$  and a low ATP to ADP ratio that is induced by exercise. The end product of glycolysis is pyruvate.

When considering aerobic metabolism, pyruvate is oxidised to  $CO_2$  and  $H_2O$ , producing energy through the Krebs cycle and oxidative phosphorylation. A key enzyme in this process is pyruvate dehydrogenase which contributes transforming pyruvate into acetyl-CoA. It has been reported that ingesting  $8 \text{ ml} \cdot \text{kg BM}^{-1}$  of a 5.5 % CHO-E solution immediately before exercise increases the exercise-induced activation of muscle pyruvate dehydrogenase during the transition from rest to steady-state exercise (Tsintzas, Williams, Constantin-Teodosiu, Hultman, Boobis & Greenhaff, 2000). During exercise the intracellular accumulation of  $Ca^{2+}$ , ADP and a high NAD/NADH ratio stimulate the activity of key enzymes in the Krebs cycle and enzymes of oxidative phosphorylation. When muscle is at rest, high concentrations of ATP act to inhibit the activity of these enzymes.

When the rate of pyruvate production exceeds that which it can be taken into the Krebs cycle it can be converted to lactate. The enzyme lactate dehydrogenase catalyses the interconversion of pyruvate to lactate and can proceed in both directions. The conversion of pyruvate to lactate regenerates stores of NAD by the oxidation of NADH. This reaction maintains the balance between NAD and NADH so that

glycolysis can continue to supply energy. The accumulation of lactate in the cell, causes it to diffuse out and accumulate in the blood. As a consequence, hydrogen ions also pass out of the cell into surrounding cells and muscle interstitial fluid. The hydrogen ions lower pH and can interfere with cell function through inhibiting enzyme activity.

### *Hepatic regulation of glucose*

Normal blood glucose concentrations (euglycaemia) range between 4-5.5 mmol·l<sup>-1</sup> and are required for the optimal function of the central nervous system. As discussed previously, exercise causes an increased uptake of glucose from the blood. Thus, during prolonged exercise blood glucose concentrations fall and if not replenished can result in hypoglycaemia. Symptoms of hypoglycaemia include loss of co-ordination, muscle weakness and mental confusion and therefore are detrimental to exercise performance. Blood glucose concentrations can be maintained externally by dietary sources or internally by hepatic glucose regulation. At the onset of exercise, hepatic glucose production increases due to an increase in glycogenolysis and gluconeogenesis.

Hepatic glycogenolysis is activated at the onset of exercise by the actions of glucagon and adrenaline. These hormones stimulate glucose release by activating glycogen phosphorylase, while simultaneously inactivating glycogen synthase activity through a series of phosphorylation reactions (Suh, Paik & Jacobs, 2007). Evidence of glycogenolysis is evident by an exercise induced reduction in hepatic glycogen concentration (Nilsson & Hultman, 1973). Hepatic glycogen content has been shown to be higher in trained compared with sedentary males (Galbo, Holst & Christensen, 1975). In addition, when exercising at the same relative intensity hepatic glucose output was higher in endurance trained male athletes compared to untrained men (Kjaer, Secher, Bach & Galbo, 1987). Therefore, during endurance exercise, the release of glucose into the circulation is dependent upon the glycogenolytic activity and liver glycogen concentrations.

Gluconeogenesis is the synthesis of glucose from non-CHO sources and has been estimated to account for approximately 20% of glucose production at rest and during

moderate intensity ( $\sim 45\% \dot{V}O_2\text{max}$ ) cycle exercise 30-50 min in duration (Stanley, Wisneski, Gertz, Neese & Brooks, 1988). During prolonged exercise gluconeogenesis becomes increasingly important, converting glycerol, lactate, and amino acids into glucose in order to delay depletion of hepatic and skeletal muscle glycogen concentrations (Stanley et al., 1988). Tappy et al. (2000) review the liver's role in glucose homeostasis, detailing how glucose production is controlled by hormones and regulatory processes that occur within the liver (Tappy, Jequier & Schneiter, 2000)

### *Centrally governed*

As mentioned, muscular contraction is resultant of neural innervation of the working muscles by the central nervous system. Glycolytic flux has been shown to be independent of the oxygenation state and metabolic feedback in stimulated human skeletal muscle, but proportional to muscle activation (Conley, Kushmerick & Jubrias, 1998). Therefore, the control of glycolysis by a factor related to muscle activation is an example of forward control or open loop control (Conley et al., 1998). Muscle glycogen may have a signalling role, relaying information about the quantity of available substrate to the upper motor centres in the brain that control muscle contraction. One study investigated the influence of muscle glycogen concentration and exercise intensity on the rate of whole body CHO oxidation. It was reported that when subjects completed 1 h of low intensity exercise ( $45\% \dot{V}O_2\text{max}$ ) with high pre-exercise muscle glycogen concentrations the rates of whole body CHO oxidation was greater compared to exercising performed at moderate intensities ( $70\% \dot{V}O_2\text{max}$ ) with low muscle glycogen concentrations. Thus, the authors suggested that muscle glycogen concentration is above exercise intensity in the hierarchy of metabolic control (Arkininstall, Bruce, Clark, Rickards, Burke & Hawley, 2004). Muscle glycogen may have a role in the regulation of pace, i.e. energy expenditure during prolonged exercise. In one study, cyclist performed 2 h of sub-maximal exercise interspersed with sprints every 20 min, followed by a 1 h time-trial. It was reported that cyclists finished with virtually identical muscle glycogen concentrations despite starting exercise with significantly different glycogen concentrations i.e. high or low. The small intra-subject co-efficient of variation (10 %) in muscle glycogen concentration at fatigue led authors to suggest that each individual had their own set "end" glycogen concentration, which was achieved by "constraining" power output

over the time-trial (Rauch et al., 2005). The authors postulate that central processing appeared to calculate, then initiate the cessation of exercise to prevent absolute glycogen depletion. This intramuscular monitoring of the muscle glycogen concentration and its rate of decline has been initially termed the “glycostat” (Rauch et al., 2005).

Although the concept of a “glycostat” would fit with these observations, it is important to note that the study reported no physiological evidence that the muscle is releasing any chemical or neural message to the brain conveying its glycogen concentration. It is also important to note that the “glycostat” concept of pace regulation has been primarily championed by a single research group. One argument against the “glycostat” hypothesis comes from observations from marathon performances when athletes “hit the wall”. Under these conditions the endogenous store of CHO in the muscle are completely depleted and only fat is being oxidised for energy metabolism. If the “glycostat” were true then runners would have regulated their running speed as to prevent the complete depletion of their muscle glycogen. It would appear that other factors independent of the muscle glycogen concentrations are involved in the regulation of running speed.

In 2001 Pedersen and colleagues reported that muscle fibres produce and release the cytokine interleukin-6 (IL-6) into the circulation during exercise (Pedersen, Steensberg & Schjerling, 2001). A review by Pedersen et al. (2005) proposes that IL-6 and other cytokines, which are produced and released by skeletal muscles, should be named 'myokines.' Pedersen et al. (2005) speculate that myokines could represent the link from working muscle to other organs such as the liver. In addition it is suggested that myokines may also have a role in influencing mood, performance, and cognitive function during exercise (Pedersen & Febbraio, 2005). The myokine IL-6 may therefore be a candidate which researchers could explore as the linking messenger which relays information of endogenous stores of CHO in the muscle with the brain and thus impacting on self-selected exercise.

## **2.6 Pacing strategies: Regulating factors in the control of energy expenditure**

Pacing strategies are universal. Whether it be walking to work, jogging to catch a bus,



climbing stairs or running the Olympic marathon. It is, in fact, most probable that every active individual would have at some point adopted a pacing strategy to achieve their desired task or goal. Pacing strategy has been defined as the conscious or subconscious regulation of work output according to predetermined plan (Ansley, Schabort, St Clair Gibson, Lambert & Noakes, 2004). For endurance athletes, pacing strategies are employed to optimize performance during distance running (Albertus, Tucker, St Clair Gibson, Lambert, Hampson & Noakes, 2005), whilst avoiding irreparable damage to the physiological systems (Ansley et al., 2004). With regard to this, an athlete will spontaneously select and modulate their pace prior and during a race to avoid premature fatigue (Billat, Slawinski, Danel & Koralsztein, 2001). Considering the majority of sports involve running of variable intensity there is relatively little research literature on the topic (Foster et al., 2003).

#### *Constant vs. freely chosen speed*

The metabolic and physiological responses to prolonged (>90 min) constant load exercise (<75%  $\dot{V}O_2\text{max}$ ) have been extensively studied. In comparison, much less is known about the metabolic and physiological response to variable intensity exercise, in which work rate frequently fluctuates. The reasons for this are unclear. It has been proposed that it may be due to concerns that non steady state exercise will lead to inaccurate, non reliable estimates of substrate metabolism or that steady state conditions are common in sport (Palmer, Borghouts, Noakes & Hawley, 1999). In running, it is most likely that appropriate equipment, sophisticated enough to respond and record changes in running speed throughout exercise has been unavailable to assess variable intensity exercise in controlled laboratory environments. This is most certainly the reason why much research in this area is performed on cycle ergometers where cadence and power output can be constantly and accurately recorded and why studies into running have been dominated by prolonged steady state exercise. The metabolic and performance responses to constant load versus variable intensity exercise have been studied in trained cyclists (Palmer et al., 1999). In cycling competitions cyclists work over a range of exercise intensities. For example, cyclists may maintain a constant power output for prolonged periods of a race, interspersed with brief periods of high power output during breakaway sprints. In one study, cyclists were required to complete a set amount of work in 140-min, at either a

constant load or 5 x 20-min blocks of variable intensity exercise interspersed with 10-min blocks of constant exercise. After the 140-min subjects were asked to complete a 20-km time-trial in the fastest time possible. This was the first study to examine both the metabolic and performance responses to prolonged variable intensity or constant load exercise of the same average intensity. Oxygen uptake was remarkably steady throughout both constant load and variable intensity work and the cyclists did not perceive any difference in average effort between the two work loads. There was a tendency for a reduction in total muscle glycogen utilisation (16 %) during the 140-min variable intensity cycles compared with constant load exercise that produced the same work load (Palmer et al., 1999). Despite the similar whole body responses of  $\dot{V}O_2$ , heart rate, RPE and energy expenditure the evidence suggests that during the variable intensity exercise there may be glycogen sparing in the type 1 muscle fibres. This was evident through muscle biopsy analysis and high lactate concentrations reported during the variable intensity trial. The small differences in CHO metabolism had no significant effect on the subsequent 20-km time-trial performance (Palmer et al., 1999). Unfortunately no muscle biopsies were taken post time-trial and therefore glycogen concentration at the absolute end of exercise was not reported.

#### *Pattern of energy expenditure*

Observation of athletic performance during competition shows that sports records are performed with variable rather than constant pace running (Sandals, Wood, Draper & James, 2006). This even applies to distance running, where variations in running speed have been reported to have coefficients of variation of 1-5% (Billat et al., 2001). Indeed, in any event lasting more than 2 min athletes will have the opportunity to use the energy available from the cleavage of phosphocreatine (PCr) and glycolysis in a flexible manner (Fukuba & Whipp, 1999). It is therefore of interest to study the physiological responses to changes in running speed.

A common observation amongst accomplished, well trained and recreational athletes is that energy resources appear to be distributed over the duration of an event in order to allow a sprint finish (Foster et al., 2003). This is reinforced by both laboratory (Palmer et al., 1999; Rauch et al., 2005; Weltan, Bosch, Dennis & Noakes, 1998a; Weltan, Bosch, Dennis & Noakes, 1998b) and field (Billat et al., 2001; Sandals et al.,

2006) investigations that consistently observe an increase in power output over the final stages of exercise. It has been suggested that athletes may be engaging in monitoring processes that allows them to optimize the distribution of their metabolic resources (Foster et al., 2003).

Tucker et al. (2009) reviewed a hypothetical model of anticipatory regulation of performance. It was proposed that muscle activation is regulated by the brain during exercise to avoid achieving physiological disturbances which would be harmful to the body. Specifically, it was reported that the perception of effort (rating of perceived exertion: RPE) is generated as a result of numerous afferent signals during exercise and serves as a mediator of any subsequent alterations in exercise intensity. It is reported that RPE mediates feed forward and anticipatory regulation of exercise performance. Interestingly, the model incorporates both feed forward as well as feedback components. Knowledge of the exercise duration is used to set initial exercise intensity and in addition to generate a so called “template” for the rate of increase in the RPE. Thus, during exercise, afferent feedback from numerous physiological systems will result in the generation of a conscious RPE (which can be recorded), which is continuously matched with the sub-conscious “template” by altering muscle activation throughout exercise (Tucker, 2009).

With respect to metabolism, if a runner selects a speed which is too fast they will fatigue quickly and not finish the race. However, if they run slowly they will not achieve their best performance. Thus during exercise, the athlete constantly alters their energy expenditure per unit of time with respect to a “end point” (Ulmer, 1996). The pattern of energy expenditure is somewhat general and does not appear to evolve greatly with repeated performance (Foster et al., 2003).

### ***Teloanticipation***

Observations from variable intensity exercise, highlight a key question in the physiology of running performance i.e. “what determines the self-selected running speed during exercise? ”. A study investigating pacing strategies in cycling reported that providing cumulative mismatching feedback on the completed distance during a 20 km time-trial did not alter the self-selected power output or time to complete the performance ride (Albertus et al., 2005). It was concluded that factors unrelated to the

distance feedback to the cyclists were responsible for pacing strategy. Instead, it has been reported that the body has the ability to feedback the intensity of the metabolic rate to the motor control system (Ulmer, 1996). An interesting model which offers an explanation for the self-selection of running speed during exercise is called teloanticipation. Prior to exercise, analysis of previous experience, via central nervous system feed forward control, pre-sets the exercise intensity. This integrative process, believed to occur at the level of the subconscious, culminates in the selection of a specific workload or exercise intensity and has been coined teloanticipation (Albertus et al., 2005). However, if it were just prior experiences that influenced exercise behaviour, performance would be easy to predict. Instead it appears that teloanticipation is complex and the central nervous system processes various factors before selecting intensity and regulating energy expenditure during exercise. These components can be categorised as intrinsic or extrinsic. Intrinsic factors include all physiological factors that underpin the ability of the runner to produce and maintain a given running speed through the duration of an event. Thus, the physiological determinants of endurance running performance are reviewed in section 2.7.

Extrinsic factors are numerous, but for running races knowledge of the task (experience of the race distance or knowledge of the course) and environmental factors such as temperature, wind and humidity will be critical. A recent study reported that increasing the ambient temperature from moderate (~ 14 °C) to hot (~ 31 °C) had no influence on the repeated performance of a 15 min running time-trial. The time-trial followed a 90 min run at constant speed on a motorised treadmill (Tyler & Sunderland, 2008). Interestingly, the authors reported there were no significant differences in any physiological responses between the ambient and hot conditions, which is perplexing given the additional thermoregulatory strain when exercising in the heat (Burke, 2001).

### *Interoception*

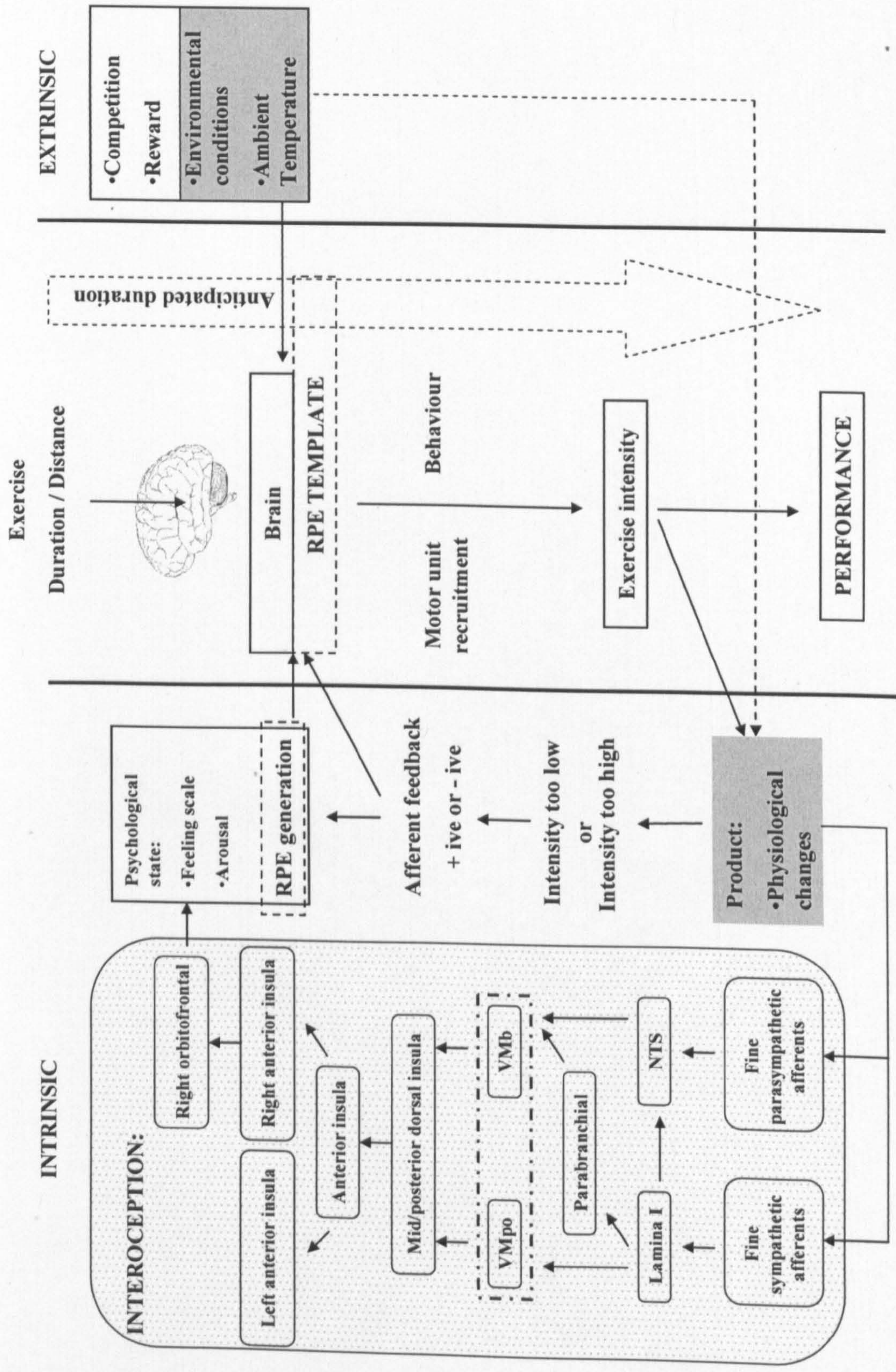
Feelings that we perceive from our bodies include temperature, pain, itch, tickle, sensual touch, muscular and visceral sensations, vasomotor flush, hunger, thirst and others related to the bodies state (Craig, 2002). Interoception functions through the lamina I spinothalamocortical system and acts as a homeostatic afferent pathway that conveys signals from small diameter primary afferents that represent the physiological

status of all tissues of the body. Thus, interoception constitutes the basis for subjective evaluation of one's condition, that is, "how you feel". These feelings have not only a sensory, but also an affective, motivational aspect (Craig, 2002). In relation to exercise, interoception may have an important role in governing the selection of running speed.

Several studies have investigated the psychological response to CHO supplementation during prolonged exercise. In one study, participants completed a 2 h cycle at 70 %  $\dot{V}O_2$ max either ingesting water or a 6.4 % CHO-E solution immediately before ( $5 \text{ ml} \cdot \text{kg BM}^{-1}$ ) and at every 15 min thereafter ( $2 \text{ ml} \cdot \text{kg BM}^{-1}$ ) (Backhouse et al., 2005). It was reported that pleasure initially improved and was subsequently maintained in the CHO-E trial, in contrast to a decline reported when water was ingested. Ratings of perceived exertion increased over the 2 h cycle, but were lower at 75 min into exercise when the CHO-E solution was ingested. In running, the effects of ingesting a CHO-E solution on affective states and RPE were assessed during prolonged intermittent high-intensity exercise (Backhouse et al., 2007). Participants consumed either a 6.4% CHO-E ( $0.6 \text{ ml} \cdot \text{kg BM} \cdot \text{h}^{-1}$ ) or an artificially sweetened placebo solution immediately before ( $8 \text{ ml} \cdot \text{kg BM}^{-1}$ ) and every 15 min ( $3 \text{ ml} \cdot \text{kg BM}^{-1}$ ) during exercise. In addition to the metabolic benefits, CHO ingestion during prolonged high-intensity exercise was reported to elicit enhanced perceived activation that may impact upon task persistence and performance (Backhouse et al., 2007). Considering the concept of interoception, the results of Backhouse et al. (2005, 2007) highlight the importance of assessing not only "what," but also "how" a person feels.

Figure 2.3 is a schematic model combining the concepts of teloanticipation, interoception and an anticipatory RPE on self-selected exercise behaviour/ selection of exercise intensity (Albertus et al., 2005; Craig, 2002; Tucker, 2009). Intrinsically, the organisational chart of interoception is listed. Small diameter afferents that innervate tissues in parallel with sympathetic afferents provide input into the lamina I. The lamina I are small diameter afferents that innervate tissues in parallel with parasympathetic efferents, provide input to the nucleus of the solitary tract (NTS). In mammals such activity is integrated in the parabrachial nucleus, which projects to insular cortex by way of the ventromedial thalamic nucleus (VMb). In humans, these

stimuli are re-represented in the anterior insula on the same side of the brain. The parasympathetic activity is then re-represented in the left side (dominant) hemisphere, whereas the sympathetic activity is re-represented in the right (non-dominant) hemisphere. These re-representations provide the foundation for a subjective evaluation of interoceptive state which is forwarded to the orbitofrontal cortex where hedonic valence is represented in mammals (Craig, 2002).



**Figure 2.3.** Schematic model combining the concepts of teloanticipation, interoception and an anticipatory RPE on self-selected exercise behaviour / selection of exercise intensity (ventromedial thalamic nucleus; VMb, nucleus of the solitary tract; NTS, ventromedial nucleus; VM po).

## 2.7 Physiological determinants of endurance running performance

If runners want to achieve their personal best race performances, they must select a pace that they can maintain for most of the race and then be able to increase it as they approach the finishing line. There are several key physiological factors that appear to be fundamental to running success which will be covered in the following section.

### *Maximal oxygen uptake and Fractional utilisation*

An athlete's maximal oxygen uptake ( $\dot{V}O_{2\max}$ ) is determined by the respiratory and cardiovascular systems ability to deliver oxygen rich blood to the muscle and the muscles ability to utilise the oxygen delivered. The  $\dot{V}O_{2\max}$  represents the maximal rate that energy can be generated by aerobic metabolism. Thus runners with high  $\dot{V}O_{2\max}$  values should be able to run at faster speeds than runners with lower  $\dot{V}O_{2\max}$  values, assuming that they are equally well trained. However,  $\dot{V}O_{2\max}$  alone is not the best indicator of 'endurance fitness' *per se* because it is the ability to sustain a high percentage of  $\dot{V}O_{2\max}$  i.e.  $\% \dot{V}O_{2\max}$  that reflects the training status or fitness of an individual not simply how fast they can run.

There are numerous physiological factors that determine  $\dot{V}O_{2\max}$ . The most important factor determining  $\dot{V}O_{2\max}$  is the delivery of oxygen to the muscle (Richardson, Grassi, Gavin, Haseler, Tagore, Roca & Wagner, 1999). Athletes with high  $\dot{V}O_{2\max}$  values are usually reported to have high stroke volumes during exercise and hence large cardiac outputs. The higher the cardiac output the greater the delivery of oxygenated blood to working muscles. Cardiac output is increased when there is an increase in blood volume and even more so when the haemoglobin concentrations are optimum. It is well known that endurance training increases cardiac output, blood volume and the total amount of haemoglobin. Endurance training also increases the number of capillaries around the slow contracting, slow fatiguing muscle fibres (Type 1) and therefore helps accelerate the delivery of oxygen to the muscle cells. Within the muscle fibres the mitochondria density dictates the aerobic capacity of the cells because it is within the mitochondria that substrates are degraded aerobically which increases energy production (ATP turnover). Thus endurance training influences the



whole of the oxygen delivery system from the lungs to the mitochondria and the improvement in the process is reflected as an increase in  $\dot{V}O_{2\max}$ .

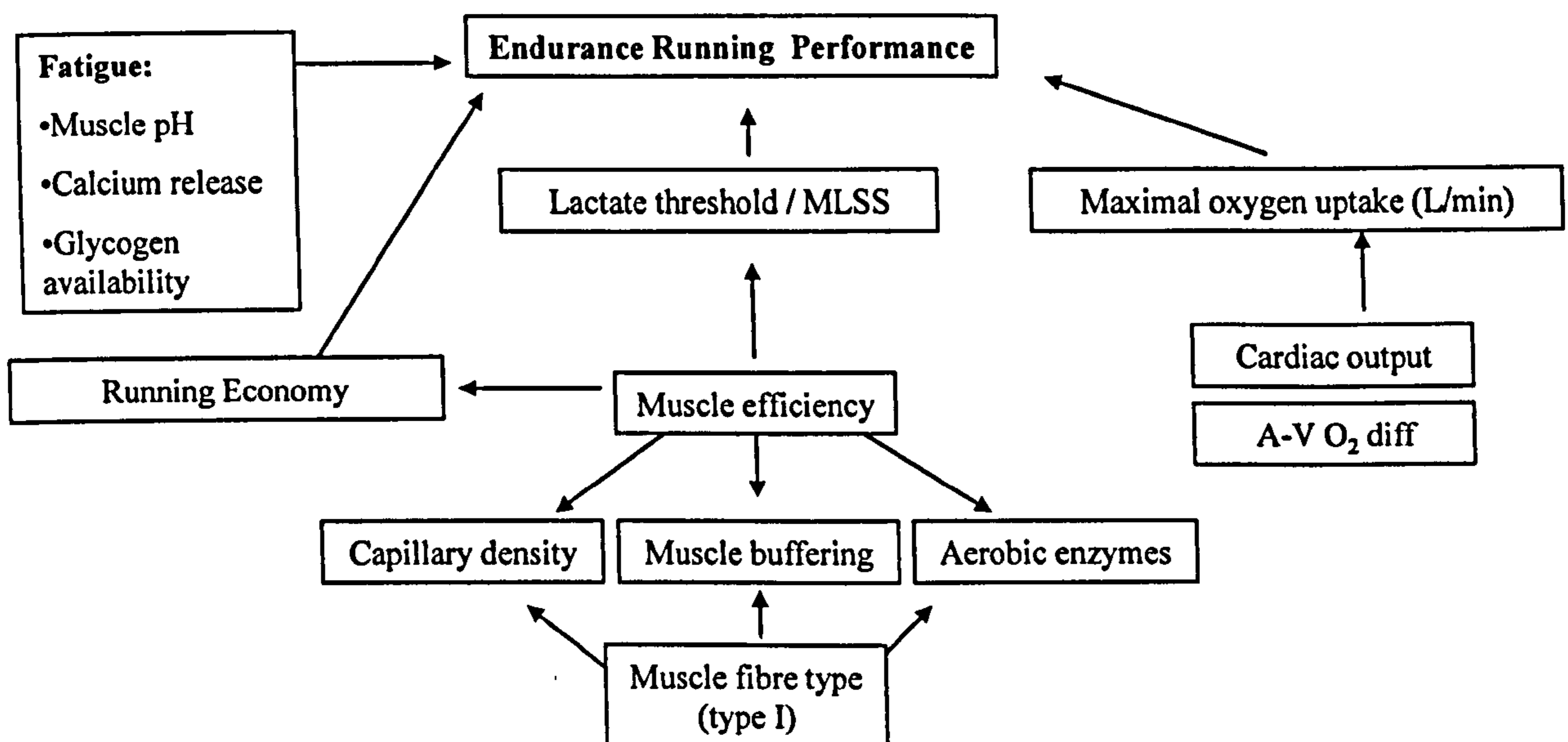
Success in middle and long distance events has been reported to be closely associated with athlete's with high maximal oxygen uptakes (Costill, Branam, Eddy & Sparks, 1971). For example, Ramsbottom et al. (1989) reported a strong correlation between maximal oxygen uptake ( $\dot{V}O_{2\max}$ ) and 5 km run performance ( $r^2 = 0.89$ ). Costill et al. (1973) reported a similarly strong correlation ( $r^2 = 0.91$ ) between  $\dot{V}O_{2\max}$  and times to complete a 16 km (10 mile) road race (Costill, Thomason & Roberts, 1973). Although possessing a high  $\dot{V}O_{2\max}$  appears key in determining endurance performance other factors which influence the physiological response to sub-maximal exercise are often better predictors of endurance performance (Williams, 1990) (Costill et al., 1973). For example, when runners possess the similar  $\dot{V}O_{2\max}$  values, the relative running economy was reported to be the discriminatory factor determining run performance during the 5 km time-trial (Ramsbottom, Nute & Williams, 1987) and during longer races (Costill et al., 1973; Farrell, Wilmore, Coyle, Billing & Costill 1979). In endurance events where the demand for energy is met almost entirely by aerobic metabolism, runners with a high  $\dot{V}O_{2\max}$  can meet the oxygen requirement by using a relatively low fraction of their maximum oxygen uptake ( $\% \dot{V}O_{2\max}$ ). Thus conversely, runners with a low  $\dot{V}O_{2\max}$  have to work at higher relative intensities to run at the same speed. In general the runners who are able to run at fast speeds are those who are able to run at a high fraction of their  $\dot{V}O_{2\max}$  (Maughan & Gleeson, 2004).

One of the physiological factors determining a runners ability to run at a high fraction of their  $\dot{V}O_{2\max}$  is the high proportion of type I and type IIa muscle fibres (characteristic of endurance athletes). The activity of enzymes involved in oxidative metabolism is high in type I fibres enhancing the ability of the muscle fibre to utilise oxygen and also utilise fat as a fuel. At the same running intensity an athlete with a greater proportion of type I fibres will oxidise a greater proportion of fat and thus spare muscle glycogen concentrations. Therefore, having a high  $\dot{V}O_{2\max}$  accompanied with the ability to exercise at a high fraction of that  $\dot{V}O_{2\max}$  without

excessive rates of CHO oxidation and lactate production are key for success in endurance performance.

The effects of accumulating lactate and associated acidosis of the muscle cell is discussed below in section 2.8. A major determinant of running performance is believed to be “lactate threshold” (Farrell, Wilmore, Coyle, Billing & Costill, 1979; Jones, 1998; Sjodin & Svedenhag, 1985; Tanaka & Matsuura, 1984). For the purpose of this review “lactate threshold” is defined as blood lactate concentrations equal to  $4.0 \text{ mmol}\cdot\text{l}^{-1}$ . “Lactate threshold” has been strongly correlated with 3 km ( $r^2 = 0.93$ ) (Grant, Craig, Wilson & Aitchison, 1997), 10 km, ( $r^2 = 0.86$ ) (Nicholson & Sleivert, 2001) and marathon ( $r^2 = 0.80-0.92$ ) (Noakes, Myburgh & Schall, 1990) performance. In a study investigating the physiological demands of a half-marathon, self-selected running speed was reported to have a greater correlation with running speeds at which blood lactate concentrations reached  $4.0 \text{ mmol}\cdot\text{l}^{-1}$  compared with  $\dot{V}O_{2\text{max}}$  values (Williams & Nute, 1983).

More recently, researchers have reported that the running speed at maximum lactate steady state is a more accurate predictor of endurance performance than running speed at lactate threshold “ $4.0 \text{ mmol}\cdot\text{l}^{-1}$ ” (Tolfrey, Hansen, Dutton, McKee & Jones, 2009). The maximal lactate steady state is the highest blood lactate concentration that can be maintained during a prolonged constant speed sub-maximal run. The running speeds recorded at maximal lactate steady state are usually slightly faster than those recorded for lactate threshold. Although maximum lactate steady state does appear to be a more accurate predictor of running performance, this test does have the disadvantage of being more time consuming and demanding on the athlete. For example determining maximum lactate steady state requires the athlete to complete a series of 30 min runs whereas “lactate threshold” can be estimated in a single visit to the laboratory. In summary, the relationship between blood lactate concentration and running speed reflects a host of underpinning physiological and metabolic characteristics of the individual. Figure 2.4 summarises the main physiological factors determining endurance running performance.



**Figure 2.4.** Schematic model of physiological determinants of endurance running performance (MLSS: maximal lactate steady state). (Figure adapted from physiological basis of sports performance, Hargreaves & Hawley, 2003).

## 2.8 Fatigue during prolonged exercise

Prolonged exercise can be described as the maintenance of continuous or intermittent physical exertion for durations equal to or greater than 30 min. In running events, this would equate to completing distances of 10 000 m upwards. Fatigue during prolonged exercise has been defined as the failure to maintain the required or expected force (Edwards, 1981). Thus in running, fatigue would be reflected as a reduction in running speed over the duration of an event.

Fatigue during prolonged exercise is complex and is influenced both by the physical and psychological makeup of an individual. Exercise usually induces acute fatigue, from which the athlete can recover relatively quickly depending upon the exercise load. Physiologically, fatigue can be classified into two categories, namely peripheral and central. Peripheral fatigue involves an impaired force generation by the working muscles as a consequence of impaired functioning of regions from the peripheral nerve down to the myofilament cross bridge formation (Gibson & Edwards, 1985).

During glycolysis,  $\text{NAD}^+$  is converted to NADH. If the NADH formed by glycolysis is not reoxidised to  $\text{NAD}^+$  at an equal rate, the rate of glycolysis and thus the energy supply of the cell will be reduced. During aerobic metabolism pyruvate undergoes completed oxidation to  $\text{CO}_2$  and water. However, when rates of glycolysis are high the availability of  $\text{NAD}^+$  becomes limiting and a potential cause of fatigue (Maughan & Gleeson, 2004). The reduction of pyruvate to lactate allows the regeneration of  $\text{NAD}^+$  and allows glycolysis to continue. However, a negative effect of the conversion of pyruvate to lactate is acidosis of the cell and can also be a potential cause of fatigue. When lactate accumulates within the muscle cell the associated hydrogen ions causes a reduction in the intramuscular pH. The fall in pH have been reported to be cause of fatigue by a number of mechanisms including inhibition of the glycolytic enzymes, inhibition of calcium release from sarcoplasmic reticulum and direct inhibition of cross bridge formation between actin and myosin (Erdogan & Kurdak, 1999).

However, during prolonged sub-maximal exercise the runner will slow down and stop without high blood or muscle lactate concentrations. Therefore the hydrogen ion is probably not a cause of fatigue under these circumstances. The most important variable linked with fatigue is muscle glycogen depletion. The association between glycogen depletion and fatigue was determined in the 60s, with the use of invasive muscle biopsy techniques. It was found that muscle glycogen concentrations are almost completely depleted at exhaustion. Importantly it was reported that increasing the CHO content of subjects' diet increased muscle glycogen concentrations and prolonged exercise performance (Bergstrom et al., 1967). Muscle glycogen depletion not only occurs in the type I muscle fibres but also in the type II muscle fibres (Tsintzas et al., 1996a).

Numerous studies reported that fatigue during prolonged exercise is associated with the depletion of muscle glycogen and that CHO ingestion during exercise can delay fatigue by maintaining CHO availability, CHO oxidation and under certain circumstance delaying the depletion of muscle glycogen (Coyle et al., 1986; Tsintzas & Williams, 1998). One cause of fatigue may be linked with the reduction in muscle glycogen around the muscle sarcoplasmic reticulum. Glycogen is the preferential fuel used for calcium release and uptake. As glycogen stores are depleted the compromised ability to release and uptake calcium impair muscle contraction, causing

fatigue (Stephenson, Nguyen & Stephenson, 1999).

Central fatigue is caused by impaired neural drive involving the brain, spinal cord and peripheral nerve (Gibson et al., 1985). Thus, a reduction in muscle recruitment may prevent permanent physiological damage to the muscle or tissue. The depletion of the bodies CHO stores during prolonged exercise results in hypoglycaemia which would contribute to central fatigue. However another central fatigue hypothesis that is still being explored is brain serotonin (5-HT). During exercise there is an increase in free tryptophan (pre-cursor of serotonin) which crosses the blood brain barrier. Serotonin has been the focus of research due to its role in depression and mood. The central fatigue hypothesis suggests that an increase in central ratio of serotonin to dopamine is associated with feelings of tiredness and lethargy, accelerating the onset of fatigue, whereas a low ratio would favour improved performance through the maintenance of motivation and arousal (Meeusen, Watson, Hasegawa, Roelands & Piacentini, 2006).

Studies have shown that training (Hardman & Williams, 1983; Legaz Arrese, Serrano Ostariz, Jcasajus Mallen & Munguia Izquierdo, 2005; Ramsbottom, Williams, Fleming & Nute, 1989) and nutrition are vital in delaying fatigue and achieving optimal performance during prolonged running.

### *Electrolytes*

During prolonged exercise the thermoregulatory response causes water and electrolytes to be lost from the body through sweating. The electrolytes lost in sweat include potassium, calcium, magnesium and chloride. However, the main electrolyte lost in sweat is sodium. For a review of the fluids and electrolytes lost during exercise see (Maughan, 1991). The concentration of sodium in sweat varies in each individual. However, the concentration of sodium in sweat has been reported to range between 20 to 80 mmol·l<sup>-1</sup>. The addition of sodium chloride to solutions ingested during exercise not only helps replace the sodium lost through sweating but also has other important functions. The addition of sodium (approximately 20 mmol·l<sup>-1</sup>) to a CHO solution has been reported to increase the voluntary drive to drink (Wilk & Bar-Or, 1996) and maintain the plasma sodium concentrations (Vrijens & Rehrer, 1999).

## 2.9 The effects of carbohydrate ingestion before and during exercise on metabolism

### *Blood glucose and serum insulin response*

The glyceamic index (GI) of a food is believed to be a sign of the rate of digestion and entry of glucose in the peripheral circulation. The blood glucose and insulin response is different when complex CHO or simple CHO are ingested. Such CHO can be classified based upon the relative rise in plasma glucose after the ingestion of a single CHO load, as compared to the same amount of glucose. Foods such as white bread, rice, cornflakes, and jam are classified as having a high glyceamic index (HGI) and therefore produce a high postprandial insulin and glucose response. Conversely foods such as lentils, figs and milk do not produce high postprandial blood glucose and insulin response and are therefore classified as low glyceamic foods (LGI) (Jenkins, Wolever, Jenkins, Josse & Wong, 1984; Jenkins, Wolever, Taylor, Barker, Fielden, Baldwin, Bowling, Newman, Jenkins & Goff, 1981).

However, the concentration of blood glucose reflects the balance of glucose entry into and removal out of the blood, into tissues of the body. A study by Schenk et al. (2003) investigated the plasma glucose kinetics in response to the ingestion of LGI and HGI breakfast cereals. Following an overnight fast, participants consumed either corn flakes or bran cereal containing 50 g of available CHO. The GI of the corn flakes ( $131.5 \pm 33.0$ ) was more than twice that of bran cereal ( $54.5 \pm 7.2$ ). However, the study reports that there were no significant differences in the rate of appearance of glucose into the plasma during a 180 min postprandial period. Interestingly, postprandial hyperinsulinemia occurred earlier following the ingestion of bran cereal, resulting in a 76 % higher plasma insulin concentration at 20 min ( $20.4 \pm 4.5$  compared with  $11.6 \pm 2.1 \mu\text{U}\cdot\text{ml}^{-1}$ ). The rise in plasma insulin was associated with a 31 % higher rate of disappearance of glucose when ingesting bran cereal compared to ingesting corn flakes between 30 min and 60 min post prandial. Thus the authors concluded that the low GI response to the bran cereal was not due to a lower rate of appearance of glucose in the plasma. Instead, the earlier postprandial hyperinsulinemia and earlier increase in the rate of disappearance of glucose reduced the increase in the plasma glucose concentration (Schenk, Davidson, Zderic, Byerley & Coyle, 2003).

Nevertheless, studies have used the GI classification of CHO to investigate the effect of consumption of HGI and LGI meals on exercise metabolism. The ingestion of a HGI meal has been reported to result in greater muscle glycogen storage compared to consuming a LGI meal. However, during a 30 min treadmill run at 70 %  $\dot{V}O_2$ max ingesting a LGI meal was reported to shift substrate oxidation towards fat and reduce the rate of muscle glycogen utilisation (Wee, Williams, Tsintzas & Boobis, 2005). Substrate metabolism during 3 h postprandial periods and during a 1 h run at 70 %  $\dot{V}O_2$ max was investigated following the consumption of either a HGI or LGI meal. In this study it is reported that fat oxidation was higher and CHO oxidation is lower during a 3 h resting postprandial period following the LGI meal compared to the consumption of the HGI meal. Although, no significant differences in substrate oxidation were reported during the 60 min run, blood plasma glucose concentrations were better maintained following the consumption of the LGI meal (Stevenson, Williams & Nute, 2005).

#### *Disposal of glucose load at rest*

The human body has huge storage potential for CHO. The storage capacity has been suggested to be approximately  $15 \text{ g} \cdot \text{kg BM}^{-1}$  and can accommodate an increase of approximately 500 g of CHO before net lipid synthesis begins contributing to increasing body fat mass (Acheson, Schutz, Bessard, Anantharaman, Flatt & Jequier, 1988). Studies have reported mixed results when investigating the resting response to ingesting large glucose loads. Felig et al. (1973) reported that only 15 g out of 100 g of glucose ingested escaped the splanchnic bed 3 h after ingestion. Therefore, the authors concluded that approximately 85% of the load remained in the liver (Felig, Wahren & Hendler, 1975). However, in contrast Katz et al. (1983) reported 65 g from an ingested load of 92 g was taken up by the muscles. Thus, two thirds of the initial glucose load had escaped the splanchnic bed (Katz, Glickman, Rapoport, Ferrannini & DeFronzo, 1983). Despite these discrepancies, direct measurement of liver glycogen has shown that the majority of CHO when provided orally (Nilsson et al., 1973) or intravenously (Nilsson & Hultman, 1974) is deposited in the liver.

A study by Chryssanthopoulos et al. (2004) examined the influence of a CHO meal on postprandial metabolic responses and skeletal muscle glycogen concentrations. Of note, is that this study used “normal” foods that would be commonly ingested in a typical western diet breakfast. The ingestion of  $2.5 \text{ g CHO} \cdot \text{kg BM}^{-1}$  provided by the meal resulted in a  $10.6 \pm 2.5 \%$  increase in muscle glycogen, 3 h post consumption. Further analysis, resulted in the authors estimating that approximately 20 % of the ingested CHO was converted into muscle glycogen and 12 % was oxidised. The remaining 67 % of the ingested CHO was unaccounted for. However, in line with Nilsson & Hultman (1974), the authors suggest that it may have been stored in the liver and/or remained in the G.I tract still undergoing digestion (Chryssanthopoulos et al., 2004).

A review by Jentjens & Jeukendrup (2003) reports that the amount, timing, type of CHO, rate of gastric emptying, intestinal absorption of the ingested CHO, hepatic glucose storage and output and muscle glucose transport and oxidation are all limiting factors in the rate of muscle glycogen re-synthesis. However, that the rate of glycogen re-synthesis can be increased up to three fold when glucose is infused intravenously following exercise compared to when CHO is ingested, suggests that it is the delivery of glucose to the peripheral circulation rather than uptake of glucose by the muscle that is main limiting factor (Jentjens & Jeukendrup, 2003).

One of the most recent areas of interest has been developing the type of CHO ingested post-exercise. Recent studies have reported that ingesting a high molecular weight (approximately  $500,000 - 700,000 \text{ g} \cdot \text{mol}^{-1}$ ) low osmolality ( $62-84 \text{ mOsmol} \cdot \text{kg}^{-1}$ ) glucose polymer solutions can increase gastric emptying (Leiper, Aulin & Soderlund, 2000) and subsequent muscle glycogen resynthesis (Piehl Aulin, Soderlund & Hultman, 2000). These benefits of high molecular weight solutions have been reported following glycogen depleting exercise and when compared to ingesting low molecular weight (approximately  $500-900 \text{ g} \cdot \text{mol}^{-1}$ ) high osmolality ( $124 - 350 \text{ mOsmol} \cdot \text{kg}^{-1}$ ) glucose polymer solutions. Stevens et al. (2007) investigated the influence of ingesting high molecular weight and low molecular weight solutions immediately after a cycle to exhaustion at  $75\% \dot{V}O_{2\text{max}}$  on a 15 min cycle time-trial performed 2 h later. In this study total work done in 15 min was reported to be significantly improved when cyclists ingested 1 l of fluid containing 100 g of high



molecular weight glucose polymer compared to the ingestion of the same volume and concentration of low molecular weight glucose polymer or CHO free flavoured water. The authors suggest that the improved performance was due to the high molecular weight glucose to improving the recovery of glycogen stores between exercise bouts. The mechanism by which the high molecular weight CHO improves glycogen resynthesis appears to be an increased rate of emptying from the stomach. Thus, increasing the available CHO for glycogen resynthesis (Stephens, Roig, Armstrong & Greenhaff, 2007).

### *Disposal of glucose during exercise*

When performing exercise after a prolonged or overnight fast the rate of blood glucose utilisation will be influenced by the intensity (Wahren, Felig, Ahlborg & Jorfeldt, 1971) and the duration of exercise (Ahlborg & Felig, 1982). Blood glucose becomes increasingly important to CHO oxidation as the duration of exercise increases. Specifically, when muscle glycogen concentrations are low, blood glucose can account for up to 75 % of total rate of CHO oxidation. Thus, utilisation of blood glucose has been shown to reach rates of up to  $1.2 \text{ g} \cdot \text{min}^{-1}$  during the later stages of prolonged cycle exercise at  $70\% \dot{V}O_2\text{max}$  (Coggan & Coyle, 1987; Coggan & Coyle, 1991). These findings highlight the value of maintaining blood glucose concentrations and the importance of blood glucose as an energy substrate during exercise which depletes endogenous glycogen stores. Numerous studies have shown that feeding CHO during strenuous exercise can successfully maintain blood glucose concentrations (for review see (Coyle, 1992)).

The effect of ingesting CHO-E solutions on glycogen degradation in type I and type II muscle fibres has been investigated during a run to volitional fatigue at  $70\% \dot{V}O_2\text{max}$ . In this study the ingestion of a  $5.5\%$  CHO-E solution  $8 \text{ ml} \cdot \text{kg BM}^{-1}$  immediately before and  $2 \text{ ml} \cdot \text{kg BM}^{-1}$  at 20 min intervals during the run was reported to reduce glycogen utilisation by 25% in type I fibres compared to when a placebo solution was ingested (Tsintzas et al., 1996a). However, when muscle glycogen utilisation was investigated during a 1 h treadmill run performed at  $70\% \dot{V}O_2\text{max}$  it was reported that ingesting a CHO-E solution during exercise had no

influence on muscle glycogen use when a pre-exercise meal was consumed 3 h before exercise (Chryssanthopoulos, Williams & Nowitz, 2002a).

The ingestion of CHO has also been investigated during high intensity cycle exercise approximately 1 h in duration. In this study cyclists ingested either a 6 % glucose solution or flavoured placebo at volumes equivalent to  $7 \text{ ml} \cdot \text{kg BM}^{-1}$  immediately before and  $3.5 \text{ ml} \cdot \text{kg BM}^{-1}$  every 15 min during exercise. It was reported that of the 84 g of glucose ingested, only 22 g appeared in the peripheral circulation. In addition, ingesting CHO had no effect on CHO oxidation, muscle metabolism, or time to exhaustion ( $83 \% \dot{V}O_2\text{peak}$ ) (McConell, Canny, Daddo, Nance & Snow, 2000).

Carbohydrate ingestion and its subsequent utilisation during exercise can be studied extensively using  $^{14}\text{C}$  (Peronnet, Adopo, Massicote, Hillaire-Marcel, Brisson & Guezennec, 1992) or  $^{13}\text{C}$  (Peronnet, Rheume, Lavoie, Hillaire-Marcel & Massicotte, 1998) labelled exogenous CHO. The quantity of CHO oxidised can be calculated from the ratio of the tracer released in the form of  $^{13}\text{CO}_2$  or  $^{14}\text{CO}_2$  to the tracer administered. The amount of tracer trapped within the body and the amount of tracer released in the form of  $\text{CO}_2$  but which comes from the oxidation of endogenous substrate should also be taken into account.

Although  $^{14}\text{C}$  has negligible background interference from endogenous stores of CHO (Peronnet et al., 1992), as  $^{13}\text{C}$  is a natural stable isotope which is used more commonly in studies, mainly due to ethical consideration. The main limitation of the  $^{13}\text{C}$  method is that the isotopic composition of  $\text{CO}_2$  arising from endogenous substrate oxidation can change during exercise independent of CHO ingestion and has also been shown to be influenced by the participants diet (Wagenmakers, Rehrer, Brouns, Saris & Halliday, 1993). Nevertheless  $^{13}\text{C}$  has been used extensively as a measure of exogenous CHO oxidation.

Adopo et al. (1994) used  $^{13}\text{C}$  labelling to measure exogenous CHO oxidation in response to ingesting different concentrations and mixtures of CHO during 2 h cycling at approximately  $60 \% \dot{V}O_2\text{max}$ . It was found that increasing the total amount of CHO ingested from 50 to 100 g (diluted in 500 ml of water) increased the total amount of exogenous CHO oxidation ( $37.8 \pm 2.2$  to  $58.3 \pm 8.1$  g). The total quantity

of CHO oxidised was lower when fructose was provided in the same quantities in comparison to glucose ( $32.2 \pm 1.2$  and  $45.8 \pm 2.6$  g). However, when 50 g of glucose and fructose were supplied in the same drink the total amount of CHO oxidised was similar to those observed when the drinks were ingested separately ( $39.5 \pm 4.8$  and  $34.1 \pm 1.5$  g, respectively). Thus, the cumulative amount of exogenous CHO oxidation was 21 % larger ( $73.6 \pm 6.6$  g) than when 100 g of glucose was ingested (Adopo, Peronnet, Massicotte, Brisson & Hillaire-Marcel, 1994). From these observations the authors suggested that the route for glucose and fructose absorption and metabolism maybe different. Therefore, combining different mixtures of CHO to sports drinks may be of considerable benefit to endurance performance. A more recent study has shown that supplying a glucose fructose solution in greater quantities (glucose  $1.2 \text{ g}\cdot\text{min}^{-1}$  and fructose  $0.6 \text{ g}\cdot\text{min}^{-1}$ ) result in higher rates of exogenous CHO oxidation with mean values reaching up to  $1.3 \text{ g}\cdot\text{min}^{-1}$ . The rate of oxidation was approximately 50 % higher when ingesting a glucose and fructose drink in comparison to glucose alone when cycling for 2 h at 50 %  $\dot{V}O_2\text{max}$  (Jentjens, Moseley, Waring, Harding & Jeukendrup, 2004).

Rowlands et al. (2005) investigated CHO oxidation following the ingestion of high molecular weight and low molecular weight CHO solution prior to exercise. In this study, cyclists exercised at approximately 64 %  $\dot{V}O_2\text{max}$  for 150 min. On three separate occasions either 600 ml of a 11.25 % high molecular weight ( $500\text{-}750 \text{ kg}\cdot\text{mol}^{-1}$ ,  $21 \text{ mOsm}\cdot\text{kg}^{-1}$ ), low molecular weight ( $8 \text{ kg}\cdot\text{mol}^{-1}$ ,  $110 \text{ mOsm}\cdot\text{kg}^{-1}$ ) solution providing  $1.8 \text{ g CHO}\cdot\text{min}^{-1}$  or plain water was ingested in the first 3 min of exercise, followed by 200 ml every 15 min. The study reported that CHO oxidation, determined by stable-isotope and indirect calorimetry methods, was not affected by CHO molecular weight. Instead, the results confirmed previous reports that that ingested glucose polymers can only be oxidized up to a rate of approximately  $1.0 \text{ g}\cdot\text{min}^{-1}$  during exercise (Rowlands, Wallis, Shaw, Jentjens & Jeukendrup, 2005).

## **2.10 The effect of carbohydrate ingestion on exercise performance**

Running is one of the most prevalent and popular modes of exercise for both recreational activity and competitive sport. A common dietary practice employed by both recreational and professional runners is the ingestion of CHO both before and during exercise in the pursuit of improved performance. This phenomenon is most likely a consequence of two factors. First, the large body of evidence which reports the benefit of CHO ingestion in a variety of exercise protocols (laboratory, field, intermittent and continuous exercise) and second, the promotion of CHO products by large business corporations in pursuit of profit. This section will review the available studies which have investigated the effect of CHO feedings on exercise performance.

### *Performance studies*

Tests to investigate CHO feedings on exercise performance can be classified into two categories. First, laboratory tests that use constant-pace exercise to fatigue for example in cycling (Maughan, Fenn & Leiper, 1989a) or treadmill running (Brewer, Williams & Patton, 1988b) assess endurance capacity. Second, those tests that require the completion of a pre-set amount of external mechanical work (cycling) (Widrick, Costill, Fink, Hickey, McConnell & Tanaka, 1993b) or distance (cycling or running) (Chryssanthopoulos, Williams, Wilson, Asher & Hearne, 1994d; Jeukendrup, Brouns, Wagenmakers & Saris, 1997a) in as fast a time as possible or ask athletes to complete as much work as possible in a specified time (Schabort et al., 1998) assess endurance performance.

The key studies investigating the influence of CHO ingestion on cycling capacity are displayed in Table 2.1. Studies investigating the effect on CHO ingestion on cycling endurance performance are reported in Table 2.2. Those studies which have investigated the influence of CHO ingestion on running endurance capacity and performance are reviewed in detail below:

Table. 2.1

Reference	Mode or sport	n =	Protocol (Intensity) (Duration)	Preparation	Fluid volume	Beverage	Performance
Ingestion before prolonged cycling exercise							
Felig et al. 1982	Cycle	19 males	60-65% $\dot{V}O_2$ max to exhaustion	Fasted	40-80 g·h <sup>-1</sup>	Glucose	ns
Coyle et al. 1983	Cycle	10 males	70-79% $\dot{V}O_2$ max to exhaustion	Fasted	121 g	Glucose Placebo	157 ± 5 min* 134 ± 6 min
Bjorkman et al. 1984	Cycle	8 males	68 ± 3 % $\dot{V}O_2$ max	Fasted	250 ml every 20 min	Glucose 7% Fructose 7% Water	137 ± 13 min* 114 ± 12 min 116 ± 13 min
Coyle et al. 1986	Cycle	7 males	70 ± 1 % $\dot{V}O_2$ max	Fasted	2 g·kg BM <sup>-1</sup> after 20 min 0.4 g·kg BM <sup>-1</sup> at 20 min intervals (total CHO = 406 g)	Glucose Placebo	4.02 ± 0.33 h* 3.02 ± 0.19 h
Coggan & Coyle 1989	Cycle	6 male	70 ± 1 % $\dot{V}O_2$ max	Fasted	3 g·kg BM <sup>-1</sup> (after 135 min)	Glucose 50% Placebo	205 ± 17 min* 169 ± 12 min

Time to exhaustion:

Reference	Mode or sport	n =	Protocol (Intensity) (Duration)	Preparation	Fluid volume	Beverage	Performance
<b>Ingestion before prolonged cycling exercise</b>							
Maughan et al. 1989	Cycle	6 male	70 % $\dot{V}O_2$ max	Fasted	100 ml Immediately before 10 min intervals	Glucose (36 %) Fructose (36 %) Glucose-Fructose (36 %) Glucose-electrolyte (4%) Water No drink	90.8 ± 12.4 min* 70.2 ± 8.3 min
Wright et al. 1991	Cycle	9 males	70 % $\dot{V}O_2$ max	Pre exercise Meal or Fasted	Meal : 5 g·kg BM <sup>-1</sup> 3 h before (CHO; 333 ± 26 g)	Meal + Glucose (8%) (5% Glucose 3% fructose) Meal + Placebo	289 ± 17 min** 237 ± 13 min*
				During exercise:	20 min intervals 0.2 g·kg BM <sup>-1</sup> (Total CHO 175 ± 11 g)	Placebo + Glucose (8%) Placebo +Placebo	266 ± 17 min* 201 ± 14 min
Sherman et al. 1989	Cycle	9 male 1 female	95 min @ 50 % $\dot{V}O_2$ max + 70 % % $\dot{V}O_2$ max 15 min intervals TT: 45 min @ 70 %	Pre ex meal 4 h	PLA CHO 45 g CHO 156 g CHO 312 g	Water	56.2 min 54.1 min 54.0 min 47.9 min*

(Bjorkman, Sahlin, Hagenfeldt & Wahren, 1984; Coggan & Coyle, 1989; Coyle et al., 1986; Coyle, Hagberg, Hurley, Martin, Ehsani & Holloszy, 1983; Felig, Cherif, Minagawa & Wahren, 1982; Maughan, Fenn & Leiper, 1989b; Sherman, Brodowicz, Wright, Allen, Simonsen & Dermbach, 1989; Wright et al., 1991)

Table 2.2.

Reference	Mode or sport	n =	Protocol (Intensity) (Duration)	Preparation	Fluid volume when consumed	Beverage	Performance
<b>Ingestion before high intensity (&gt;70% <math>\dot{V}O_2</math> peak) cycling performance approximately 60 min in duration</b>							
Bonen et al. 1981	Cycling	8	30 min 80% continuous	Glycogen depleted	565 ml 15 min before	a = 20% glucose b = no CHO 1.5 g·kg BM <sup>-1</sup>	Time to exhaustion: a = 26.6 min b = 29.8 min  No difference
El-Sayed et al. 1997	Cycling	8	60 min TT	Glycogen depleted	4.5 mg·kg <sup>-1</sup> ·min <sup>-1</sup> 25 min before	a = 8% b = PLA 0.4 g·kg BM <sup>-1</sup>	Distance covered: a = 41.5 km * b = 41.0 km  CHO improved performance
Foster et al. 1979	Cycling	8	5 or 60 min 80 or 100% $\dot{V}O_2$ max	3 h fast	300 ml 30 min before	a = 25% glucose b = water 1.1 g/kg	Time to exhaustion: 80% 100% a = 53 min * 360 s b = 43 min 345.6 s  CHO improved performance at 80%
Koivisto et al. 1981	Cycling	9	30 min 70% continuous	Controlled diet Overnight fast	250 ml 45 min before	a = 30 % Glu b = 30% Fruc c = PLA 1 g·kg BM <sup>-1</sup>	Time to exhaustion: a = 29.3 min b = 30.1 min c = 30.4 min

No difference

Table 2.2.

Reference	Mode or sport	n =	Protocol (Intensity) (Duration)	Preparation	Fluid volume when consumed	Beverage	Performance
Neufer et al. 1987	Cycling	10	60 min : 45 min 77% continuous 15 min TT.	12 h fast	400 ml 5 min before	a = 11.25% 8.25% Glu poly 3% Fruc b = PLA	Work in final 15 min: a = 175 kNm* b = 159 kNm  CHO improve performance-Greatest improvement was observed when a 200g CHO mean was provided 4 h prior to exercise + CHO feeding 5 min prior to exercise.
Snyder et al. 1993	Cycling	10	25 min 4 x 1.6 km TT Seaprated by 4.8 km At 80%	10 h fast liquid meal 4hr before	5 ml·kg BM <sup>-1</sup> 15 min before	a = 19.7% glu poly b = PLA 1 g·kg BM <sup>-1</sup>	TT performance: a = 25.2min b = 25.6 min
Anantaraman et al. 1995	Cycling	5	60 min 90% continuous Allowed decrease in Power output	4 h fast	300 ml immediately before, 300 ml every 15 min	a = 10% glucose b = PLA 2.14 g·kg BM <sup>-1</sup>	Total work a = 599 kJ* b = 560 kJ  CHO improved performance with pre- exercise feeding. No further benefit by supplying CHO at 15 min intervals.



Reference	Mode or sport	n =	Protocol (Intensity) (Duration)	Preparation	Fluid volume when consumed	Beverage	Performance
Ball et al. 1995	Cycling	8	50 min Followed by Wingate test	Overnight fast	2 ml · kg BM <sup>-1</sup> 10 min	a = 6% (Gatorade) b = PLA 0.90 g·kg BM <sup>-1</sup>	Peak, mean and minimum power were significantly improved with CHO.
Below et al. 1995	Cycling	8	60 min 50 min at 80% 10 min TT	Overnight fast	ad libitum 1330 ml · h <sup>-1</sup>	a = 6% (Gatorade) b = PLA	Performance in last 10 min a = 10.2 min b = 10.9 min  CHO improved performance
Jeukendrup et al. 1997	Cycling	19	60 min TT	Overnight fast	8ml·kg BM <sup>-1</sup> warm-up 2 ml·kg BM <sup>-1</sup> at 25, 50, 75% of total work done	a = 7.6% b = PLA + 5 g·l <sup>-1</sup> Fruc	Time to complete task a = 58.7 min* b = 60.2 min  CHO improved performance
Powers et al. 1990	Cycling	9	40 min 85% continuous Until 10% drop off	6 h fast	210 ml before and every 15 min	a = 6% Glu poly b = PLA (non-elec) c = elec PLA	Time to exhaustion: a = 39.2 min b = 35.8 min c = 40.2 min  No difference

Reference	Mode or sport	n =	Protocol (Intensity) (Duration)	Preparation	Fluid volume when consumed	Beverage	Performance
Mitchell et al. 1998	Cycling	8	60 min 7 x 12 min at 70% with 3 min rest sprint final 12 min	4-6 h fast	2.1 ml · kg BM <sup>-1</sup> during rest period	a = 5% Glu b = 6% (36% glu, 31% fruc, 33% sucrose) c = 7.5% (74% glu, 26% fruc) d = PLA	All CHO trials improved total amount of work done compared to PLA. Exact values not given. No difference between CHO trials.

(Anantaraman et al., 1995; Ball et al., 1995; Below et al., 1995; Bonen, Malcolm, Kilgour, MacIntyre & Belcastro, 1981; el-Sayed, Balmer & Rattu, 1997; Foster, Costill & Fink, 1979; Jeukendrup et al., 1997b; Koivisto, Karonen & Nikkila, 1981; Mitchell, Costill, Houmard, Flynn, Fink & Beltz, 1988; Neuffer et al., 1987; Powers et al., 1990; Snyder, Moorhead, Luedtke & Small, 1993).

## *The influence of increased dietary intake of CHO on running capacity*

Increasing the dietary intake of CHO can be achieved by the ingestion of high CHO foods, such as spaghetti, rice and bread or the ingestion of confectionary (Brewer et al., 1988a). It is important to note that increasing the intake of CHO can be difficult for many athletes as intense exercise *per se* has been reported to suppress appetite (Burns, Broom, Miyashita, Mundy & Stensel, 2007). However, confectionary offers a convenient option to increase the daily intake of CHO for athletes who are either too busy or do not possess appropriate nutritional information / skills. For example, increasing the dietary intake of CHO during recovery from exhaustive exercise was reported to benefit subsequent running capacity 3 days later. In this study, runners “normal” CHO intake was increased to 70 % by either using normal food such as bread and pasta or confectionary. In the control group an equivalent increase in energy intake was achieved by ingesting additional protein and fat. The runners who consumed normal CHO foods and confectionary improved run time to exhaustion at 70 %  $\dot{V}O_2\text{max}$  by 26 % and 23 % respectively. It is important to note that there was no improvement in running capacity when runners consumed the “control” isoenergetic diet (Brewer et al., 1988a).

Increasing dietary intake of CHO has also been reported to improve running capacity 22.5 h after completing a prolonged bout of exercise. In this study, male runners were asked to complete a constant paced run at 70 %  $\dot{V}O_2\text{max}$  for 90 min or until volitional fatigue, which ever occurred first. Runners then completed a run to fatigue at 70 %  $\dot{V}O_2\text{max}$  22.5 h later. During the 22.5 h recovery period runners either consumed a diet containing  $8.8 \pm 0.1 \text{ g CHO} \cdot \text{kg}^{-1} \text{ BM}$  or an isoenergetic diet in which additional energy intake was provided in the form of fat and protein. Those runners consuming a diet rich in CHO were able to replicate their first run performance. However, run capacity was reduced by approximately 15 min in the runners who consumed a low CHO, isoenergetic diet (Fallowfield & Williams, 1993).

It has been reported that a diet consisting of LGI CHO during recovery from prolonged running may offer additional benefits to running capacity. In a further study from the same laboratory, following the completion of a 90 min run at 70 %  $\dot{V}O_2\text{max}$ ,

runners consumed either a HGI or LGI CHO diet providing 8 g CHO · kg BM<sup>-1</sup>. Run time to fatigue at 70 %  $\dot{V}O_2$ max the following day was improved on the LGI diet (108.9 ± 7.4 min) in comparison to when runners consumed HGI foods (96.9 ± 4.8 min). The improved running capacity may have been a consequence of an increased rate of fat oxidation during exercise following the LGI diet (Stevenson, Williams, McComb & Oram, 2005).

### *The influence of increased dietary intake of CHO on endurance performance*

The effect of a high CHO diet on 30 km treadmill time-trial performance has been investigated in endurance trained runners. Following the completion of a 30 km time trial, runners were either assigned to either a control or CHO group. In the CHO group runners were provided with confectionary which increased their daily intake of CHO to 566 ± 29 g CHO · day<sup>-1</sup> for the first 3 days and 452 ± 26 g CHO · day<sup>-1</sup> for the remaining 4 days of recovery. In the control group runners consumed additional fat and protein to match the additional energy intake of the CHO group. Runners ran faster during the final 5 km following the high CHO diet. Although, 6 out of the 9 runners improved performance following the high CHO diet, there were no significant differences in time taken to complete the 30 km run between trials (Williams, Brewer & Walker, 1992a). Unfortunately, this study did not use a homogenous group of participants. In this study, 3 of 9 runners who failed to improve performance were female. Muscle fibre composition and enzyme activity are similar in male and female runners when matched according to their preferred competitive distance (Costill, Fink, Flynn & Kirwan, 1987). However, care must be taken when interpreting the results given the differences between men and women in running performance.

The time taken to complete a 10 km treadmill time-trial was investigated in six male runners following either a high or low CHO diet. In the low CHO trial, the participants diet was manipulated for 7 days to so that only 40 % of total energy intake came from CHO. The high CHO diet contained 55 % CHO for the first 4 days followed by 70 % CHO for the remaining 3 days. Running speeds were similar between trials and thus there were no differences in 10 km run performance between the high CHO (48.6 ± 2.7 min) and low CHO (48.6 ± 2.3 min) diets. The rate of CHO

oxidation was greater on the high CHO diet compared to the low CHO, although there were no significant differences in any metabolites measured during exercise i.e. blood lactate, blood glucose and plasma free fatty acids (Pitsiladis et al., 1996). It is important to note that the 10 km runs were performed at a gradient of 4 percent, which would have reduced the self-selected running speed. The authors state that the gradient of 4 percent was used to reduce muscle damage associated with treadmill running. However, there is no evidence that running on a treadmill induces greater muscle damage than running outside. In fact, muscle damage may actually be reduced when running on a level treadmill (or 1 percent gradient) due to the reduction in eccentric muscle contractions compared to running outside over uneven terrain.

Sherman et al. (1981) investigated the effect of altering the daily intake of CHO over 6 days on muscle glycogen concentrations and subsequent performance during a 20.9 km run. This study used three dietary interventions which were either 3 days low CHO (15 %) followed by three days high CHO (70 %), 3 days medium CHO (50 %) and three days high CHO (70 %) or 6 days on a medium CHO diet (50 %). It was reported that all diets increased muscle glycogen. However, there were no differences in 20.9 km run performance (Sherman et al., 1981). Unfortunately, in this study there was not a trial which provided CHO-E solutions during exercise. It is therefore unknown whether performance would have been improved if runners ingested additional CHO during the 20.9 km run.

In addition to exercise performance it is important to consider the importance of CHO ingestion during training. During eleven days of intensified training increasing the dietary intake of CHO from  $5.5 \text{ g CHO} \cdot \text{day}^{-1}$  to  $8.5 \text{ g CHO} \cdot \text{day}^{-1}$  allowed runners to maintain a higher training intensity and improved time to complete a 16 km run (Achten, Halson, Moseley, Rayson, Casey & Jeukendrup, 2004). Furthermore, increased daily CHO intake was associated with an improved mood state. An improved mood state during exercise is an important factor for elite athletes in preserving motivation. However, a common problem in health promotion is the high incidence of drop out when sedentary individuals become active. Therefore an improved mood state may therefore be just as important in novice individuals as it may increase their adherence to an exercise program.

*The influence of pre-exercise meals with and without CHO supplementation during exercise on running capacity*

The ingestion of pre-exercise meals providing approximately  $2.5 \text{ g CHO} \cdot \text{kg BM}^{-1}$  has been reported to increase muscle glycogen concentrations by approximately 11 % (Chryssanthopoulos et al., 2004). The foods provided in the majority of pre-exercise meal studies were mainly HGI CHO i.e. white bread, jam and orange squash. The consumption of HGI food has been reported to cause a rapid rise in blood glucose concentrations and concentrations of insulin are still elevated 3 h after ingestion (Chryssanthopoulos et al., 2004). High insulin concentrations, accompanied by an increased uptake of glucose by muscle at the start of exercise could potentially result in a rapid decline in blood glucose concentrations. Thus, it has been suggested that consuming meals with LGI food may result in more stable blood glucose concentrations before and during exercise. In addition, the ingestion of LGI CHO has been reported to shift substrate oxidation to towards fat metabolism as a result of the lower insulin response to these CHOs. Thus, endurance capacity could theoretically be improved by reducing the oxidation of the limited stores of muscle glycogen.

At present there are conflicting results regarding the effectiveness of HGI versus LGI CHO on endurance running capacity assessed by run time to fatigue at 70 %  $\dot{V}O_2\text{max}$ . Wee et al. (1999) report that providing isoenergetic meals ( $850 \pm 21 \text{ kcal}$ , 67 % CHO, 30% protein, and 3% fat) providing  $2.0 \text{ g}$  of either HGI or LGI  $\text{CHO} \cdot \text{kg BM}^{-1}$  did result in lower rates of CHO oxidation and an increase in fat oxidation in the LGI trial. However, performance between the HGI ( $113 \pm 4 \text{ min}$ ) and LGI trials ( $111 \pm 5 \text{ min}$ ) did not significantly differ (Wee et al., 1999). However, Wu & Williams (2006) did report that run time was significantly improved ( $108.8 \pm 4.1 \text{ min}$ ) when a LGI meal was ingested 3 h before exercise compared to an isoenergetic HGI meal ( $101.4 \pm 5.2 \text{ min}$ ). Similar to previous observations fat oxidation was increased during exercise when LGI foods were ingested. An interesting observation that may account for the differences in performance between the two studies may be the type of LGI food provided in the pre-exercise meals. The study by Wee et al. (1999) provided LGI food in the form of lentils, whereas Wu et al. (2006) used food more representative of the runners' normal diet (All bran cereal, apples and peaches).

Endurance running capacity has been reported to be improved when combining a pre-exercise CHO meal with the ingestion of CHO-E solutions during exercise. In one study runners either consumed a pre-exercise meal 3 h before exercise and a CHO-E solution during exercise, a liquid placebo meal 3 h before and CHO-E solution during exercise, or a liquid placebo meal and placebo solution during the run. Running capacity was assessed by run time to volitional fatigue at 70%  $\dot{V}O_2\text{max}$  on a motorised treadmill. Endurance capacity was reported to be improved by over 20 min when the CHO meal was ingested in combination with the CHO-E solution during exercise ( $147.4 \pm 9.6$  min) compared to when the CHO-E solution was ingested alone ( $125.3 \pm 7$  min). Furthermore, endurance capacity was significantly reduced on the double placebo trial ( $115.1 \pm 7.6$  min) (Chryssanthopoulos & Williams, 1997). In another study by Chryssanthopoulos et al. (2002) run time to volitional fatigue at 70%  $\dot{V}O_2\text{max}$  was assessed on three occasions. In this study, runners received a pre-exercise meal providing  $2.5 \text{ g CHO} \cdot \text{kg BM}^{-1}$  3 h before exercise and either a 6.9% CHO-E solution or water. On a separate occasion runners received a liquid placebo pre-exercise meal and water during exercise. The authors reported that a CHO meal before exercise improved endurance running capacity ( $111.9 \pm 5.6$  min) compared to the placebo trial ( $102.9 \pm 7.9$  min). However, the combination of the meal and CHO-E solution during exercise resulted in a further improvement in running capacity ( $125.1 \pm 5.3$  min) (Chryssanthopoulos et al., 2002b).

*The influence of pre-exercise meals with CHO supplementation during exercise on running performance*

Performed in a laboratory environment, ten endurance-trained males completed two 30 km runs on a motorised treadmill. Running speeds were controlled by a device which was held in the hand of the participant during the time-trial. In one trial runners consumed a placebo solution 4 h before exercise and a 6.9% CHO-E solution,  $8 \text{ ml} \cdot \text{kg BM}^{-1}$  immediately before and  $2 \text{ ml} \cdot \text{kg BM}^{-1}$  every 5 km. In the other trial, runners consumed a pre-exercise meal ( $2 \text{ g CHO} \cdot \text{kg BM}^{-1}$ ) 4 h before exercise and ingested water in the same volumes and timings as in the CHO-E trial. The total quantity of CHO consumed during the pre-exercise meal was greater (135 g) compared to the quantity of CHO consumed during the CHO-E trial (84 g). However,

the mean 30 km performance times were virtually identical for CHO-E ( $121.7 \pm 4.1$  min) and the pre-exercise meal trials ( $121.8 \pm 3.6$  min). Blood glucose concentrations were higher during the first 20 km of the CHO-E trial. Thus, it appears that the ingestion of the CHO-E drink immediately before and during exercise was sufficient to meet the demands placed upon CHO metabolism during the 30 km run (Chryssanthopoulos et al., 1994c). It is also important to note that blood glucose concentrations were maintained above  $4.5 \text{ mmol} \cdot \text{l}^{-1}$  during exercise following the pre-exercise meal.

#### *The influence of CHO intake during recovery from prolonged running on subsequent running capacity*

The ingestion of a 6.9 % CHO-E solution following prolonged constant-pace running has been reported to improve endurance capacity 4 h later. On one occasion runners ingested a 6.9 % CHO-E solution which provided  $1.0 \text{ g CHO} \cdot \text{kg}^{-1} \text{ BM}$  immediately after a prolonged run and again 2 h later. On a separate occasion, runners ingested equal volumes of a placebo solution. The run time during the first run was not different between the CHO-E and placebo trials. However, when runners ingested the CHO-E solution during recovery, running capacity was improved by  $22.2 \pm 3.5$  min compared to when the placebo solution was ingested (Fallowfield, Williams & Singh, 1995). Another study by Fallowfield et al. (1997) investigated the influence of increasing CHO intake on recovery and subsequent running capacity in male and female runners. In this study runners completed a 90 min run at  $70\% \dot{V}O_{2\text{max}}$  followed by a run to fatigue at  $70\% \dot{V}O_{2\text{max}}$  4 h later. Runners ingested approximately 1 L of either a 6.9 % or 19.3 % glucose solution immediately after and at 2 h into the recovery period after the first 90 min run. It was reported that no further improvements in running capacity were achieved when CHO intake was increased from  $1.0 \text{ g CHO} \cdot \text{kg}^{-1} \text{ BM} \cdot 2 \text{ h}^{-1}$  ( $58.5 \pm 5.2$  min) to  $3.0 \text{ g CHO} \cdot \text{kg}^{-1} \text{ BM} \cdot 2 \text{ h}^{-1}$  ( $57.6 \pm 6.3$  min) (Fallowfield & Williams, 1997).

Interestingly, it has been reported that ingesting a prescribed volume of CHO-E solution is more effective during recovery from prolonged exercise, compared to *ad libitum* ingestion. Following a 90 min treadmill run at  $70\% \dot{V}O_{2\text{max}}$ , runners rested



for 4 h before completing a second run at 70 %  $\dot{V}O_2$ max to volitional fatigue. In the 4 h recovery period runners were provided a 6.9 % CHO-E solution, which was either ingested *ad libitum* or provided to replace the equivalent BM losses occurred during the first run. Running capacity was improved by 16 % on the prescribed intake trial ( $69.9 \pm 9.1$  min) in comparison to *ad libitum* ingestion ( $60.2 \pm 10.2$  min). Although, the total volume of solution ingested was the same between trials, a significantly greater volume and amount of CHO was ingested during the final hour of recovery when fluid intake was prescribed (Wong, Williams, Simpson & Ogaki, 1998).

In a study using the same exercise model (90 min run at 70 %  $\dot{V}O_2$ max, 4 h recovery, 70 %  $\dot{V}O_2$ max run to volitional fatigue) the ingestion of a CHO-E solution was reported to be more effective in restoring endurance capacity compared to ingesting the same volume of a placebo solution. Despite, both trials achieving complete rehydration in the runners, run time was  $24.3 \pm 4.4$  min longer in the CHO-E trial ( $69.3 \pm 5.5$  min) compared to when placebo was ingested ( $45.0 \pm 4.2$  min) (Wong, Williams & Adams, 2000). However, there does not appear to be a dose dependent relationship between the quantity of CHO ingested during recovery and subsequent running capacity. A study by Wong and Williams (2000) reported that increasing the quantity of CHO ingested during a 4 h recovery period by 3-fold had no additional benefit to run capacity. In this study, runners ingested a 6.5% CHO-E solution providing 50 g of CHO immediately after a prolonged treadmill run on two occasions. Runners then ingested either a 6.5 % CHO-E solution (providing approximately 167 g CHO) or a placebo solution every 30 min at volumes equivalent to 150 % body mass losses occurred during the first run. The running capacity of the runners were no different following the ingestion of a CHO-E solution ( $56.9 \pm 8.1$  min) compared to when the placebo solution was ingested ( $65.4 \pm 7.8$  min) (Wong & Williams, 2000).

The ingestion of a CHO-E solution has also been reported to delay the onset of fatigue during a subsequent bout of prolonged sub-maximal running in a warm environment. In this study runners completed a run at 60 %  $\dot{V}O_2$ max for 90 min or until volitional fatigue. The mean ambient temperature was 35 degrees C, 40 % relative humidity. In the 4 h recovery period, before a second run to fatigue, runners ingested either a 6.9 % CHO-E solution or sweetened water solution. The solutions provided a volume

equivalent to 140 % of body mass losses occurred during the runners first run. It was reported that running capacity was improved by approximately 15 min during the second run to fatigue following the ingestion of the CHO-E solution ( $60.9 \pm 5.5$  min) in comparison to the ingestion of placebo ( $44.9 \pm 3.0$  min) (Bilzon, Allsopp & Williams, 2000). A subsequent study investigated the effect of ingesting different quantities of glucose during recovery on substrate storage and utilisation during recovery and subsequent exercise in a warm environment. Runners ingested 55 g of CHO provided in a 7.5 % CHO-E solution immediately following a run to fatigue at 60 %  $\dot{V}O_2$ max. The runners then ingested either a CHO-E solution or placebo solution during a 3 h recovery period. It was reported that glycogen resynthesis was greater during recovery and total CHO oxidation was increased when runners ingested the CHO-E solution (providing 220 g CHO). However, endogenous stores of glycogen were not spared and fat oxidation was suppressed during the second run (Bilzon, Murphy, Allsopp, Wootton & Williams, 2002). It is important to note that running capacity in the warm environment was limited by thermoregulatory incapacity of the runners rather than substrate availability. In addition, the importance of CHO ingestion post exhaustive exercise is dependant upon the timing of the next event. Few, prolonged running events requires the participant to repeat their effort within 24 h. However, these results of the studies above are relevant to ultra endurance events, for example the “marathon de sables” which requires runners complete approximately 5 marathon distances over 6 days.

### *The influence of fluid ingestion versus no fluid ingestion on running capacity*

In running, endurance capacity has been reported to be improved when runners were provided with water or a 7 % CHO-E solution in comparison to a no fluid trial. When asked to run at 85 % their of maximum heart rate, runners mean time to volitional fatigue were reported to be 56 min, 78 min and 102 min for the no fluid, water and CHO-E solution trials respectively (Macaraeg, 1983). Water ingestion has also been reported to improve running capacity at 70 %  $\dot{V}O_2$ max. In this study runners either ingested no fluid or ingested an initial bolus of water  $3.0 \text{ ml} \cdot \text{kg BM}^{-1}$  immediately before and  $2.0 \text{ ml} \cdot \text{kg BM}^{-1}$  at 15 min intervals during exercise. Run time to volitional fatigue was reported to be improved on the fluid replacement trial ( $103 \pm 12.4$  min)

compared to when no fluid was ingested ( $77.7 \pm 7.7$  min) (Fallowfield, Williams, Booth, Choo & Grows, 1996). To our knowledge no running studies have compared the influence of ingesting fluid versus no fluid ingestion on running performance.

### *The influence of CHO ingested in the hour before and during exercise on running capacity*

Few studies have investigated the influence of CHO feeding in the hour before exercise. This is due to fears that CHO ingestion will cause rebound hypoglycaemia and negatively effect performance. In one study, a group of female runners ingested 300 ml of fluid 45 min prior to exercise containing either 100 g of glucose, 100 g of fructose, saccharin (sweetener), or plain water. The running test involved a constant speed treadmill test at 80 %  $\dot{V}O_2$ max for 85 min or to volitional fatigue, whichever occurred first. The performance times for the glucose ( $63.9 \pm 8.5$  min) and fructose trials ( $61.9 \pm 8.3$  min) were improved compared to the placebo solution ( $52.2 \pm 5.5$  min). However, the best performance time was achieved on the water only trial ( $65.6 \pm 7.6$  min). In this study, the authors recommend fructose as a preferred sugar supplement as blood glucose concentrations were reported to be more stable in comparison to glucose. However, the authors questioned the value of pre-feeding CHO during high intensity exercise less than 85 min in duration (McMurray, Wilson & Kitchell, 1983). Nevertheless, these results are surprising given the intensity at which the runs were performed. For example, non-elite female runners would not be expected to run at such a high percentage of their maximal oxygen uptake for such long durations. This is highlighted by a similar study in which 11 male runners ran to volitional fatigue at 82 %  $\dot{V}O_2$ max on a motorised treadmill. In this study mean performance times were only approximately 10 minutes in duration. On three separate occasions the runners ingested 100 ml of water containing either 75 g of glucose, 75 g of fructose or flavoured water 30 min before exercise. All solutions were sweetened with aspartame to a similar level. The mean running capacity times reported were greater when ingesting glucose ( $644 \pm 261$  s) compared to the ingestion of fructose ( $611 \pm 227$  s) or water ( $584 \pm 189$  s) (Ventura, Estruch, Rodas & Segura, 1994).

A study by Chryssanthopoulos et al. (1994) report that run to exhaustion at 70 %  $\dot{V}O_2$ max was similar when runners ingested either a solution containing 75 g of glucose in 300 ml of water, or 300 ml of sweetened water (no CHO) 30 min before exercise. Blood glucose concentrations were 55 % higher at the beginning of the CHO trial compared with those recorded for the sweetened water (Chryssanthopoulos, Hennessy & Williams, 1994a). Although a transient decrease in blood glucose concentrations were reported at the start of exercise, this did not negatively affect running capacity.

Ingesting CHO-E solutions during the first hour of exercise has been reported to improve running capacity in comparison to ingesting water. In this study runners ingested approximately 850 ml of either a 5.5 % or 6.6 % CHO-E solution during the first hour of a run to volitional fatigue at 70 %  $\dot{V}O_2$ max. Each solution provided a  $47.8 \pm 2.0$  g and  $58.8 \pm 2.9$  g of CHO respectively. Water was ingested during the remainder of the run. During a third trial water only was ingested. The authors reported that running capacity was similar when runners ingested either the 5.5 % solution ( $124.5 \pm 8.4$  min) or 6.9 % CHO solution ( $121.4 \pm 9.4$  min). Both CHO trials improved running capacity in comparison to the water only trial ( $109.6 \pm 9.6$  min). However, differences in running capacity only reached significance between the 5.5 % CHO and water trial (Tsintzas, Williams, Wilson & Burrin, 1996b).

The ingestion of a 5.5 % CHO-E solution has also been shown to improve running capacity at 70 %  $\dot{V}O_2$ max when ingested before and during the run. In this study runners ingested  $8 \text{ ml} \cdot \text{kg BM}^{-1}$  of either a placebo solution or a 5.5 % CHO-E solution immediately before the start of the run and  $2 \text{ ml} \cdot \text{kg BM}^{-1}$  at 20 min intervals during the run. Run time to volitional fatigue was improved by approximately 30 min following the ingestion of the CHO-E solution ( $132.4 \pm 12.3$  min) compared to the ingestion of the placebo solution ( $104.3 \pm 8.6$  min) (Tsintzas et al., 1996a). The majority of time to fatigue protocols clearly demonstrated the benefit of ingesting CHO-E solutions during prolonged exercise. However, there applicability to running events is questionable given that no competitive events are designed specifically to run the competitor to exhaustion.

### *The influence of CHO ingested in the hour before exercise on running performance*

The ingestion of CHO-E solutions has been reported to improve 15 km run performance the heat. On three separate occasions runners ingested 1 L of fluid which contained either a 6 % or 8 % CHO or water 1 h before a 15 km self-selected run on a motorised treadmill. The authors reported that oxygen uptake and run time were not significantly different between trials over the first 13.4 km of the run. However, running performance during the final 1.6 km was significantly improved when ingesting both the 6 % and 8 % solutions in comparison to the ingestion of water (Millard-Stafford, Roskopf, Snow & Hinson, 1997). Unfortunately, in this study the time-trial protocol was intermittent. In addition, the solutions were ingested *ad libitum* whilst the runners were stationary during the collection of blood samples. Therefore, the volume of solution ingested by the runners was not representative of that which can actually be consumed whilst running.

### *The influence of CHO ingested immediately before and during on running endurance performance*

Williams et al. (1990) investigated the influence of drinking water versus two CHO based solutions (50 g glucose-glucose polymer or 50 g glucose-fructose polymer) on 30 km run performance performed on a motorised treadmill. In this study, runners ingested 1 L of experimental drink during each run and controlled running speed by means of a hand held device. Overall performance times for the water ( $129.3 \pm 17.7$  min), glucose ( $124 \pm 14.9$  min) and glucose-fructose ( $125.9 \pm 17.9$  min) were not different between trials. However the ingestion of both CHO solutions prevented the decrease in running speed which was observed over the final 10 km of the run when drinking water (Williams, Nute, Broadbank & Vinall, 1990). This study was one of the first to recognise the limitation of the runner having to interact with the treadmill console to change running speed during running performance tests. Although the hand held device advanced the testing method, it still involved manually pressing buttons to increase or decrease speed. In a unique study, the effect of drinking two different CHO-E solutions versus water was investigated on marathon running performance (42.2 km) under controlled laboratory conditions. On three occasions separated by 4 weeks runners either consumed  $3 \text{ ml} \cdot \text{kg BM}^{-1}$  of either water, a 6.9 % CHO-E

solution, or a 5.5 % CHO-E solution immediately before and 2 ml · kg BM<sup>-1</sup> at 5 km intervals during the run. The mean performance times were fastest when ingesting the 5.5 % solution (190 ± 3.9 min) compared to the ingestion of the 6.9 % solution (192.4 ± 3.3) or water (193.9 ± 5.0) (Tsintzas, Williams, Singh, Wilson & Burrin, 1995b). Unfortunately, few studies investigating effect of ingesting CHO-E solutions on endurance performance have included trials in which no fluid is ingested.

*The influence of CHO supplementation during exercise on endurance performance  
“real life” running events*

Outside of the laboratory, Tsintzas et al. (1993) investigated the effect of CHO supplementation on 30 km road race performance. Runners completed thirty, 1 km circuits while ingesting either a 5 % CHO solution or non-flavoured tap water immediately prior to the start of the race (250 ml), and every 5 km thereafter (150 ml). The time taken to complete the 30 km was significantly faster (128.3 ± 19.9 min) on the CHO trial in comparison to when water was ingested (131.2 ± 18.7 min). The speed of the runners slowed in the final 5 km of the 30 km on the water trial. This decline in running speed was not observed when the runners ingested CHO. Instead runners were able to maintain their chosen running speed up to the completion of the 30 km (Tsintzas et al., 1993).

Over a half marathon distance (21 km) the ingestion of CHO gel providing 1.1 ± 0.2 g CHO · kg BM<sup>-1</sup> was shown to have a negligible effect on performance. Runners were provided with *ad libitum* intake of either a flavoured placebo drink or water and the CHO gel (Burke et al., 2005). An interesting observation from this study was that runners ingesting the CHO gel suffered from G.I discomfort, which resulted in a significant reduction in race performance. This study highlights the sensitivity of the G.I tract to nutritional interventions. Therefore, studies should take all possible precautions in avoiding G.I discomfort of the participants which may mask any possible effect of the intervention. Few studies report the habitual drinking strategies of their participants which may be an important factor when ingesting large volumes of fluid during exercise tests.

One study investigated the influence of CHO-E solution ingestion on 40 km run performance in the heat. In this study runners ingested 400 ml of either a 7 % CHO-E solution or placebo (sweetened water) 30 min prior to exercise and 250 ml every 5 km during the run. The runners were instructed to self-select a running speed equivalent to training pace for the first 35 km and complete the final 5 km of the run at race pace. The reported incidence of G.I discomfort i.e. stomach upset, bloating, or nausea was not different between the two trials. There were also no reported differences in any of the physiological variables between trials. However the ingestion of the CHO-E solution did increase blood glucose concentrations during the run. Time to complete the final 5 km was significantly faster following the ingestion of the CHO-E solution (21.9 min) compared with the ingestion of the placebo solution (24.4 min) (Millard-Stafford, Sparling, Roskopf & DiCarlo, 1992). It should be noted that a benefit to time-trial performance of approximately 2.5 min over 5 km is a huge performance gain. This substantial effect is surprising as up to the completion of 35 km there were no differences in the self-selected “training pace” between the CHO and placebo trials. In addition, there was no significant difference in the perception of effort during the two trials. Blood glucose was also well maintained during the 40 km run on the placebo trial, suggesting that runners did not experience significant fatigue leading into the final 5 km of the run.

In another field study, van Nieuwenhoven et al. (2005) investigated the effect of ingesting sports drinks on G.I complaint and 18 km run performance. Ninety-eight well-trained runners performed three competitive 18 km races ingesting either water, a sports drink (CHO-E) or a sports drink with added caffeine ( $150 \text{ mg}\cdot\text{l}^{-1}$ ). Each runner ingested 150 ml of the experimental solution immediately before, at 4.5 km, 9 km, and 13.5 km during the race. The run times to complete the 18 km were virtually identical for the water (78 min 3 s  $\pm$  8 min 30 s) CHO-E (78 min 23 s  $\pm$  8 min 47 s) and CHO-E plus caffeine (78 min 3 s  $\pm$  8 min 42 s) trials. It is important to note, that the ingestion of CHO-E solutions led to higher incidences of all types of G.I complaints compared to water. Specifically, the incidence of flatulence and reflux were greater in comparison to the water trial. The addition of caffeine to the CHO-E solution had no effect on G.I complaint compared to the ingestion of the CHO-E solution. Therefore the authors concluded that the ingestion of sports drinks offered no benefit to performance over that of water (van Nieuwenhoven et al., 2005). Advice

to distance runners about fluid ingestion during exercise is to experiment during training to avoid incidence of discomfort during competition. As this study did not allow habituation with the fluid ingestion procedures this may have contributed to the high incidence of reported G.I discomfort.

### *The influence of mouth- rinsing a CHO solution on endurance performance*

Intriguingly, studies investigating the impact on CHO-E solutions on endurance performance have recently reported that benefits may be gained without ingestion (Place, 2009). Carter et al. (2004) were the first to investigate the possible role of CHO receptors in the mouth influencing exercise performance. The study reported that time to complete a set amount of work as quickly as possible (approximately 1 h, 914 +/- 40 kJ) was significantly improved ( $59.57 \pm 1.50$  min versus  $61.37 \pm 1.56$  min) when mouth-rinsing a 6.4 % maltodextrin solution in comparison to water. The solution was rinsed around the mouth for 5 s before being expectorated. Therefore no solution was believed to be ingested. The authors speculated that CHO in the mouth may have a “central” effect increasing central drive or motivation toward exercise (Carter et al., 2004a). It has also been reported that mouth-rinsing both a glucose and maltodextrin solution improved 1 h cycle time-trial performance in comparison to a placebo containing saccharin and artificially sweetened placebo respectively (Chambers et al., 2009).

In support of these findings, Pottier et al. (2008) reported significant improvements in cycle time-trial performance when mouth-rinsing a 6 % CHO-E solution in comparison to a placebo. Intriguingly, mouth-rinsing with a CHO-E solution improved 1 h cycling performance compared to the ingestion ( $14 \text{ ml} \cdot \text{kg} \cdot \text{h}^{-1}$ ) of the same solution (Pottier et al., 2008). This finding may have direct implications for running performance, which can be influenced by G.I discomfort brought on by CHO-E solution ingestion during exercise. However, a recent study that provided a meal 2 h before exercise ( $2.4 \text{ g CHO} \cdot \text{kg BM}^{-1}$ ) reported that mouth-rinsing a 6.4% maltodextrin solution had no impact on 1 h cycle performance in comparison to mouth-rinsing a placebo (Beelen, Berghuis, Bonaparte, Ballak, Jeukendrup & van Loon, 2009). The pre-exercise status of the participant may therefore be an important factor when investigating central / performance responses to mouth-rinsing CHO.



Whitham & McKinney (2007) is the only published study to have investigated the impact of mouth-rinsing a CHO solution on running performance. Following a 4 h post-prandial period, runners completed a 15 min warm-up run at 65%  $\dot{V}O_2$ max, followed by a 45 min time-trial in which the aim was to achieve the greatest distance possible. Overall distance covered was not significantly different when runners mouth-rinsed either a 6 % maltodextrin (9333 m  $\pm$  988 m) or flavoured matched placebo (9309 m  $\pm$  993) (Whitham et al., 2007).

### *Limitations to published research*

Despite the popularity of running events and sports associated with running (10 km, half-marathon, marathon, triathlon, Iron-man) the most common mode of exercise used to investigate the influence of CHO-E solution ingestion has been cycling. In addition, the majority of studies investigating the effect of CHO ingestion both before and during exercise have assessed running capacity. In comparison to running capacity there are relatively few studies that have assessed running performance. Other limitations include the small number of participants in some studies. Thus, care must be taken when extrapolating these results to the wider population. Few studies investigating performance report the repeatability of the performance test performed in their own laboratory. Thus, small changes in exercise performance may be masked by the day-to-day variation of the testing method. Finally, the energy intake of the participant prior to exercise can have a significant effect on performance. In some studies authors failed to specify the exact composition of a pre-exercise meal or the duration the fasting period prior to exercise. Therefore it is difficult to determine whether the effect observed was due to the intervention or simply a result of other nutritional factors.

# **CHAPTER 3**

## **GENERAL METHODS**

### **3.1 Introduction**

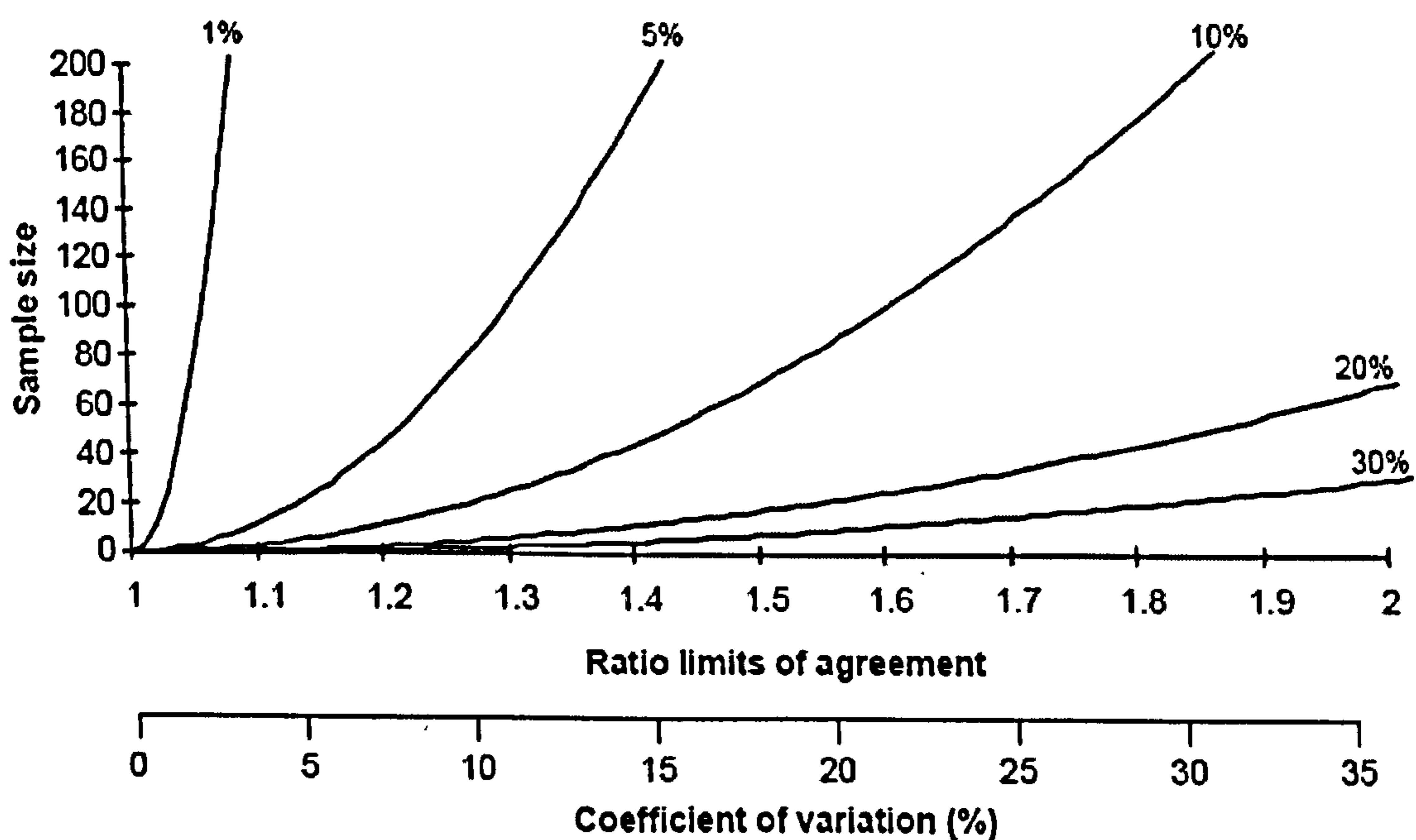
In the present thesis, two experimental exercise models were used to investigate the effects of CHO-E feedings on self-selected treadmill running speed. The main test was a 1 h time-trial, which assessed endurance running performance by recording the total distance achieved during 1 h of treadmill running (Chapter 4). The second experimental model asked runners to self-select a running speed which equated to a rating of perceived exertion (RPE) of “15 - hard” during a 30 min treadmill run.

This chapter has been divided into five sections. Section one describes the preliminary tests which were performed prior to each study. The second section describes the experimental protocols used in the main studies. The third section describes the psychological scales used during trials. The fourth section reports the nutritional controls of the studies. The final section describes the methods of collection, treatment and analysis of expired air and blood samples.

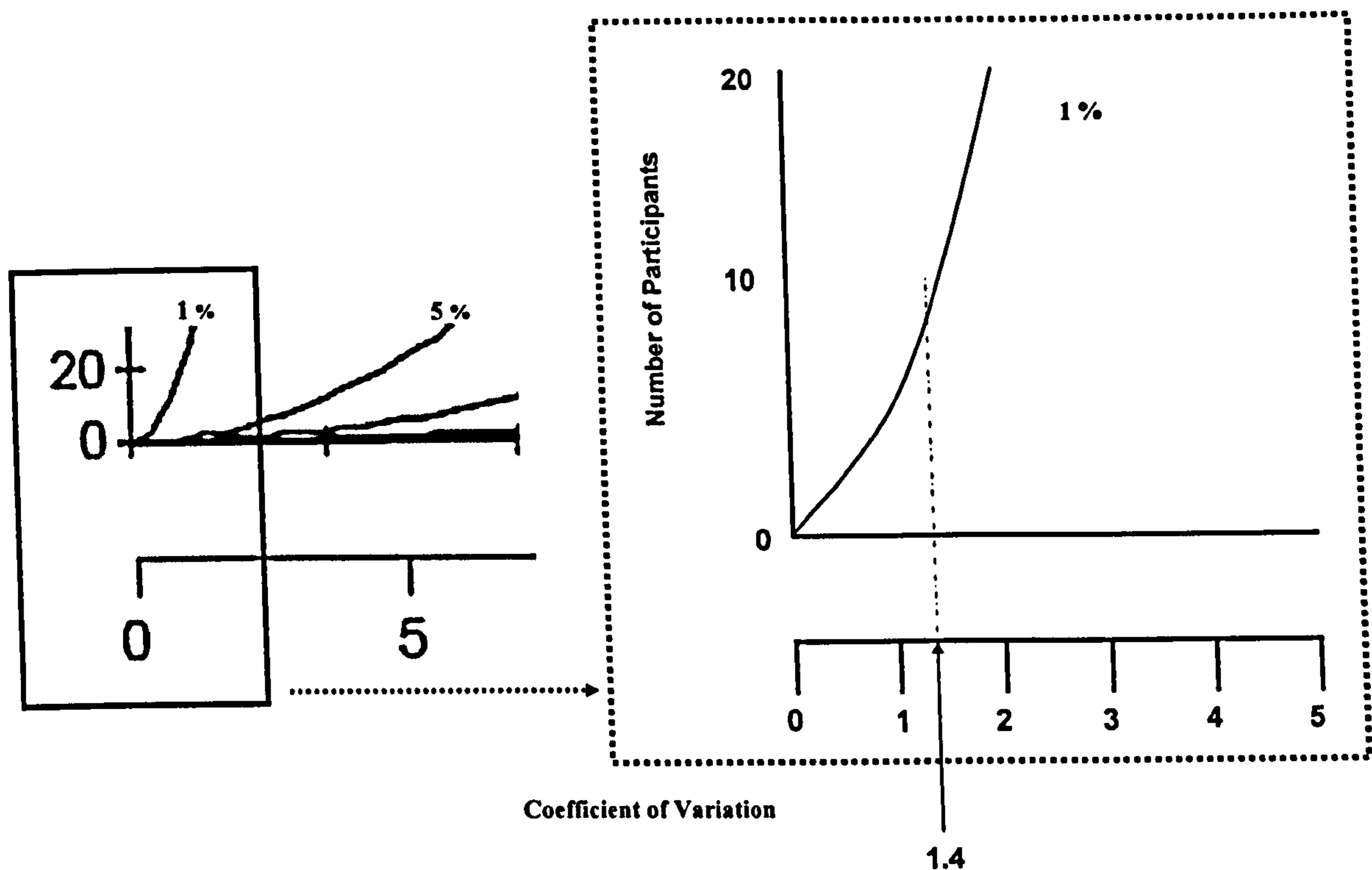
## 3.2 Preliminary measurements

### 3.2.1 Participants

The participants used in the study were recruited from local running clubs and from the student population. All the participants were healthy males who regularly undertook endurance based running training (3-5 times a week) and competed in endurance races i.e. 10 km, half-marathon, marathon and triathlon. Information about what was required to complete the studies was given to each participant verbally and in writing before they participated in the studies. No financial reward was offered to the participants, who were also told that they were free to withdraw from the study at any time, without need of an explanation. All participants completed a health history questionnaire and signed a statement of informed consent. All studies included in this thesis were approved by the Loughborough University Ethical Advisory Committee. The number of participants selected for each study was determined using a nomogram based upon the ratio limits of agreement (Nevill & Atkinson, 1997).



**Figure 3.1.** A nomogram replicated from Batterham & Atkinson (2005). The nomogram estimates the effects of measurement repeatability error on whether 'analytical goals' are attainable or not in exercise physiology. Statistical power is 90 %. The different lines represent different worthwhile changes of 1, 5, 10, 20 and 30 % due to some hypothetical intervention (Batterham & Atkinson, 2005).



**Figure 3.2.** A magnified view of the nomogram, with the co-efficient of variation (CV) of the exercise test set at 1.4 % (Chapter 4).

### 3.2.2 Body mass, height and heart rate

In all studies, body mass (BM), was determined to the nearest 0.1 kg using the beam balance scales (Model 3306ABV, Avery Industrial Ltd, UK). Body mass was recorded before exercise and immediately post exercise, after a period of cooling and towel drying to remove the sweat from the skin. Height was measured to the nearest 0.01 m using a wall mounted stadiometer (Body Care, UK).

Heart rate was monitored during preliminary tests and exercise trials. Heart rate was recorded at 5 s intervals using short range telemetry (Polar Electro, Kempele, Finland). This system involves the use of a transmitter, i.e. an electrode belt fastened around the chest and a wrist-mounted receiver that records and stores momentary heart rate at the predetermined intervals. Stored data was downloaded and analysed using the corresponding computer software (Polar heart rate analysis software, version 5.04). During preliminary tests and experimental trials the wrist-mounted receiver was

covered, so that runners were unaware of their heart rate during exercise. Preliminary and experimental trials were conducted with the principle investigator present at all times.

### **Ambient temperature, Humidity and barometric pressure**

Dry and wet bulb temperatures were measured using a whirling hygrometer (Brannan Thermometers Ltd, Cumberland). Humidity was then calculated from these measurements using conversion tables. Barometric pressure was measured using a wall mounted barometer (Fisher Scientific UK, Loughborough, Leicestershire) prior to each experimental trial.

### **3.2.3 Sub-maximal oxygen uptake**

Participants ran on a treadmill set at a 1 % gradient (to reflect the energetic cost of outdoor running (Jones & Doust, 1996)) for 20 min. The treadmill speed was increased every 4 min. Expired air samples were collected between 2.45 and 3.45 min.sec of each 4 min period. Heart rate was monitored continuously throughout the run (Appendix B).

### **3.2.4 Peak oxygen uptake**

The peak oxygen uptake ( $\dot{V}O_{2\text{peak}}$ ) for each participant was determined using an uphill treadmill running test, adapted from Taylor et al. (1955). The treadmill speed was kept constant throughout the test and gradient was increased from an initial incline of 3 % by 3 % every 3 min (Taylor, Buskirk & Henschel, 1955). Expired air samples were collected during 1:45-2:45 min of each 3 min stage. A final expired air sample was taken during the last min of the test immediately after the participant signaled that the current intensity i.e. running speed and incline could be maintained for one more minute. Verbal encouragement was given throughout the test (Appendix C). As the test was not repeated, values are not reported as the maximum oxygen uptake. Therefore a more conservative description for the highest value for oxygen uptake achieved during this test is reported to be the  $\dot{V}O_{2\text{peak}}$  of the participant. The

values of  $\dot{V}O_{2\text{peak}}$  were verified according to criteria equivalent to an R value  $\geq 1.10$  (Howley, Bassett & Welch, 1995).

### **3.3 Experimental protocols**

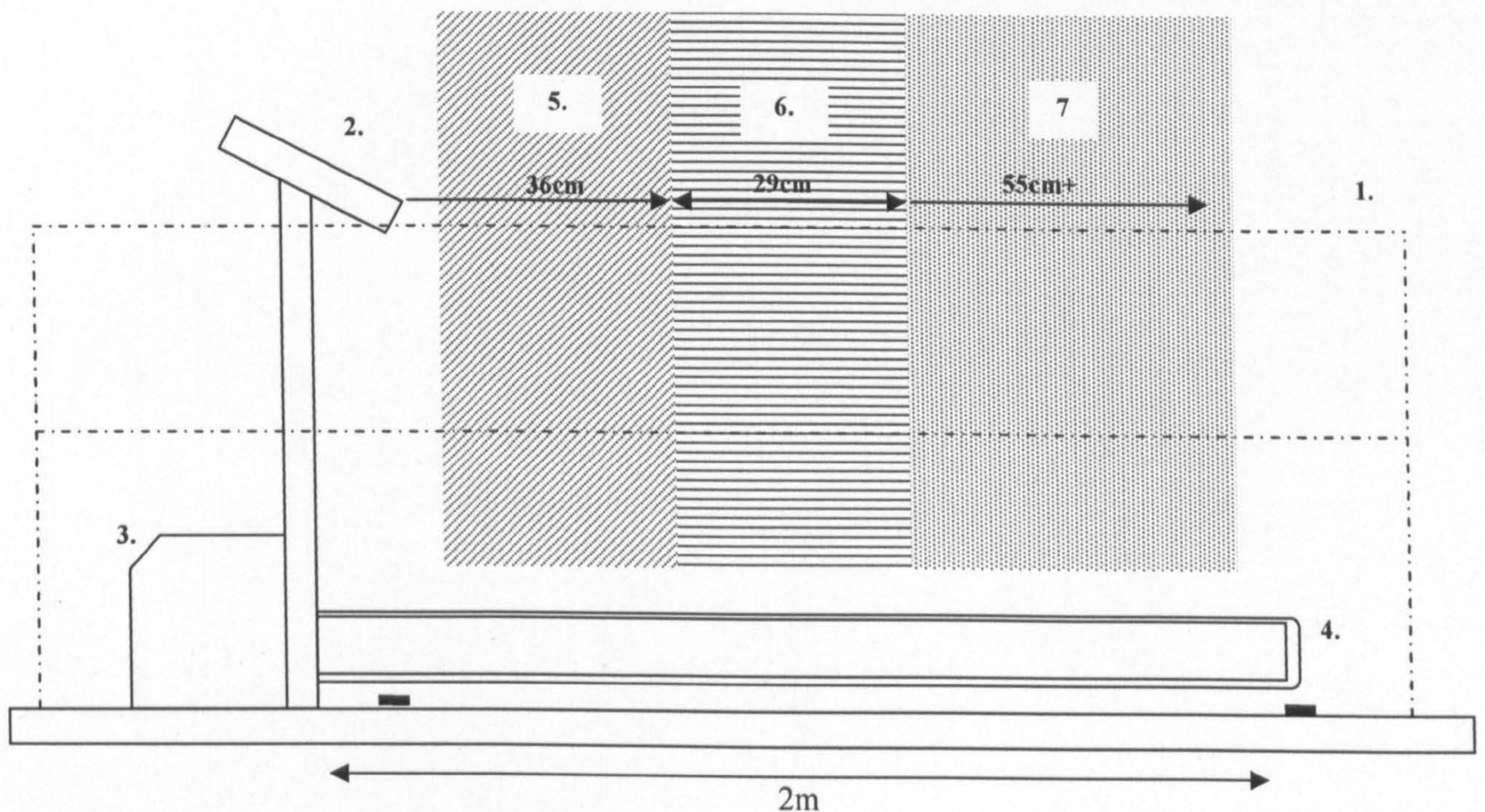
#### **3.3.1 Habituation**

Prior to the beginning of main data collection for experimental trials participants completed a full habituation with testing procedures and exercise durations. Thus, participants completed the 1 h run ingesting volumes of fluid equivalent to those consumed in the main trials. The participants ingested / mouth-rinsed plain water. No blood samples were taken during the habituation. However, blood collection procedures were simulated. The habituation trials were performed to ensure that the runners were fully habituated and comfortable running on and using the automated treadmill and to also verify the selection of speed equivalent to 60 %  $\dot{V}O_{2\text{peak}}$  for the warm-up run prior to the performance runs.

All experiments were conducted in the laboratories of the School of Sport and Exercise Sciences Loughborough University. During the performance phase of the running trials, participants were monitored continuously via closed circuit television by the principal investigator in an adjacent room (Chapters 4-8) or via a window looking into the exercising room (Chapter 9). The principle investigator was accompanied by a second person, who was present for safety, as required by the ethical committee. The laboratory contained the treadmill and fan positioned 1 m in front of the runner to provide cooling. During trials, blinds were drawn and no music was allowed. Only the principal investigator entered the laboratory to collect samples during the trials so that the runner was not distracted by the environment or extraneous activities. The treadmill display panel and heart rate monitor were covered so that feedback to the runner was limited to a clock displaying the running time.

### 3.3.2 Automated treadmill

All studies reported in this thesis used an automated treadmill which allowed changes in speed without manual input (Chapter 4). All main trials were carried out on a motorised treadmill (Runner MT2000, Bianchini and Draghetti, Cavezzo, Italy) that has an ultrasonic feedback-controlled radar modulator that spontaneously regulates treadmill belt velocity corresponding to the changing position of the runner on the treadmill belt (Minetti, Boldrini, Brusamolin, Zamparo & McKee, 2003). Thus, the treadmill velocity increases or decreases as the runner moves to the front or the back of the treadmill belt respectively. Therefore changes in velocity are achieved without the need for manual input or visual feedback from the runner. More specifically, when the runner moves to the front section of the treadmill (<36 cm from treadmill console) the speed increases ( $0.8 \text{ m}\cdot\text{s}^{-1}$ ). If the runner stays in the middle of the treadmill (between 36 and 65 cm from treadmill console) the speed remains constant. When the runner moves to the rear of the treadmill (>65 cm from treadmill console) the speed decreases ( $1.1 \text{ m}\cdot\text{s}^{-1}$ ). Consequently the runner will always be brought back to the centre of the treadmill belt (Appendix D). Self-selected running speeds were recorded in real time, every 15 s from the treadmill console to a computer adjacent to the treadmill. The computer screen was turned off so that the runner was unaware of their performance. The self-selected speeds during the experimental trials were downloaded on completion of the runners' trials and incorporated into a spreadsheet (Excel, Microsoft windows, 1998) to calculate distance covered in the 1 h. Prior to each study, the treadmill was calibrated by measuring the both the treadmill belt length and the time it required to complete 50 revolutions at various speeds whilst a participant (70 kg) was running. The speed shown on the treadmill display panel was validated against the calculated speeds of the treadmill.



**Figure 3.3** : Schematic of treadmill (not to proportion for clarity). 1 = support bars; 2 = console; 3 = motor; 4 = 2m treadmill bed; 5 = acceleration; 6 = constant speed; 7 = deceleration.

### 3.3.3 One hour (1 h) run protocol

Endurance running performance was measured by the total distance covered when running on the automated treadmill at 1 % gradient for 1 h (Chapter 4). Prior to each trial, runners completed a 5 min warm up at 60%  $\dot{V}O_2$ peak. During the 5-min warm up expired air was collected between 4-5-min and then analysed using the Douglas bag method. Ratings of perceived exertion (RPE) were taken after 3 min into the warm-up. On completion of the warm-up runners were allowed 2 min to prepare for the run and empty their bladder again if required (on these occasions, urine was collected and the volume taken into account in weight loss calculations). Runners began the trial by standing at the front of the treadmill (1% gradient) and received the exact following instruction from the same investigator:

“*name* this is a running performance test, run as far as you can in 60 minutes. Control the speed using the automated treadmill system. Stand at the front of the treadmill to increase the speed, the test will begin in 3. 2. 1.”



Runners received no encouragement or instruction throughout the rest of the trial. Runners received no feedback about their performance, i.e. distance covered, running speed and heart rate until they had completed all main trials. Runner stride length was determined by dividing the number of strides by the distance covered between 9-10 min, 19-20 min, 39-40 min, 55-56 min.

### **3.3.4 Self-selected running speed**

The self-selection of running speed was measured by asking runners to select speeds equivalent to a set rating of perceived exertion. The test comprised a 2-min walk at 4 km·h<sup>-1</sup> followed by a 10-min warm-up run at a speed equivalent to 60%  $\dot{V}O_2$ peak. Immediately after the 10-min warm-up the runners began the 30-min trial. The runners were asked to select the speed (which varied throughout the test) which they perceived to be equivalent to a “hard pace” RPE of “15” for the duration of 30 min and were free to adjust the speed using the automated treadmill system. On completion of 30-min run, the runners walked for a further 2-min at 4 km·h<sup>-1</sup>. Runners received no feedback about the distance covered over the 30-min run until the completion of all experimental trials.

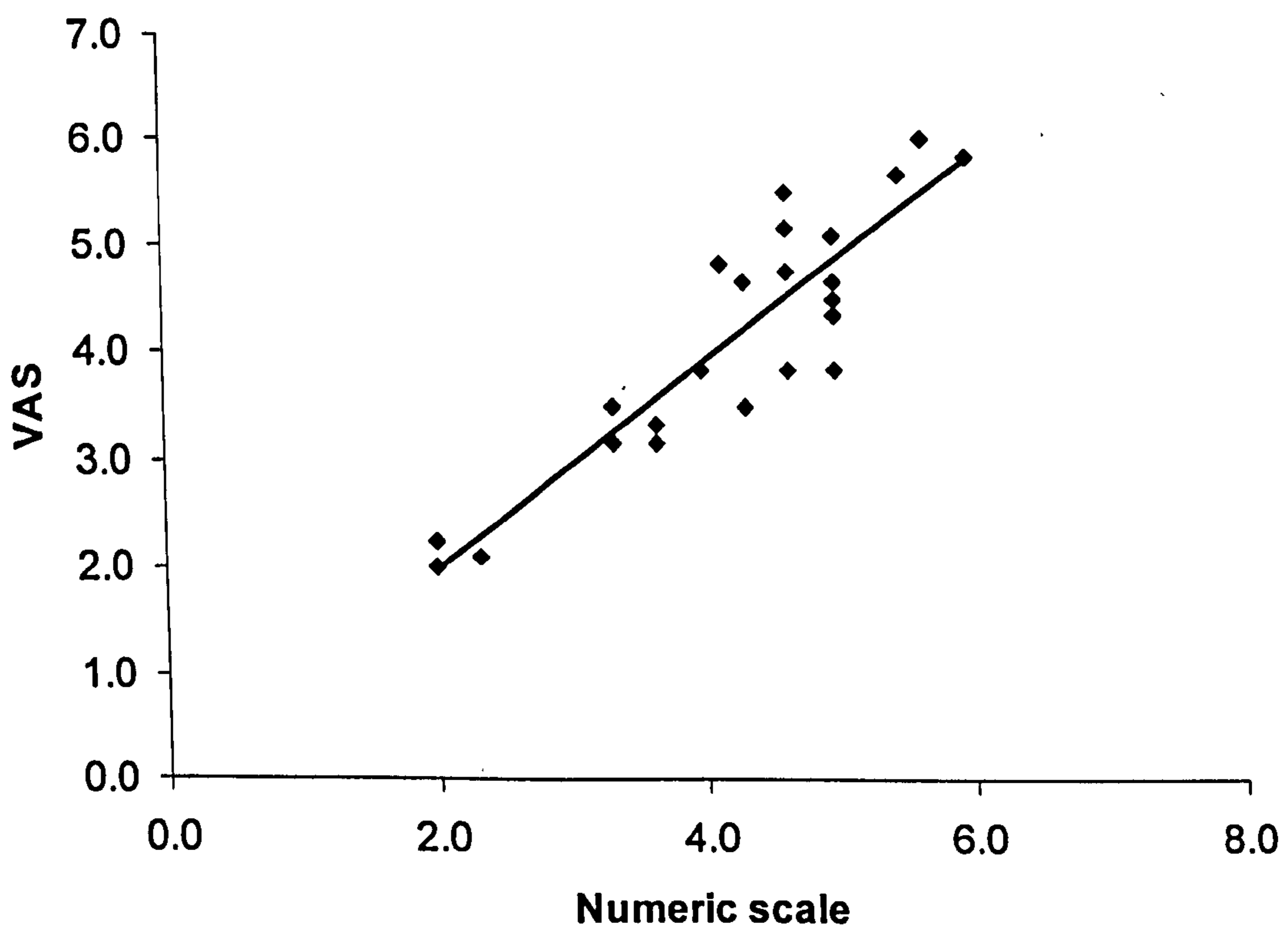
Self-selected running speeds were recorded in real time from the treadmill console to a computer adjacent to the treadmill. The computer screen was turned off so that the runner was unaware of their performance. The self-selected speeds during the experimental trials were downloaded on completion of the runners' trials and incorporated into a spreadsheet (Excel, Microsoft windows, 1998) to assess the self-selected speed and calculate distance covered in the 30 min.

### 3.4 Psychological scales

#### 3.4.1 Perceived activation

Perceived activation is a measure of the participant's state of arousal. Perceived activation was assessed using the 6-point single item Felt Arousal Scale (FAS). The perceived activation scale (FAS, (Svebak & Murgatroyd, 1985) is a six point, single item measure of perceived activation/arousal (Appendix E). The scale ranges from 1 to 6, with anchors at 1 ("low arousal") and 6 ("high arousal"). Runners were asked to rate how they felt at that particular moment. Instructions for the scale were:

"Estimate here how aroused you actually feel. By "arousal" here is meant how "worked-up" you feel. You might experience high arousal in one of a variety of ways, for example as excitement or anxiety or anger. Low arousal might also be experienced by you in a number of ways, for example as relaxation or boredom or calmness".

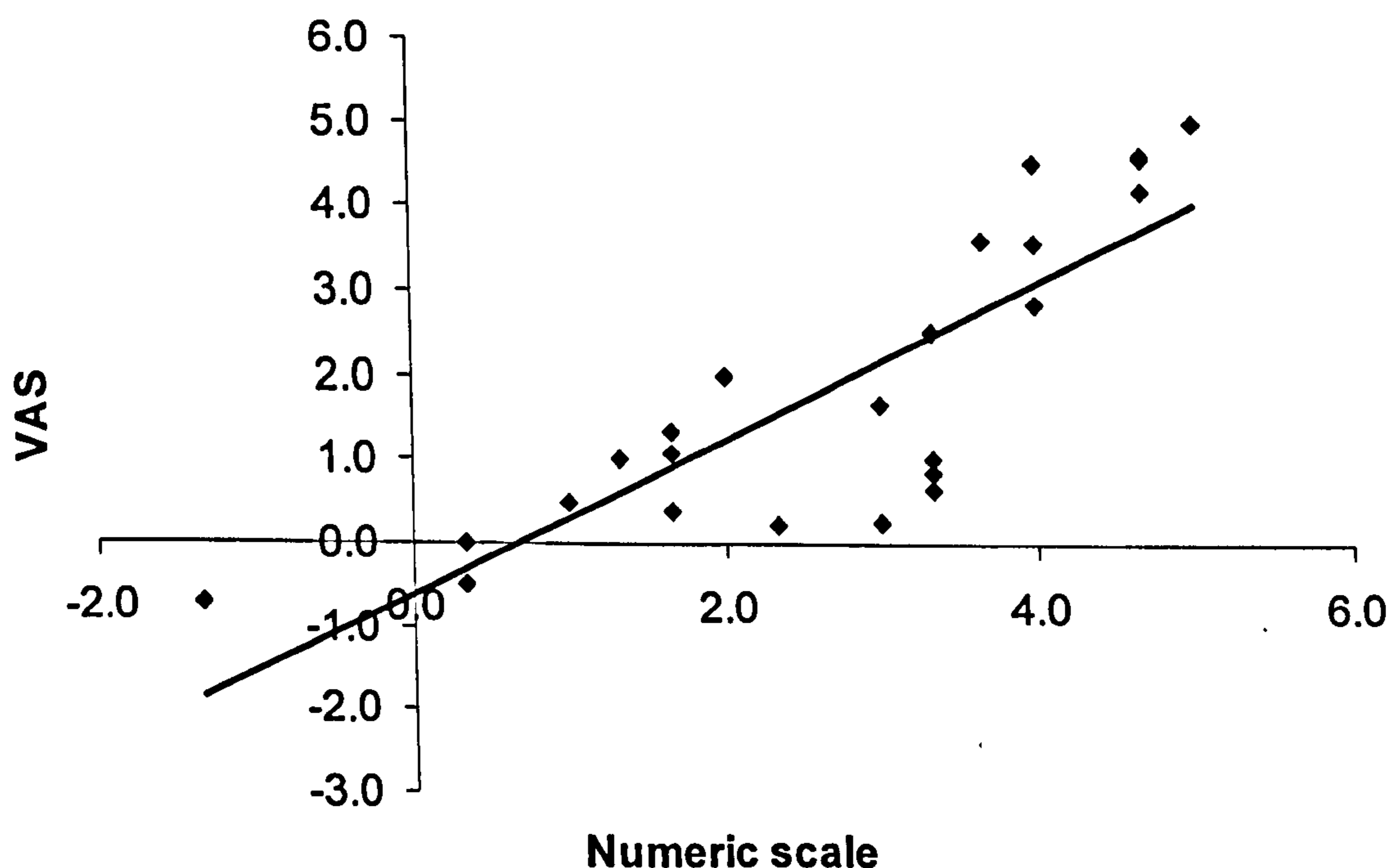


**Figure 3.4** The correlation between subjective scores of perceived activation when participants verbally stated score (Numerical scale) and when participants recorded scores on a visual analogue scale (VAS) at rest, immediately before and after a 1 h run,  $n = 24$ ,  $r^2 = 0.81$ .

### 3.4.3 Feeling scale

The Feeling Scale (FS) (Hardy et al., 1989) was used to assess the feelings of the runners i.e. the affective dimension of pleasure-displeasure (Appendix E). This FS scale is an 11-point single item bipolar rating scale that ranges from - 5 to + 5. Anchors are provided at the “0” point (“neutral”) and at all odd integers, ranging from “very good” (+5) to “very bad” (-5) (Ekkedakis, Backhouse, Gray & Lind, 2008). Runners were asked to rate how they felt at that particular moment. Instructions for the scale were:

“It is quite common to experience changes in mood while participating in exercise. Some individuals find exercise pleasurable, whereas others find it to be unpleasurable. Additionally feelings may fluctuate across time. That is one might feel good and bad a number of times during exercise. Scientists have developed a scale to measure such responses. Select the number that best represents your true feelings using the FS”

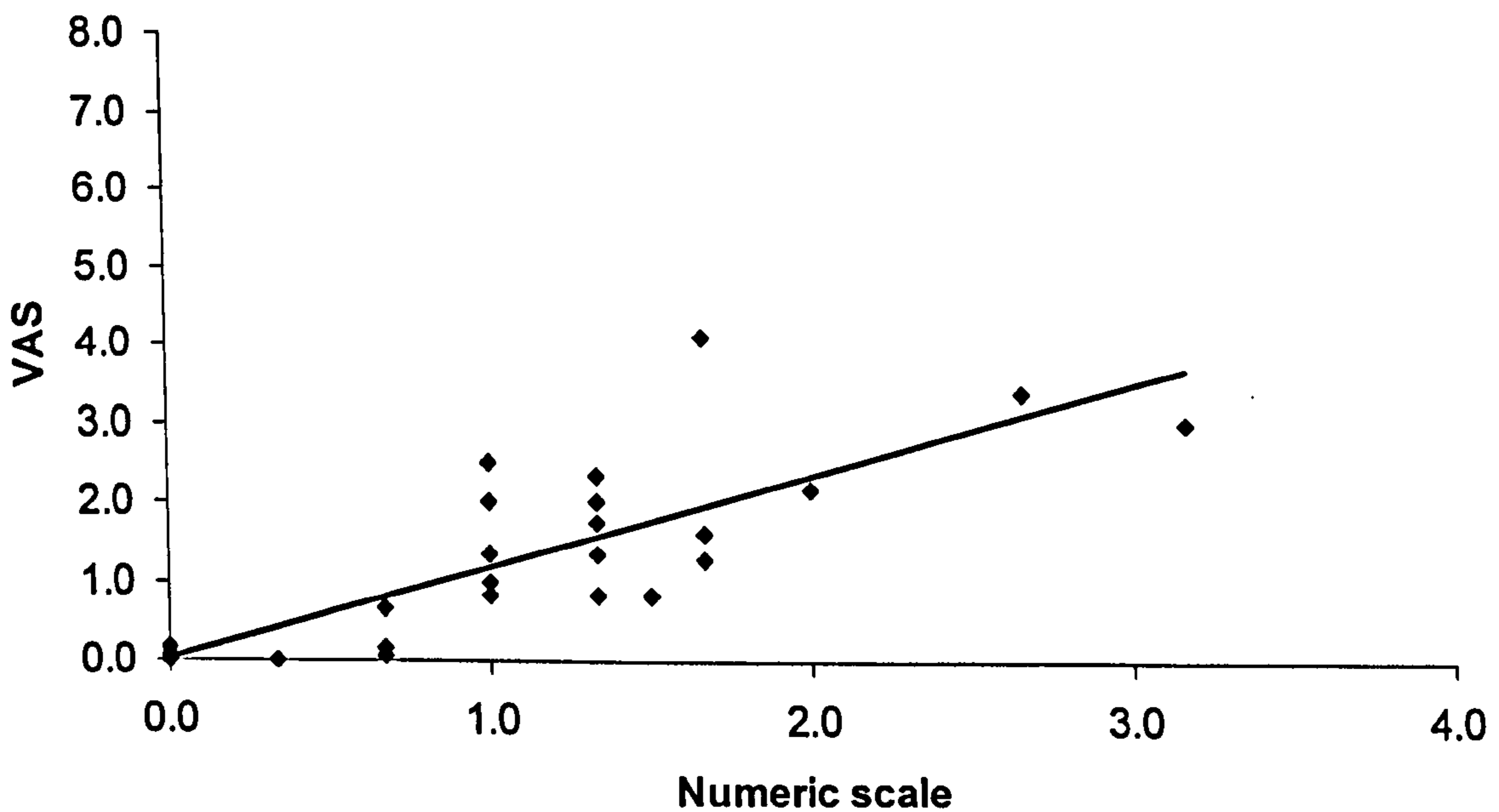


**Figure 3.5.** The correlation between subjective scores for feeling scale when participants verbally stated score (Numerical scale) and when participants recorded scores on a visual analogue scale (VAS) rest, immediately before and after a 1 h run,  $n = 24$ ,  $r^2 = 0.70$ .

### 3.4.4 Gastrointestinal discomfort

Gastrointestinal (G.I) discomfort was rated using a single item 12 point scale with anchors provided at 0 “neutral” , 4 “uncomfortable” , 8 “very uncomfortable” and 12 “painful” (Appendix E). The gastrointestinal tract was explained to the participant before being instructed:

“The G.I scale is used as a measure of gastrointestinal discomfort during exercise; it is common for athletes to experience G.I discomfort during exercise and for this to change throughout exercise. I would like you to rate your G.I discomfort using the scale saying a number between 0 and 12 for how you feel at that particular time”



**Figure 3.6.** The correlation between subjective scores for G.I discomfort when participants verbally stated score (Numerical scale) and when participants recorded scores on a visual analogue scale (VAS) rest, immediately before and after a 1 h run,  $n = 24$ ,  $r^2 = 0.60$ .

### **3.4.5 Rating of perceived exertion (RPE)**

The Rating of Perceived exertion Scale (Borg, 1982) was used as a measure of perceived effort during exercise (Appendix F). The RPE is a 15-point scale, which has been found to be a valid and reliable measure of perceived exertion during exercise (Borg 1982a). The scale ranges from 6 to 20, with anchors ranging from “very, very light” to “very, very hard”.

## **3.5 Nutritional control**

### **3.5.1 Preparation**

Runners were asked to refrain from heavy exercise and to consume a standardised diet 48 h before each trial. Participants were provided with food diaries to record their energy intake over this period. Food diaries were analysed using CompEat Pro version 5.8.0. No caffeine or alcohol was consumed during this period. This is because caffeine and alcohol have been found to have transient effects on exercise performance and affective states (Rogers, Edwards, Green, & Jas, 1992; Burke, 2008). All trials were conducted in the morning at the same time of day and there was 7 days between each trial.

### **3.5.2 Carbohydrate feedings**

The studies reported in this thesis provided CHO both before and during exercise. Prior to exercise CHO was provided in the form of a meal (3 h before exercise) and a CHO-E solution (30 min before exercise). During exercise CHO was provided in the form of a CHO-E solution.

The composition of the CHO-E and placebo solutions used in this thesis is shown in Table 3.1. The main reason for selecting these specific drinks was that they were similar in composition to those solutions which have previously been shown to improve endurance performance. The drinks were provided by Lucozade Sport, Brentford, England, with the placebo solutions identical in formulation to the CHO-E

solutions except they contained no CHO. In addition the drinks used were typical of commercially available sports drinks widely used in a variety of sport and exercise activities.

The pattern of drinking used in each study is described in detail in the individual chapters. During exercise, all drinks were administered to the participants in plastic volumetric syringes (Kendal monoject, UK). The use of plastic volumetric syringes reduced the risk of spillage and ensured the correct volume of solution was ingested. In addition, this method of fluid provision caused minimal interruption to treadmill running performance. Following the completion of trials participants were asked if they could identify which solution they had ingested. On the occasion when runners said “yes” they were asked to state on which trial they believed they were ingesting (Chapter 5, 6, 9) or mouth-rinsing (Chapter 7, 8, 9) the CHO-E solution.

**Table 3.1.** Nutritional value of experimental drinks, per 100 ml. Both solutions contained sweeteners aspartame and acesulfame K. The CHO in the CHO-E solution consisted of a blend of glucose syrup and maltodextrin.

	CHO-E	PLA
Flavour	Orange	Orange
Carbohydrate (g)	6.4	Nil
Protein (g)	Trace	Trace
Fat (g)	Nil	Nil
Sodium (mg·100 ml <sup>-1</sup> )	50	50
Potassium (mg·100 ml <sup>-1</sup> )	9.0	9.0
Osmolality (mOsm/kg)	282	61
Energy (kcal)	28	2

### 3.5.3 Pre-exercise meal

In Chapter 6 a high CHO meal was consumed following an overnight fast. The runners arrived at the laboratory and consumed a pre-exercise meal 3 h before the start of the 1 h run. The pre-exercise meals were designed to provide 2.5 g of CHO

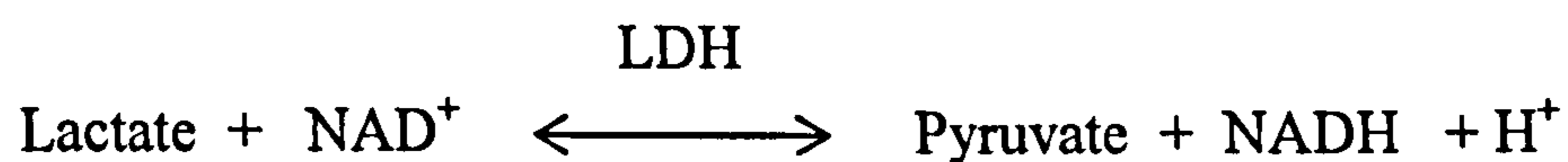
per kg of BM. The meals included typical breakfast foods and consisted of white bread, jam, corn flakes, skimmed milk, water and orange juice, which amounted to approximately 85 % of energy intake from CHO, 12% from protein and 3% fat (Appendix H). The CHOs included in the meal were obtained from foods classified as having a high glycaemic index (Jenkins et al., 1984). The meal provided has been previously shown to increase muscle glycogen concentrations by  $10.6 \pm 2.5\%$  (Chryssanthopoulos et al., 2004). Runners were required to eat the meal within 15 min and asked to consume the various components of the meal in the same order for both trials.

### **3.6. Collection and analysis of expired air samples**

The Douglas Bag method was used during preliminary and main trials to collect and analyse expired air. The participants breathed through a low resistance respiratory valve and light weight, wide bore (40 mm) tubing (Falconia Ltd) into a 150-litre Douglas bag through a two way tap (Harvard equipment). Resting and exercising expired air was collected for 5 and 1 min respectively. The oxygen ( $O_2$ ) and carbon dioxide ( $CO_2$ ) content of expired air was analysed using a single unit incorporating both a paramagnetic oxygen analyser, operating on the basis of the susceptibility of oxygen to a paramagnetic gas and an infra-red carbon dioxide analyser (Servomex, Model 1440C, Crowborough, Essex). The instruments were calibrated against nitrogen and a known gas mixture of  $O_2$  and  $CO_2$  before each trial and checked against room air. A dry gas meter (Harvard Apparatus Ltd, Edenbridge, Kent) calibrated against a 600-litre Tissot spirometer (Collins Ltd, USA) and a thermometer (Edale Instruments, Model C) was used to measure the volume and temperature of the expired air samples. Minute oxygen uptake ( $\dot{V}O_2$ ), carbon dioxide expired ( $\dot{V}CO_2$ ), ventilation rate ( $\dot{V}E$ ) and respiratory exchange ratio (RER) were calculated. All gas volumes were corrected to STPD conditions using the Haldane transformation formula. Whole body percentage substrate oxidation rates were calculated using indirect calorimetry (Peronnet & Massicotte, 1991) (Appendix I).

### 3.7 Collection and analysis of blood samples

Capillary blood samples were obtained in duplicate (20 µl) from a finger prick incision. All blood samples were deproteinised in 200 µl of perchloric acid, frozen and later analysed for the concentrations of glucose and lactate (Maughan, 1982). The concentration of blood lactate was determined via fluorimetric analysis (Locarte 8.9, UK) using 20 µl of supernatant derived from the previously deproteinised whole blood samples. A spectrophotometer (UV-mini 1240, Shimadzu, Europe) was used to determine blood glucose concentrations (Randox, Ireland). The principle of this method is the amount of light absorbed by any sample will be proportionate to the concentration of analyte. The fluorimetric analysis is dependent on the interconversion of metabolites linked to a change in the oxidation state of  $\text{NAD}^+$ . Thus the metabolite to be measured (NADH) is linked by the following reaction:



In the resting study (Chapter 7), finger tip blood samples were collected in 300 µl microvettes (Microvette, CB 300, Sarstedt Ltd, UK). Following the collection of 2 x 20-µl, the remainder of the blood was centrifuged and plasma collected for the determination of plasma insulin concentrations using an ELISA kit (Merckodia, Insulin ELISA, Sweden) and plate reader (Expert Plus, ASYS Atlantis, Eugendorf, Austria). The ELISA is a solid phase two site enzyme immunoassay. During incubation insulin in the sample reacts with peroxidase-conjugated anti-insulin antibodies and anti-insulin antibodies bound to a microtitration well. A washing step removes the unbound enzyme labelled antibody. The bound conjugate is detected by reaction with 3,3',5,5'-tetramethylbenzidine (TMB). The reaction is stopped by adding acid to give a colourmetric endpoint that is read spectrophotometrically (Merckodia, Insulin ELISA, Sweden).



**Table 3.2.** Coefficient of Variation ((SD/ mean) \* 100) for the measures of oxygen uptake ( $\dot{V}O_2$ ) and blood assays as determined from subjects and blood samples in the present studies (n. =10).

5 min resting $\dot{V}O_2$	7.4 %
Sub-maximal: speed $\dot{V}O_2$	6.7 %
$\dot{V}O_{2peak}$	5 %
Blood glucose	1.1 %
Blood lactate	1.9 %

## CHAPTER 4

### REPEATABILITY OF SCORES ON A NOVEL TEST OF ENDURANCE RUNNING PERFORMANCE

#### 4.1 SUMMARY

The aim of the present study was to determine the repeatability of a running endurance test using an automated treadmill system that requires no manual input to control running speed. On three separate occasions, seven days apart, 10 experienced male endurance trained runners (age  $32 \pm 10$  y,  $\dot{V}O_{2\text{peak}}$   $61 \pm 7$  ml  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup>) completed a treadmill time trial, in which they were instructed to run as far as possible in 1 h. The treadmill was instrumented with an ultrasonic feedback-controlled radar modulator that spontaneously regulated treadmill belt speed corresponding to the changing running speed of each runner. Estimated running intensity was  $70 \pm 11$  %  $\dot{V}O_{2\text{peak}}$  and distance covered  $13.5 \pm 2$  km, with no difference in mean performances between trials. The coefficient of variation (CV), estimated using ANOVA with subject and trial as main effects, was 1.4%. In summary, using an automated treadmill system, improved the repeatability of a 1 h treadmill time trial compared to time trials where speed is controlled manually. The present protocol is a reliable method of assessing endurance performance in endurance trained runners.

## **4.2 INTRODUCTION**

The most common method of replicating running performance under laboratory conditions involves the use of a motorised treadmill that allows the manipulation of speed and/or gradient to control running intensity. For example, researchers have successfully used treadmill running to investigate the physiological responses to 800m, 1500m (Sandals et al., 2006), endurance capacity (time to fatigue) (Tsintzas et al., 1993; Tsintzas, Williams, Boobis, Symington, Moorehouse, Garcia-Roves & Nicholas, 2003; Tsintzas et al., 1996b; Wee et al., 1999), 30 km time trials (Williams, Brewer & Walker, 1992), half (Williams et al., 1983) and even full marathon distances (Tsintzas et al., 1995b).

Atkinson and Nevill (1998) define the term repeatability as the consistency of measurements. Alternatively, others have defined reliability as the “absence of measurement error” (Safrit & Wood, 1989) although it is important to note that some error will always be present in continuous measurements (Atkinson & Nevill, 1998).

Laursen et al. (2007) have recently reported that performance during treadmill time trials have a greater repeatability than time to fatigue running tests. However the limiting factor in treadmill running methods, identified by Laursen et al. (2007) and other treadmill time trial studies (Hickey et al., 1992; Schabert et al., 1998; Whitham et al., 2007) is the inability of runners to spontaneously alter running speed. Instead, runners must manually press buttons on the treadmill console to change their running speed. This is a comparatively blunt response compared with cycling, where changes in power output can be achieved simply by altering pedal cadence. In an attempt to overcome this limitation, an automated treadmill system was designed (Minetti et al., 2003). The automated treadmill system allowed rapid and spontaneous alterations in treadmill speed, without the need for manually altering the speed. The treadmill system was used to determine the self-selected running and walking speeds at different gradients. However, to date the automated treadmill system has not been used to investigate time-trial running performance.

The aim of this study was therefore to attempt to improve the methods used for treadmill time-trials by using an automated treadmill system. Using a treadmill with the same technology as that described by Minetti et al. (2003), i.e. which allowed runners to rapidly change their running speed with no manual input. This study examined the repeatability of a 1 h time-trial.

### **4.3 METHODS**

#### **Participants**

Ten endurance trained male athletes ( $32 \pm 10$  years, body mass  $72.0 \pm 6.0$  kg, stature  $1.78 \pm 0.07$  m,  $\dot{V}O_{2\text{peak}}$   $61.0 \pm 7$  ml  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup>) gave their written consent before participating in this study approved by Loughborough University Ethical Advisory Committee. All participants were experienced runners accustomed to training and/or competitions lasting at least one hour. All runners had completed either a half or full marathon distance (ultra marathon 36 h  $n = 1$ ) within the last year.

#### **Preliminary tests**

A series of preliminary tests were conducted prior to the main experimental trials to determine (i) the relationship between running speed and oxygen uptake (ii) the relationship between running speed and blood lactate concentration. Finger tip blood samples (20- $\mu$ l) were taken in duplicate immediately after the expired air collection during the 20 min sub-maximal run, deproteinised, frozen and later analysed for the concentrations of lactate (Maughan, 1982) (iii) the peak oxygen uptake ( $\dot{V}O_{2\text{peak}}$ ) (Chapter 3).

#### **1 hour run protocol**

Runners were fully habituated with testing procedures before the completion of three 1 h running trials (T1, T2, T3) as described in Chapter 3. The runners were asked to refrain from heavy exercise and to consume their normal diet 48 h before each trial. No caffeine or alcohol was consumed during this period. All trials were conducted at the same time of day (morning 7.00 am – 9.00 am) and separated by seven days.

Runners arrived at the laboratory following an overnight fast (12-13 h), emptied their bladder before body mass (BM) was recorded. All trials were conducted in a laboratory ( $20 \pm 1$  °C, 55% relative humidity) containing only the treadmill and fan positioned 1 m in front of the runner to provide cooling. Runners were monitored throughout exercise via closed circuit television by an investigator in an adjacent room.

Runners drank water *ad libitum* during their first trial, the quantity of which was recorded and provided for all subsequent trials. No expired air or blood samples were collected during the 1 h trials. Runners did not receive any feedback from their 1 h run performance until the completion of the study.

Runner stride length was determined by dividing the number of strides by the distance covered between 9-10, 19-20, 29-30, 39-40, 49-50 min. The relative intensity at which each trial was performed was determined by extrapolating heart rate and oxygen uptake from the preliminary sub-maximal running tests.

### Statistics

The agreement (repeatability) between the three 1 h run trials was examined using a repeated-measures analysis of variance (ANOVA) (see Nevill & Atkinson, 1998). The ANOVA estimates the main effect of trial bias and participants and also provides a within-subject measurement error ( $s_w^2$ ). From the within-subject measurement error we can estimate the standard deviation of differences between two trial measurements ( $s$ ) as follows,  $s = \sqrt{2} \times s_w$ . Provided the residual errors are normally distributed and are not related to the size or level of the measurements, some authors recommend reporting this error as the '95 % limits of agreement', defined as  $\pm 1.96 \times s$  (Bland & Altman, 1986).

The presence or absence of a relationship between residual errors and the size of measurement can be assessed by plotting absolute residual errors against the predicted measurements. If evidence of heteroscedasticity (evidence of a greater error variation with larger measurements) is detected, a log transformation can be performed to overcome such an effect. Under such circumstances, the analysis described above

should be re-applied to the log-transformed measurements. By taking antilogs of the resulting errors ( $s$ ), we obtain a dimensionless ratio that indicates the measure of unexplained variation, which can be reported as a coefficient of variation (CV), i.e. the variation of the test when it is repeated, expressed as a percentage. From this error ratio we can obtain, what Nevill and Atkinson (1997) describe as the 95 % ratio limits of agreement that should contain 95% of the observed ratios (obtained by dividing one trial measurement by a second). All data reported as mean  $\pm$  standard deviation ( $s$ ).

## **4.4 RESULTS**

### **Warm-up**

The mean 5 min warm up speed was  $11 \pm 2 \text{ km} \cdot \text{h}^{-1}$ , mean  $\dot{V}\text{O}_2$  was  $36 \pm 4 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for T1 and T2 and  $37, \pm 5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  for T3, equivalent to 59, 59 and 60 %  $\dot{V}\text{O}_{2\text{peak}}$  for trials 1-3 respectively. Rating of perceived exertion for the warm-up was  $10 \pm 2$  and was consistent for the three trials.

### **1 hour run performance**

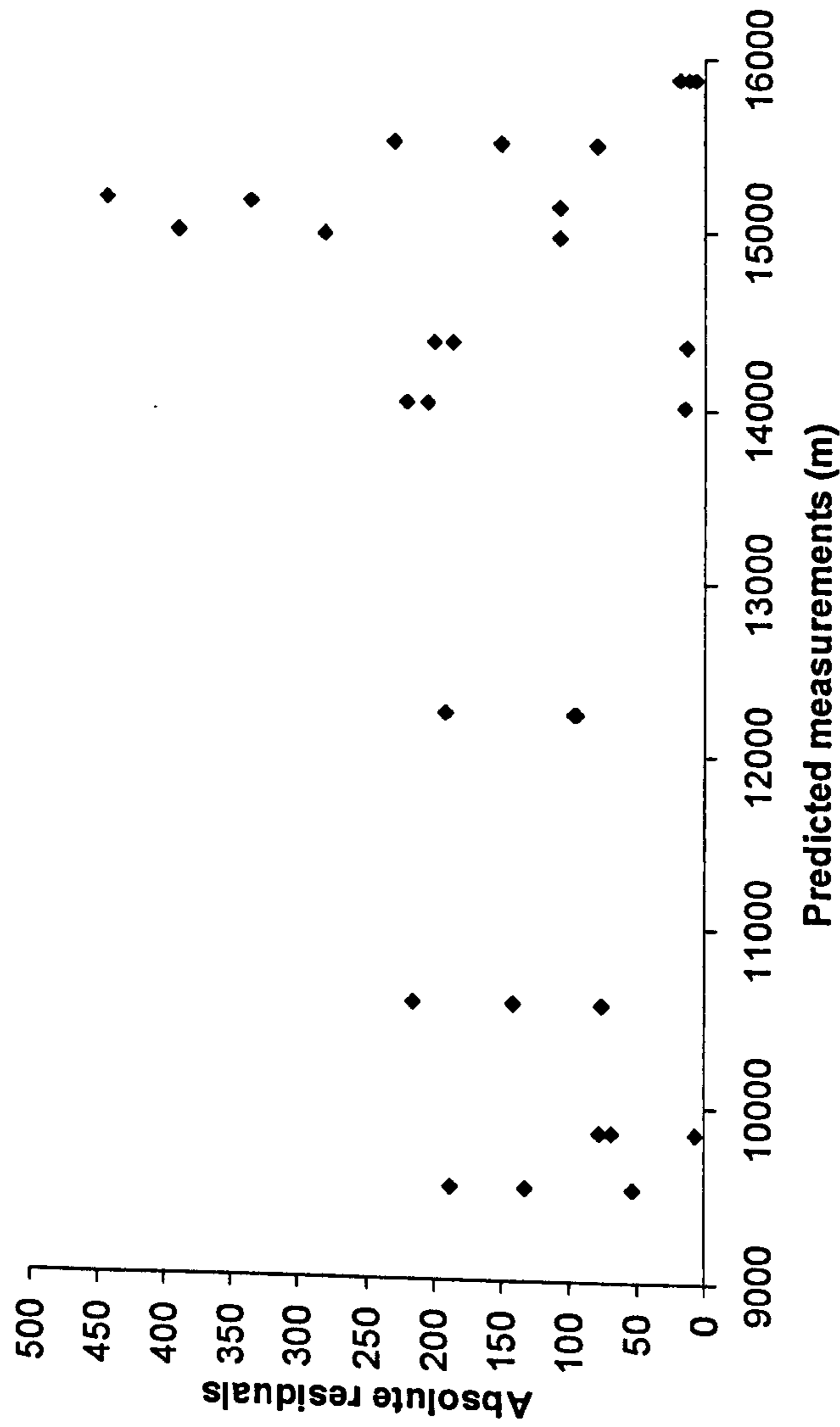
Each 1 h time-trial was run at an intensity of  $70 \pm 11 \%$   $\dot{V}\text{O}_{2\text{peak}}$ , heart rate was  $156 \pm 14 \text{ beats} \cdot \text{min}^{-1}$  and runners lost  $1.0 \pm 3 \%$  of their BM over the 60 min. Total distance covered by each runner during each 1 h trial, together with the mean distance covered for the three trials can be seen in Table 4.1.

The mean running speed for each trial was T1:  $13.5 \pm 2.5 \text{ km} \cdot \text{h}^{-1}$ ; T2:  $13.5 \pm 2.4 \text{ km} \cdot \text{h}^{-1}$ ; T3:  $13.5 \pm 2.4 \text{ km} \cdot \text{h}^{-1}$  (Figure 4.2). Runners increased their running speed from 0 to 2 min, until it was stable. Runners maintained a constant pace until approximately 59 min, before increasing their speed to the end of the run.

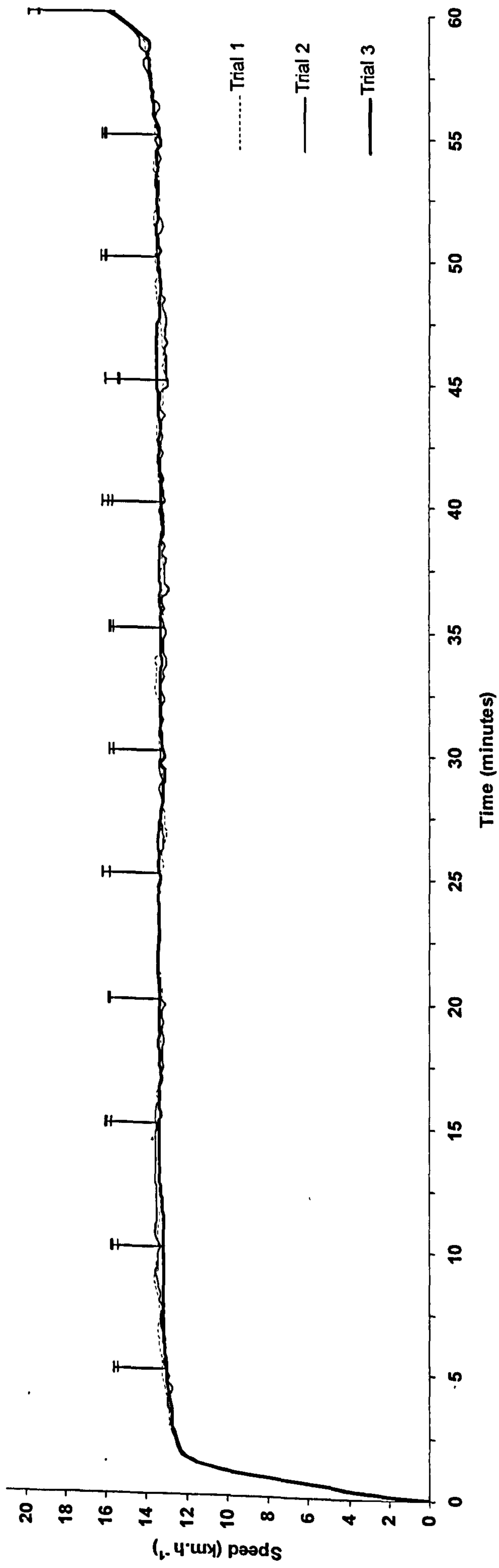
Total distance covered during the 1 h run was strongly associated with mean stride length, coefficient of determination given as  $r = 0.86$  (Figure 4.5, stride frequency =  $180 \pm 48 \text{ strides} \cdot \text{min}^{-1}$ , stride length  $1.34 \pm 0.28 \text{ m}$ ). Extrapolation from the preliminary sub-maximal running tests suggests that the 1 h runs were performed at a blood lactate concentration of  $\sim 2 \text{ mmol} \cdot \text{l}^{-1}$  (Figure 4.6).

Subject	1	2	3	4	5	6	7	8	9	10	Mean (m)	s
Trial 1 (m)	15202	15178	9895	15875	12138	14427	15583	13928	9419	10593	13224	2493
Trial 2 (m)	15308	14791	9825	15887	12235	14228	15354	14148	9553	10453	13178	2449
Trial 3 (m)	14866	14898	9818	15868	12329	14414	15504	13944	9606	10668	13192	2395
<b>Mean</b>	<b>15125</b>	<b>14956</b>	<b>9846</b>	<b>15877</b>	<b>12234</b>	<b>14356</b>	<b>15480</b>	<b>14007</b>	<b>9526</b>	<b>10571</b>	<b>13198</b>	<b>2446</b>
<b>s</b>	<b>231</b>	<b>200</b>	<b>43</b>	<b>9</b>	<b>96</b>	<b>111</b>	<b>116</b>	<b>123</b>	<b>96</b>	<b>109</b>		

**Table 4.1:** Total distance covered (m) by the ten runners during each of the three trials, together with the mean distance covered for the three trials.



**Figure 4.1.** Relationship between absolute residuals and predicted distances covered in 1 h.



**Figure 4.2:** Treadmill speed ( $\text{km}\cdot\text{h}^{-1}$ ) over the 1 h run with standard deviation shown at 5 min intervals for clarity ( $n=10$ , mean  $\pm$  SD).



## Heteroscedasticity and 95 % ratio limits of agreement

Figure 4.3 shows the association between the residual errors plotted against the predicted distances run in 1 h. When the absolute residuals were correlated against the predicted measurements, there was evidence of heteroscedasticity,  $r = 0.210$  ( $P > 0.05$ ). For this reason the data was log transformed the dependant variable, distance (Figure 4.4). The resulting within-subject measurement error, obtained from the repeated-measures ANOVA, was  $(s_w^2) = 0.00000969$ . Note that the units associated with this error ratio term are dimensionless as explained in the methods section. The anticipated difference between two trial measurements becomes  $s = 0.013927$ . The correlation between the absolute residuals and predicted values for log transformed distance was  $r = 0.031$  ( $P < 0.05$ ). The unexplained error variation calculated with both participants and trials as main effects, results in a CV of 1.4 %. The equivalent 95 % ratio limits of agreement were obtained as an error ratio ( $\pm 1.028$ ), i.e. 95 % of the ratios (one trial measurement, divided by a second) should lie between 0.973 and 1.028.

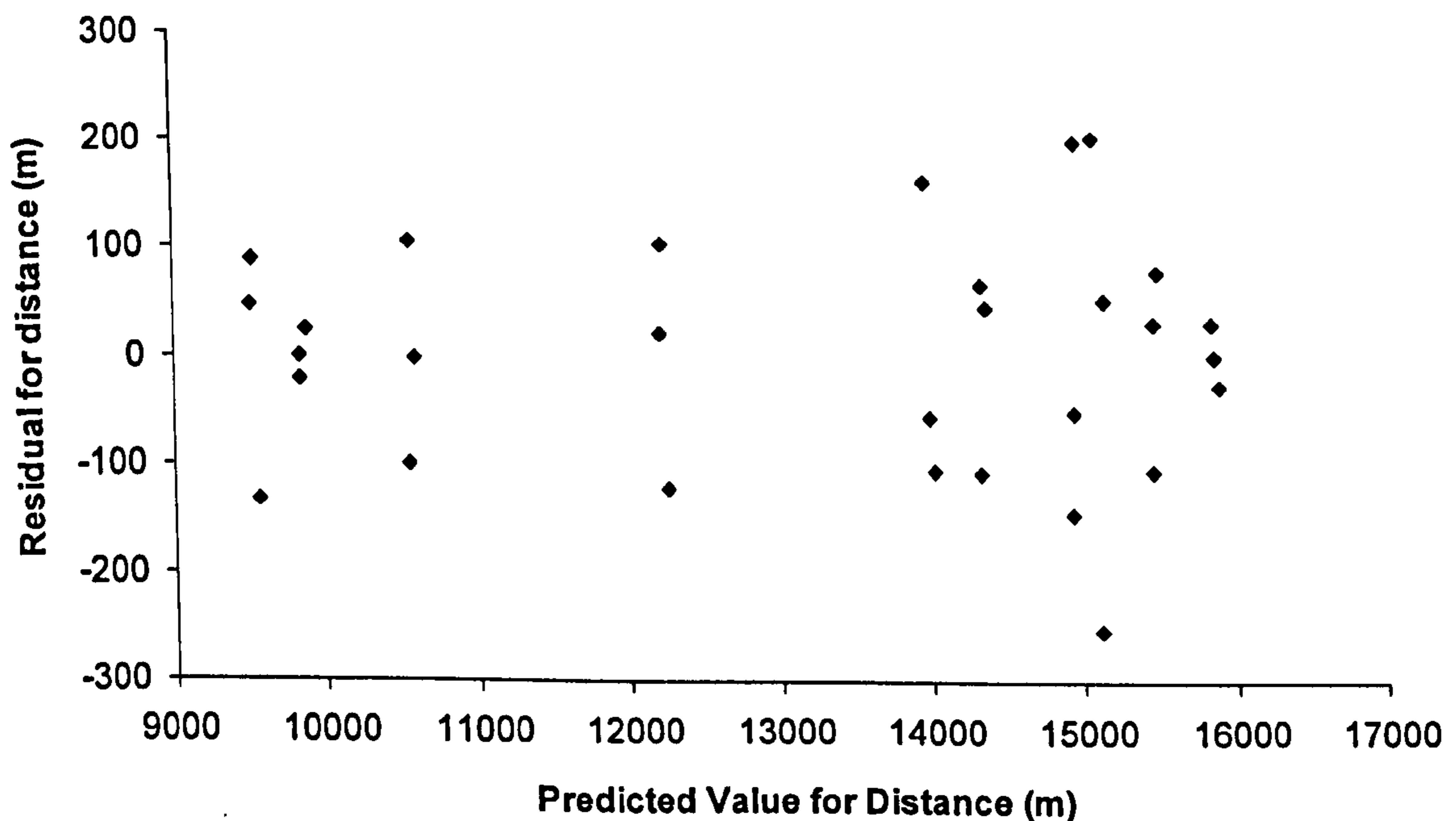


Figure 4.3 :Relationship between the residuals and predicted distances covered in 1 h.

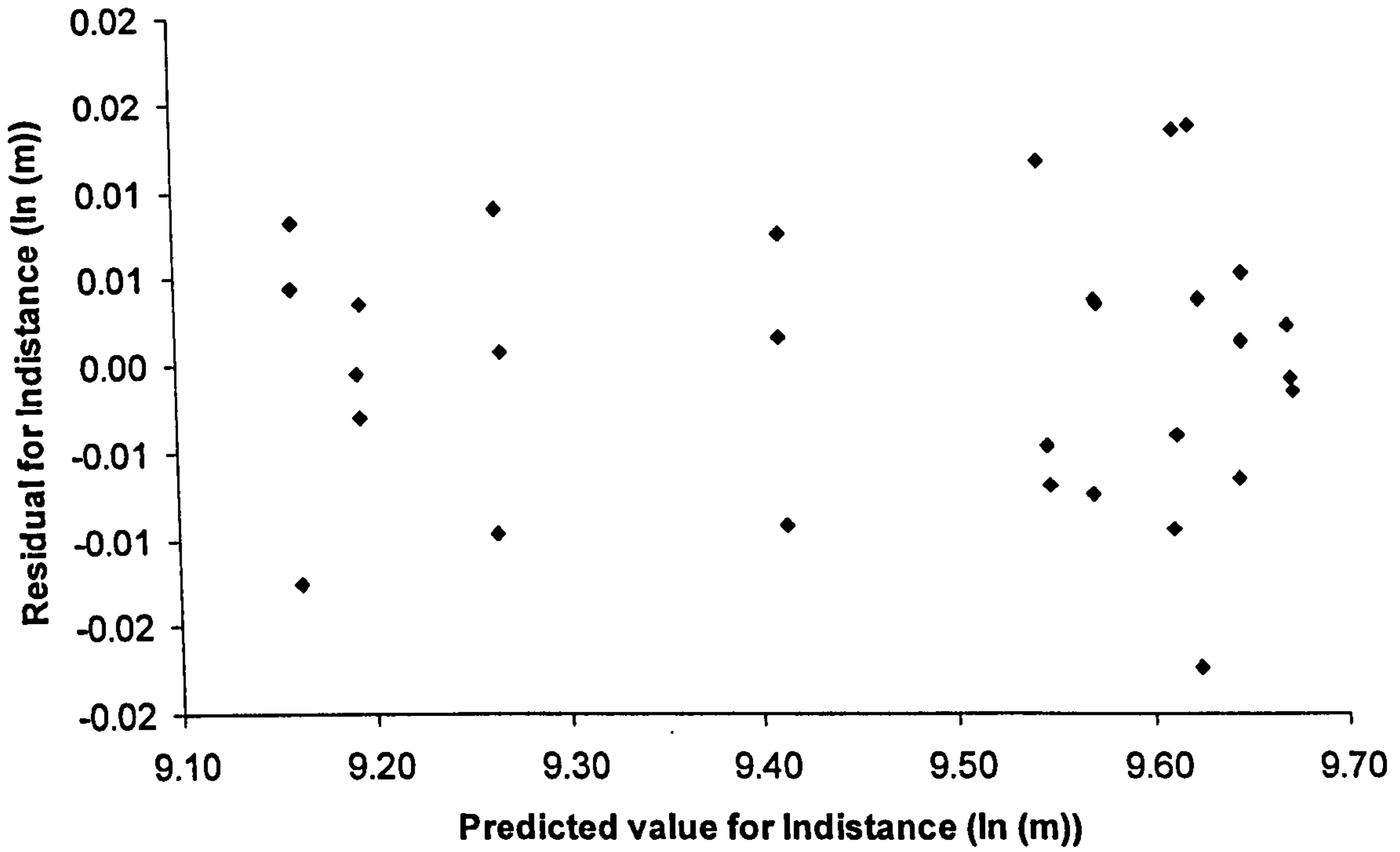


Figure 4.4: Relationship between the residuals and predicted distances covered in 1 h, all measurements log transformed.

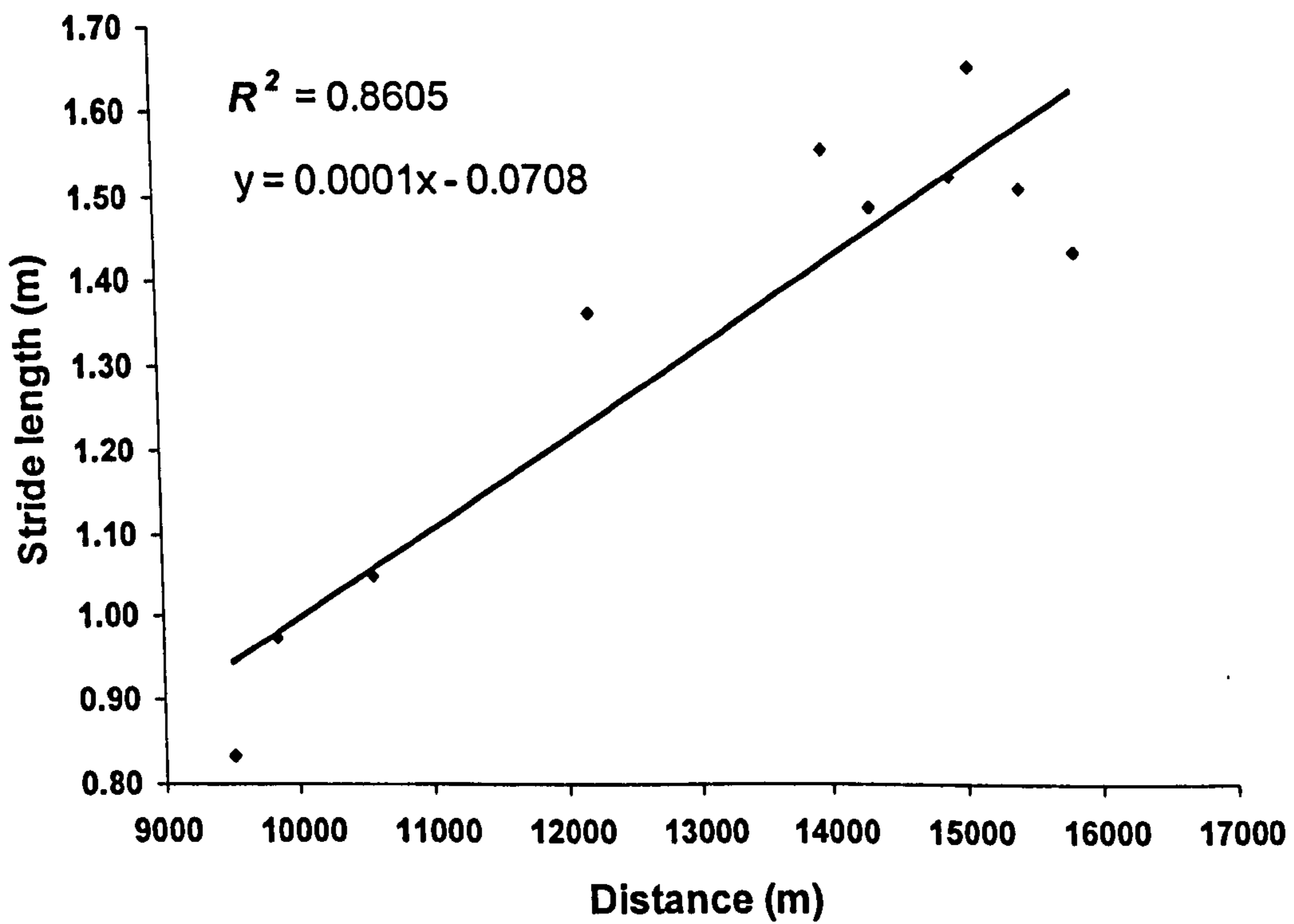
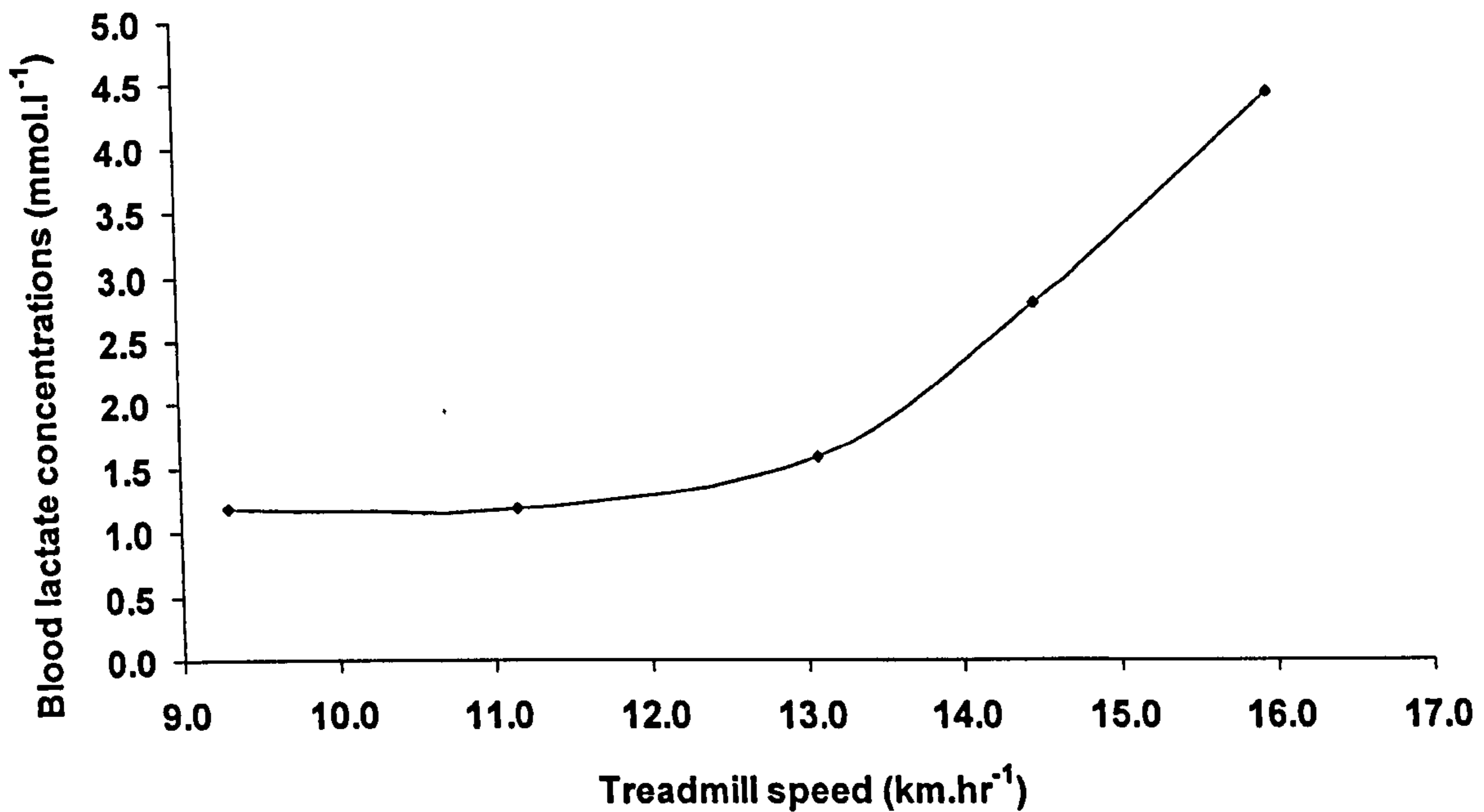


Figure 4.5 : Relationship between mean stride length and total distance covered (n.=10)



**Figure 4.6:** Relationship between blood lactate concentrations ( $\text{mmol}\cdot\text{l}^{-1}$ ) and running speed ( $n=10$ ).

#### **4.5 DISCUSSION**

Research into the influence of nutrition and training on exercise performance, requires simulating the demands of the event under controlled laboratory conditions. The most common method of achieving this in running involves the use of motorised treadmills. The major limitation with traditional treadmill running, however, is the inability to replicate the free and spontaneous changes in speed that occur during actual running events. In response, an automated treadmill system was developed that allows runners to freely control their running speed, removing the limitations associated with manual changes in speed (Minetti et al., 2003).

The main finding of this study was that runners were able to replicate their selection of speed using an automated treadmill. The CV was 1.4 % when runners were asked to run as far as possible in 1 h. Interestingly, the results of the repeated measures ANOVA identified no significant difference in total distance covered between the three trials. However, the plot of the residuals against the fitted values did identify evidence of heteroscedasticity (a positive correlation between the absolute residuals and the fitted values,  $r = 0.210$  (Figure 4.1) ( $P > 0.05$ ). This evidence of heteroscedasticity was eliminated by taking logarithms (see Figure 4.4) and, as such,

the unexplained error was reported as a percentage error, in the form of a CV equivalent to 1.4 %. Hopkins et al. (2001) have previously found running tests require a CV of 1.5 % or less, in order to detect small changes in running performance. The CV of 1.4 % obtained in the present study is therefore sufficiently reliable to detect worthwhile changes in running performance.

The CV of 1.4 % is an improvement on the CV of 2.7% reported previously by Schabort et al. (1998b) who used the same instruction i.e. to run as far as possible in 1 h, but who used a manually controlled treadmill. Laursen et al. (2007) have previously stated that manually changing the treadmill speed by pressing the appropriate console buttons is dependent upon the runners perception of their ability to run faster or slower. This method of controlling the treadmill speed may not be sufficiently sensitive to detect small differences in performance (Whitham et al., 2007). In the present study, the runners' ability to make spontaneous, accurate adjustments to their speed over the 1 h might be responsible for the improved CV with the automated system in comparison with manually altering the treadmill speed. A smaller CV of approximately 1% has been reported in a study on 10 km treadmill time-trials (Russell, Redmann, Ravussin, Hunter & Larson-Meyer, 2004). In this study, however, runners ran for 90-min at 65 %  $\dot{V}O_2$ max before completing the 10 km time-trial. Though reliable, this test and others that employ prolonged pre time-trial runs (Doyle & Martinez, 1998) might not be appropriate when investigating physiological responses during shorter (~1 h) more intense (~70 %  $\dot{V}O_2$ peak) run or cycle performance (Carter et al., 2004a; Jeukendrup et al., 1997b; Whitham et al., 2007).

Although the present study reports a greater reproducibility than previous running time-trials (Doyle et al., 1998; Schabort et al., 1998; Whitham et al., 2007) and time to fatigue tests (Billat, Renoux, Pinoteau, Petit & Koralsztein, 1994; Jeukendrup, Saris, Brouns & Kester, 1996), it still falls short of the 1 % - 1.1 % CV reported for cycle time-trials (Hickey et al., 1992; Palmer, Dennis, Noakes & Hawley, 1996). The exact reason for this is unclear. One possible explanation might be the differences between the automated treadmill and cycle ergometers. Though responsive, the automated treadmill does not have the same degree of sensitivity as does the cycle

ergometer, which allows rapid changes in power output simply by altering pedal cadence.

The time a runner takes to cover a certain distance is determined by stride length and stride frequency (Brandon & Boileau, 1992). Day-to-day stride length and frequency have been shown to be very reproducible in well-trained runners (Brisswalter & Legros, 1994) and therefore it is not surprising that stride length is strongly correlated with distance covered in 1 h. In the present study, neither stride length or frequency decreased during the 1 h run, suggesting that the runners did not experience a marked amount of fatigue. The relatively low blood lactate concentration during the run ( $\sim 2 \text{ mmol} \cdot \text{l}^{-1}$ ) would suggest that the chosen running speeds were supported largely by aerobic metabolism. It is important to note that analysis of the physiological data revealed there was variation in the intensity which the 1 h run was performed between runners. It has been estimated that runners can run at approximately 80 % of their  $\dot{V}O_2\text{max}$  for a duration of 1 h. However, the estimated running intensity was  $70 \pm 11$  %  $\dot{V}O_2\text{peak}$  and estimated blood lactate concentration was low ( $\sim 2 \text{ mmol} \cdot \text{l}^{-1}$ ). Therefore, although all runners were instructed to run “maximally” it would appear that some runners self-selected running speeds which were “comfortably” maintained for the 1 h. Future studies should record oxygen uptake during the 1 h time trials which will provide more accurate information on the intensities which the runners are self-selecting.

The central governor concept states that athletes have the ability to regulate their metabolic response towards an “anticipated” end point (Noakes, 2007; Rauch et al., 2005). Previously, it has been suggested that athletes might be engaging in monitoring processes that allows them to optimize the distribution of their metabolic resources over the duration of the race or exercise task (Foster, De Koning, Hettinga, Lampen, La Clair, Dodge, Bobbert & Porcari, 2003). Consistent with this and previous research in both laboratory (Palmer et al., 1999; Rauch et al., 2005; Weltan et al., 1998a; Weltan et al., 1998b) and field (Billat et al., 2001; Sandals et al., 2006) investigations it appears that the runners distributed their energetic resources over the 1 h run, in order to be able to sprint during the last minute of exercise. Therefore, the knowledge of specific exercise duration appears to be important when repeating endurance

performance tests. Finally, it is acknowledged that while in the present study the 1 h time-trial has been shown to have good repeatability, this may not be the case when using time to complete a fixed distance.

In conclusion, asking runners to cover as much distance as possible in 1 h, using an automated treadmill system that allows runners to control the speed without manual input, is a reliable method of assessing endurance performance in endurance trained male runners.

## CHAPTER 5

### INFLUENCE OF INGESTING A CARBOHYDRATE-ELECTROLYTE SOLUTION BEFORE AND DURING 1 HOUR RUNNING PERFORMANCE

#### 5.1 SUMMARY

The aim of this study was to investigate the influence of ingesting a CHO-E solution on performance during a 1 h treadmill run. Eight male endurance trained runners (age  $31 \pm 8$  y: mean  $\pm$  SD) completed three 1 h performance runs separated by 1 week. The study used a double-blind placebo (P1, P2) controlled design. On two occasions (P1, P2) runners consumed a placebo solution,  $8 \text{ ml} \cdot \text{kg BM}^{-1}$ , 30 min prior to and  $2 \text{ ml} \cdot \text{kg BM}^{-1}$  at 15 min intervals throughout the 1 h run. On a separate occasion runners consumed the same quantity of a 6.4% CHO-E solution (C). Total distance covered for P1, P2 and C trials was  $13685 \pm 1116$  m,  $13715 \pm 1143$  m and  $14046 \pm 1104$  m respectively. Although there was no difference between the two PLA trials (P1, P2) ( $P > 0.05$ ), the distance covered during the C trial was significantly greater than both PLA trials ( $P < 0.05$ ). The ingestion of CHO resulted in a higher blood glucose concentration only at the onset of exercise ( $P < 0.05$ ), compared to the PLA trials. Blood lactate, RER and CHO oxidation were similar in all three trials. In conclusion, the ingestion of a 6.4% CHO-E solution before and during exercise was associated with improved running performance in runners compared to the ingestion of colour and taste matched placebo.

## **5.2 INTRODUCTION**

There are relatively few studies examining the effects of ingesting CHO during road races because of the difficulties of controlling variables that may influence the outcome of races, for example, the day-to-day differences in environmental conditions. Of those studies that have created road races to examine the efficacy of ingesting CHO on running performance only some (Millard-Stafford et al., 1992; Tsintzas et al., 1993) but not all (Burke et al., 2005) have reported a performance benefit. Therefore, it is not surprising that most studies on nutrition and performance have been designed to try to create race conditions in well-controlled laboratory environments.

Although there are clear advantages of conducting running studies in a well-controlled laboratory environment treadmill running does not normally allow runners to change their pace spontaneously. Treadmill speed is usually changed manually by the runner or by the investigator i.e. at the request of the runner. Chapter 4 describes a 1 h running performance test that required runners to complete the greatest distance in the set time. This method allowed performance trials, conducted under controlled laboratory condition, to be more representative of “free running”. This performance test has a co-efficient of variation (CV) of 1.4 % which is smaller than those reported earlier studies (Chapter 4). Taking advantage of this advancement in treadmill performance testing, the aim of the this study was to investigate the influence of ingesting a 6.4% CHO-E solution on the total distance completed during a 1 h running performance test.



## **5.3 METHODS**

### **Participants**

Eight endurance trained male runners gave their written consent before participating in this study approved by Loughborough University Ethical Advisory Committee. The runners' physiological characteristics and running experience are reported in table 1. The number of participants was determined using a nomogram based upon the ratio limits of agreement (Nevill et al., 1997). All were experienced runners accustomed to training and/or competitions lasting at least 1 h in duration.

**Table 5.1** Physiological characteristics and training status of the runners (n.=8)

Age (years)	31 ± 8
Height (m)	1.80 ± 0.07
Weight (kg)	74.9 ± 5.3
$\dot{V}O_{2peak}$ (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	62.0 ± 4.7
Experience (years)	10 ± 5 (5-18)
Training frequency / week	4 ± 1 (3-5)
Miles/ week	43 ± 17 (30-70)

### **Preliminary tests**

A series of preliminary tests were conducted prior to the main experimental trials to determine (i) the relationship between running speed and oxygen uptake and (ii) the peak oxygen uptake ( $\dot{V}O_{2peak}$ ) (Chapter 3).

### **Experimental design**

All trials were conducted in the morning at the same time of day (7.00 am- 10.00 am) and separated by 7 days. Runners reported to the laboratory following an overnight fast (12 - 15 h). Running performance was assessed using the 1 h run protocol described in Chapter 3. The study utilised a double-blind, placebo controlled design. The runners first trial was single blind i.e. all runners ingested a placebo solution (P1).

The next two trials were double-blind i.e. runners ingested either a CHO-E (C) or the same placebo solution (P2) as they ingested during their first trial.

Runners were asked to refrain from heavy exercise and to consume their normal diet for 48 h before each trial. No caffeine or alcohol was consumed during this period. There were no significant differences between the three trials in the average daily energy intake, or composition in terms of CHO, protein or fat consumed in the 48 h before each trial.

### Beverages

The first trial, (P1), was single blinded. All runners ingested the equivalent of 8 ml·kg BM<sup>-1</sup> of a colour and taste matched placebo solution 30 min before the 1 h run and at 15 min intervals during the run they ingested the equivalent of 2 ml·kgBM<sup>-1</sup> i.e. at 15 min, 30 min, 45 min. Each 2 ml · kg BM<sup>-1</sup> bolus of solution was served in two separate plastic volumetric syringes (Kendal monoject). The solutions were weighed using an electronic balance (Mettler, Toledo AB54-s, Switzerland) to ensure the correct volume was consumed 30 min before exercise. A container with the solution was placed on the same electronic balance, to ensure the correct volume of solution was taken up into the plastic syringe. All syringes were empty following the ingestion of the solution during the run. The feeding schedule was designed to provide the runner with approximately 60 g CHO · h<sup>-1</sup> (quantities shown previously to improve performance). The ingestion of large volumes of fluid immediately prior to the run was avoided to minimise the risk of G.I discomfort impacting on performance. The following two experimental trials adopted a double blind random cross over design. Runners followed the protocol described above ingesting either a placebo (P2) or a commercially available 6.4 % CHO-E solution (C) (Lucozade Sport, Brentford, England). The placebo solutions were matched in formulation to the CHO-E solution except that they contained no CHO.

Finger tip blood samples (20- $\mu$ l) were taken in duplicate at rest, immediately before the 1 h run and at 15, 30, 45 and 60-min, during the run. All blood samples were deproteinised, frozen and later analysed for the concentrations of glucose and lactate (Maughan, 1982). One minute expired air samples were collected using the Douglas

bag method at approximately 15 min, 30 min and 45 min into the 1 h run. Runners' stride lengths were determined by dividing the number of strides by the distance covered between 9-10-min, 19-20-min, 39-40-min and 55-56-min.

The feeling scale (FS), felt arousal scale (FAS), together with the gastrointestinal (G.I) scale were administered at rest, immediately prior to and at 15-min, 30-min, 45-min and 60-min during the 1 h run (Chapter 3). Runners RPE was collected using the Borg Rating of Perceived Exertion scale (Borg, 1982) during the warm-up and at 15 min, 30 min and 45 min during the 1 h run.

### **Statistical analysis**

All data were analysed using SPSS (version 16.0). The mean differences in performance (total distance covered, running speed, stride length and frequency) psychological variables (RPE, FS, FAS and G.I) and trial order were detected using one-way within measures analyses of variance (ANOVA) with Bonferroni pair-wise comparison when significance was identified. Significant main effects for individual time points were further analyzed using paired *t*-tests and the Bonferroni adjustment for the number of pair-wise comparisons employed. Mean differences in self-selected running speed and comparisons over time (analysed in 5-min blocks over the 1 h run) were detected using a 3 x 20 (trial by time) within measures ANOVA. All data are presented as mean  $\pm$  SD and a critical alpha level was set at  $P \leq 0.05$  *a priori*.

## **5.4 RESULTS**

### **Performance**

Total distances covered during the three trials were  $13685 \pm 1116$  m;  $13715 \pm 1143$  m and  $14046 \pm 1104$  m for the P1, P2 and C trials respectively ( $F_{(2,14)} = 12.1$ ,  $P = 0.001$ ). The mean difference in distance covered between the P1 and P2 trials was 30.7 m ( $P = 1.000$ ). The mean differences in distance covered between the C trial and placebo trials were 362 m (2.6%,  $P = 0.027$ ) and 331 m (2.4%,  $P = 0.011$ ) for P1 and P2 trials respectively.

Of the eight runners that participated in the study, seven increased the total distance covered during the 1 h C trial. There was no trial order effect between the three trials

( $F_{(2,14)} = 3.0, P = 0.085$ ). If we were to interpret our results as a fixed distance i.e. the mean distance run during the CHO trial (greatest distance), runners would have had to run for 93.6 s longer during the P1 trial and 86.4 s longer in the P2 trial to cover the same distance.

The mean running speeds for the three trials were P1:  $13.7 \pm 1.1 \text{ km} \cdot \text{h}^{-1}$ ; P2:  $13.7 \pm 1.1 \text{ km} \cdot \text{h}^{-1}$ ; C:  $14.1 \pm 1.1 \text{ km} \cdot \text{h}^{-1}$  ( $F_{(2,14)} = 14.1, P = 0.0004$ ). The mean difference in running speed between the P1 trial and P2 trial was  $0.03 \text{ km} \cdot \text{h}^{-1}$  ( $P = 1.000$ ). Mean difference self-selected running speed for the C trial was  $0.39 \text{ km} \cdot \text{h}^{-1}$  faster compared to the P1 trial ( $p = 0.022$ ) and  $0.36 \text{ km} \cdot \text{h}^{-1}$  faster compared to the P2 trial ( $P = 0.005$ ). When analysed in 5-min blocks, no significant difference in running speed was observed between P1 and P2 at any time point ( $P > 0.05$ ). Self-selected running speed was significantly faster between 25-50 min in the C trial in comparison to both P1 and P2 trials ( $P < 0.05$ ). Pacing strategy was similar between runners and consistent between trials; a significant effect of time on running speed was observed between 0-5 min, 45-50 min and 55-60 min (Figure 5.1).

The treadmill performance test used in the present study has a coefficient of variation of 1.4 % (Chapter 4). Therefore, the overall improvement in running performance, after accounting for between run variability, was 1.2 % and 1.0% for the C trial in comparison to P1 trial and P2 trial respectively. In real terms, we estimate that the participants ran 164 m further than in the P1 trial and 137 m further in the P2 trial. Thus, with respect to a fixed distance improvements in time during the C trial would equate to 43.2 s in comparison to the P1 trial P1 and a 36.0 s improvement in comparison to the P2 trial.

The 5 min warm up speed was  $11 \pm 0.8 \text{ km} \cdot \text{h}^{-1}$ ,  $\dot{V}O_2$  was  $37 \pm 4 \text{ ml} \cdot \text{kg BM} \cdot \text{min}^{-1}$  equivalent to 60 %  $\dot{V}O_{2\text{peak}}$  for all trials. Rating of perceived exertion for the warm-up was  $9 \pm 1$  for trials P1 and P2 and  $10 \pm 2$  for trial C.

There were no differences in the mean physiological responses (Table 5.2) or psychological responses (Table 5.3) between the main trials. The rates of CHO oxidation, estimated from indirect calorimetry, were similar in each trial. Mean

weight loss over each 1 h trial was  $1 \pm 0.3$  kg, which represents less than 1 % of mean BM.

**Table 5.2.** Mean physiological response during the CHO-E (C) and two placebo trials (P1, P2) at a self-selected pace (n.= 8, mean  $\pm$  SD).

	Trial		
	C	P1	P2
CHO oxidation (g $\cdot$ min <sup>-1</sup> )	$3.8 \pm 0.2$	$3.3 \pm 0.4$	$3.8 \pm 0.4$
Lipid oxidation (g $\cdot$ min <sup>-1</sup> )	$0.44 \pm 0.07$	$0.42 \pm 0.13$	$0.60 \pm 0.14$
RER	$0.93 \pm 0.01$	$0.93 \pm 0.02$	$0.90 \pm 0.02$
$\dot{V}O_2$ (ml $\cdot$ kg $\cdot$ min <sup>-1</sup> )	$49.5 \pm 0.5$	$49.5 \pm 0.1$	$48.9 \pm 0.2$
% $\dot{V}O_2$ peak	$80 \pm 6$	$79 \pm 8$	$80 \pm 6$
Heart rate (beats $\cdot$ min <sup>-1</sup> )	$157 \pm 13$	$158 \pm 10$	$158 \pm 8$
Sweat rate (l $\cdot$ h <sup>-1</sup> )	$1.3 \pm 0.4$	$1.4 \pm 0.3$	$1.3 \pm 0.4$

Mean blood glucose concentrations were  $4.7 \pm 0.6$  mmol  $\cdot$  l<sup>-1</sup>,  $4.6 \pm 0.6$  mmol  $\cdot$  l<sup>-1</sup> and  $5.3 \pm 0.6$  mmol  $\cdot$  l<sup>-1</sup> for the P1, P2 and C trials respectively ( $F_{(2,14)} = 19.6$ ,  $P < 0.001$ , Figure 5.2). No difference was observed between trials for blood lactate concentrations at any time point ( $F_{(2,14)} = 2.5$ ,  $P = 0.154$ , Figure 5.3).

**Table 5.3:** Mean psychological scores for rating of perceived exertion (RPE), feeling (FS), felt arousal (FAS) and gastrointestinal (G.I) scales during the CHO-E trial (C) and two placebo trials (P1, P2).

	Trial		
	C	P1	P2
RPE	$14 \pm 1$	$15 \pm 1$	$15 \pm 1$
FAS	$4.5 \pm 0.7$	$4.6 \pm 0.6$	$4.5 \pm 0.6$
FS	$3.3 \pm 0.5$	$3.3 \pm 0.5$	$2.7 \pm 0.8$
G.I	$1.1 \pm 0.3$	$1.9 \pm 0.6$	$1.2 \pm 0.7$

Energy intake in the 48 h prior to the trials was  $2676 \pm 166$  Kcal  $\cdot$  day<sup>-1</sup>, representing a daily intake of  $426 \pm 69$  g CHO ( $5.5$  g  $\cdot$  kg BM<sup>-1</sup>),  $86 \pm 18$  g of protein ( $1.2$  g  $\cdot$  kg BM<sup>-1</sup>) and  $76 \pm 25$  ( $1.0$  g  $\cdot$  kg BM<sup>-1</sup>) of fat. The mean volume of solution ingested 30 min prior to exercise was  $600 \pm 42$  ml providing  $38 \pm 3$  g of CHO. The mean volume

of fluid administered at 15-min intervals was  $150 \pm 11$  ml. This provided a total of  $450 \pm 32$  ml ingested within the 1 h run, equating to  $29 \pm 2$  g of CHO. The total quantity of CHO consumed during the C trial was  $67 \pm 5$  g.

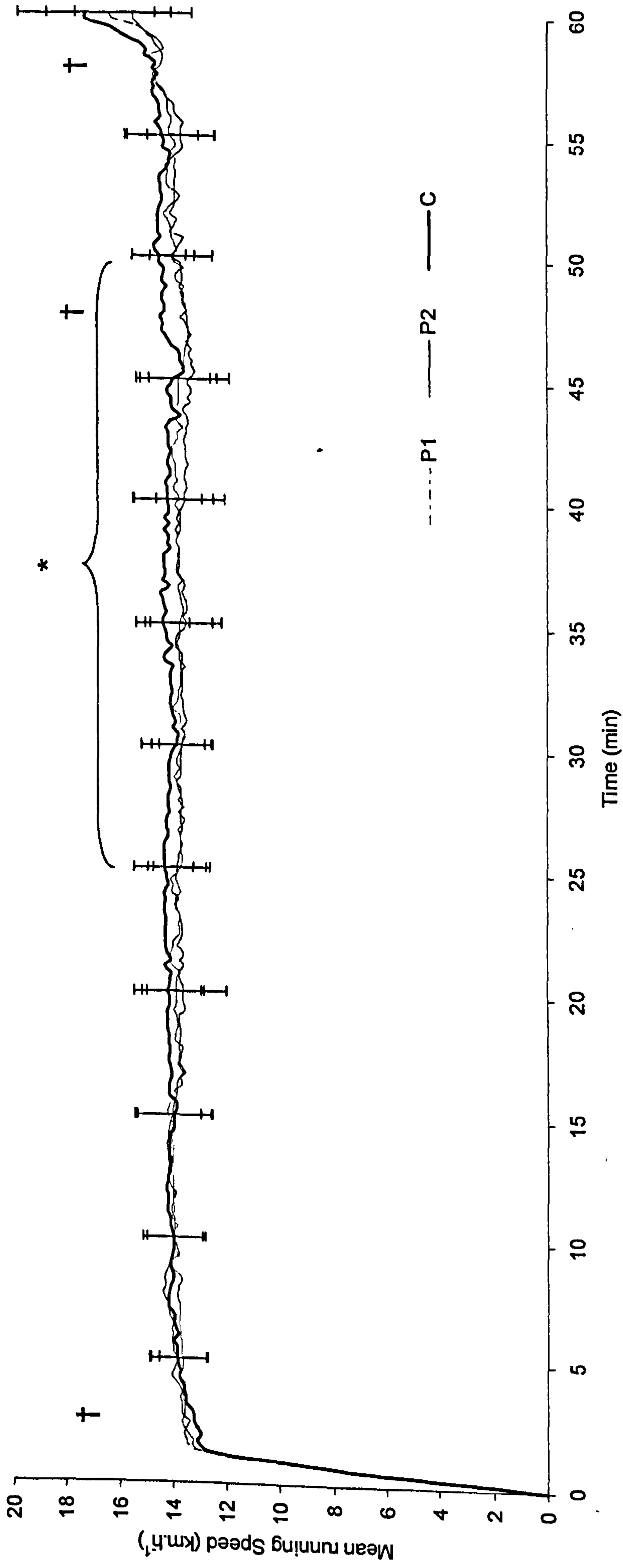
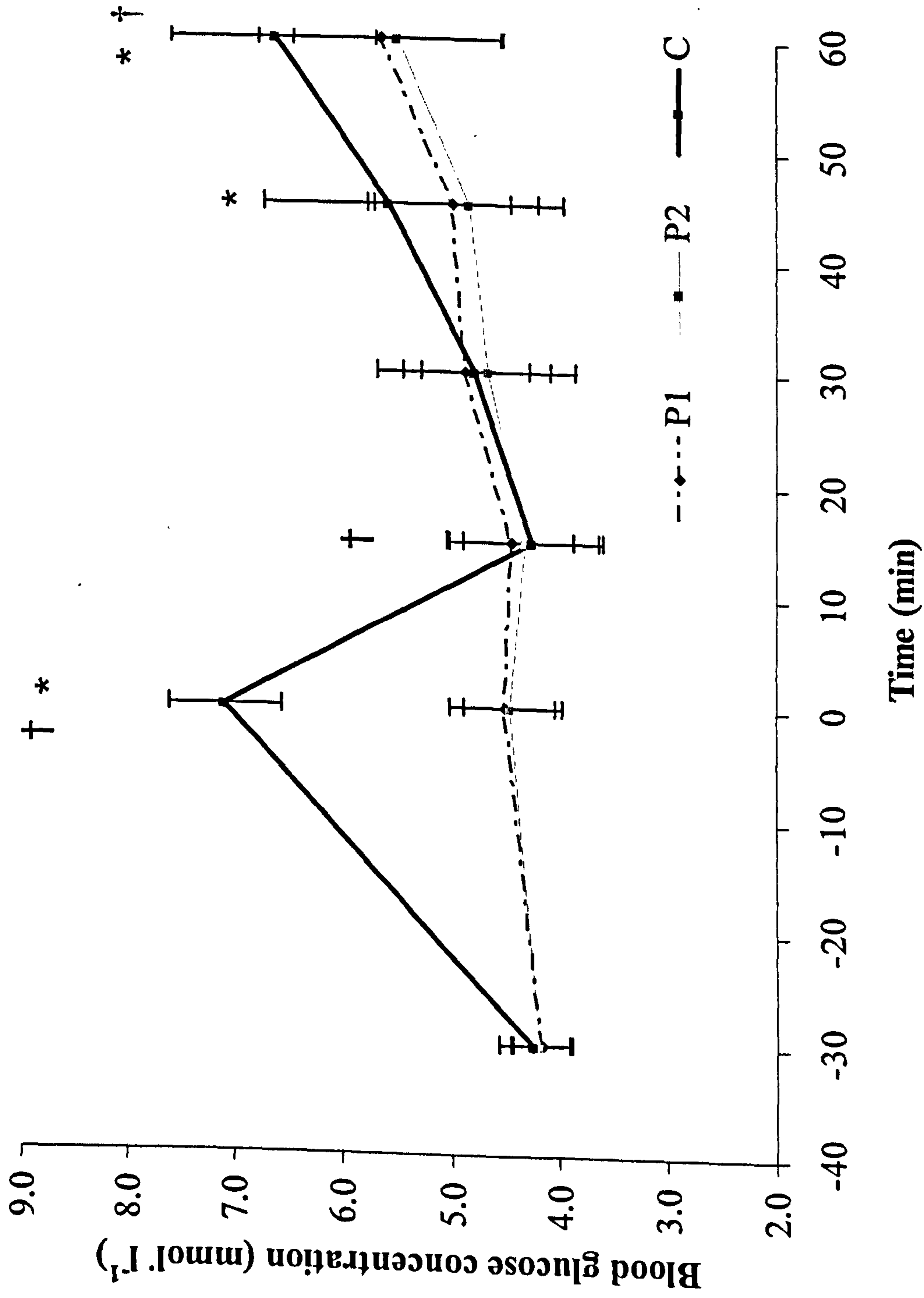
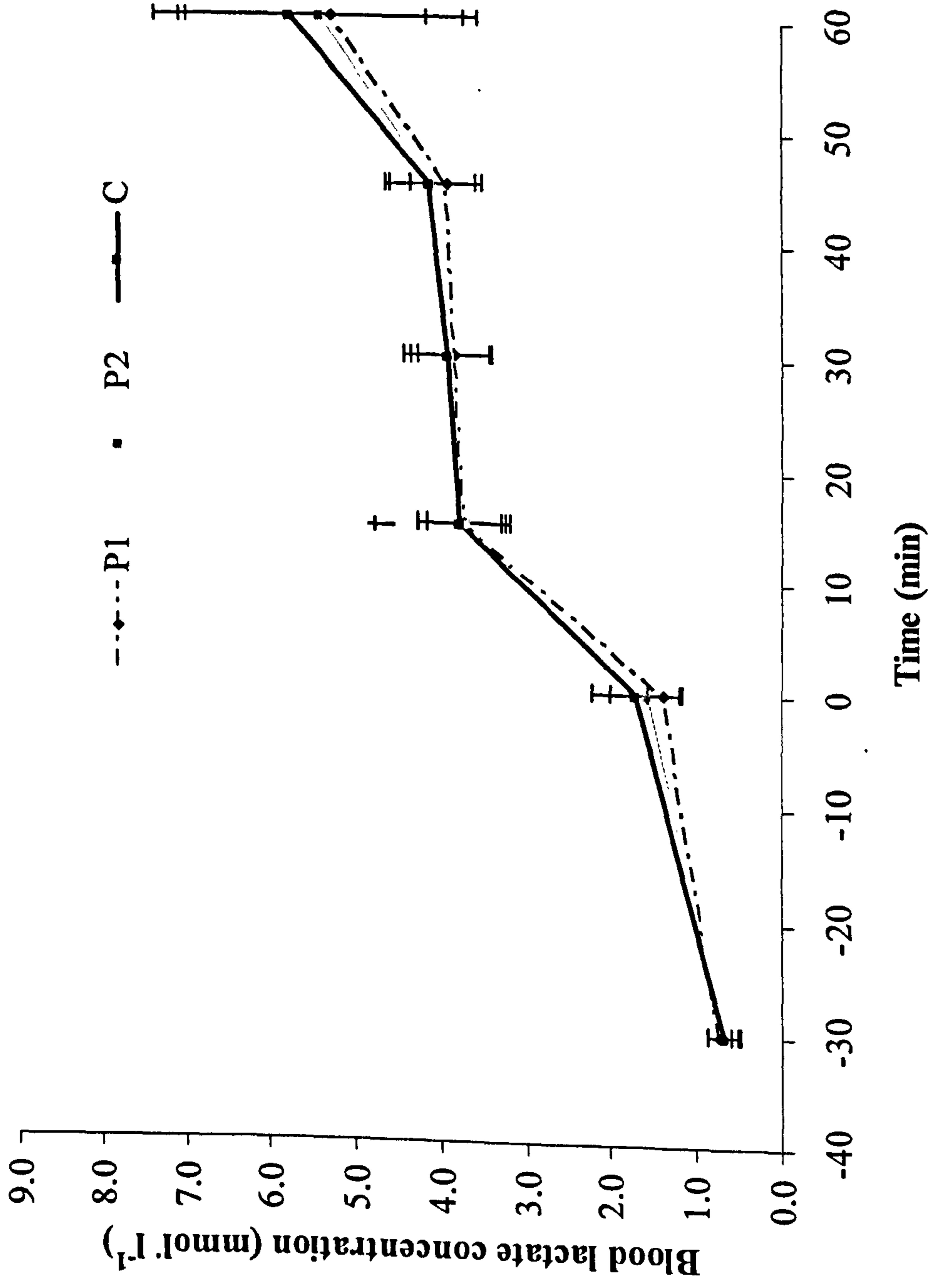


Figure 5.1: Running speed ( $\text{km} \cdot \text{h}^{-1}$ ) over the 1 h run with standard deviation shown at 5 min intervals for clarity. \* indicates significant difference of C vs. P1 and P2 ( $P < 0.05$ ). † indicates a significant effect of time ( $P < 0.05$ ).



**Figure 5.2:** Mean blood glucose concentration ( $\text{mmol} \cdot \text{l}^{-1}$ ) for each trial before and during each 1 h running trial. \* indicates significant difference between C and P1 and P2 ( $P < 0.05$ ). † indicates a significant effect of time for trial C ( $P < 0.05$ ) ( $n = 8$ , mean  $\pm$  SD).





**Figure 5.3** Mean blood lactate concentration ( $\text{mmol} \cdot \text{l}^{-1}$ ) at rest and during each 1 h running trial. † indicates a significant effect of time ( $P < 0.05$ ) ( $n = 8$ , mean  $\pm$  SD).

## **5.1 DISCUSSION**

The main finding of this study was that the ingestion of a 6.4% CHO-E solution significantly improved 1 h running performance in comparison to the ingestion of a colour and taste matched placebo. When asked to run as far as possible in 1 h 7 out of 8 runners improved total distance covered when consuming the CHO-E solution. These results add to those of other studies that show that CHO ingestion improves endurance capacity (Chryssanthopoulos et al., 1994a; Tsintzas et al., 1995b; Williams, Brewer & Walker, 1992b), and also improves endurance performance of runners during more intense time-trial tests (Millard-Stafford et al., 1997).

To our knowledge, this is the first study to investigate the influence of CHO-E solution ingestion on 1 h endurance running performance and so it is difficult to make direct comparisons with other running studies of a similar duration. However, improvements in 1 h exercise performance following the ingestion of CHO have been shown in several cycling studies using time-trial protocols (Anantaraman et al., 1995; Ball et al., 1995; Below et al., 1995; Jeukendrup et al., 1997b; Mitchell et al., 1988).

In the present study, the uncorrected overall improvement in distance covered of 2.6 % and 2.4 % following the ingestion of CHO is similar to the improvement observed previously in 1 h cycle time-trial performance. Jeukendrup et al. (1997) reported the time-trial performance of their cyclists was improved by 2.3% following the ingestion of a CHO-E solution in comparison with the ingestion of placebo. However, the 2.3 % improvement in cycle time-trial performance was within the known variation of the cycle time-trial method (3.35 %) (Jeukendrup et al., 1996). In the present study the known variation in the testing methodology i.e. 1.4 % was subtracted from the overall difference between the CHO (C) and two placebo trials (P1 and P2). Thus, it is possible to report with confidence that the performance benefit of CHO ingestion was an effect of the intervention and not simply variation in the testing method. Another way of interpreting the results would be to consider that if the runners had been in a race, those on the placebo trials would have been approximately 150 m behind those on the CHO trial as they crossed the finish line.

These findings are in agreement with Millard-Stafford et al. (1997) who, under control laboratory conditions, found the ingestion of both a 6 % and 8 % CHO solution improved 15 km running performance, compared to the ingestion of water. Runners consumed 1-L of experimental solution 60-min prior to exercise, before *ad libitum* intake throughout the time-trial. Unlike the present study, the performance benefit of CHO ingestion was only evident towards the end of the run. Self-selected running speed was approximately 5% faster over the final 1.5 km of the run, during both CHO trials. Despite the similar duration, the present study differs from Millard-Stafford et al. (1997) in several aspects that may have influenced running performance. First, the 15 km run was performed in a warm environment (27°C, 76% RH). Exercising in a warm environment has been shown to cause a shift in substrate metabolism toward CHO oxidation and place a greater strain on the cardiovascular system as a result of the demands of thermoregulation (Burke, 2001). Thus, the benefits of providing CHO and fluid on performance may be magnified when exercising in a hot environment. This may explain, in part, why a large benefit was not observed in the present study, which was conducted in a thermo-neutral environment (16 ± 1 °C, 58 % relative humidity). Second and key to performance testing, Millard-Stafford et al. (1997) used an intermittent running protocol, using a manually controlled treadmill. The 15 km run was stopped on the completion of 7 km and 13.4 km for blood collections and *ad libitum* fluid intake. Since no warm-up was permitted, runners were instructed to perform a “moderate” running speed for the first 5-min, before being asked to select speeds representing a “hard training pace”. Following the last break (approximately 5-min) runners were then instructed to give an “all out effort” to complete the final 1.5 km. Thus, the intermittent protocol may have prevented runners from following their “natural” or “optimum” pacing strategy towards the 15 km run. Interestingly, Millard-Stafford et al. (1997) observed no difference in running speed over the middle section of the run (7-13 km). In the present study, it is clear that improved performance was not a consequence of large differences in speed selection. Instead, the increase in distance covered with CHO-E solution ingestion was the result of runners selecting slightly faster speeds between 25-50 min (Figure 5.1). This faster speed at 25-50 min was possibly a consequence of the automated treadmill being more responsive to spontaneous changes in self-selected running speed during the time-trial. Thus these performance improvements may be masked when treadmill running speed is controlled manually.

Relatively few studies have investigated the influence of CHO ingestion under “real life” race conditions. Interestingly, the uncorrected performance benefit of the present study (2.6 % and 2.4 %) was similar to the performance improvement found with CHO ingestion during a 30 km road race (2.3 %) (Tsintzas et al., 1993). In this “real life” study, runners performed thirty 1 km outdoor circuits, drinking either a 5 % CHO solution or water, immediately prior and at 5 km intervals during the run. Unlike the present study, CHO ingestion did not appear to increase running speed. Instead, improved performance was attributed to runners being able to maintain their chosen running speed over the final 5 km of the run. In contrast to these earlier findings, Burke et al. (2005) found that providing approximately  $1 \text{ g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  CHO along with *ad-libitum* water intake during a half marathon had a negligible effect on race time in comparison to a placebo trial. Despite providing the CHO in the form of sports gels, the study failed to reduce the incidence of G.I discomfort. In fact, the ingestion of sports gels induced some degree of G.I discomfort in a small number of runners which clearly impaired run performance. Drinking mineral water has been found to induce less symptoms of G.I complaint in comparison to the consumption of a 6.8% CHO or CHO caffeinated drink during an 18 km run (van Nieuwenhoven et al., 2005). Despite not having a water only trial, the ingestion of a CHO-E solution in the present study appeared to induce no greater risk to G.I discomfort than the ingestion of placebo.

It has been suggested that taking physiological measures during time-trials may interfere with the runners' concentration (Jeukendrup et al., 1997b) and therefore affect time-trial performance. In the present study all efforts were made to minimise interaction with the runner during the 1 h run. Interaction was limited to the collection of expired air, delivery of drinks and the collection of finger prick blood samples on three occasions during the run. In the present study, it was not possible to establish whether or not run performance would have been further improved had no measurement interventions been included. However, the similarity between the two PLA trials suggests that interaction with the runners was consistent. With respect to disrupting running performance, CHO feedings have been shown to slow the time for a runner to pass through feeding zones during a half marathon (Burke et al., 2005). Unpublished observations from our laboratory have shown that even drinking from

easily accessible bottles causes a marked reduction in running speed on the automated treadmill. The reduction in speed is a consequence of the runner slowing in order to perform the action of tilting their head to drink. In the present study, we found that administering drinks in volumetric syringes allowed the runner to maintain running speed whilst drinking. In addition, providing the solutions in syringes ensured the exact volume of fluid was consumed by the runner. The plastic syringe also reduced the risk of spillage, a common problem associated with providing drinks in cups.

The finding that CHO feedings improved 1 h endurance running performance in the present study is difficult to explain because endogenous stores of CHO are unlikely to be limiting during exercise of 1 h duration (Tsintzas, Williams, Boobis & Greenhaff, 1995a). Tsintzas et al. (1995a) found that ingesting a 5.5 % CHO solution immediately before and during a constant pace 1 h treadmill run (70%  $\dot{V}O_2$ max) resulted in a decrease in muscle glycogen utilisation in type-1 fibres in comparison to the ingestion of water. Despite elevated blood glucose, total CHO oxidation rates were similar between trials and muscle glycogen was not limiting on completion of the 1 h run. In the present study, any alteration in substrate metabolism, with CHO ingestion is likely to have been masked by the intensity at which the run was performed, i.e. approximately 80%  $\dot{V}O_2$ peak (Coggan et al., 1991). Consequently, in comparison to the study reported by Tsintzas et al. (1995a) it is likely that CHO oxidation was elevated as a consequence of the increased demand on skeletal muscle glycogen for energy provision (Arkininstall, Bruce, Clark, Rickards, Burke & Hawley, 2004). The similarity in estimated substrate oxidation between trials in the present study was not surprising, as running speed and thus intensity was not significantly different over the three collection periods ( $P > 0.05$ ). Pre-exercise feedings of CHO have been shown to cause hypoglycaemia during exercise (Koivisto et al., 1981). The hypoglycaemic response, however, has been shown to disappear within 10 min of exercise (Moseley, Lancaster & Jeukendrup, 2003). Therefore, although the results of the present study did not show a pronounced hypoglycaemic response to CHO ingestion in the present study, this occurrence could have been missed by the blood collection sampling times. If there was transient hypoglycaemia in the present study during the first 10 min of exercise it did not appear to affect the selection of running speed. This finding is consistent with previous studies that reported that

hypoglycaemia during the first 10 min of exercise did not influence RPE or subsequent exercise performance (Moseley et al., 2003).

In conclusion the ingestion of a 6.4 % CHO-E solution was associated with an increase in distance completed during 1 h of treadmill running. There was no evidence that CHO ingestion provided any physiological or psychological advantage above that of ingesting a placebo solution, leaving the mechanisms responsible for improved performance unknown.

## CHAPTER 6

### INFLUENCE OF INGESTING A CARBOHYDRATE-ELECTROLYTE SOLUTION BEFORE AND DURING 1 HOUR RUNNING PERFORMANCE IN FED ENDURANCE TRAINED RUNNERS

#### 6.1 SUMMARY

This study investigated whether the ingestion of a CHO-E solution would improve 1 h running performance in runners who had consumed a CHO meal 3 h before exercise. Ten endurance-trained male runners completed two trials that required them to run as far as possible in 1 h on an automated treadmill that allowed changes in running speed without manual input. Before each run, runners consumed a CHO meal ( $2.5 \text{ g} \cdot \text{kg BM}^{-1}$ ) 3 h before exercise. They then consumed either a 6.4 % CHO-E solution or placebo (PLA) solution,  $8 \text{ ml} \cdot \text{kg BM}^{-1}$ , 30 min prior to and  $2 \text{ ml} \cdot \text{kg BM}^{-1}$  at 15 min intervals throughout the 1 h run. There were no difference in total distance covered i.e.  $13680 \pm 1525 \text{ m}$ ; and  $13589 \pm 1635 \text{ m}$  for the PLA and CHO-E solution trials respectively ( $P > 0.05$ ). Blood glucose and lactate concentration, RER, and CHO oxidation during exercise were similar between trials ( $P > 0.05$ ). There were also no differences in perceived exertion (RPE), felt arousal (FAS) or pleasure-displeasure (FS) between trials ( $P > 0.05$ ). In conclusion the ingestion of a 6.4% CHO-E did not improve 1 h running performance when a high CHO meal was consumed 3 h before exercise.

## **6.2 INTRODUCTION**

When a high CHO pre-exercise meal is combined with the ingestion of a CHO-E solution during exercise then the improvements in endurance capacity (time to fatigue) during cycling (Wright et al., 1991) and during running (Chryssanthopoulos et al., 1994b) are greater than when either of these CHO interventions are adopted alone. In Chapter 5 it was reported ingesting a CHO-E solution was associated with improved 1 h running performance. However, consistent with the results of cycling time-trial studies that have shown performance benefits with CHO ingestion, the runners in who participated in the study in Chapter 5 performed the 1 h run following an overnight fast.

Although many athletes avoid eating immediately before early morning training or competition, given the choice most athletes would prefer to have a meal a few hours before competition rather than to fast before exercise. Therefore, studies on the performance benefits of pre-exercise meals are worthwhile because they may give a better insight into the 'real world' responses of athletes to various nutritional strategies. Therefore, the aim of the present study was to investigate the effect of ingesting a CHO-E solution on performance during a 1 h running time-trial following the consumption of a high CHO pre-exercise meal.

## **6.3 METHODS**

### **Participants**

Ten recreational club runners ( $34 \pm 9$  y; body mass  $75.4 \pm 6.1$  kg, height  $1.81 \pm 0.07$  m,  $\dot{V}O_{2\text{peak}}$   $62.0 \pm 6.6$  ml  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup>) gave their written consent before participating in this study approved by Loughborough University Ethical Advisory Committee. All the runners who were selected to participate in this study were completing 3-5 training session per week of at least 1 h in duration and had completed a half or full marathon within the last year.



## **Preliminary tests**

A series of preliminary tests were conducted prior to the main experimental trials to determine (i) the relationship between running speed and oxygen uptake and (ii) the peak oxygen uptake ( $\dot{V}O_{2\text{peak}}$ ) (Chapter 3).

## **Experimental design**

All trials were conducted in the morning at the same time of day (7.00 am- 9.00 am) and separated by 7 days. Runners reported to the laboratory following an overnight fast (12 - 15 h). Running performance was assessed using the 1 h run protocol described in Chapter 3. The study utilised a double-blind, random cross-over design. Runners were asked to refrain from heavy exercise and to consume a standardised diet 48 h before each trial i.e. they recorded their food intake in the 48 h before the first trial and replicated it before the next trial. There were no significant differences between trials in the average daily energy intake, or quantities of carbohydrate, protein or fat consumed in this 48 h period (Dietary composition analysed by CompEat Pro 5.8.0). All trials were conducted in the morning and there was 7 days between each trial.

### *Pre-exercise meal and drinks*

Runners arrived at the laboratory following an overnight fast (12 h) and consumed a pre-exercise meal 3 h before the start of the 1 h run. The pre-exercise meal consisted of white bread ( $121 \pm 10\text{g}$ ), jam ( $77 \pm 4\text{g}$ ), corn flakes ( $62 \pm 5\text{g}$ ), skimmed milk ( $312 \pm 34\text{g}$ ), water ( $315 \pm 0\text{ ml}$ ) and orange juice ( $174 \pm 28\text{g}$ ), which amounted to approximately 86% of energy intake from carbohydrate, 11% from protein and less than 3% fat (Appendix H). The carbohydrates included in the meal was obtained from foods classified as having a high glyceamic index (Jenkins et al., 1984). The meal, ( $2.5\text{ g CHO} \cdot \text{kg BM}^{-1}$ ) has previously been shown to increase muscle glycogen concentrations by  $10.6 \pm 2.5\%$  (Chryssanthopoulos et al., 2004). Runners were required to eat the meal within 15-min and asked to consume the various components of the meal in the same order for both trials. Runners then sat at rest for 3 h before commencing the 1 h performance run. A 5-min resting expired air sample was collected 45-min prior to the start of the 1 h run.

Runners ingested  $8 \text{ ml} \cdot \text{kg BM}^{-1}$  of a commercially available 6.4% CHO-E solution (C) (Lucozade Sport, Brentford, England) or placebo solution (P) 30 min prior to the start of the 1 h run. Runners ingested a volume of the same solution  $2 \text{ ml} \cdot \text{kg BM}^{-1}$  at 15-min intervals throughout the 1 h run. Each  $2 \text{ ml} \cdot \text{kg BM}^{-1}$  bolus of solution was served in two separate plastic volumetric syringes (Kendal, monoject, UK). The placebo solution was matched in formulation to the CHO-E solution except that it did not contain CHO. The feeding schedule used in the present study replicated that of Chapter 5, in which significant improvements in 1 h running performance were reported with CHO-E solution ingestion.

#### *Blood collection, heart rate and expired air*

Finger tip blood samples (20- $\mu\text{l}$ ) were taken in duplicate on arrival at the laboratory and then at 30 min before, immediately before the 1 h run and at 15-min intervals during the run. All blood samples were deproteinised in perchloric acid, frozen and later analysed for the concentrations of glucose and lactate (Maughan, 1982). Expired air was collected by Douglas bag method between 13.40 - 14.40 min, 28.40 - 29.40 min and 43.40 - 44.40 min. Heart rate was recorded continuously at 15 s intervals during the warm-up and during the 1 h run (Polar Electro, Kempele, Finland).

#### *Psychological scales*

The feeling scale (FS), felt arousal scale (FAS), together with the gastrointestinal (G.I) discomfort scale were administered at rest, immediately prior to and at 15-min, 30-min, 45-min and 60-min during the 1 h run (Chapter 3). Runners RPE was collected using the Borg Rating of Perceived Exertion scale (Borg, 1982) during the warm-up and at 15 min, 30 min and 45 min during the 1 h run.

#### **Statistical analysis**

All data were analysed using SPSS (version 16.0). A paired samples t-test was performed to identify differences in running performance (total distance covered, trial order) between the two trials. The differences in psychological (RPE, FS, FAS, G.I) and physiological (bloods, expired air, heart rate) variables were studied using a two way repeated measures ANOVA (trial x time). Significant main effects for individual time points were further analyzed using paired *t*-tests and the Bonferroni adjustment for the number of pair-wise comparisons employed. Mean differences in self-selected

running speed and comparisons over time (analysed in 5-min blocks during the 1 h run) were detected using a (trial by time) within measures ANOVA. The critical alpha level was set at  $P \leq 0.05$  *a priori*. All data reported as mean  $\pm$  standard deviation.

## 6.4 RESULTS

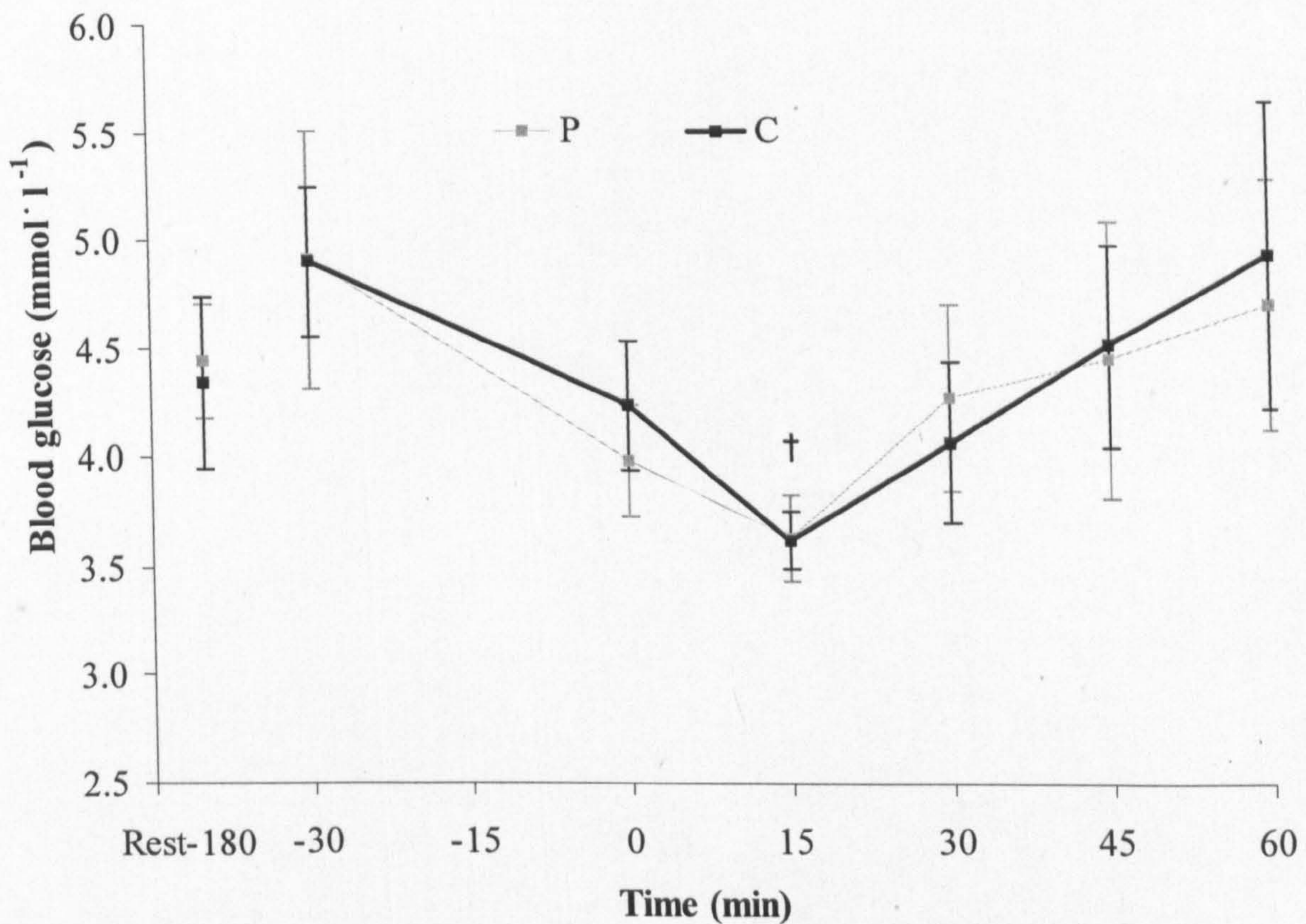
### *Performance*

There were no differences in the mean distances covered which were  $13680 \pm 1525$  m and  $13589 \pm 1635$  m for the P and C trials respectively ( $P = 0.360$ ). The range of total distances covered achieved by the runners in the present study was similar to that achieved in the study which reported the 1.4% CV when completing the 1 h run (Chapter 4). The percentage difference in total distance covered between the P and C trials was 0.5 %, which is within the previously reported day-to-day variation of the 1 h run test protocol (CV: 1.4%) (Chapter 4). The mean running speeds were  $13.7 \pm 1.5$   $\text{km} \cdot \text{h}^{-1}$  and  $13.6 \pm 1.6$   $\text{km} \cdot \text{h}^{-1}$  for the P and C trials respectively ( $P = 0.264$ ). When analysed in 5-min blocks there were no significant difference in running speed between the two trials at any time point. Pacing strategy was similar between runners and consistent between trials ( $P < 0.05$ ) (Figure 6.2).

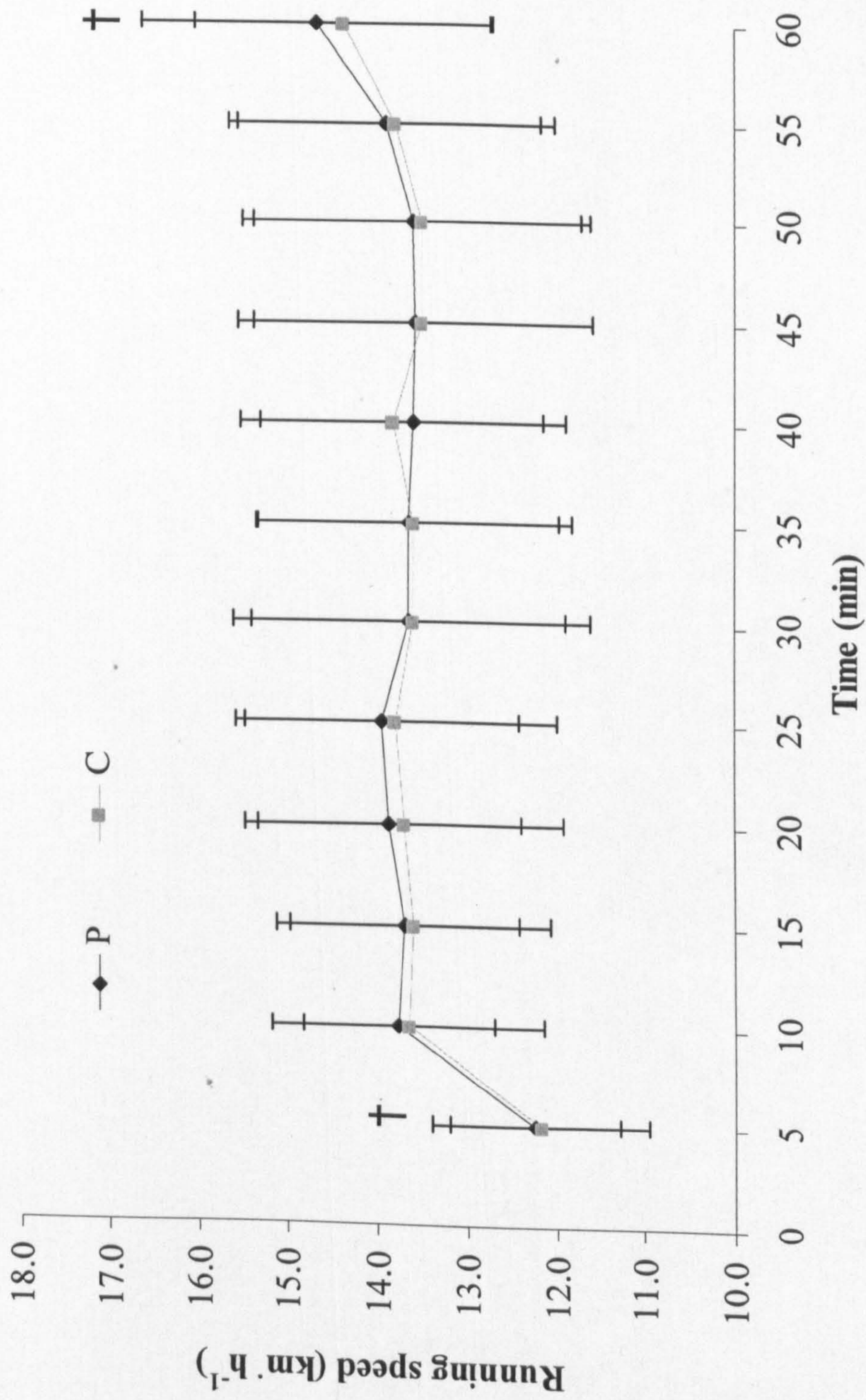
The mean physiological responses and psychological scores during P and C trials are listed in Table 6.1. There was no difference in blood glucose concentrations at rest or throughout exercise between trials ( $P > 0.05$ ). However, there was a significant effect of time from 30-min before, to the start of the 1 h run (Figure 6.1). The mean volume of fluid ingested 30-min prior to exercise was  $590 \pm 52$  ml which provided  $38 \pm 3$  g of CHO in the C trial. All runners successfully used the plastic syringes to ingest the drink provided during exercise. There was no incidence of spillage. The mean volume of fluid ingested at 15-min intervals in each trial was  $148 \pm 13$  ml. This provided a total of  $443 \pm 39$  ml during the 1 h run supplying  $28 \pm 2$  g of CHO in the C trial. Thus the total quantity of CHO consumed during the C trial was  $66 \pm 6$  g. Calculated sweat rates were  $1.5 \pm 0.3$   $\text{l} \cdot \text{h}^{-1}$  and  $1.6 \pm 0.2$   $\text{l} \cdot \text{h}^{-1}$  for the P and C trials respectively.

The 5-min warm-up speed was  $11.2 \pm 0.9$   $\text{km} \cdot \text{h}^{-1}$  and the  $\dot{V}\text{O}_2$  was  $35.5 \pm 3.8$   $\text{ml} \cdot \text{kg BM} \cdot \text{min}^{-1}$  equivalent to  $61 \pm 8$  %  $\dot{V}\text{O}_2$  peak for both trials. During the warm-up the

RPE values were  $9 \pm 2$  and  $9 \pm 1$  for the P and C trials respectively. There were no difference in any psychological scores (FAS; P:  $2.8 \pm 1$ , C:  $2.8 \pm 1$ , FS; P:  $1.8 \pm 1.8$ , C:  $1.4 \pm 1.7$ , G.I; P;  $1 \pm 1$ , C:  $1 \pm 1$ ,  $P > 0.05$ ) or oxygen uptake (P;  $4.2 \pm 0.2 \text{ ml} \cdot \text{kg BM} \cdot \text{min}^{-1}$ , C;  $4.4 \pm 1.1 \text{ ml} \cdot \text{kg BM} \cdot \text{min}^{-1}$ ,  $P > 0.05$ ) at rest.



**Figure 6.1:** Mean blood glucose concentration ( $\text{mmol} \cdot \text{l}^{-1}$ ) for each trial before and during both 1 h running trials. † indicates a significant effect of time for both trials ( $P < 0.05$ ) ( $n=10$ , mean  $\pm$  SD).



**Figure 6.2:** Running speed (km·h<sup>-1</sup>) over the 1 h run with standard deviation shown at 5-min intervals for clarity. † indicates a significant effect of time over 5-min (n.=10, mean ± SD).

	Physiological response						Psychological scores			
	Exercise intensity (% $\dot{V}O_2$ peak)	Heart rate (beats·min <sup>-1</sup> )	RER	CHO oxidation (g·min <sup>-1</sup> )	Blood lactate (mmol·L <sup>-1</sup> )	RPE	G.I comfort	FAS	FS	
P	74 ± 7	159 ± 11	0.96 ± 0.01	4.1 ± 0.2	3.6 ± 0.5	14 ± 1	1.5 ± 0.6	4.5 ± 0.3	1.8 ± 0.4	
C	75 ± 7	160 ± 11	0.94 ± 0.04	3.7 ± 0.3	3.5 ± 0.6	13 ± 1	1.6 ± 0.6	4.3 ± 1.0	1.6 ± 0.4	
P=	0.730	0.202	0.159	0.162	0.602	0.338	0.810	0.343	0.122	

**Table 6.1:** Mean physiological responses and psychological scores during the 1 h run when consuming a 6.4% CHO-E solution (C) or a colour and taste matched placebo (P) (n.=10, mean ± SD).

## **6.5 DISCUSSION**

The main finding of this study was that the ingestion of a 6.4% CHO-E solution did not influence running speed and total distance covered during the 1 h time-trial, when the runners consumed a CHO meal 3 h before exercise. These findings, the first to report on running performance, are consistent with several cycling studies that have shown that the ingestion of CHO-E solution during exercise has no benefit during shorter (approximately 1 h) more intense ( $> 70\% \dot{V}O_2\text{peak}$ ) time-trials in fed individuals (Desbrow et al., 2004; Neufer et al., 1987; Widrick et al., 1993a).

The increase in CHO in liver and muscle following a pre-exercise meal that is also supplemented with a CHO-E solution during exercise has clear performance benefits when performance is assessed as constant pace exercise to fatigue (Chryssanthopoulos et al., 1997). However, in a study on methods of CHO loading and running performance Sherman et al. (1981) showed that elevated glycogen stores prior to a 20.9 km track race produced no better finishing times than those achieved without glycogen loading (83 min). Thus, the normal muscle glycogen stores of these fasted well trained runners were sufficient to meet the demands of the race. Similarly, when a CHO-E drink was ingested during prolonged cycling time-trials with high pre-exercise muscle glycogen stores there were no improvements in performance (Neufer et al., 1987; Widrick et al., 1993a). Nevertheless, it is important to note, when pre-exercise muscle glycogen stores were less than optimal, the ingestion of a CHO-E solution during exercise improved time-trial performance, compared with the performance in the absence of CHO-E solution (consistent with the results of Chapter 5).

The ingestion of a high CHO meal ( $2.5 \text{ g} \cdot \text{kg BM}^{-1}$ ) 3 h before exercise has been reported to increase muscle glycogen concentration only by about 11 to 15 % (Chryssanthopoulos et al., 2004; Wee et al., 1999). Therefore, it is reasonable to assume that in the present study the majority of CHO ingested increased liver and muscle glycogen stores and there was probably a small amount remaining within the gastrointestinal tract undergoing digestion and absorption.

In the present study blood glucose concentrations fell below resting values 15-min into exercise in both trials (Figure 6.1). These changes reflect a greater rate of blood glucose disappearance than the release of hepatic glucose. Chryssanthopoulos et al. (2004) has shown

that the consumption of the same meal as adopted in the present study, significantly raised serum insulin concentrations during the 3 h post prandial period, but the subsequent ingestion of a CHO-E solution during exercise did not produce a further increase in serum insulin concentrations. A high pre-exercise insulin concentration, accompanied with muscle contraction would have facilitated the uptake of exogenous glucose early in exercise (Ivy, 1987). Furthermore the ingestion of a CHO-E solution has been shown to decrease hepatic glucose production and so this may have also contributed to the early transient decrease in blood glucose concentrations (Jeukendrup, Wagenmakers, Stegen, Gijsen, Brouns & Saris, 1999; McConell, Fabris, Proietto & Hargreaves, 1994). The ingestion of a CHO solution (71 g/120 min) by fasting individuals has been shown to reduce hepatic glucose output and even greater amounts of CHO (354 g/120 min) appear to completely block hepatic glucose production (Jeukendrup et al., 1999). In the presence of a reduced hepatic glucose output, the ingested CHO contributes to maintaining blood glucose concentrations in particular and to CHO metabolism in general.

Although the present study shows that there was no performance benefit from drinking a CHO-E solution during the 1 h time-trial, the previous study, using the same methods, showed that fasted runners improved their performance as a result of ingesting the same CHO-E solution (Chapter 5). The obvious difference between the fasting and the fed study was the greater CHO status of the runners in the present study i.e. the fed subjects has a greater quantity of endogenous CHO. Therefore, it is reasonable to ask how the CHO status of runners' might influence the selection of running speed during time-trials / races.

It has been suggested that the selection of pace during exercise is directly linked with pre-exercise muscle glycogen concentrations (Rauch et al., 2005). Thus, according to this suggestion, runners will select faster initial speeds when their pre-exercise glycogen stores are high than when they are low. If this were the case then it may help explain, at least in part, the results of previous time-trial studies that have shown improved performance in fasting cyclists who ingested CHO-E solutions immediately before and during exercise (Ball et al., 1995; Below et al., 1995; Jeukendrup et al., 1997b) but no improvements in performance when they consumed pre-exercise CHO meals (Desbrow et al., 2004; Neuffer et al., 1987; Widrick et al., 1993a). The assumption being that after a CHO pre-exercise meal the pace selected would already be at its most appropriate and thus additional CHO-E solution feedings would have little further influence on pace selection. However, Johnson et al. (2006) reported that the



selection of exercise intensity by well-trained cyclists was independent of pre-exercise glycogen concentrations. In their study cyclists selected the same pace at the beginning of a 3 h time trial when they had high or low pre-exercise muscle glycogen concentrations. However, the cyclists in both trials ingested a 7% glucose polymer solution at a rate of  $15 \text{ mL} \cdot \text{kg} \cdot \text{BM} \cdot \text{h}^{-1}$  (approximately  $74 \text{ g} \cdot \text{h}^{-1}$ ) throughout exercise which may have influenced the selection of power outputs at the start of exercise on the low glycogen trial. It is also important to note the duration of exercise in the low glycogen trial. Cyclists fatigued after 2 h whereas when they had high pre-exercise glycogen stores they completed the required 3 h.

During exercise of 1 h duration there appears to be sufficient endogenous CHO to fuel muscle metabolism such that ingesting a CHO-E solution has little apparent benefit. Specifically, in cycling it is reported that only a small quantity of total CHO ingested (approximately 26%) is absorbed during high intensity ( $\sim 80\% \dot{V}O_2\text{max}$ ) exercise. In addition, the exogenous CHO contributes minimally to total CHO oxidation (McConnell et al., 2000). Thus, observations that CHO ingestion improved time-trial performance without any apparent benefit to metabolism led authors to speculate that CHO may exert a “central” response. The use of functional magnetic resonance imaging (fMRI) has recently provided evidence that both glucose and maltodextrin in the mouth activate regions in the brain associated with reward, such as the insula/frontal operculum, orbitofrontal cortex and striatum (Chambers et al., 2009). Ingesting CHO during exercise may activate these brain regions and result in altered behaviour e.g. improved 1 h run performance (Chapter 5). Interestingly, however, Chambers et al. (2009) recognised that the central response to tasting CHO would be modulated by the physiological status of the body, i.e. a fasted or fed state. Thus, speculatively, that the CHO status of the runners’ had been “optimised” by the ingestion of the pre-exercise meal, may explain why 1 h running performance was not affected by the ingestion of a CHO-E solution in the present study. While only speculation, the idea of central monitoring of the whole body CHO status which in turn influences the self-selection of exercise intensity is worthy of further investigation.

In conclusion, the circumstantial evidence from the present study suggests that when endogenous CHO stores are adequate then experienced runners select running speeds that are the most appropriate for the completion of the required task. Thus, ingesting a CHO-E solution during a 1 h performance run resulted in no further performance benefit in runners who consumed a high CHO meal 3 h before exercise.

## CHAPTER 7

### THE INFLUENCE OF MOUTH-RINSING A CARBOHYDRATE SOLUTION ON 1 HOUR RUNNING PERFORMANCE

#### 7.1 SUMMARY

**Purpose:** To investigate the influence of mouth-rinsing a CHO-E solution on 1 h running performance. A second study determined whether mouth-rinsing a CHO-E solution altered the blood glucose and plasma insulin concentrations at rest. **Methods:** Following a 13 h fast, ten endurance-trained male runners completed two 1 h performance runs on an automated treadmill, whilst mouth-rinsing 25 ml of either a 6.4% CHO-E (C) or placebo (P) solution immediately before and at 15-min intervals during the 1 h run. An additional ten healthy active males followed the same mouth-rinsed procedure during a 1 h resting period. Finger prick blood samples were obtained for determination of blood glucose and plasma insulin concentrations. **Results:** Runners covered 211 m (90 % CI = 42 m to 380 m,  $P = 0.048$ ) further during the C trial ( $14298 \pm 685$  m, mean  $\pm$  SD) in comparison to the P trial ( $14086 \pm 732$  m). There was no change in blood glucose concentrations during the 1 h run (P; pre,  $4.3 \pm 0.2$  mmol·L<sup>-1</sup>, post,  $4.3 \pm 0.3$  mmol·L<sup>-1</sup> C: pre,  $4.3 \pm 0.4$  mmol·L<sup>-1</sup>, post,  $4.3 \pm 0.3$  mmol·L<sup>-1</sup>). At rest there was no change in blood glucose (P;  $4.3 \pm 0.1$  mmol·L<sup>-1</sup>, C:  $4.3 \pm 0.2$  mmol·L<sup>-1</sup>) or plasma insulin (P;  $6.2 \pm 1.1$  mU·L<sup>-1</sup>, CHO;  $5.9 \pm 1.0$  mU·L<sup>-1</sup>) concentrations ( $P > 0.10$ ). **Conclusion:** Mouth-rinsing a 6.4% CHO-E solution was associated with increased distance covered during a 1 h performance run in comparison to mouth-rinsing a placebo solution. Mouth-rinsing a CHO-E solution was not associated with changes in blood glucose concentration during exercise or at rest.

## **7.2 INTRODUCTION**

It has been reported that infusing glucose into cyclists ( $60 \text{ g} \cdot \text{h}^{-1}$ ) had no influence on their 1 h cycle time-trial performance, yet mouth-rinsing the CHO solution without ingestion resulted in significant improvements in their performance (Carter et al., 2004a; Carter et al., 2004b). These findings are also supported by Pottier et al. (2008) who reported the benefits of mouth-rinsing a CHO-E solution on 1 h cycle time-trial performance in comparison to the ingestion of the same solution (Pottier et al., 2008). Thus, a key variable, impacting on performance, appears to be the method by which CHO enters the circulation during exercise performance i.e. ingestion or infusion. These observations have led authors to speculate on possible link between CHO in the mouth and the brain during exercise, a relationship which has been recently explored by using functional magnetic resonance imaging (Chambers et al., 2009).

Nevertheless, mouth-rinsing a CHO solution has recently been shown not to have an influence on performance during a 1 h treadmill running test (Whitham et al., 2007). However, the authors of this study acknowledged that a limitation to treadmill time-trials was the runners' inability to spontaneously alter running speed. Thus, these authors suggested that treadmill tests which require conscious alterations of running speed, may not be sufficient to detect any potentially sub-conscious central effect of a CHO mouth-rinse on performance (Whitham et al., 2007).

Using the advancement in treadmill performance testing (Chapter 4), the aim of the present study was to investigate the influence of mouth-rinsing a 6.4% CHO-E solution on self-selected running speed and distance covered in 1 h. In order to confirm that the mouth-rinsing procedure did not lead to changes in blood glucose concentrations an additional study was conducted that examined blood glucose and plasma insulin concentrations during a 1 h resting period.

## **7.3 METHODS**

### **Participants**

The physiological characteristics of the participants for both the performance and resting study are reported in table 1. Participants were healthy active men who gave their written consent before participating in this study approved by Loughborough University Ethical Advisory Committee. The number of participants was determined using a nomogram based upon the ratio limits of agreement (Nevill et al., 1997). All participants in the performance study were experienced runners accustomed to training and/or competitions lasting at least 1 h in duration.

**Table 7.1:** Participant characteristics for the resting and performance study (mean  $\pm$  SD and range).

<b>Study</b>	<b>Resting</b>	<b>Performance</b>
n. =	10	10
Age (years)	26 $\pm$ 3	25 $\pm$ 4
Body mass (kg)	76.3 $\pm$ 5.2	74.3 $\pm$ 5.5
Height (m)	1.74 $\pm$ 0.13	1.79 $\pm$ 0.06
$\dot{V}O_{2peak}$ (l $\cdot$ min <sup>-1</sup> )		4.7 $\pm$ 0.3
$\dot{V}O_{2peak}$ (ml $\cdot$ kg BM <sup>-1</sup> $\cdot$ min <sup>-1</sup> )		63.9 $\pm$ 4.3
Running experience (years)		6 $\pm$ 2 (3-10)
Training frequency / week		4 $\pm$ 1 (3-6)
Training volume: Approximate miles / week		43 $\pm$ 13 (30-70)

### **Preliminary tests**

A series of preliminary tests were conducted prior to the main experimental trials to determine (i) the relationship between running speed and oxygen uptake and (ii) the peak oxygen uptake ( $\dot{V}O_{2peak}$ ) (Chapter 3).

## **Experimental design**

All trials were conducted in the morning at the same time of day (7.00 am- 9.00 am) and separated by 7 days. Running performance was assessed using the 1 h run protocol described in Chapter 3. Participants fasted for 13-15 h overnight (no food, only water). There were no significant differences between trials in the average daily energy intake ( $14.4 \pm 1.5$  MJ), or quantities of CHO ( $6.5 \pm 1.5$  g · kg BM<sup>-1</sup>), protein ( $2.0 \pm 0.4$  g · kg BM<sup>-1</sup>) or fat ( $1.5 \pm 0.5$  g · kg BM<sup>-1</sup>) consumed in the 48 h before each trial (Dietary composition analysed by CompEat Pro 5.8.0). For the resting study participants sat quietly in a laboratory recovery room for 20 min before the start of the 1 h resting period.

## **Solution and rinse procedure**

The solutions used in this study were a commercially available 6.4% CHO-E beverage (C) (Lucozade Sport, Brentford, England) and a colour and taste matched placebo (P) solution. The P solution was matched in formulation to the CHO-E solution except that it contained no CHO. Both the exercise and resting studies utilised double-blind random cross-over design. In both studies participants' mouth-rinsed the solutions immediately before and at 15-min, 30-min and 45-min during the 1 h. Each 25 ml of solution was delivered in a plastic volumetric syringe (Kendal monoject) a total of 4 times, equating to a total volume of 100 ml of solution rinsed and expectorated over the duration of the trial. In the resting study participants were asked to swallow twice to clear the oral cavity of saliva before each rinse. The solution was mouth-rinsed for 5 s before being expectorated into a pre-weighed plastic container. The syringe and plastic container were weighed before and after each rinse using an electronic balance (Mettler, Toledo AB54-s, Switzerland) to determine the volume of rinsed solution and expectorate respectively. The volume of expectorate was subtracted from the known volume of solution rinsed to determine if any solution had been ingested or remained in the oral cavity. On completion of the studies participants were asked if they could distinguish between the solutions that they had mouth-rinsed during the two trials.

## Psychological scales

The feeling scale (FS), felt arousal scale (FAS), together with the gastrointestinal (G.I) discomfort scale were administered at rest, immediately prior to and at 15-min, 30-min, 45-min and 60-min during the 1 h run. The RPE was collected from the runner during the warm-up and at 15-min, 30-min and 45-min during the 1 h run. During the resting trial a one-dimensional bi-polar hedonic scale with a numeric anchors ranging from -4 (extremely unpleasant) through 0 (neither pleasant nor unpleasant) to +4 (extremely pleasant) was used to assess the hedonic response to the rinsed solution (Appendix G). This scale has been used previously to assess the hedonic tone of a taste stimulus at rest (Just et al., 2008).

## Blood analysis

In the performance and resting trials all blood samples (20- $\mu$ l) were taken in duplicate, deproteinised, frozen and later analysed for the concentrations of glucose (Maughan, 1982). For the performance trials finger prick blood samples were collected at rest and immediately after the 1 h run. In the resting study, finger tip blood samples were collected in 300  $\mu$ l microvettes (Microvette, CB 300, Sarstedt Ltd, UK) at rest, immediately before the first rinse and at 2, 4, 10, 15, 30, 45 and 60 min. Following the collection of 2 x 20- $\mu$ l, the remainder of the blood was centrifuged and plasma collected for the determination of plasma insulin concentrations using an ELISA kit (Mercodia, Insulin ELISA, Sweden) and plate reader (Expert Plus, ASYS Atlantis, Eugendorf, Austria).

## Statistical analysis

All data were analysed using SPSS (version 16.0). The mean differences in performance (total distance covered and trial order) were detected using a paired samples t test. The quantitative approach to likelihoods of benefit, triviality and negative effect on running performance was further enriched by dividing the range of substantial values into more finely graded magnitudes. Using a spreadsheet by Hopkins *et al.*, (Hopkins, 2007) the *P* value was converted into 90 % confidence intervals (CI) for, and inferences about, the true value of the effect statistic. It has been previously reported that distance runners and support professionals need to be concerned about changes in performance of between ~0.5% and ~1.0% (Hopkins et al., 2001). Thus, the set threshold value for possible beneficial or negative effects on performance was set at 1 % of the mean distance covered over the two trials. Mean

differences in self-selected running speed (analysed in 5-min blocks during the 1 h run) and psychological scores were detected using a repeated measures factorial ANOVA (trial x time). Significant main effects for individual time points were further analyzed using paired *t*-tests and the Bonferroni adjustment for the number of pair-wise comparisons employed. For the resting study, blood glucose and plasma insulin concentrations were analysed using a repeated measures factorial ANOVA (trial x time) with significant main effects for individual time points further analyzed using paired *t*-tests and the Bonferroni adjustment. All data are presented as  $M \pm SD$ , the confidence interval was set at  $P \leq 0.10$  *a priori* (Hopkins, 2002).

## 7.4 RESULTS

### Performance study

The runners completed a greater distance during the C trial ( $14298 \pm 685$  m) than during the P trial ( $14086 \pm 732$  m). Runners completed 211 m (90 % CI of difference = 42 m to 380 m,  $P = 0.048$ ) more during the C trial than during the P trial, representing 1.5 % of the total distance covered. The threshold value was set at 142 m, thus the chance that the true value of the effect is beneficial, negligible or negative to running performance was 76.3 %, 23.5 % and 0.2 % respectively. Individual running performance for the P and C trials are shown in Figure 7.1; there was no trial order effect on distance covered ( $P = 0.527$ ). The change in distance covered  $\pm$  the 90 % confidence limit (169 m) is shown Figure 7.2.

The mean running speed in the P trial was  $14.1 \pm 0.7$  km·h<sup>-1</sup> and in the C trial it was  $14.3 \pm 0.7$  km·h<sup>-1</sup> ( $P = 0.054$ ). The mean difference in running speed was 0.2 km·h<sup>-1</sup> (CI: 0.04 km·h<sup>-1</sup> to 0.4 km·h<sup>-1</sup>). Pacing strategy was similar between runners and consistent between trials. Mean self-selected running speed during the 1 h run is shown in Figure 3. Calculated sweat rate was  $1.5 \pm 0.2$  L·h<sup>-1</sup> for the P trial and  $1.6 \pm 0.2$  L·h<sup>-1</sup> for the C trial ( $P = 0.531$ ), equivalent to a loss of 1.1 % mean BM.

At rest, there was no significant difference in perceived activation (FAS; P:  $3.1 \pm 1.0$ , C:  $3.1 \pm 0.1$ ), runners pleasure-displeasure (FS; P:  $1.5 \pm 1.9$ , C:  $1.2 \pm 2.0$ ), G.I comfort (P;  $0.8 \pm 1.0$ , C:  $0.5 \pm 1.1$ ) or oxygen uptake values (P:  $3.8 \pm 0.5$  mL·min<sup>-1</sup>·kg<sup>-1</sup>, C:  $3.7 \pm 0.4$  mL·min<sup>-1</sup>·kg<sup>-1</sup>). The 5-min warm-up speed was  $11.7 \pm 1.1$  km·h<sup>-1</sup> and  $\dot{V}O_2$  was  $38 \pm 3$  mL·min<sup>-1</sup>·kg<sup>-1</sup> equivalent to 60 %  $\dot{V}O_{2peak}$  for both trials.

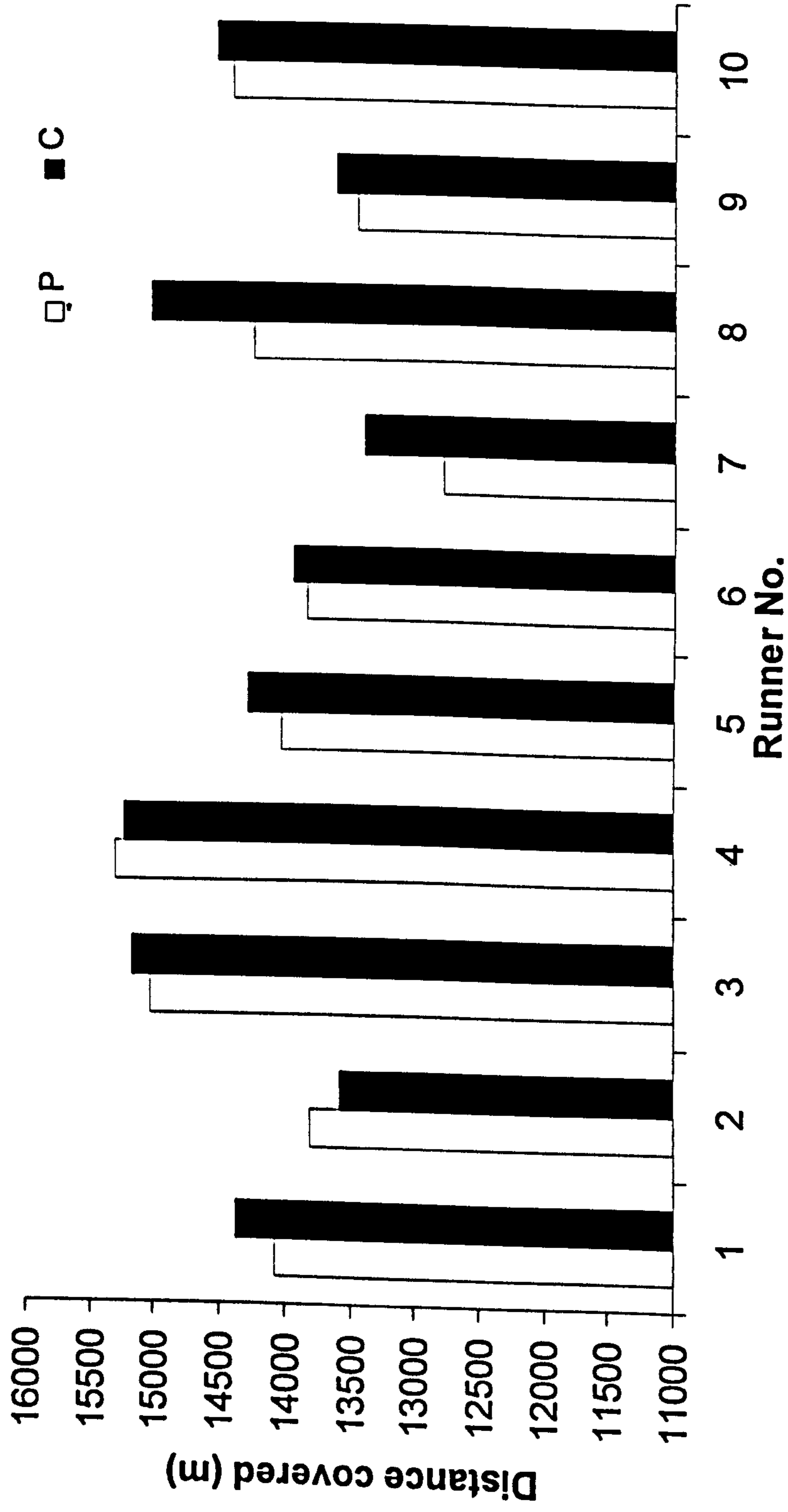
Rating of perceived exertion for the warm-up was  $9 \pm 2$  for the P trial and  $10 \pm 1$  for the C trial. The mean volume of expectorate for the P trial ( $25.5 \pm 1.0$  mL) and the C trial ( $26.2 \pm 1.0$  mL) was greater than the mean volume of solution rinsed ( $25.1 \pm 0.1$  mL) in both trials ( $P < 0.05$ ). The mean physiological responses and psychological scores during the C and P trials are reported in Table 7.2. None of the 10 runners were able to distinguish between the solutions they had mouth-rinsed in the two trials.

### *Resting study*

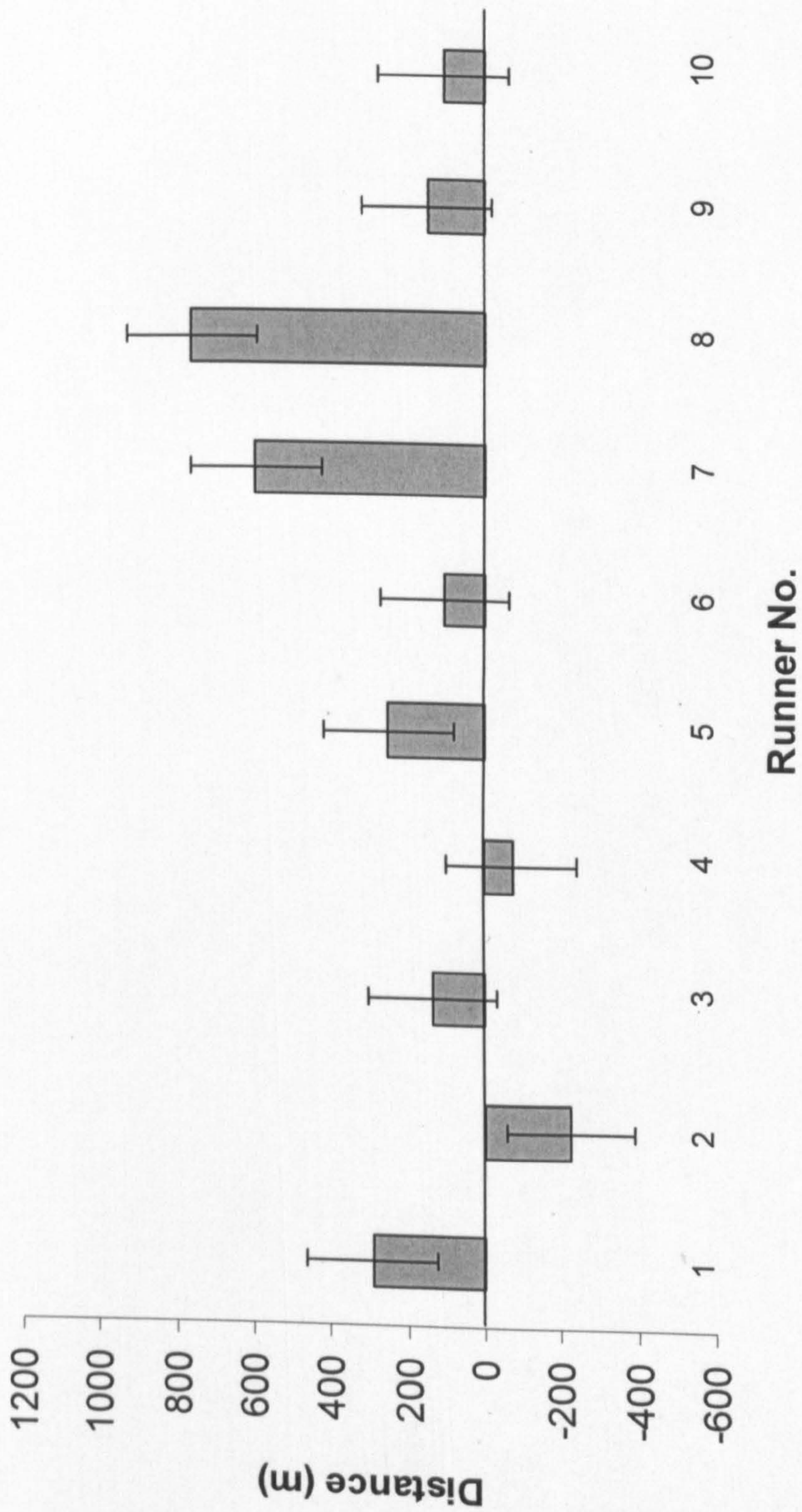
There was no change in blood glucose concentrations during the 1 h in the C trial or in the P trial ( $P = 0.277$ ). The mean blood glucose concentrations were  $4.3 \pm 0.1$  mmol·L<sup>-1</sup> for the P trial and  $4.3 \pm 0.2$  mmol·L<sup>-1</sup> for the C trial ( $P = 0.619$ ). There were also no differences in plasma insulin concentrations during the 1 h resting period ( $P = 0.302$ , n. = 6) or between trials (P;  $6.2 \pm 1.1$  mU·L<sup>-1</sup>, C;  $5.9 \pm 1.0$  mU·L<sup>-1</sup>).

The mean volume of fluid rinsed during the P trial was  $26.0 \pm 0.3$  mL and the mean volume of expectorate was  $25.7 \pm 0.7$  mL ( $P = 0.004$ ). Thus, the mean difference between the rinse and expectorate was 0.3 mL. The mean volume of solution rinsed during the C trial was  $26.6 \pm 0.9$  mL and the mean volume of expectorate was  $26.5 \pm 0.9$  mL ( $P = 0.394$ ). The mean scores for the hedonic scale at rest were  $1.6 \pm 1.6$  for the P trial and  $2.1 \pm 1.2$  for the C trial ( $P = 0.339$ ).

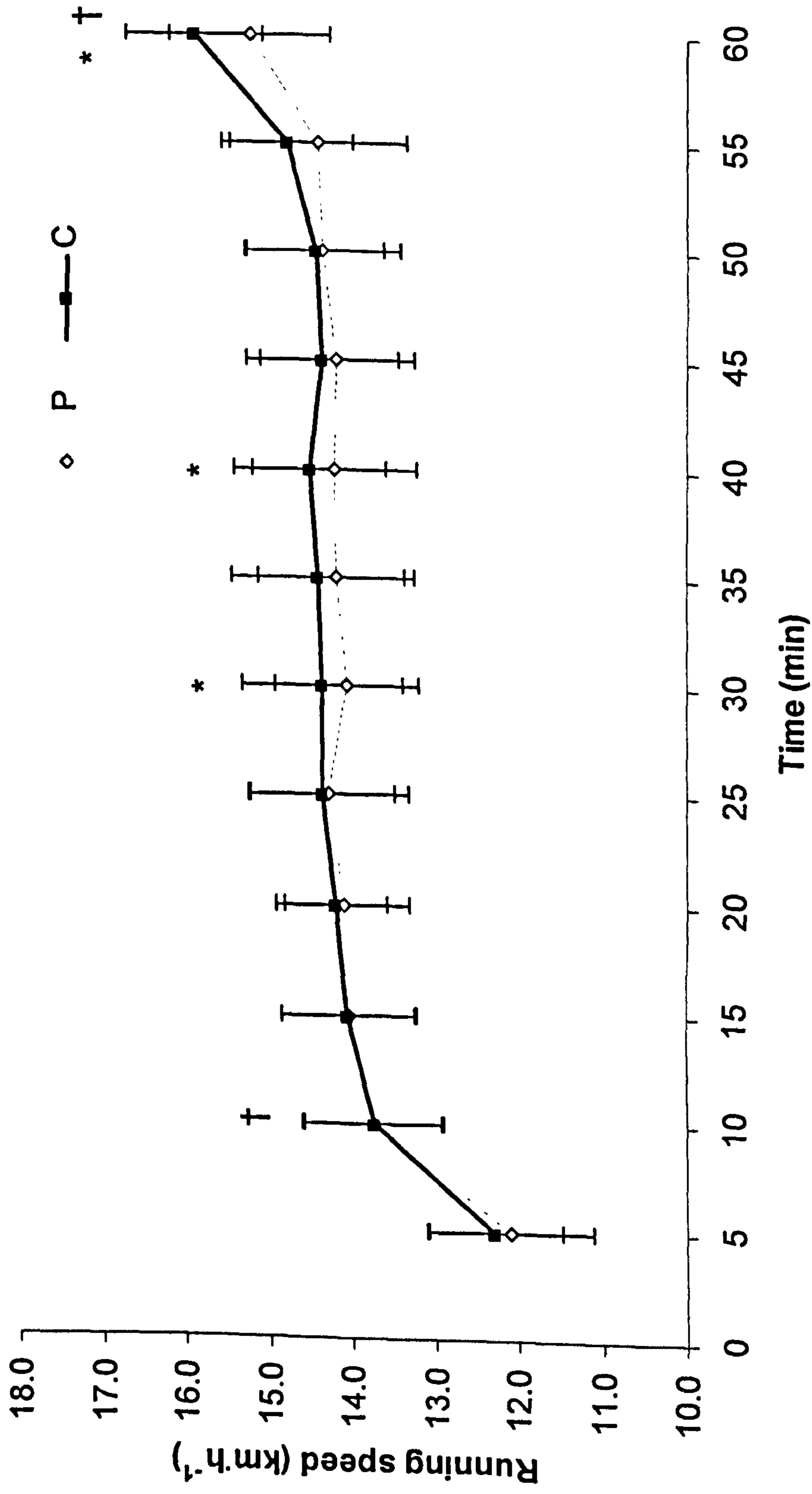




**Figure 7.1:** Individual running performance (total distance covered (m)) by the ten runners during the 1 h run during the P trial (open bars) and C trial (filled bars).



**Figure 7.2** The change in distance covered between trials  $\pm$  the 90 % confidence limit (169 m).



**Figure 7.3.** Mean self-selected running speed ( $\text{km}\cdot\text{h}^{-1}$ ) during the 1 h run. † indicates a significant effect of time over 5-min. \* indicates a significant difference between trials ( $P < 0.10$ ) ( $n=10$ , mean  $\pm$  SD).

	Physiological response					Psychological scores			
	Exercise intensity (% $\dot{V}O_2$ peak)	Heart rate (beats·min <sup>-1</sup> )	RER	CHO oxidation (g·min <sup>-1</sup> )	Blood glucose mmol·L <sup>-1</sup> Pre      Post	RPE	G.I comfort	FAS	FS
P	78 ± 7	163 ± 12	0.88 ± 0.04	2.9 ± 0.7	4.3 ± 0.2      4.3 ± 0.3	14 ± 1 <sup>†</sup>	1.7 ± 0.7*	3.5 ± 0.2	0.9 ± 0.7
C	77 ± 7	163 ± 13	0.89 ± 0.03	3.0 ± 0.7	4.3 ± 0.4      4.3 ± 0.3	14 ± 1 <sup>†</sup>	1.2 ± 0.6*	3.4 ± 0.2	0.9 ± 0.9
P =	0.825	0.563	0.279	0.329	.460      .370	0.591	0.014	0.589	0.959

**Table 7.2:** Physiological response and psychological scores for the P and C trials during the 1 h run. \* denotes a significant effect of time from 0 to 45 min, † denotes a significant effect of time from 15 -30 min and 30-45 min ( $P < 0.10$ ) (n.=10, mean ± SD).

## **7.5 DISCUSSION**

The main finding of the present study was that runners who mouth-rinsed a 6.4% CHO-E solution covered a greater distance during a 1 h running performance test than when they mouth-rinsed a colour and taste matched placebo.

These findings are in agreement with several studies which have shown the benefit of mouth-rinsing CHO solutions on time-trial performance during cycling (Carter et al., 2004a; Chambers et al., 2009; Pottier et al., 2008). Carter et al. (2004b) were first to report that compared to mouth-rinsing with water, mouth-rinsing a 6.4 % maltodextrin solution improved 1 h cycling performance by 2.9 %. The findings of the present study provide evidence that participants are able to mouth-rinse a solution during exercise without ingestion. However, it is important to note that salivary secretions during the mouth-rinse procedure would have contributed to the volume of expectorate. Nevertheless, despite not being able to ascertain absolutely whether any solution was ingested, the results of the present confirm assumptions made by Carter et al. (2004b) that mouth-rinsing a CHO-E solution has no influence on blood glucose concentrations. Pottier et al. (2008) have also reported that mouth-rinsing a 6 % (sucrose 5.4 g·100 ml<sup>-1</sup>, glucose 0.46 g·100 ml<sup>-1</sup>) CHO-E solution improves time-trial performance during cycling. However, they also reported that mouth-rinsing had a greater performance benefit (3.7 %) than when their cyclists ingested (14 ml · kg BM· h<sup>-1</sup>) the same solution. Despite the mouth being exposed to CHO in both trials, the discrepancy in performance was attributed to the short oral transit time when ingesting the CHO-E solution (Pottier et al., 2008). In two separate investigations, Chambers et al. (2009) reported that mouth-rinsing both a sweet 6.4 % glucose solution and non-sweet 6.4% maltodextrin solution improved 1 h cycling performance by approximately 2 % and 3 % respectively. Each cycling study used the same performance test in which cyclists were required to complete a set amount of external mechanical work as quickly as possible (approximately 1 h in duration). This performance test has a reported CV of 3.35 % (Jeukendrup et al., 1996). Consequently, only Pottier et al. (2008) report an improvement in performance greater than that of the known variation of performing the cycling time-trial i.e. 3.7 %. In the present study the difference in distance covered between the C and P trials was greater than the 1.4 % (CV) i.e. the day-to-day variation of the testing procedure (Chapter 4).

Therefore, it is possible to report with confidence that the improvement in distance covered during the C trial was an effect of the intervention and not simply a consequence of the daily variation in the participants running performance.

Analysis of the true value of the effect statistic revealed that mouth-rinsing a CHO-E solution is likely (76 %) to have a substantially positive effect on 1 h running performance (Batterham & Hopkins, 2005). In Figure 7.1, it is clear that two of the ten runners (No. 7 & 8) increased their performance substantially on the C trial. Interview with these runners revealed that they did not habitually ingest sports drinks during training or competition. Therefore an interesting question, would be to discover whether such individuals are more sensitive to CHO interventions i.e. responders, non-responders. However, it is important to note that caution should be taken when interpreting data with low participant numbers. When runners 7 & 8 were removed from the analysis the C trial was likely to have a 77.8 % negligible effect and 21.9 % positive effect. Nevertheless, in both the analysis of 8 and 10 runners mouth-rinsing a CHO-E solution had a 0.3 % and 0.2 % chance having a substantial negative effect on performance respectively. The results of the present study contribute to the evidence reported by 4 out of the 5 studies that mouth-rinsing a CHO solution is associated with improvements in performance. Therefore, athletes who suffer from gastrointestinal discomfort when ingesting CHO-E while running may want to consider the mouth-rinsing strategy because there are no adverse effects and it may lead to an improvement in performance.

In the studies that employed cycling time-trials, each reports that the mean power output during the time-trial was increased when mouth-rinsing a CHO solution (Carter et al., 2004a; Chambers et al., 2009; Pottier et al., 2008). However, only Carter et al. (2004b) reported significant changes in power output during the time-trial i.e. during the first three quarters (approximately 45-min) of the performance test. In the present study, running speed reached statistical significance between 25-30 min and 35-40 min (Figure 7.3). An explanation as to why mouth-rinsing CHO influenced performance at different time points between the present study and previous cycling studies is hard to establish given the two different modes of exercise. However, it is most likely a consequence of the different pacing strategies adopted by runners and cyclists in time-trials. Runners typically maintain their self-selected running speed for

the majority of the test and tend to sprint towards the end of the time-trial (see Chapters 4, 5 and 6). In cycle studies, power output gradually declines during the first three quarters of the time-trial before being increased to the completion of the set amount of external work (Carter et al., 2004a; Chambers et al., 2009; Jeukendrup et al., 1997b; Pottier et al., 2008). These observations suggest that care must be taken when making comparisons between cycling and running protocols. Thus, there is a distinct difference between the present study and earlier studies using cycling as the exercise mode. In the present study mouth-rinsing a CHO-E solution improved running performance by increasing self-selected running speed, whereas in cycling the benefit to performance appeared to be achieved by reducing the decline in power output during the time-trial.

In contrast to the results of the present study, Whitham & McKinney (2007) reported that the distance covered during 45-min of treadmill running was not improved when runners mouth-rinsed a 6 % maltodextrin or flavoured matched placebo solution at 6-min intervals. Runners completed a 15 min warm-up run at 65%  $\dot{V}O_2$ max before being asked to run as far as possible in 45 min. Unlike the present study Whitham & McKinney (2007) found no differences in mean running speed at any time point during the 45-min run. Nevertheless, the study confirmed previous observations that runners were able to accurately replicate their running performance when asked to cover as much distance as possible in a given time (Chapter 4). That Whitham & McKinney (2007) observed no difference in running speed with a CHO mouth-rinse is possibly a consequence of two key aspects of their treadmill running procedure. The first difference is that although runners had complete control of the treadmill speed they had to make changes manually. Therefore, when the runners felt like changing their running speed they had to engage in the process of manually altering the treadmill speed. Whitham & McKinney (2007) recognised that using a performance test that required a manual control of pace may not be optimal for detecting a potentially sub-conscious central effect of CHO mouth-rinse. This may explain why differences in speed were observed in the present study, which used an automated treadmill system that allowed spontaneous changes in speed without manual input (Chapter 4). Thus, the automated treadmill may be more sensitive in detecting the small effect size that mouth-rinsing a CHO-E may induce.

Observations from cycling studies suggest that CHO supplementation has little ergogenic effect when endogenous glycogen stores are sufficient to maintain exercise intensity over the duration of the exercise period (Desbrow et al., 2004; Widrick et al., 1993a). In support of this observation Chapter 6 reported no benefit of CHO-E solution ingestion on 1 h running performance when a pre-exercise meal was consumed 3 h before exercise. Therefore, a further reason why performance differences were seen in the present study but not by Whitham & McKinney (2007) is the pre-exercise CHO status of the individual. The 4 h post prandial period reported by Whitham & McKinney (2007) may not have been long enough to reduce endogenous CHO stores to show the benefit of the mouth-rinsing procedure. Nevertheless, it is important to note that both Carter et al. (2004b) and Pottier et al. (2008) who imposed a 4 h and 3 h fast respectively, did find significant performance benefits from mouth-rinsing a CHO solution. However, the amount and composition of the pre-exercise meals were not reported.

The mechanism(s) by which mouth-rinsing with a CHO-E solution increased self-selected running speed in the present study are, as yet, unknown. Previous authors have speculated that mouth-rinsing CHO may trigger reward centres in the brain (Carter et al., 2004b) or suppress fatigue signals to the brain from working muscles (Pottier et al., 2008). Chambers et al. (2009) recently used functional magnetic resonance imaging (fMRI) to explore the influence of oral exposure to glucose and maltodextrin on the brain. The study reports that both glucose and maltodextrin in the mouth activate regions in the brain associated with reward, such as the insula/frontal operculum, orbitofrontal cortex and striatum (Chambers et al., 2009). These regions of the brain are believed to mediate behavioural responses to rewarding stimuli, such as taste (Rolls, 2007). With respect to performance, mouth-rinsing a CHO-E solution during exercise may activate these brain regions and result in altered behaviour i.e. increased in self-selected running speed. For example, one possible mechanism to explain this phenomenon might involve the ratings of perceived exertion. In the present study runners report the same relative perceived exertion despite selecting slightly faster running speeds. Activation of reward centres in the brain stimulated by mouth-rinsing CHO may reduce the sense of effort towards exercise. The runners then



increase their self-selected speeds to match their expected sense of effort and thus self-selected running speed is increased.

However, it is important to note that the glucose and maltodextrin solutions used for the *f*MRI scans were far more concentrated (18 %) than those used during exercise (6 % - 6.4 %). In the present study blood glucose and plasma insulin concentrations were unchanged during the 1 h resting period in response to mouth-rinsing CHO. In a study by Just et al. (2008), which reported a release of insulin in response to glucose in the mouth, participants held the solution in the mouth for a much longer duration (45 s) compared to rinse times in the present and previous exercise studies (5 s). Thus, just as with the question of the influences of solution concentration, the duration which the CHO-E solution is in the mouth may also have an influence on a centrally governed feed forward mechanism.

Future research is needed to establish whether the same central activation, reported by Chambers et al. (2009), can be achieved when mouth-rinsing CHO solutions with similar concentrations to those which have been shown to influence exercise performance. In addition, of interest would be to discover what overall contribution a centrally governed “feed forward” response makes when performance benefits are observed following the ingestion of CHO-E solutions.

In conclusion, mouth-rinsing a 6.4% CHO-E solution was associated with increased self-selected running speed and distance covered during a 1 h performance run. Mouth-rinsing with a 6.4% CHO-E solution did not alter blood glucose or plasma insulin concentrations at rest. These findings suggest that mechanisms independent of glucose delivery to the systemic circulation may be responsible for the improved 1 h running performance.

## CHAPTER 8

### THE INFLUENCE OF CARBOHYDRATE-ELECTROLYTE MOUTH-RINSE ON SELF-SELECTED SPEEDS DURING A 30 MINUTE TREADMILL RUN

#### 8.1 SUMMARY

The purpose of this study was to examine the influences of a CHO-E mouth-rinse on self-selected running speeds during a 30-min treadmill run. Ten endurance trained males performed two trials, each involving a 10-min warm-up at 60%  $\dot{V}O_2$  peak followed by a 30-min run. The run was performed on an automated treadmill that allowed the spontaneous selection of speeds without manual input. Participants were asked to run at speeds which equated to a Rating of Perceived Exertion (RPE) of "15", mouth-rinsing either a 6% CHO-E (C) or taste matched placebo (P) solution. In addition to recording self-selected speeds and total distance covered we also assessed the subjective feelings of the runners. The total distance covered was greater during the C than during the P trial ( $P < 0.05$ ). Faster speeds selected during the first 5-min of exercise corresponded with enhanced feelings of pleasure when mouth-rinsing the CHO-E solution. mouth-rinsing with a CHO-E solution increased total distance covered during a self-selected 30-min run in comparison to mouth-rinsing a colour and taste matched placebo.

## **8.2 INTRODUCTION**

The results reported in Chapter 7 were consistent with other studies, in which authors have speculated that simply tasting CHO may exert a positive influence on the brain and central nervous system (Carter et al., 2004a; Chambers et al., 2009; Pottier et al., 2008). The concept that CHO may exert a central effect is of interest because it is consistent with findings that runners 'feel better' and report lower ratings of perceived exertion while ingesting a CHO-E solution during prolonged running (Backhouse et al., 2007; Backhouse et al., 2005).

If there is central recognition of CHO in the mouth, it is reasonable to ask whether this alters the runners' perception towards exercise and if so, whether this translates into faster self-selected running speeds. However, the 1 h time-trial run may not be the optimal test to use in order to answer this question. This is because the test requires the participant to perform at their maximal effort in order to achieve their best performance. For example, if a runner was feeling "bad" this may not be reflected in their selection of running speed due to the overriding motivation to perform well. Thus, changing from a time-trial to a less demanding running test would allow more focus on the psychological response to mouth-rinsing a CHO-E solution. If the runners 'feel better' following mouth-rinsing the CHO-E solution then this may be reflected in their choice of running speeds.

To this end, to determine if fasted runners 'felt any better' while mouth-rinsing the CHO-E solution they were simply asked to change their speed according to 'how they feel' during a 30-min run. In order for the runners to self-select the same range of running speeds they were asked to select a pace that represented a rating of 15 (hard) on the Borg RPE scale (Borg, 1982).

## **8.3 METHODS**

### **Participants**

Ten endurance trained male recreational runners ( $23 \pm 4$  years; body mass  $75 \pm 7$  kg, height  $181 \pm 7$  cm,  $\dot{V}O_{2\text{peak}}$   $62 \pm 3$  ml·kg<sup>-1</sup>·min<sup>-1</sup>) (mean  $\pm$  SD) gave their written consent before participating in this study approved by Loughborough University Ethical Advisory Committee. The number of participants was determined using a nomogram based upon the ratio limits of agreement (Nevill et al., 1997). Participants were regularly running for 30-60 min, 3-5 days a week. Hence, the duration of the run in the present study was within their normal training programme.

### **Preliminary tests**

A series of preliminary tests were conducted prior to the main experimental trials to determine (i) the relationship between running speed and oxygen uptake and (ii) the peak oxygen uptake ( $\dot{V}O_{2\text{peak}}$ ) (Chapter 3).

### **Experimental design**

Experimental trials were separated by 7 days and conducted at the same time of day (7.00 am - 9.00 am), utilising a randomised double blind cross over design. In the 48 h period before the main trials runners were asked to consume and record their habitual diet that was similar to that which they adopted prior to preparing for a race. Runners were also asked not to consume caffeine or alcohol during this period. The same diet was repeated prior to each trial. On the morning of each trial runners reported to the laboratory following an overnight fast (12-13 h) and sat quietly in a comfortable environment (20°C, 55% Relative Humidity (RH)) for 30-min. After 25-min at rest, a 5-min expired air sample was collected. Thereafter, participants emptied their bladder before their body mass was recorded. The runners completed both 30 min trials in the fasted state.

All tests were conducted in a laboratory maintained at  $19 \pm 1$  °C, RH  $62 \pm 8\%$ , containing only the treadmill and fan positioned 1m in front of the runner with constant air speed to provide cooling throughout the run. Human interaction was limited to the collection of expired air samples, ratings of subjective scales and the

delivery of solutions. Runners were monitored throughout exercise via closed circuit television by an investigator in an adjacent room. The treadmill display panel was covered during each trial so that runners were only able to see a clock displaying the time remaining during each phase of the run. All trials involved 40-min of treadmill running using a 1% treadmill gradient in order to simulate the energetic cost of outdoor running.

The tests comprised of a 2-min walk at  $4 \text{ km}\cdot\text{h}^{-1}$  followed by a 10-min warm-up run at a speed equivalent to  $60\% \dot{V}\text{O}_2\text{peak}$ . Immediately after the 10-min warm-up run the runners began the 30-min trial. The runners were asked to select speed which they perceived to be equivalent to a “hard pace” RPE of “15” for the 30-min run and were free to adjust the speed using the automated treadmill system. On completion of 30-min run, the runners walked for a further 2-min at  $4 \text{ km}\cdot\text{h}^{-1}$  before being towel dried and their body mass recorded. Runners received no feedback about the distance covered over the 30-min run until the completion of the study. On completion of the study runners were asked if they could identify the C trial. If runners said yes, they were asked to state on which trial they believed they were mouth-rinsing with the CHO-E solution.

### **Solution and rinse protocol**

During the two main trials either a  $6.0 \text{ g}\cdot 100 \text{ ml}^{-1}$  CHO-E solution (C) or a taste matched placebo (P) was rinsed round the oral cavity for 5-s before being expectorated into a pre-weighed plastic bag. Plastic bags were re-weighed using an electronic balance (Mettler, Toledo AB54-s, Switzerland) to help determine whether or not any of the solution had been ingested. Each mouth-rinse solution ( $20 \pm 1^\circ\text{C}$ ) was administered immediately after resting expired air collection, immediately prior to and at 3-min, 6-min and 9.5-min during the warm-up run. During the 30-min run the mouth-rinse was administered at 5-min intervals. Each 25 ml bolus of solution was served in a plastic syringe (Kendal monoject) a total of 10 times, equating to 250 ml bolus of solution rinsed and expectorated over the duration of the trial.

## **Subjective scales**

The feeling scale (FS), felt arousal scale (FAS), together with the gastrointestinal (G.I) discomfort scale were administered on arrival at the laboratory, 25-min into the rest period, immediately prior to and at 3-min, 6-min and 9.5-min into the 10-min warm-up run. During the 30-min run the scales were presented to the runners every 5-min i.e. before the delivery of the solutions. The runners also completed the FS, FAS and GI scales on completion of the 30-min run and at the end of the 2-min cool-down walk.

## **Statistical Analysis**

All data were analysed using SPSS (version 13.0). The mean differences in self-selected running speed and comparisons over time (analysed in 5-min blocks over the 30-min run) were detected using a two-factor (trial x time) repeated measures analysis of variance (ANOVA) with repeated measures on both factors used to analyse for main effects and interaction of these two factors. Significant interaction between the trial and time factors in the ANOVA were explored using the Holm-Bonferroni step-wise method. For comparisons between single normally distributed data, paired Student's t-tests were used to examine differences between trials. Psychological and G.I scales were analysed using a two-factor ANOVA for repeated measures on two factors (experimental condition and sampling time). Significant main effects for individual time points were further analyzed using paired t-tests and the Bonferroni adjustment for the number of pairwise comparisons was employed. All data are presented as mean  $\pm$  SD. Significance alpha level was set at 0.05 to indicate statistical significance.

## **8.4 RESULTS**

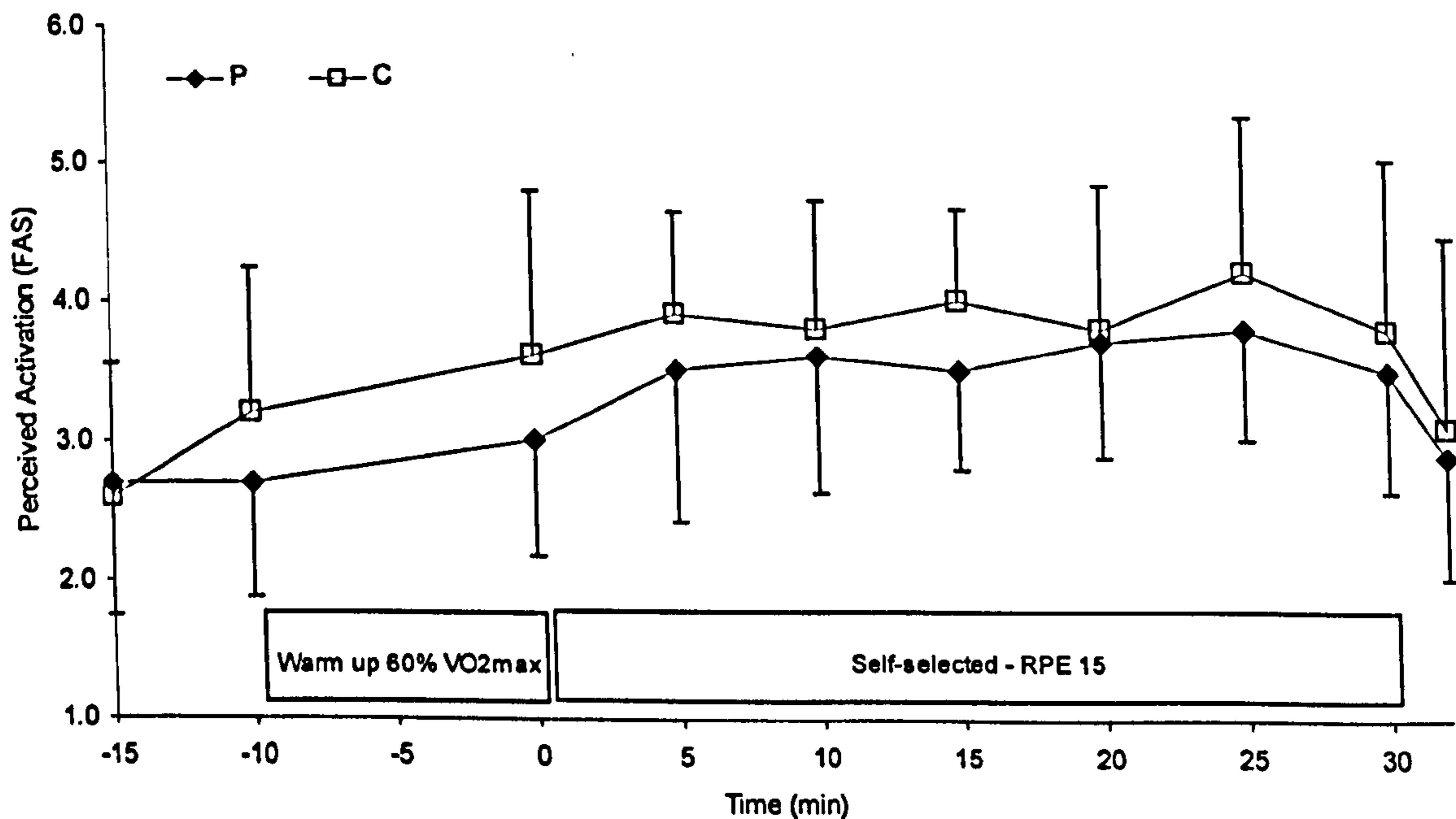
### **Physiological responses**

There was no difference between trials in heart rate response to exercise: mean heart rate during the 10-min warm-up was  $130 \pm 6$  beats $\cdot$ min<sup>-1</sup> for both C and P trials. Heart rates analysed at 15 s intervals in 5-min blocks, revealed a significant increase during

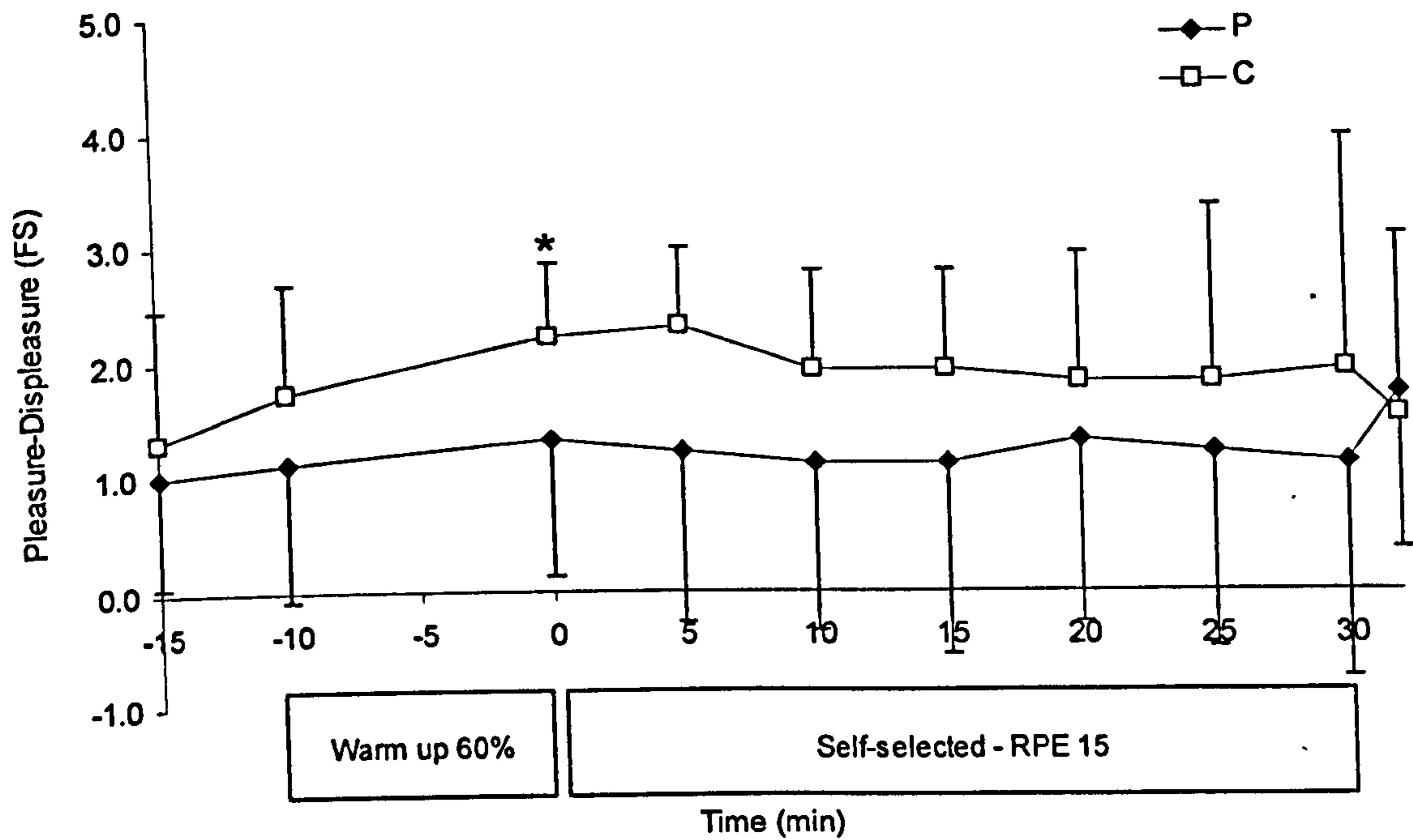
the 30-min run ( $P < 0.05$ ). However, there were no differences between C and P trials (C  $162 \pm 7$  beats·min<sup>-1</sup> vs. P  $161 \pm 7$  beats·min<sup>-1</sup>,  $P > 0.05$ ). The mean loss of body mass as sweat was  $0.9 \pm 0.4$  kg and  $0.8 \pm 0.2$  kg for the P and C trials ( $P > 0.05$ ).

### Subjective response

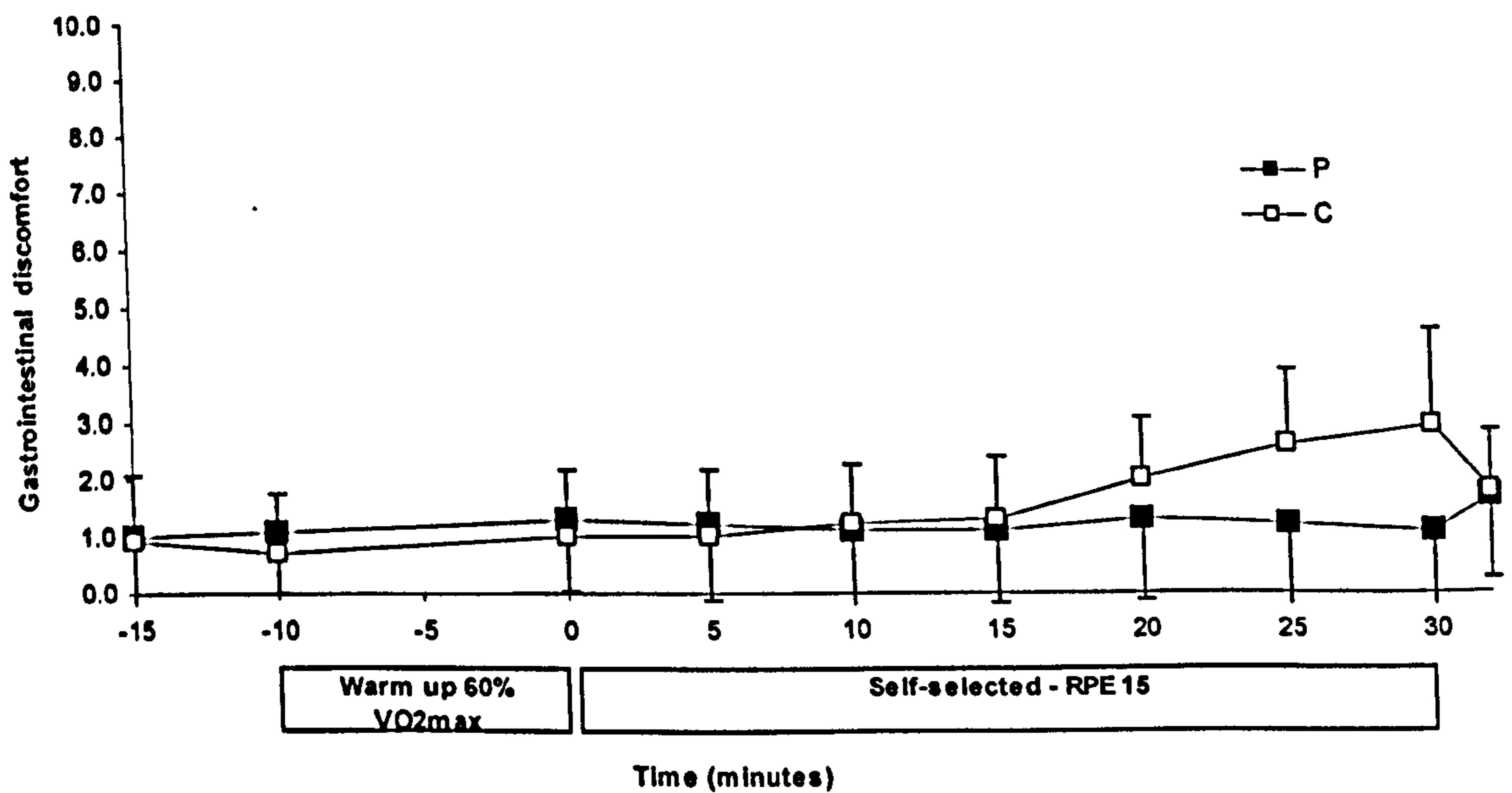
Rating of perceived activation (FAS) increased from rest (C  $2.2 \pm 1.0$ ; P  $2.7 \pm 0.7$ ) to the start of exercise ( $P < 0.05$ ). No difference was observed over the 30-min run and there were no differences between trials (C  $3.8 \pm 0.2$  vs. P  $3.6 \pm 0.1$ , Figure 8.1). There were no differences in ratings of pleasure-displeasure (FS) at rest (C  $1.1 \pm 1.0$  vs. P  $1.0 \pm 1.2$ ). However, FS was elevated at the start of the 30-min run while rinsing the CHO-E solution (P:  $1.3 \pm 1.2$  vs. C:  $2.2 \pm 0.6$ ,  $P < 0.05$ , Figure 8.2), but there were no differences between trials during the remainder of the 30-min run (C  $1.9 \pm 0.2$  vs. P  $1.6 \pm 0.1$ ,  $P > 0.05$ ). Although, there was no differences in G.I discomfort at rest (C  $0.8 \pm 1.1$  vs. P  $0.7 \pm 1.1$ ), it did increase over the 30-min run, but with no differences between trials (C  $1.6 \pm 0.8$  vs. P  $1.5 \pm 0.6$ , Figure 8.3).



**Figure 8.1** Perceived activation, felt arousal scale (FAS) (mean± SD) during the C and P trials. -10 to 0 = warm up at 60 %  $\dot{V}O_2$ peak. 1 = low activation, 6 = high activation (n.= 10, mean ± SD).



**Figure 8.2** Pleasure-displeasure, feeling scale (FS) (Mean  $\pm$  SD) during the C and P trials. -10 to 0 = 10 minute warm up at 60 %  $\dot{V}O_2$ peak. Pleasure-Displeasure scale ranges from +5 very good to -5 very bad. \* denotes a significant difference  $P < 0.05$  (n = 10, mean  $\pm$  SD).



**Figure 8.3** Gastrointestinal discomfort during the C and P trials. -10 to 0 = 10 min warm-up at 60 %  $\dot{V}O_2$ peak. 0 = neutral, 4 = uncomfortable, 8 = very uncomfortable, 12 = painful (n = 10, mean  $\pm$  SD).



## Self-selected running speed

The self-selected running speed for P was  $12.9 \pm 1.3 \text{ km} \cdot \text{h}^{-1}$  vs.  $13.2 \pm 1.1 \text{ km} \cdot \text{h}^{-1}$  for the C trial ( $P > 0.05$ ) (Figure 8.4). Analysed at 15-s intervals in 5-min blocks self-selected speed was faster in the first 5-min of the 30-min run when rinsing with the CHO-E solution ( $P < 0.05$ ). Consequently runners covered a significantly greater distance during the first 5-min of the 30-min run (C 1039 m vs. P 1016 m). The total distance covered was  $6469 \pm 515 \text{ m}$  during the P trial and  $6584 \pm 520 \text{ m}$  during the C trial. Thus during the C trial the distance run was 115 m further, i.e. 1.7% of the total distance, than on the placebo trial ( $P < 0.05$ ). Post-trial interviews with the runners revealed that only two of the ten runners were able to correctly identify the solution rinsed during the tests. The other 8 runners were unable to distinguish between the two solutions.

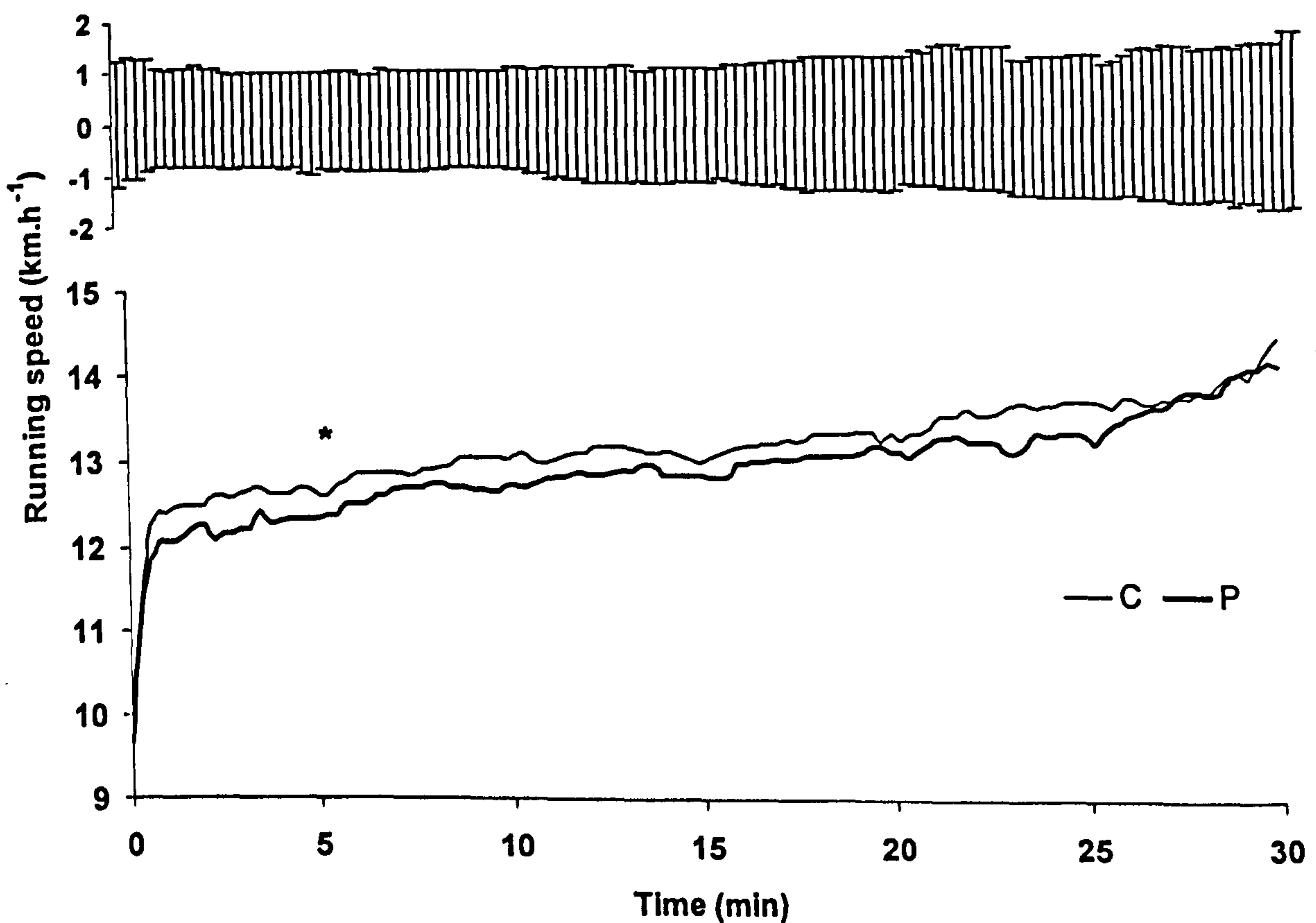
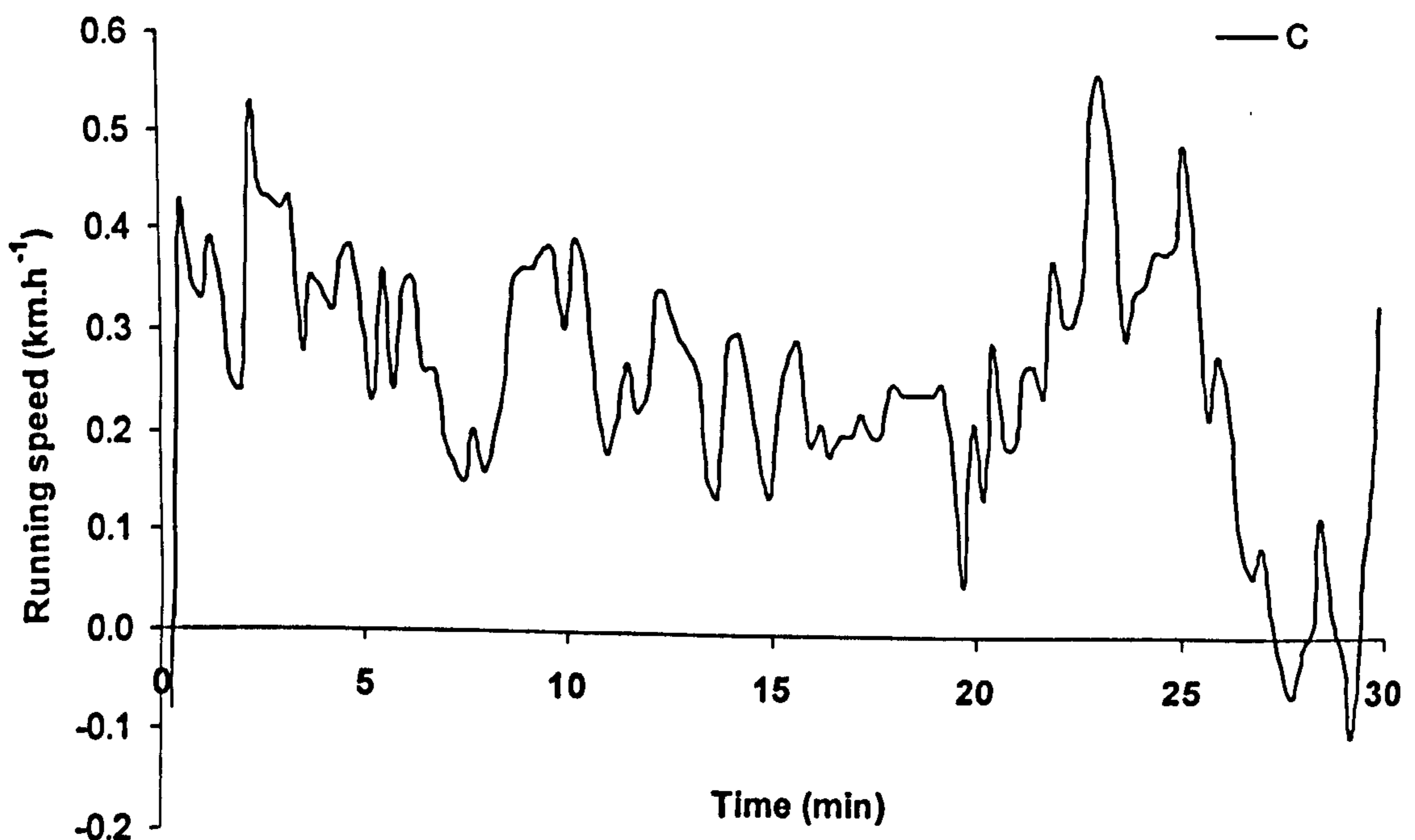


Figure 8.4 Mean running speed ( $\text{km} \cdot \text{h}^{-1}$ ) for the C and P trials during the 30-min performance test. For clarity (SD) is shown on separate ordinate. Mean running speed ( $\text{km} \cdot \text{h}^{-1}$ ) for P =  $12.9 \pm 1.3 \text{ km} \cdot \text{h}^{-1}$  vs.  $13.2 \pm 1.1 \text{ km} \cdot \text{h}^{-1}$  for C. \* denotes significant difference in speed analysed in 5-min blocks ( $P < 0.05$ ) ( $n = 10$ , mean  $\pm$  SD).

## Metabolism

No difference was observed in resting oxygen uptake between the two trials ( $\dot{V}O_2$ : P =  $0.30 \pm 0.04$  l·min<sup>-1</sup>, C =  $0.31 \pm 0.06$  l·min<sup>-1</sup>) or RER (P =  $0.84 \pm 0.03$ , C =  $0.85 \pm 0.03$ ). During the 10-min warm up,  $\dot{V}O_2$  and RER (P =  $2.5 \pm 0.2$  l·min<sup>-1</sup>,  $0.92 \pm 0.06$ , C =  $2.5 \pm 0.2$  l·min<sup>-1</sup>,  $0.91 \pm 0.04$ ) were not different between the C and P trials. The quantity of solution expectorated was equal to or greater than the quantity rinsed (P  $252.5 \pm 2.4$  ml vs. C  $251.5 \pm 1.7$  ml,  $P > 0.05$ ).

There was no difference in estimated CHO oxidation rate during the resting period prior to exercise (C  $0.19 \pm 0.05$  g·min<sup>-1</sup> vs. P  $0.22 \pm 0.05$  g·min<sup>-1</sup>) or during the 10-min warm-up (C  $2.6 \pm 0.5$  g·min<sup>-1</sup> vs. P  $2.3 \pm 0.6$  g·min<sup>-1</sup>). Estimated fat metabolism did not differ at rest (C:  $0.06 \pm 0.07$  g·min<sup>-1</sup> vs. P:  $0.07 \pm 0.02$  g·min<sup>-1</sup>) or during the 10-min warm-up (C:  $0.31 \pm 0.23$  g·min<sup>-1</sup> P:  $0.43 \pm 0.23$  g·min<sup>-1</sup>). Estimated energy expenditure (calculated by 1 kcal x kg BM x (distance covered) km) over the duration of the 30-min run was significantly greater when rinsing with the CHO solution (C  $493 \pm 54$  kcal vs. P  $485 \pm 55$  kcal,  $P < 0.05$ ).



**Figure 8.5** Mean difference in running speed (km·h<sup>-1</sup>) between the C (Line) and P (0) trials during the 30-min performance test. Mean running speed (km·h<sup>-1</sup>) for P =  $12.9 \pm 1.3$  km·h<sup>-1</sup> vs.  $13.2 \pm 1.1$  km·h<sup>-1</sup> for C ( $P > 0.05$ ) (n = 10, mean  $\pm$  SD).

## **8.5 DISCUSSION**

The results of the present study showed that mouth-rinsing a CHO-E solution significantly increased the self-selected running speed during the first 5-min of the 30-min run. As a result of the selection of an initial faster speed resulted in a greater distance covered over the first 5-min and contributed to a significant difference in total distance covered in 30-min.

In this study, rather than complete the greatest possible distance in 1 h (Chapters 4 -7), runners were asked to select a speed that equated to a “hard pace” i.e. 15 on the Borg scale (Borg, 1982) for a 30-min run. Using the automated treadmill the runners were able to spontaneously adjust their speed with no manual input, thus providing a unique insight into how mouth-rinsing a CHO-E solution influenced self-selected running speed (Chapter 4). However, we acknowledge that runners’ interpretation of an RPE of “15” (hard) would undoubtedly be influenced by the run duration. Therefore, we informed runners of the specific run time i.e. 30-min and provided them with the time remaining during the run.

Administering both the FS and FAS scale, enabled a more complete description and examination of the subjective experience following mouth-rinsing a CHO-E solution. The ingestion of a CHO-E solution has been reported to elevate perceived activation during the final 30-min of 120-min intermittent running exercise (Backhouse et al., 2007) and also attenuate the decline in pleasure-displeasure during a 120-min bout of cycling (Backhouse et al., 2005). In the present study, there was a trend for runners to experience higher perceived activation (Figure 8.1) whilst mouth-rinsing with the CHO-E solution. This effect was not evident during the 1 h time-trial (Chapter 7). Interestingly, the observation of an enhanced feeling of pleasure at the onset of the 30-min run when rinsing with the CHO-E solution, corresponded to the significant increase in speed over the first 5-min. These findings, taken together with those of Backhouse et al. (2005, 2007) suggest that the ingestion or recognition of a CHO-E solution in the mouth may induce a “feel good” effect.

The results of the present study are consistent with those reported in Chapter 7 that found mouth-rinsing a CHO-E solution was associated with runners self-selecting faster running speeds, despite performing at the same rating of perceived exertion. The results of the present study also provide some support for the results of Carter et al. (2004a), who reported that mouth-rinsing a 6.4 % maltodextrin solution improved 1 h cycle time-trial performance. In their study, the cyclists were able to maintain a higher power output for the first 75 % of the time-trial while mouth-rinsing the CHO-E solution. In addition, they completed the time-trial while recording the same ratings of perceived exertion as in the placebo trial, even though they were exercising at a higher power output. However, in contrast to Carter et al. (2004a) and the results presented in Chapter 7, improved running speed only reached statistical significance over the first 5-min of the 30 min run (Table 8.1). After 5-min the effect of the CHO-E solution mouth-rinse appeared to diminish with time (Figure 8.4).

In contrast to the results of the present study, Whitham and McKinney (2007) recently reported that mouth-rinsing a CHO solution had no effect on 45-min running performance, using a traditional treadmill. Runners completed a warm-up run at 65% of  $\dot{V}O_2$ max before being asked to run as far as possible in 45-min. Runners mouth-rinsed either a 6% maltodextrin or placebo solution before and during the time-trial. However, no difference in running speed or perceived exertion was reported at any time point during the trials. It is unclear why the findings of the present study differ from those of this recent study. Direct comparisons are difficult, as the present study was not a 'time-trial' *per se*. In the study by Whitham and McKinney (2007) treadmill speeds were recorded at 5-min intervals. In the present study treadmill speeds were recording every 15-s providing a better description of the runners' responses to the mouth-rinse solutions. In addition, studies in which runners change the treadmill speeds manually may not have the same degree of sensitivity to nutritional interventions as is the case when using an automated treadmill (Whitham et al., 2007). Thus, manually changing the speed may not be sufficient to detect any subtle effects that CHO mouth-rinses may have on the runners (Chapter 7) (Laursen et al., 2007; Whitham et al., 2007). Alternatively, as seen in the present study, the major effect of mouth-rinsing a CHO-E solution is observed at the beginning of exercise and as previously mentioned the effect diminished with time. Thus, as the exercise duration

increases, the likelihood of reporting a significant difference in distance run will decrease. Though this does not support the results reported in Chapter 7 or by Carter et al. (2004a) this may explain why Whitham & McKinney (2007) found that mouth-rinsing a CHO solution had no effect on the distance covered during 45-min of treadmill running.

Possible placebo effects may have large influences when investigating small changes in exercise performance (Clark, Hopkins, Hawley & Burke, 2000). Despite being colourless and non-sweet, Carter et al. (2004a) reported that 4 of their 9 subjects were able to identify the mouth-rinse carbohydrate solution. Whitham and McKinney (2007) resolved this potential problem by adding bitter lemon juice to the maltodextrin and placebo solutions, resulting in only 1 of the 7 runners identifying the CHO solution. In the present study only 2 of the ten runners were able to distinguish between an orange flavoured CHO-E solution and a colour, viscosity and tasted matched placebo. Although the CHO-E solution differed from maltodextrin used in previous mouth-rinse studies (Carter et al., 2004a; Whitham et al., 2007) the solutions were suitably indistinguishable without the addition of any potentially undesirable masking agents.

Interestingly all runners increased their running speed during the final 15-min of the 30-min run. This finding is consistent with both laboratory (Palmer et al., 1999; Rauch et al., 2005; Weltan et al., 1998a; Weltan et al., 1998b) and field (Billat et al., 2001; Sandals et al., 2006) studies that observe an increase in speed over the final stages of exercise. However, the observed increase in speed towards the end of the 30-min run was unexpected because the runners were asked to maintain the same perceived exertion for the duration of the run. The increases in speed were not accompanied with improved perceived activation or pleasure-displeasure that might be expected when nearing the completion of a run. Therefore it would be of interest to determine how the selection of speed and psychological response may change when the duration of the run is blinded. In this case, no increases of speed would be expected as the runner would not know when they were nearing completion of the exercise. Nevertheless, in the present study blinding runners to the exercise duration would have prevented them from repeating their perceived effort to the exercise task. This is supported by the findings that the pattern of energy expenditure during the 30-

min run did not change greatly between trials or runners (Figure 8.4, (Foster et al., 2003)). However, the amplitude of positive changes in running speeds were greater when mouth-rinsing with the CHO-E solution, i.e.  $0.3 \text{ km}\cdot\text{h}^{-1}$  faster over the 30-min (Figure 8.5).

How runners select a speed for a given duration or distance remains an unanswered question. It has been suggested that during exercise athletes use a monitoring process that allows them to complete a prescribed distance before exhausting their available sources of energy/fuel (Foster et al., 2003). The subjective evaluations of ones condition that is “how you feel” has been described as interoception (Craig, 2002). Therefore during exercise interoception maybe be involved in, for example, the selection of running speeds. Interoception functions through the lamina I spinothalamocortical system and acts as a homeostatic afferent pathway that conveys signals from small diameter primary afferents that represent the physiological status of all tissues of the body. These feelings have not only a sensory, but also an affective, motivational aspect (Craig, 2002).

Speculatively, the brain or lamina I spinothalamocortical system may be monitoring the physiological integrity of the muscle and/or whole body CHO stores. Recognition of CHO in the mouth may relay signals of an incoming energy source to the brain. This ‘promise’ of additional CHO may generate an altered response towards exercise, e.g. the selection of faster running speeds in the first 5-min of the run, as in the present study. Subsequently, when the brain receives feedback that no energy source has arrived, exercise behaviour maybe adjusted accordingly (Figure 8.4). Similarly, the “promise” of additional CHO may induce an altered psychological response towards exercise, e.g. runners experiencing enhanced feelings of pleasure at the onset of exercise. When the brain receives feedback that no energy source has arrived, mood state is adjusted accordingly (Figure 8.2). This may explain why no significant differences in perceived activation or pleasure/displeasure were observed during exercise in present study or Chapter 7 but have been reported with CHO ingestion during prolonged exercise wherein the “promise” of CHO delivery has been met, i.e. the CHO solution was ingested (Backhouse et al., 2007; Backhouse et al., 2005).

In summary, when runners mouth-rinsed a CHO-E solution they experienced elevated feelings of pleasure that was associated with the self-selection of significantly faster running speeds during the first 5 min of the 30 min run. The precise mechanisms responsible for the link between the positive feelings induced by mouth-rinsing with a CHO-E solution and the selection of faster running speed remain to be determined.

## CHAPTER 9

### THE INFLUENCE OF INGESTING VERSUS MOUTH-RINSING A CARBOHYDRATE-ELECTROLYTE SOLUTION ON 1 HOUR RUNNING PERFORMANCE

#### 9.1 SUMMARY

**Purpose:** To investigate the influence of ingesting versus mouth-rinsing a CHO-E solution on 1 h running performance. **Methods:** Following a 14-15 h fast, ten endurance-trained male runners (mean  $\pm$  SD,  $\dot{V}O_{2\text{peak}} = 65.0 \pm 4.4 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) completed three 1 h performance runs separated by 1 week. On two occasions runners ingested  $8 \text{ ml} \cdot \text{kg BM}^{-1}$  of either a 6.4 % CHO-E (C) or placebo (P) solution 30 min before and  $2 \text{ ml} \cdot \text{kg BM}^{-1}$  at 15 min intervals throughout the 1 h run. On a separate occasion runners mouth-rinsed (R) a 6.4 % CHO-E solution i.e. without ingestion, at the same times as in the ingestion trials. **Results:** Total distances covered in the C, P and R trials were  $14515 \pm 756 \text{ m}$ ;  $14190 \pm 800 \text{ m}$  and  $14283 \pm 758 \text{ m}$  respectively. Runners covered 320 m more (90% CI of difference = 140 m to 510 m,  $P = 0.01$ ) during the C trial than in the P trial and 230 m more (90% CI of difference = 83 m to 380 m,  $P = 0.019$ ) than in the R trial. The mean difference in distance covered between the R and P trials was 93 m (90% CI of difference = -13000 m to 13000 m,  $P = 1.0$ ). **Conclusion:** The ingestion of a CHO-E solution was associated with a greater distance covered during a 1 h performance run in comparison to mouth-rinsing a CHO-E solution and the ingestion of the same volume of a placebo solution.



## **9.2 INTRODUCTION**

Previous chapters have reported that both ingesting (Chapter 5) and mouth-rinsing (Chapter 7) a CHO-E solution improves 1 h running performance in comparison to mouth-rinsing/ ingesting a placebo solution.

Intriguingly, a recent study reported that mouth-rinsing a CHO-E solution had a more positive effect on exercise performance than ingesting the same solution. Pottier et al. (2008) report that mouth-rinsing a 6 % CHO-E solution (sucrose  $5.4 \text{ g}\cdot 100 \text{ ml}^{-1}$ , glucose  $0.46 \text{ g}\cdot 100 \text{ ml}^{-1}$ ) improved cycling performance (set mount of work in  $\sim 1 \text{ h}$ ) by 3.7 % than when the same solution was ingested ( $14 \text{ ml} \cdot \text{kg BM} \cdot \text{h}^{-1}$ ) (Pottier et al., 2008). The authors suggest that improved performance was due to CHO in the mouth acting on a centrally governed mechanism. However, these results are perplexing given that the mouth was exposed to the same CHO solution in both the ingestion and rinse trials. The authors speculated that the ergogenic effect of having CHO in the mouth may be lost when ingesting a CHO-E solution due to the short oral transit time. Although both mouth-rinsing and ingesting a CHO-E solution been reported to improve running performance independently in the current thesis, these two approaches have not been compared using the same group of runners. To remove any discrepancy between the time that the mouth is exposed to CHO, each solution will be held in the mouth for 5 s before being either expectorated or ingested.

Therefore, the purpose of the present study was to compare the influence of mouth-rinsing and ingesting a CHO-E solution on 1 h running performance in order to try to assess the relative impact on time-trial performance.

## **9.3 METHODS**

### **Participants**

Ten male recreational runners gave their written consent before participating in this study approved by Loughborough University Ethical Advisory Committee. Their mean ( $\pm$  SD) age, height, body mass and  $\dot{V}O_{2\text{peak}}$  were  $26 \pm 6$  years,  $1.81 \pm 0.06$  m,  $74.2 \pm 5.7$  kg, and  $65.0 \pm 4.4$  ml  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup>.

### **Preliminary tests**

A series of preliminary tests were conducted prior to the main experimental trials to determine (i) the relationship between running speed and oxygen uptake and (ii) the peak oxygen uptake ( $\dot{V}O_{2\text{peak}}$ ) (Chapter 3).

### **Experimental design**

Participants fasted for 13-15 h overnight (no food, only water). All trials were conducted in the morning at the same time of day (7.00 am- 9.00 am) and separated by 7 days. A habituation trial was completed by all runners before the completion of the three main 1 h running trials as described in Chapter 3. During each 1 h run, runners either ingested a CHO-E solution (C), a taste matched placebo solution (P) or mouth-rinsed a CHO-E solution without ingestion (R). The habituation involved the completion of the 1 h run however runners ingested water rather than ingesting the CHO-E solution. There were no significant differences in the average daily energy intake ( $13.1 \pm 1.6$  MJ), or quantities of CHO ( $465 \pm 97$  g), protein ( $128 \pm 20$  g) or fat ( $99 \pm 41$  g) consumed during the 48 h before each trial (Dietary composition analysed by CompEat Pro 5.8.0). All trials were separated by 7 days and conducted in the morning at the same time of day.

### **Beverages**

The CHO solution used in the present study was a commercially available 6.4 % CHO-E beverage (Lucozade Sport, Brentford, England). The placebo solutions were matched in formulation to the CHO-E solution except that they contained no CHO.

In the two ingestion trials, runners ingested the equivalent of  $8 \text{ ml} \cdot \text{kg BM}^{-1}$  of a CHO-E solution (C) or colour and taste matched placebo solution (P) 30-min before the 1 h run. Runners also ingested 25 ml of the CHO-E solution or placebo solution immediately prior to the 1 h run and then the equivalent of  $2 \text{ ml} \cdot \text{kg BM}^{-1}$  at 15 min intervals during the run i.e. at 15-min, 30-min, 45-min. Each  $2 \text{ ml} \cdot \text{kg BM}^{-1}$  bolus of solution was served in two separate plastic volumetric syringes (Kendal monoject). In an attempt to ensure the same transient time in the mouth, runners were instructed to rinse the last mouth-full of solution in the mouth for 5 s before ingestion. The solutions were weighed using an electronic balance (Mettler, Toledo AB54-s, Switzerland) to ensure the correct volume was ingested 30-min before exercise. A container with the solution was placed on the same electronic balance, to ensure the correct volume of solution was taken up into the plastic syringe.

For the rinse trial (R) the same CHO-E solution as used in the C trial was given to the runners. Each 25 mL of solution was delivered in a plastic volumetric syringe (Kendal monoject) at the following times; 30 min prior, immediately before and at 15 min intervals during the 1 h run. The solution was mouth-rinsed for 5 s before being expectorated into a pre-weighed plastic container. The syringe and plastic container were weighed before and after each rinse using an electronic balance (Mettler, Toledo AB54-s, Switzerland) to determine the volume of rinsed solution and expectorate respectively. The volume of expectorate was subtracted from the known volume of solution rinsed to determine if any solution had been ingested or remained in the oral cavity.

#### Blood and expired air collection

Finger tip blood samples (300- $\mu\text{l}$ ) were taken at rest, immediately before the 1 h run and at 15, 30, 45 and 60-min during the run. All blood samples were deproteinised with perchloric acid, frozen and later analysed for the concentrations of glucose and lactate (Maughan, 1982). One minute expired air samples were collected at 15 min, 30 min and 45 min into the 1 h run using the Douglas bag method.

#### Psychological scales

The feeling scale (FS), felt arousal scale (FAS), together with the gastrointestinal (G.I) discomfort scale were administered at rest, immediately prior to and at 15-min, 30-min, 45-min and 60-min during the 1 h run. Runners rating of perceived exertion was collected using the Borg Rating of Perceived Exertion scale (Borg, 1982) during the warm-up and at 15-min, 30-min and 45-min during the 1 h run.

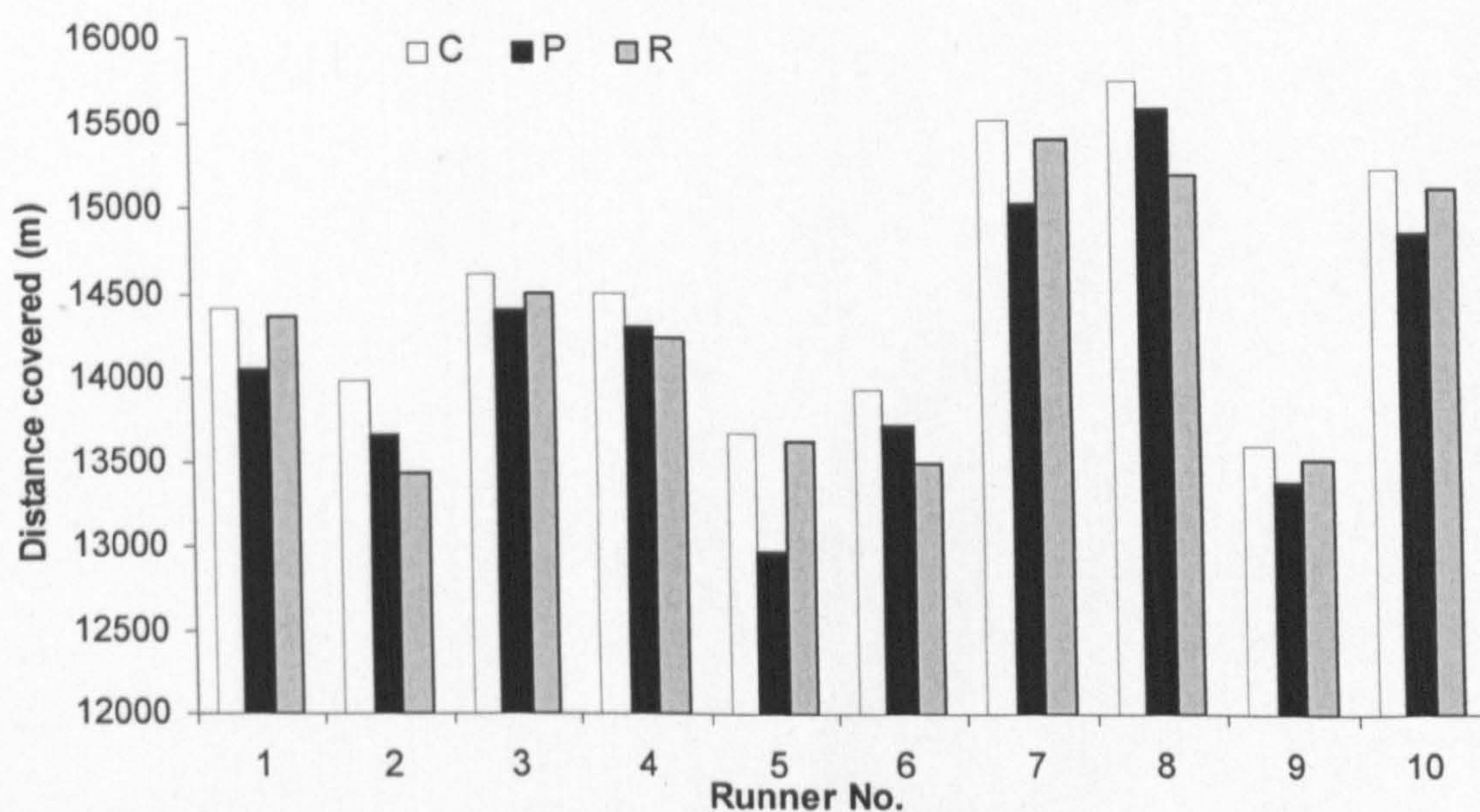
### **Statistical analysis**

All data were analysed using SPSS (version 16.0). The mean differences in performance (total distance covered and trial order) were detected using one-way within measures analyses of variance (ANOVA) with Bonferroni pair-wise comparison when significance was identified. The quantitative approach to the likelihood of the trial having a beneficial, trivial or negative effect on running performance was further enriched by dividing the range of substantial values into more finely graded magnitudes. Using a spreadsheet the P value was converted into 90 % confidence intervals (CI) for, and inferences about, the true value of the effect statistic (Hopkins, 2007). It has been previously reported that distance runners and support professionals need to be concerned about changes in performance of between ~0.5% and ~1.0% (Hopkins et al., 2001). Thus, the set threshold value for the trial to have a beneficial or negative influence on performance was set at 1 % of the mean distance covered over the three trials. Mean differences in self-selected running speed (analysed in 5-min blocks during the 1 h run) and psychological scores were detected using a repeated measures factorial ANOVA (trial x time). Significant main effects for individual time points were further analyzed using paired t-tests and the Bonferroni adjustment for the number of pair-wise comparisons employed. All data are presented as mean  $\pm$  SD, the confidence interval was set at  $P \leq 0.10$  a priori (Hopkins, 2002).

## **9.4 RESULTS**

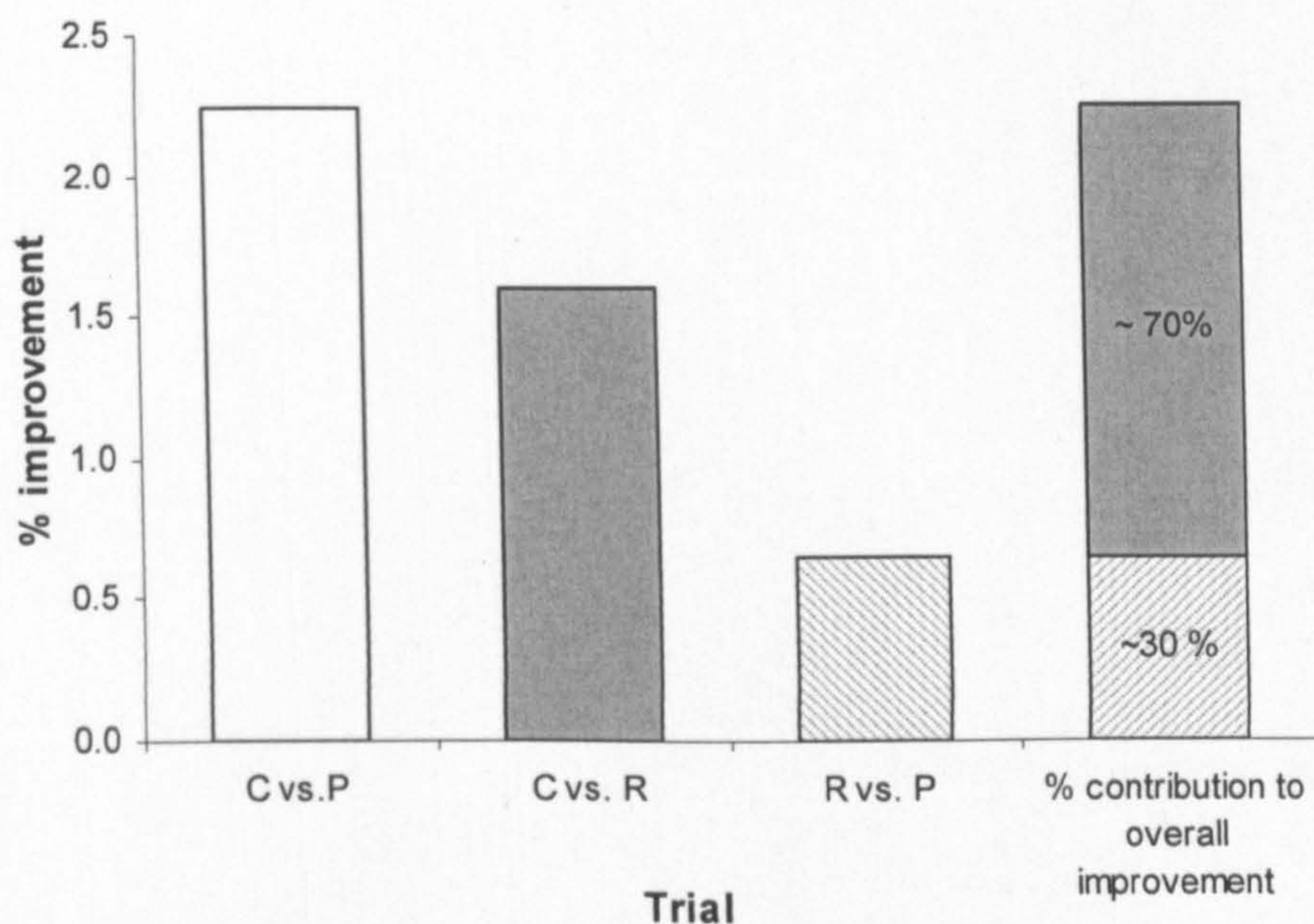
The total distances covered for the C, P and R trials were  $14515 \pm 756$  m;  $14190 \pm 800$  m and  $14283 \pm 758$  m respectively. There was no trial order effect between the three trials ( $F_{(2,18)} = 0.8$ ,  $P = 0.432$ ). The threshold value of 143 m was set as the minimum distance that would result in a meaningful difference in performance.

The mean difference in distance covered between the C trial and P trial was 320 m (90% CI of difference = 140 m to 510 m) 2.2 %,  $P = 0.01$ . The mean difference in distance covered between the C trial and R trial was 230 m (90 % CI of difference = 83 m to 380 m) 1.6 %,  $P = 0.019$ . The mean difference in distance covered between the R trial and P trial was 93 m (90 % CI of difference = -13000 m to 13000 m) 0.6 %,  $P = 1.0$ . The chance that the true value of the effect has a beneficial, trivial or negative influence on running performance was 94.8/5.1/0.1 % for the C vs. the P trial, 84.9/15/0.1 % for the C vs. the R trials and 49.7/1.5/48.7 % for the R vs. P trials, respectively.



**Figure 9.1.** Total distance covered by the 10 runners over the C trial (open blocks), P trial (filled blocks) and R trials (grey blocks).

The individual running performances can be seen Figure 9.1. From the percentage improvements in performance (Figure 9.2), mouth-rinsing a CHO-E solution contributed approximately 30 % whereas the ingestion of CHO contributed approximately 70 % to the overall performance improvement observed in the C trial. The changes in distance covered between the C trial and the P and R trials  $\pm$  the 90 % confidence limits are shown Figure 9.3. The mean running speeds for the three trials are shown in Figure 9.4.

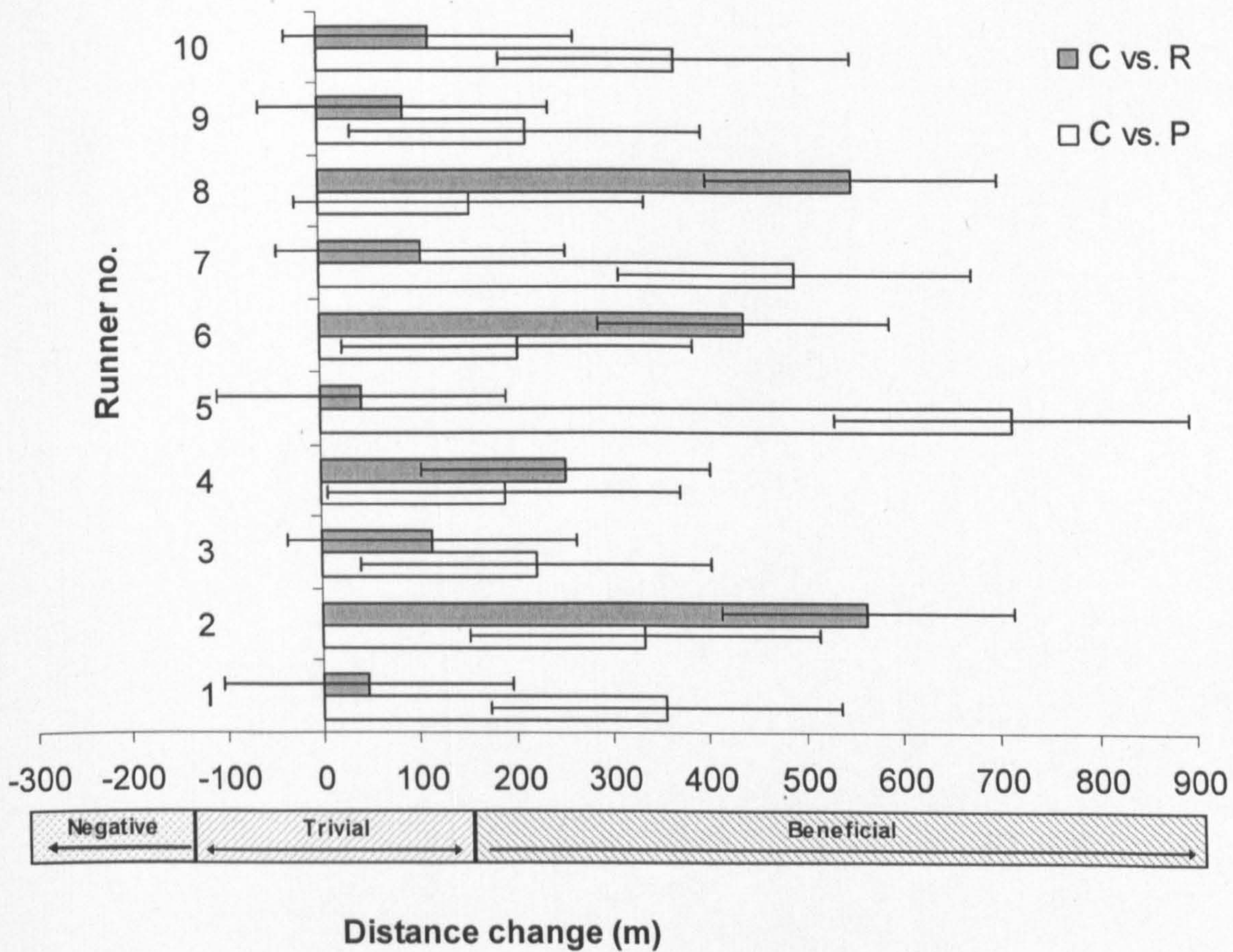


**Figure 9.2.** The percentage difference between trials and estimated contribution to overall performance benefit.

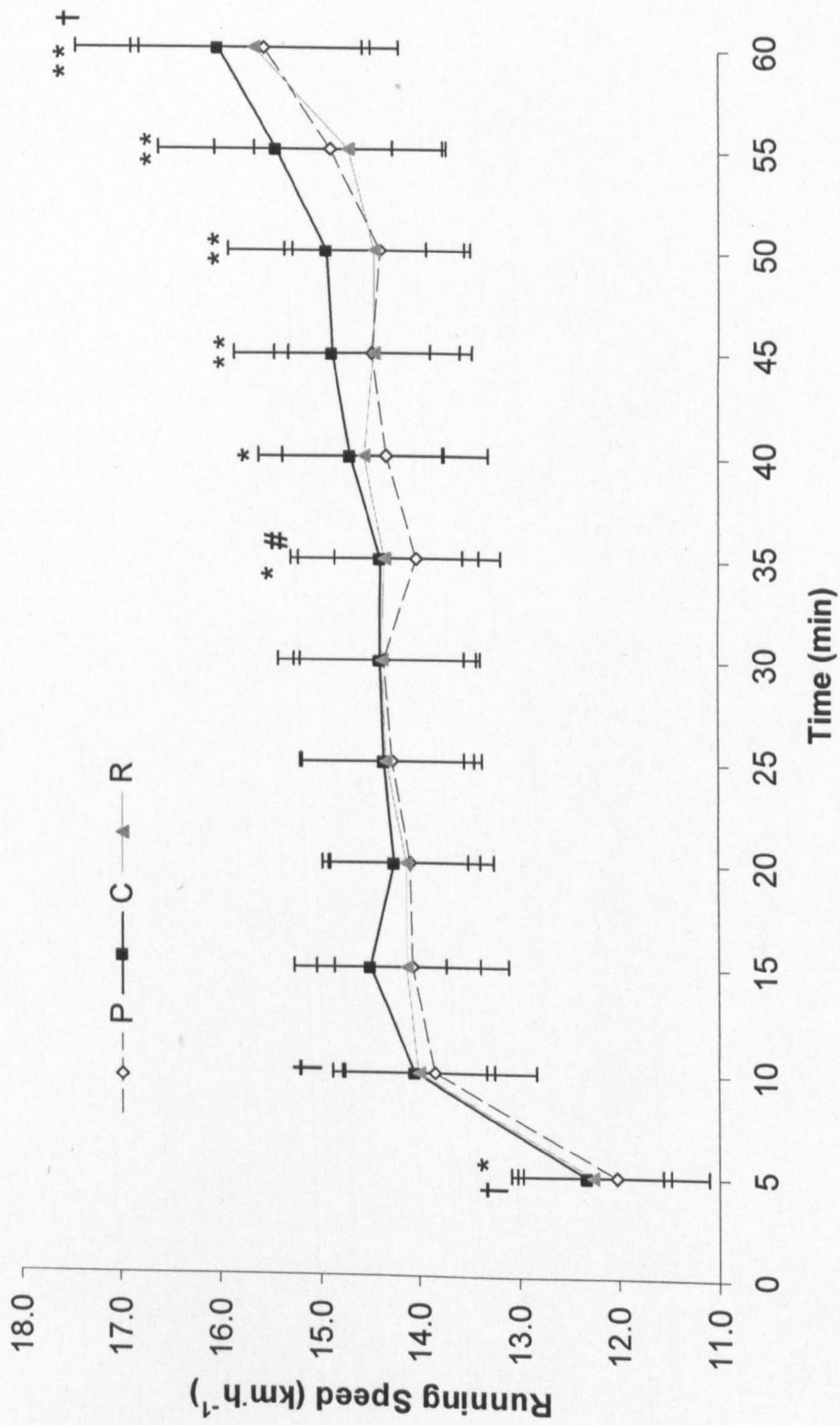
The mean physiological responses during the 1 h run for are shown in Table 9.1. Blood glucose concentrations are shown in Figure 9.5. Mean RPE and GI discomfort during the 1 h run are shown in Figure 9.6 and Figure 9.7 respectively. The mean psychological scores for FAS and FS are shown in Figure 9.8.

The mean volume of fluid ingested 30-min prior to exercise was  $594 \pm 45$  ml which provided  $39 \pm 3$  g of CHO in the C trial. All runners successfully used the plastic syringes to ingest / rinse the drink provided during exercise. The 25 ml of solution ingested immediately prior to exercise provided 2 g of CHO in the C trial. The mean volume of fluid ingested at 15-min intervals in the C and P trial was  $148 \pm 11$  ml. This provided a total of  $445 \pm 34$  ml during the 1 h run, supplying  $29 \pm 2$  g of CHO during the C trial. Thus the total quantity of CHO consumed during the C trial was  $69 \pm 5$  g. There was no difference in the volume of solution rinsed ( $25.9 \pm 0.6$  mL) and the volume of expectorate ( $25.9 \pm 1.0$  mL) during the R trial ( $P = 0.649$ ).

The mean body mass loss during the 1 h run for the C and P trials was 0.8 % and 0.9 % of pre-exercise body mass. Body mass loss was 1.1 % in the R trial. Calculated sweat rates were  $1.5 \pm 0.3 \text{ L}\cdot\text{h}^{-1}$ ,  $1.6 \pm 0.2 \text{ L}\cdot\text{h}^{-1}$ ,  $1.5 \pm 0.3 \text{ L}\cdot\text{h}^{-1}$  for the C, P and R trials respectively. The 5-min warm-up speeds were  $11.7 \pm 0.4 \text{ km}\cdot\text{h}^{-1}$  and the  $\dot{V}\text{O}_2$  was  $39.6 \pm 2.2 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  equivalent to  $60 \pm 5 \%$   $\dot{V}\text{O}_2$  peak for all trials,  $P > 0.10$ .

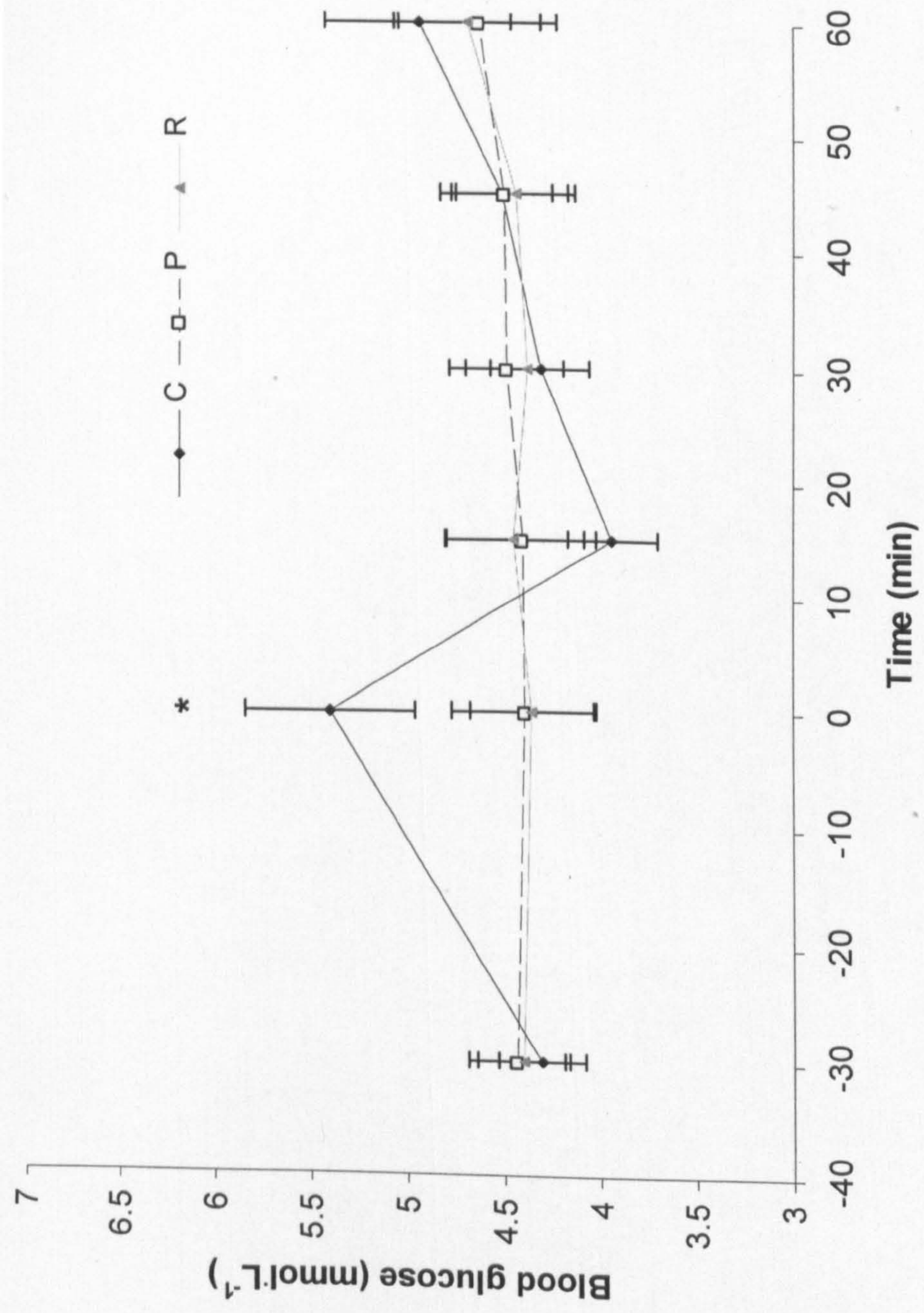


**Figure 9.3.** The change in distance covered between the C trial and the P and R trials  $\pm$  the 90 % confidence limits. Threshold limit (trivial) set at  $\pm 143 \text{ m}$ .

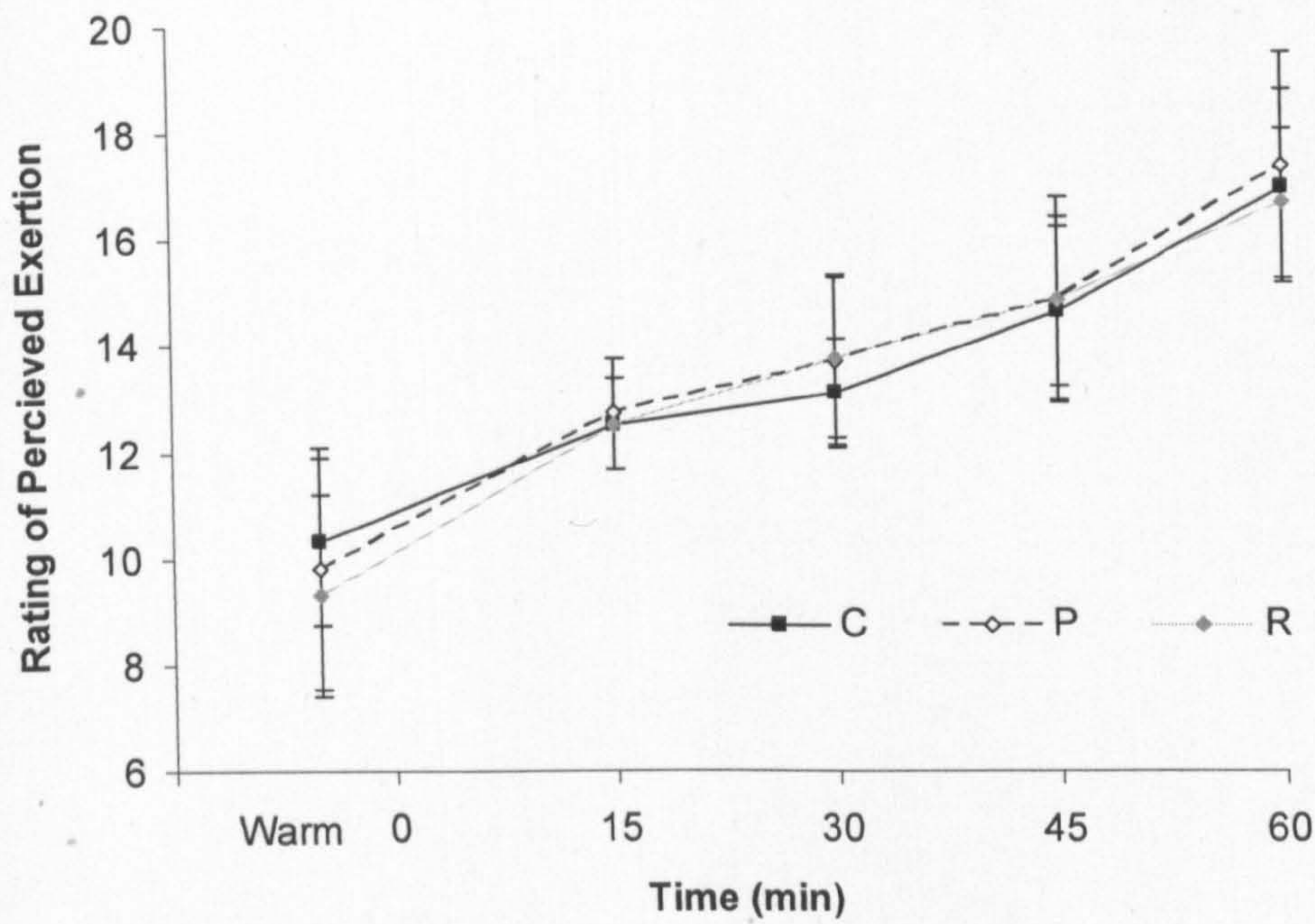


**Figure 9.4.** Mean running speed ( $\text{km}\cdot\text{h}^{-1}$ ) during the 1 h running performance test. \* indicates significant difference between C and P trials. \*\* indicates a significant difference between the C trial and P and R trials. # indicates a significant difference between R and P trials. † indicates significant effect of time,  $P < 0.10$  ( $n = 10$ , mean  $\pm$  SD).

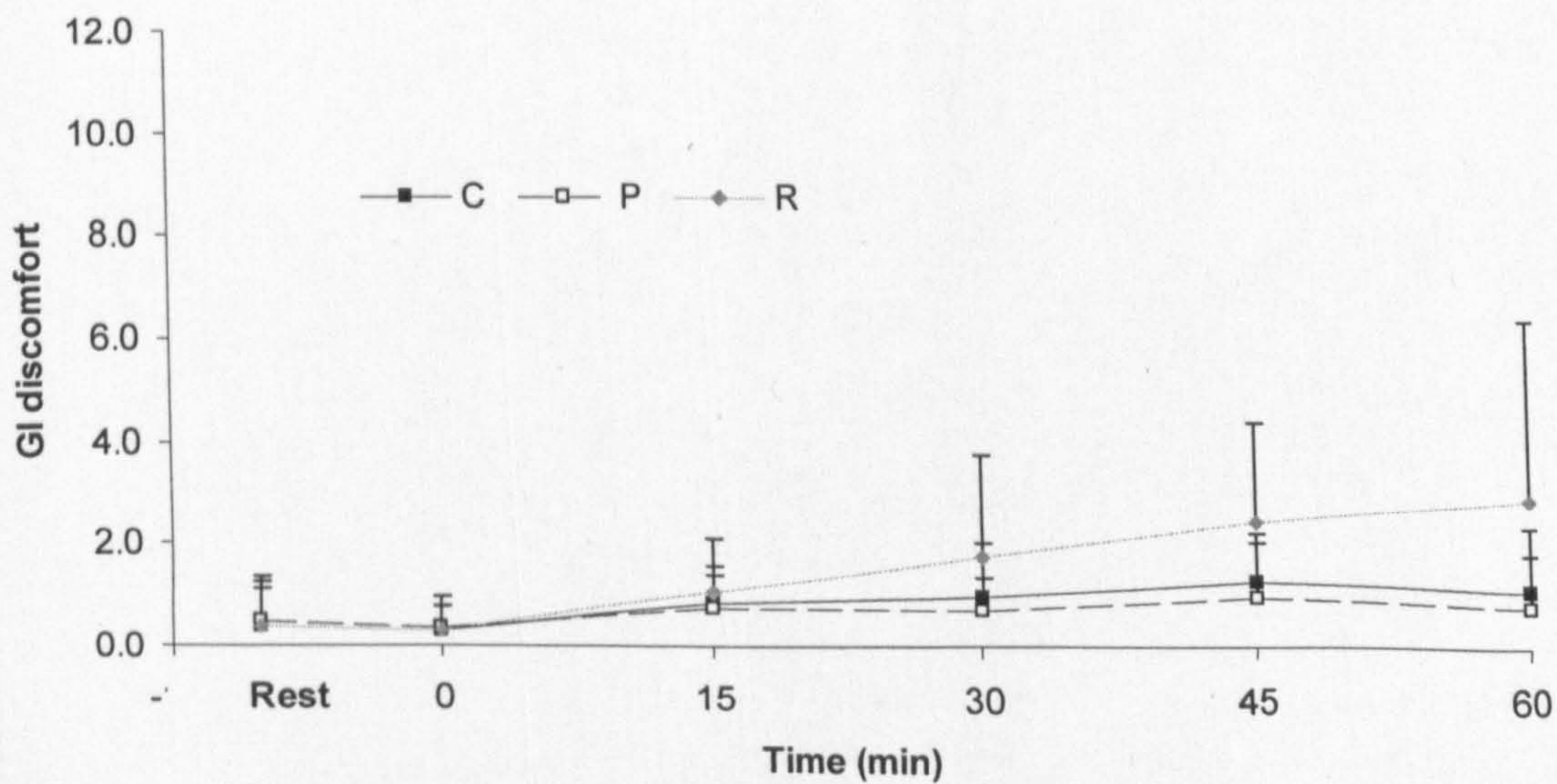




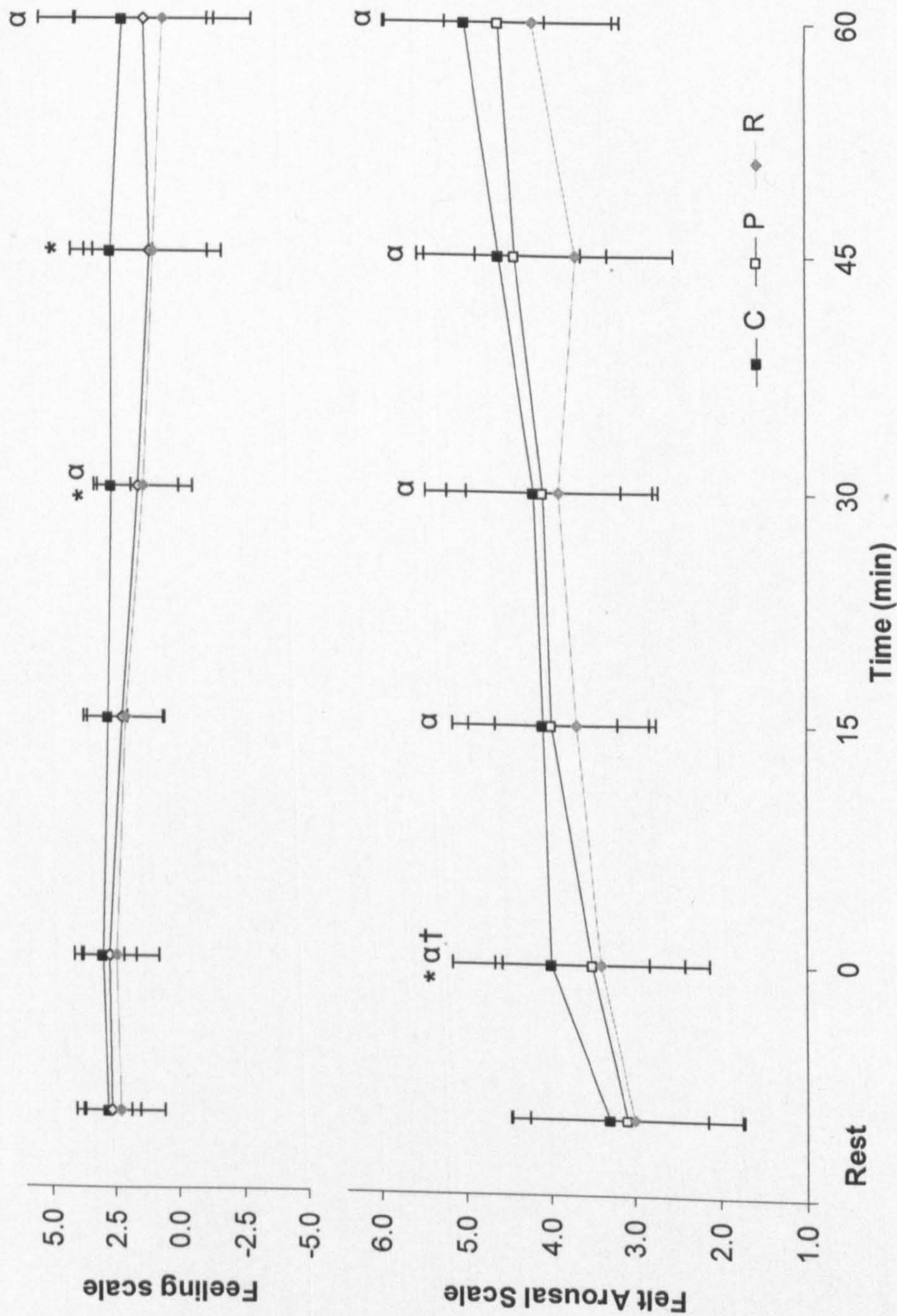
**Figure 9.5.** Mean blood glucose concentrations ( $\text{mmol}\cdot\text{l}^{-1}$ ). \* indicates significant difference between the C trial and P and R trials ( $n=10$ , mean  $\pm$  SD).



**Figure 9.6.** Mean RPE for the C, P and R trials during the 1 h run,  $P > 0.10$  ( $n = 10$ , mean  $\pm$  SD).



**Figure 9.7.** Mean GI discomfort for the C, P and R trials during the 1 h run,  $P > 0.10$  ( $n = 10$ , mean  $\pm$  SD).



**Figure 9.8.** Mean psychological scores for FAS ( $F_{(2,18)} = 7.1, P = 0.005$ ) and FS ( $F_{(2,18)} = 4.7, P = 0.023$ ) during the C, P and R trials. \* indicates a significant difference between the C trial and P trial.  $\alpha$  indicates a significant difference between the P trial and R trial ( $n = 10$ , mean  $\pm$  SD).

**Table 9.1.** Mean physiological response during the 1 h run. \* indicates significant difference between C and P. † indicates significant difference between C and R (n.= 10, mean ± SD).

Physiological response						
	Exercise intensity (% $\dot{V}O_{2peak}$ )	Heart rate (beats·min <sup>-1</sup> )	RER	CHO oxidation (g·min <sup>-1</sup> )	Blood lactate (mmol·L <sup>-1</sup> )	
<b>C</b>	74 ± 5	162 ± 6	0.92 ± 0.04	3.6 ± 0.8*	3.6 ± 0.6	
<b>P</b>	74 ± 5	161 ± 7	0.90 ± 0.03	3.2 ± 0.6	3.4 ± 0.7	
<b>R</b>	75 ± 6	162 ± 9	0.90 ± 0.03	3.2 ± 0.8	3.2 ± 0.6	
<b>F<sub>(2,18)</sub> =</b>	0.2	0.1	5.7	6.3	0.4	
<b>P =</b>	.803	.963	.012	.009	.672	

## **DISCUSSION 9.5**

The main finding of the present study was that ingesting a CHO-E solution significantly improved 1 h running performance in comparison to mouth-rinsing the same CHO-E solution or ingesting the equivalent volume of a placebo solution. The results of this study confirm previous observations (Chapter 5) that the ingestion of a CHO-E solution is associated with improved 1 h running performance.

Our findings are in agreement with studies that report the benefit of ingesting CHO solutions on self-selected running speeds during both laboratory based treadmill tests and “real life” road races. For example, in a controlled laboratory environments, the ingestion (1 L) of either a 6 % or 8 % CHO-E solution 1 h before exercise improved self-selected running speed by approximately 5 % during the final 1.6 km of a 15 km run, in comparison to the ingestion of water (Millard-Stafford et al., 1997). Interestingly, the percentage improvement in the present study (2.2 %) is similar to that reported during a “real life” 30 km race (2.3 %). In this latter study runners were asked to perform thirty circuits on a 1 km closed campus road, drinking either a 5 % CHO solution or water, immediately prior to and at 5 km intervals during the run. However, instead of the self-selection of faster running speeds as observed in the present study (Figure 9.4), improved running performance was attributed to runners being able to maintain their chosen running speed over the final 5 km of the run (Tsintzas et al., 1993).

Only one previous study has compared the influence of ingesting and mouth-rinsing a CHO-E solution on endurance performance (Pottier et al., 2008). In contrast to the results of the present study they reported a 3.7 % improvement in cycling time-trial performance when mouth-rinsing a 6 % (sucrose  $5.4 \text{ g} \cdot 100 \text{ ml}^{-1}$ , glucose  $0.46 \text{ g} \cdot 100 \text{ ml}^{-1}$ ) CHO-E solution in comparison to when their cyclists ingested ( $14 \text{ ml} \cdot \text{kg BM} \cdot \text{h}^{-1}$ ) the same solution. Several studies have reported that mouth-rinsing a CHO-E solution improves 1 h cycling time trial performance in comparison to mouth-rinsing a non-CHO taste matched placebo solution (Carter et al., 2004a; Chambers et al., 2009; Pottier et al., 2008). A possible explanation for the reported performance improvements with mouth-rinsing was investigated by Chambers et al. (2009). They found that both glucose and maltodextrin in the mouth activate regions in the brain associated with reward, such as the insula/frontal operculum, orbitofrontal cortex and striatum (Chambers et al., 2009). Thus, simply tasting a CHO solution may exert a positive influence on the central nervous system which, in turn, may result in improved performance

(Chapters 7 and 8). The results reported by Pottier et al., (2008) are therefore surprising given that the mouth (possible site of CHO receptors modulating central pathways (Chambers et al., 2009)) was exposed to CHO in both mouth-rinse and ingestion trials. However, the authors speculate that the ergogenic effect of CHO in the mouth may be lost due to the short oral transit time when ingesting CHO-E solutions (Pottier et al., 2008). In the present study the oral exposure to the CHO-E solution was standardised between trials i.e. each solution was held in the mouth for 5 s before being expectorated (R trial) or ingested (C and P trials). Thus, in contrast to Pottier et al. (2008) the combination of mouth-rinsing and ingesting a CHO-E solution was 85 % likely and 95 % likely to benefit 1 h run performance, compared to mouth-rinsing alone or mouth-rinsing and ingesting a placebo solution respectively

Analysis of the true value effect of the statistic revealed that there was almost a 50 percent chance that mouth-rinsing had either a beneficial or negative effect on 1 h running performance in comparison to ingesting a placebo solution. The ingestion of fluid during treadmill running has been reported to improve endurance capacity in comparison to no fluid intake (Fallowfield et al., 1996; Macaraeg, 1983). A study using cycling reported improved endurance capacity with fluid ingestion may be a consequence of a reduced heart rate, core temperature and utilisation of muscle glycogen compared to cycling without fluid intake (Hargreaves, Dillo, Angus & Febbraio, 1996). In contrast, ingesting water during on 1 h cycling time trial, rather than during an endurance test, has been reported to have little effect on performance. In this latter study cyclists completed 45 min of constant work followed immediately by a 15 min “all out” time-trial. Total work completed during the 15 min was similar with or without ingesting fluid or when water was ingested to prevent 50 % and 100 % loss of body mass. Furthermore, there were no difference in HR, plasma volume or body temperature between the fluid and no fluid trials (McConnell, Stephens & Canny, 1999). To our knowledge no study has investigated the influence of ingesting fluid versus no fluid on 1 h endurance running performance. In the present study the mean reduction in BM in the R trial was equivalent to 1.1 % which is similar to the findings by McConnell et al. (1999) who reported a minimal effect of fluid ingestion on cycling performance in a thermo-neutral environment.

The incidence of G.I complaints during running has been reported to be higher when ingesting CHO-E solutions than when ingesting water (van Nieuwenhoven et al., 2005). In the present study, mouth-rinsing a CHO-E solution was expected to reduce the risk of G.I

discomfort. However, the mean scores for G.I discomfort were higher on the R trial compared to both the ingestion trials (Figure 9.7). An explanation for the higher G.I discomfort scores when rinsing compared to ingestion cannot be offered at present. However, some runners reported that they felt 'empty' during the R trial. One speculation is that mouth-rinsing may have provoked stomach contractions in preparation for the "promise" of CHO (Chapter 8) that never arrived.

Studies have reported that both ingesting (Below et al., 1995; Jeukendrup et al., 1997b; Millard-Stafford et al., 1997; Tsintzas et al., 1993) and mouth-rinsing (Carter et al., 2004a; Chambers et al., 2009; Pottier et al., 2008) CHO solutions can independently improve endurance performance. These findings suggest that some of the effect observed during CHO-E solution feedings may have been attributable to a central or feed forward mechanism of CHO in the mouth. In the present study an estimated 30 % of the performance benefit in the C trial may have been due to the effect that CHO has whilst in the mouth. Therefore, the remaining 70 % of the overall performance benefit is attributed the delivery of CHO to the lower G.I tract (Figure 9.2).

The mechanism responsible for improved performance in the C trial is unlikely to be a consequence of the increased rate of oxidation (Table 9.1) of the exogenous CHO (McConnell et al 2000). Instead, reports from previous studies suggest that the increased CHO oxidation rate is a consequence of the elevated running speed i.e. exercise intensity during the C trial (Arkinstall et al., 2004; Carter et al., 2004b; McConnell et al., 2000). As discussed previously (Chapter 5 and 8) it has been proposed that the self-selection of running speed is determined by a centrally governed mechanism (Rauch et al., 2005; St Clair Gibson & Noakes, 2004) which regulates energy expenditure to use the available energy reserves economically (Foster et al., 2003).

Previous studies have reported that when a CHO solution is rinsed in the mouth the "promise" of incoming energy source is detected by, as yet, unidentified CHO receptors that trigger the reward centres in the brain (Chambers et al., 2009). Following ingestion, glucose is detected by receptors along the G.I tract i.e. in the intestinal mucosa (Berthoud, 2002) and walls of the hepatic portal veins (Hevener et al., 2000) which provide feedback to the brain of the quantitative and qualitative value of incoming energy source (Berthoud, 2002).

However, it is important to note that mouth-rinsing the CHO-E solution in the present study did not significantly improve performance compared to the mouth-rinsing and ingesting a placebo solution. A limitation of the present study was not to include a placebo mouth-rinse group without ingestion. The inclusion of this group would have allowed us to state with confidence that some of the improvement observed when the CHO-E solution was mouth-rinsed and ingested, was due to a central response in this group of runners. The reason why the present study did not report a significant improvement in performance when mouth-rinsing a CHO-E solution in comparison to placebo is unclear as the protocol mirrored that of Chapter 7 which did report a significant benefit. One possible explanation may be that the ingestion of the placebo solution had a positive effect on performance. At present there are no studies which have investigated the impact of fluid vs. no fluid on running performance. Thus, this could be a direction for future studies.

Despite the non-significant effect of mouth-rinsing CHO-E solution in the present study, several studies have used functional magnetic resonance imaging (fMRI) to investigate the central response to ingesting CHO solutions. Smeets et al., (2005a) reported that ingesting a glucose solution resulted in the activation of the hypothalamus, which was not observed with the ingestion of water, aspartame or maltodextrin solution (Smeets et al., 2005a). In addition, ingesting a more concentrated CHO solution (25 % CHO) was reported to result in a larger and more prolonged activation of the hypothalamus compared to that observed when ingesting a less concentrated solution (8 % CHO). The time course of the activation was associated with the corresponding changes in blood glucose and insulin concentrations following CHO ingestion. Interestingly, the onset of activation began before the end of glucose ingestion, i.e. before the glucose entered the blood (systemic circulation) stream (Smeets et al., 2005b). These results are consistent with a previous study which reported two separate peaks in brain activity following glucose ingestion. In this study the second peak was correlated with insulin concentrations and thus reflected the time when glucose entered the peripheral circulation. However, the first peak was not caused by swallowing or other motion artefacts during eating, suggesting that there was central response to glucose in the mouth (Liu, Gao, Liu & Fox, 2000). Speculatively, mouth-rinse followed by the ingestion of a CHO-E solution may have a “primary, feed forward” and “secondary, feedback” effect on the higher centres of the brain. This hypothesis would “fit” with the results of the Chapter 5 and Chapter 7 study and previous studies which have reported two peaks in central activation with glucose ingestion shown using fMRI techniques (Liu et al., 2000; Smeets et al., 2005b).



In conclusion the ingestion of a CHO-E solution was associated with increased distance covered during a 1 h running performance test in comparison to mouth-rinsing the same solution or ingesting a placebo solution. The relative contribution of feed forward and feedback mechanisms to overall performance benefits observed when ingesting CHO-E solutions warrant further investigation.

## CHAPTER 10

### GENERAL DISCUSSION

The purpose of this thesis was to examine the influence of CHO-E solutions on endurance running performance. Furthermore, the thesis examined the influence of CHO-E solutions on the perception of effort, mood and G.I discomfort as to explore possible links with self-selection of speeds whilst running. The main findings of this thesis were that asking runners to run as far as possible in 1 h using an automated treadmill was a sufficiently repeatable test to assess endurance running performance. The ingestion of a 6.4 % CHO-E solution significantly improved 1 h running performance in fasted but not in fed runners. Mouth-rinsing a CHO-E solution without ingestion improved 1 h running performance in fasted runners. In addition, mouth-rinsing a CHO-E solution was associated with enhanced feelings pleasure which corresponded with increased running speed when fasted runners were asked to run at a set effort. The combination of mouth-rinsing and ingesting a CHO-E solution improved 1 h running performance compared to mouth-rinsing alone or mouth-rinsing and ingesting an equal volume of placebo solution. However, mouth-rinsing a CHO-E solution without ingestion failed to improve performance compared to the ingestion of a placebo solution.

An important early part of this thesis was to develop a suitable treadmill running test that was sensitive to the relatively small changes in performance that can be achieved with nutritional interventions. The treadmill test aimed to assess endurance performance (run as far as possible in a set time) rather than endurance capacity (running for as long as possible at a set speed). The first study (Chapter 4) established that when runners are asked to cover as much distance as possible in 1 h they are able to reproduce their performance remarkably well as shown by the day to day coefficient of variation (CV) of only 1.4%. The use of an automated treadmill removed the limitation of manual interaction with the treadmill console to control running speed. Therefore, the new method advanced current treadmill running tests. The pacing strategy adopted by runners during the 1 h running performance test was consistent between studies. From a stationary start, runners increased their speed to a constant pace, which they maintained before performing a sprint in the final minute of the 1 h. It is important to note that differences in distance covered between trials in the studies reported in

this thesis were not a consequence of large differences in self-selected running speeds. Instead, differences in performance were a consequence of the selection of marginally faster or slower running speeds. Thus, the automated treadmill system appears an effective tool in detecting the influence which different nutritional interventions have on the self-selection of running speed.

In Chapters 6 and 9 of this thesis the ingestion of CHO-E solution before exercise resulted in a transient fall in blood glucose concentrations. Previous authors have suggested that ingesting CHO-E solutions in the hour before exercise could have a detrimental effect on performance. Specifically, concerns were raised about the effect on endurance capacity, as feeding CHO (75 g glucose) 45 min before exercise was reported to increase the rate of muscle glycogen utilisation. In addition the ingestion of CHO also resulted in the blood glucose concentrations of the runners falling to hypoglycaemic values during the 30 min treadmill run performed at 70 %  $\dot{V}O_2\text{max}$  (Costill, Coyle, Dalsky, Evans, Fink & Hoopes, 1977). However, in response, one study reported that a transient fall in blood glucose at the onset of exercise did not affect endurance capacity, when runners ingested 75 g of glucose in 300 ml of water or plain water 30 min before exercise (Chryssanthopoulos et al., 1994a). Nevertheless, in this study the treadmill speed was kept constant to maintain an exercise intensity equivalent to 70 %  $\dot{V}O_2\text{max}$ . Therefore, it is not clear whether, given the choice, runners would have reduced their running speed. In the present thesis, the true extent of hypoglycaemia may have been missed due to the limited blood sampling at the beginning of exercise. However, it is relevant to note that the transient fall in blood glucose concentration 15 min into exercise (Chapters 6 and 9) did not negatively affect the self-selection of running speed. This finding is consistent with studies performed in cycling which report that rebound hypoglycaemia in the first 10 min of exercise did not affect subsequent performance up to 1 h in duration (Moseley et al., 2003).

The prescribed drinking pattern adopted during the ingestion trials reported in this thesis did not induce a significant amount of G.I discomfort in the runners. This was evident from the mean scores on the G.I discomfort Scale which were consistently below the rating of “4, (uncomfortable)” in all studies. Therefore in contrast to previous findings the ingestion of CHO-E solutions did not cause G.I discomfort when compared with ingesting a placebo solution (Rehrer, Janssen, Brouns & Saris, 1989; van Nieuwenhoven et al., 2005).

Observations from the study reported in Chapter 7, where no fluid was ingested showed that G.I discomfort scores increased over time, suggesting that the action of running *per se* causes increased G.I discomfort independent of fluid ingestion. However, the study in Chapter 9 reports the mean scores of G.I discomfort were higher when mouth-rinsing the CHO-E solution in comparison to the ingestion of a CHO-E solution and ingestion of a placebo. This finding was unexpected because mouth-rinsing a CHO-E solution would be predicted not to cause G.I discomfort because no solution was ingested. It is unclear why the G.I discomfort scores were higher when rinsing compared to ingestion (Chapter 9). However, anecdotal reports from runners may offer an interesting insight to this phenomenon. Some runners on the mouth-rinse trial reported that they felt “empty” during the 1 h run. Therefore, ingesting small volumes of fluid may actually contribute to runners feelings of G.I comfort during exercise. Speculatively, holding CHO in the mouth may be a signal to the brain of an incoming source of energy and thus the body prepares for this “promise” of nutrient by the release of digestive enzymes (Berthoud, 2003). When the “promise” of food is not met, it may cause G.I discomfort and so the runners’ record increased discomfort scores.

A key finding of this thesis was that ingesting a CHO-E solution was associated with improved running performance in fasted (Chapter 5) but not in fed (Chapter 6) runners. The action of running is achieved by the recruitment of the necessary muscles as a consequence of neural innervation originating from the central nervous system. The precise mechanisms which underpin the selection of running speed for a given duration or distance are unknown. However, it is most likely a highly complex system involving both feed forward activation from the central nervous system and feedback signals from afferent tissues. One hypothesis, the central governor concept, states that exercise intensity i.e. running speed, is controlled by the brain such that the recruitment of motor units does not lead to physiological overload and cause lasting harm to the individual (Noakes, 2007; St Clair Gibson et al., 2004). It has also been speculated that the choice of exercise intensity is somehow linked to the availability of energy substrates in a way that allows the athlete to complete the prescribed distance without causing complete exhaustion (Foster et al., 2003). In the studies presented in this thesis it appears that runners distributed their energy resources over the 1 h run in such a way as to allow them to sprint during the final minute of the performance test. Therefore it is reasonable to apply the data presented in this thesis to both these constructs of running speed regulation.

With the exception of Chapter 6, the studies reported in this thesis investigated running performance following an overnight fast, preceded by a normal food intake in the 48 h before each trial. This procedure was used to standardise muscle and liver glycogen concentrations prior to exercise. Results of studies presented in Chapters 5 and 6 suggest that the CHO status of participant prior to exercise is an important variable when investigating the benefit of ingesting CHO-E solutions on endurance performance. In Chapter 5 it was reported that the ingestion of a CHO-E solution before and during the 1 h run improved running performance in comparison to the ingestion of a taste matched placebo. The mean differences in distance covered were improved by 2.6 % and 2.4 % when ingesting the CHO-E solution in comparison to the placebo solution. In addition, there were no differences between the two placebo trials. Reassuringly, when these conditions were replicated in Chapter 9 the ingestion of CHO-E solutions was also found to improve performance by a similar 2.2 %. However, when the ingestion of a CHO-E solution followed a pre-exercise meal (2.5 g CHO · kg BM<sup>-1</sup>) consumed 3 h before exercise there was no additional benefit to running performance (Chapter 6). The results of the study in Chapter 5 are consistent with other investigations performed in cycling which have shown the ingestion of CHO-E solutions can improve time-trial performance of approximately 1 h in duration. Interestingly, several reports from cycling studies have also concluded that CHO-E ingestion has no additional benefit on endurance performance in well fed individuals (Desbrow et al., 2004; Widrick et al., 1993a).

The studies reported in this thesis adopted a minimal invasive approach so as not to distract runners from their self-selection of speeds during the 1 h time-trial. Therefore we did not attempt to measure muscle glycogen of the runners before and after the performance test. However, the self-selected speeds of the runners were similar in relative exercise intensity as those used in earlier studies investigating glycogen metabolism (Chryssanthopoulos et al., 2002a; Tsintzas et al., 1995a). For example, Chryssanthopoulos et al., (2002) compared muscle glycogen utilisation during a 1 h run at 70 %  $\dot{V}O_2$ max in fed runners who consumed either water or a CHO-E solution (6.9 %) during exercise. The runners consumed the same high CHO meal (2.5 g CHO kg · BM<sup>-1</sup>) 3 h before the 1 h treadmill run as adopted in the present study. They found that ingesting the CHO-E solution did not change rate of glycogen utilisation or the accumulation of glycolytic intermediates when compared with the ingestion of water. Although this study did not include a trial in which the runners were fasted, the authors were able to compare their results with those of an earlier study that used the same exercise protocol and the same runners (Tsintzas et al., 1995a). The results of this earlier

study showed that the rate of utilization of muscle glycogen was less when the runners ingested a CHO-E (5.5 %) than when they ingested water. However, when these rates of glycogen utilization during the water trial were compared with those following the high CHO pre-exercise meal alone and water during exercise, the rate of muscle glycogenolysis was almost half of that in the fasted runners (Chryssanthopoulos et al., 2002a). This circumstantial evidence suggests that the pre-exercise CHO meal makes a significant contribution to muscle metabolism during subsequent exercise probably via an increased contribution of glucose from the increased liver glycogen stores. The ingestion of CHO-E solution (71 g/120min) by fasting individuals has been shown to reduce hepatic glucose output and even greater amounts of CHO-E solution (354 g/120 min) appear to completely block hepatic glucose production (Jeukendrup et al., 1999). In the presence of a reduced hepatic glucose output, the ingested CHO-E contributes to maintaining blood glucose concentrations in particular and to CHO metabolism in general. Therefore, it is reasonable to assume that the pre-exercise CHO meal (Chapter 6) would have increased liver and muscle glycogen stores and that the ingestion of the CHO-E solution would have contributed to CHO metabolism during the 1 h time-trials.

The mechanism by which running speed is linked with the pre-exercise CHO status of the runner is unknown. A possible explanation for the differences observed when CHO-E solutions are ingested in a fasted or fed state may be linked with the brain and the prioritisation to maintain its own homeostasis during exercise. When considering the liver as the body's glycostatic organ and its role in supplying glucose to the brain, it is reasonable to speculate that there may be central monitoring of the hepatic glycogen concentration. An overnight fast is associated with a significant reduction in liver but not muscle glycogen concentrations. Following a 3 h postprandial period it is most likely that the majority of the CHO consumed will be deposited in the liver. Thus, in contrast to an overnight fast, a pre-exercise meal would almost certainly normalise liver glycogen concentrations. Theoretically, when hepatic glycogen concentrations are low, the brain may conserve energy by selecting slower running speeds so as not to compromise a limited supply of glucose. Following a pre-exercise meal the hepatic glycogen concentrations are high. Therefore, the brain may allow an increase in energy expenditure i.e. running speed in the knowledge that there is a plentiful supply of glucose to meet the demands of the exercise challenge and maintain its homeostasis.

Interestingly, the myokine IL-6 is believed to have a role in influencing mood, performance, and cognitive function during exercise. It is known that IL-6 is released by muscle fibres

during exercise. Therefore, IL-6 could be a prospective candidate involved in providing information about the CHO status of the liver and muscles to the brain (Pedersen et al., 2005; Pedersen et al., 2001).

The results in this thesis (Chapters 5, 7) provides circumstantial evidence that a proportion of the benefit to performance observed with CHO-E solution ingestion is independent of the provision of CHO to the peripheral circulation. Under conditions of low hepatic glycogen, CHO-E solutions can have a positive influence on running performance (Chapter 5). Chapter 7 describes a resting study that showed that mouth-rinsing *per se* did not alter blood glucose or plasma insulin concentrations. However, mouth-rinsing a CHO-E solution without ingestion was associated with improved running performance in comparison to mouth-rinsing a placebo solution. Runners performed the 1 h run self-selecting faster running speeds when mouth-rinsing the CHO-E solution, even though the run was performed at the same relative perceived exertion.

However, it is important to note that mouth-rinsing a CHO-E solution failed to improve performance compared to ingesting a placebo solution. One possible explanation for why mouth-rinsing a CHO-E solution in Chapter 9 failed to improve performance was that it was compared to fluid ingestion. In comparison, Chapter 7 compared mouth-rinsing a CHO-E solution to mouth-rinsing a placebo solution without ingestion. Although there appears little evidence from cycling that providing fluid during a 1 h time-trial improves performance (McConnell et al., 1999) there are no studies reporting on the influence that fluid ingestion has on self-selected running performance. Therefore providing fluid *per se* may have a positive effect on performance so that the small effect that mouth-rinsing a CHO-E has on self-selected speed was lost. Further research is required in running performance both investigating the influence of CHO-E solution mouth-rinse and fluid ingestion so that comparisons to the findings of the present studies can be made.

Chapter 8 examined this CHO-E mouth-rinsing phenomenon in more detail by asking runners to self-select a running speed equivalent to a RPE of “15 i.e. hard” for 30 min. In this study runners consistently self-selected faster running speeds which reached levels of statistical significance during the first 5 min of the run. In addition, the increased running speeds corresponded with runners experiencing greater feelings of pleasure as scored on the feeling scale. The exact pathways by which mouth-rinsing CHO may influence mood and running

speed are unknown. However, it has been reported that regions of the brain, believed to mediate behavioural responses to rewarding stimuli, such as taste are activated by oral exposure to glucose and maltodextrin (Chambers et al., 2009). Speculatively, with respect to our findings in Chapter 8, recognition of CHO in the mouth could signal the ‘promise’ of CHO delivery to the body inducing a “feel good” effect. Thus with respect to performance, simply “feeling better” may manifest itself during exercise in the selection of faster running speeds.

Nevertheless, in the study reported in Chapter 9 the ingestion of a CHO-E solution after mouth-rinsing was found to improved 1 h run performance when compared to mouth-rinsing alone. Carbohydrate receptors are reported to exist along the G.I tract. Specifically, glucoreceptors located in the intestine and hepatic portal veins inform the brain on the nutritive value of foods (Berthoud, 2003). Speculatively, activation of these peripheral receptors in the lower G.I tract may provide “secondary” feedback to the brain which reinforces the “promise” of the initial activation achieved when the CHO is detected in the mouth. Thus, under conditions where hepatic glycogen is low, mouth-rinsing followed by the ingestion of a CHO-E solution may have a “primary” and “secondary” effect on a centrally governed response to exercise behaviour.

In conclusion, ingesting (Chapter 5 and 9) and mouth-rinsing (Chapter 7 and 8) a 6.4 % CHO-E solution was associated with improved running performance in a fasted state. The ingestion of a CHO-E solution was not associated with improved 1 h running performance when a meal providing  $2.5 \text{ g CHO} \cdot \text{kg BM}^{-1}$  was consumed 3 h before exercise (Chapter 6). The benefits to running performance associated with CHO-E solution ingestion may not be entirely attributed to the provision of exogenous substrate to the circulation because there appears to be an, as yet undermined, effect on the brain or central nervous system in general.



## **Recommendations for future research**

The following research projects may contribute to our understanding about how CHO-E solutions and pre-exercise CHO feedings influences self-selected running performance.

- As self-selected running speed appears to be influenced by the pre-exercise CHO status prior to performance, of interest would be to investigate the influence of a LGI meal on self-selected running performance.
- In addition, providing a pre-exercise meal 5-6 h before exercise may be more advantageous to performance, as it may allow insulin concentrations to return to pre-feeding concentrations.
- Studies could investigate how ingesting a different mixture of CHO, i.e. adding fructose, may influence 1 h running performance and substrate metabolism.
- To further our understanding on the effect of mouth-rinsing CHO, future studies could investigate how increasing the frequency or concentration of the rinsed solution on self-selected running speed.
- At present the majority of research investigating the influence of CHO-E solutions on endurance running performance has been performed in males. Future research could replicate the studies in the present thesis to investigate if the reported findings are evident in female runners.
- Future research could also investigate whether the concentration of CHO in CHO-E solutions can induce the same central activation when rinsed as that reported with more concentrated CHO solutions with the use of *f*MRI.

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## APPENDICES

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## APPENDIX A

### Gastrointestinal symptoms experienced during exercise

#### Upper G.I symptom:

- Bloating
- Belching
- Nausea
- Vomiting
- Reflux

#### Lower G.I symptom:

- Flatulence
- Constipation
- Diarrhoea
- Rectal blood loss
- Urge to defecate
- Abdominal cramps
- Faecal incontinence

#### Possible contributing factors:

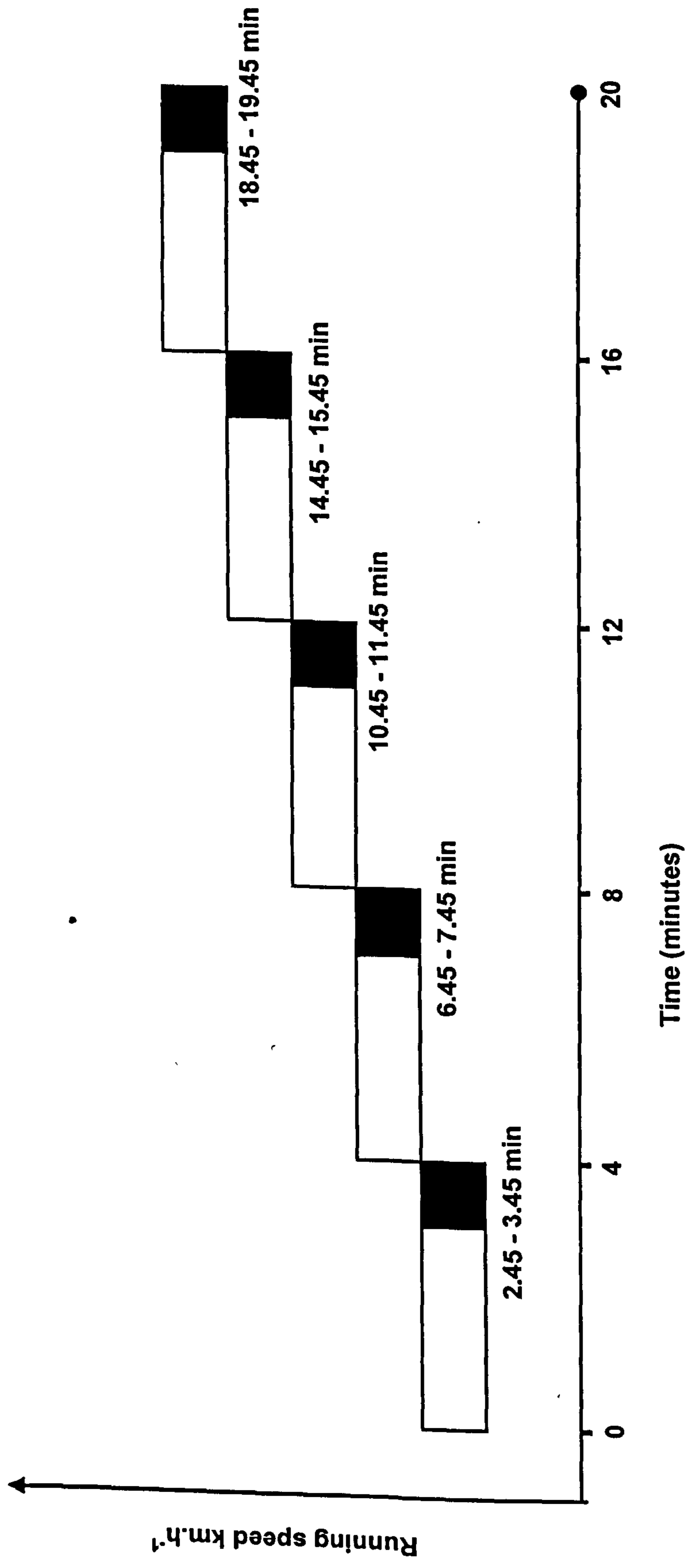
- Dehydration
- Altered G.I tract blood flow
- Altered gut permeability
- Disturbed G.I motility
- Psychological influences (nerves)

Adapted from: Nutrition in Sport. Maughan, R.J. IOC Medical Commission, International Federation of Sports Medicine. pp.248



## APPENDIX B

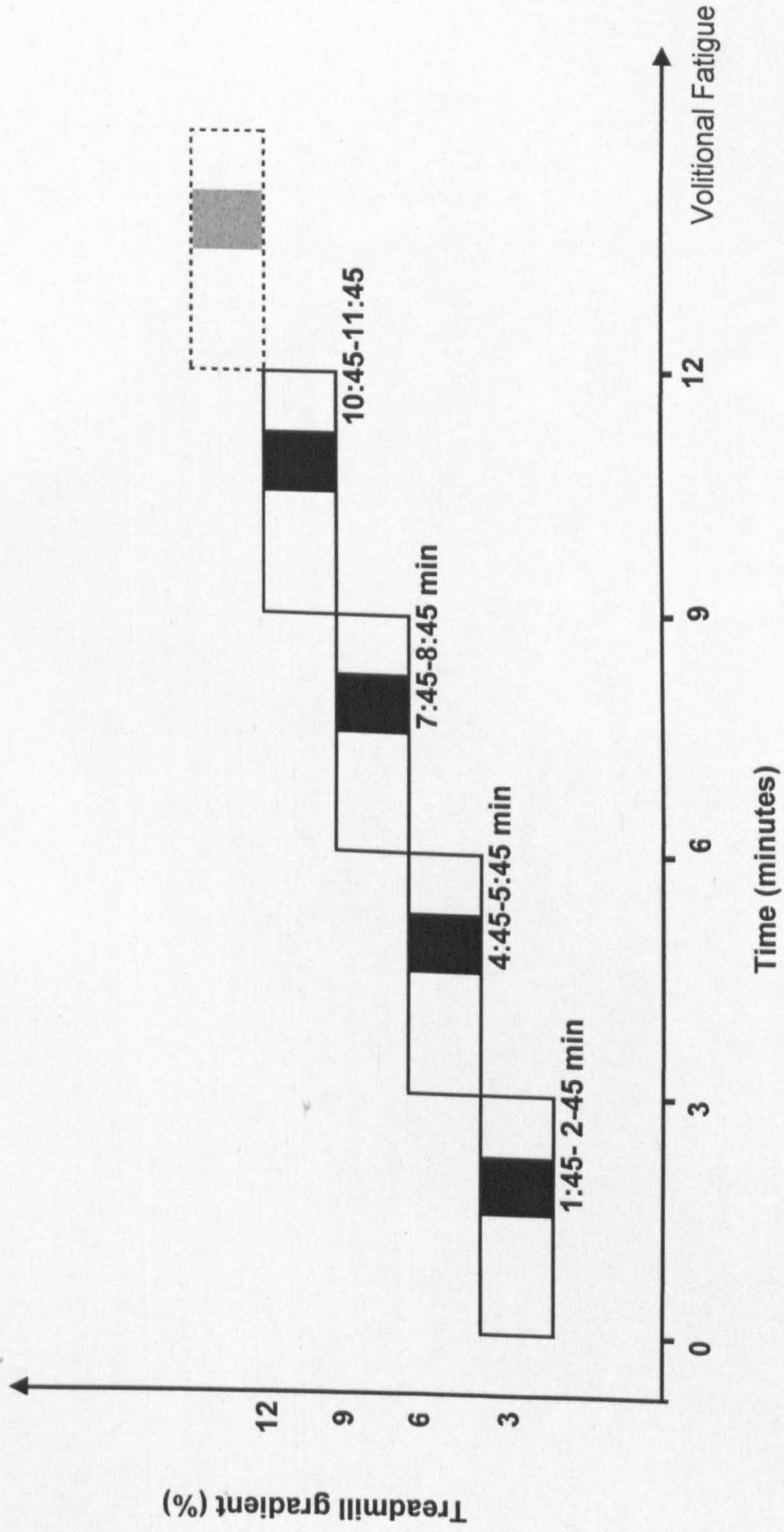
### Protocol for speed $\dot{V}O_2$ test



■ Collection period: 1 minute expireds air sample, rating of perceived exertion, heart rate

# APPENDIX C

Protocol for  $\dot{V}O_2$  peak test



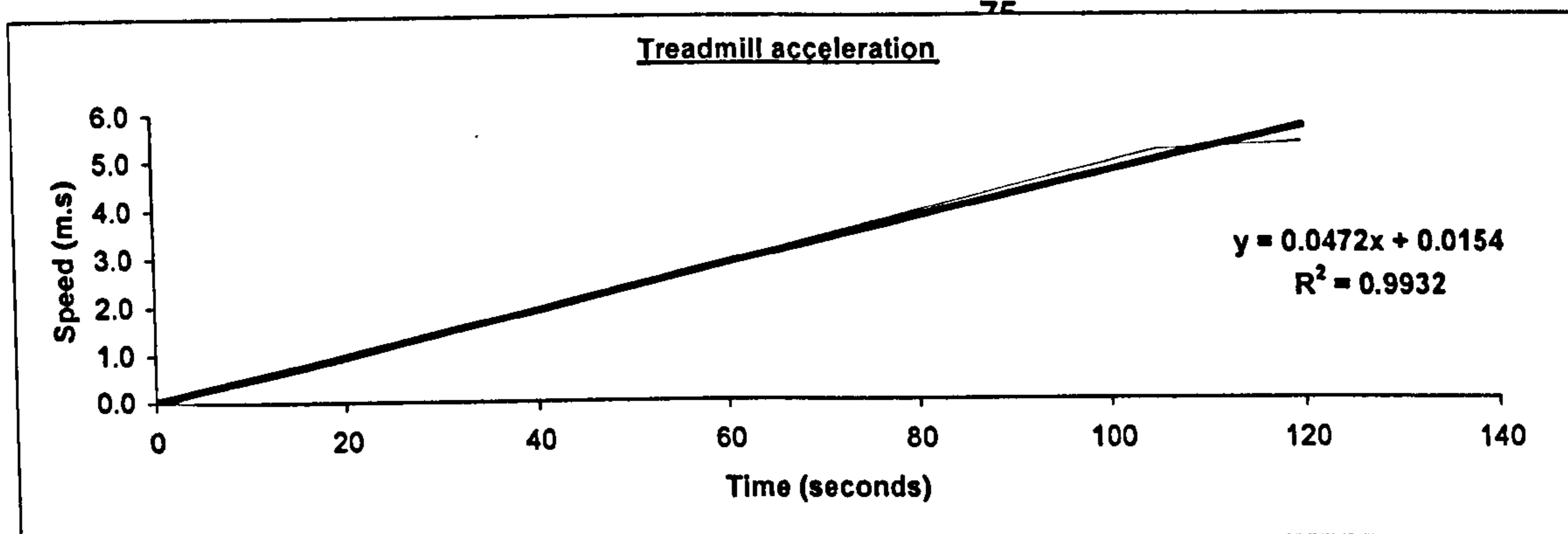
■ Collection period: 1 minute expired air sample, rating of perceived exertion, heart rate

## APPENDIX D

### Treadmill responsiveness

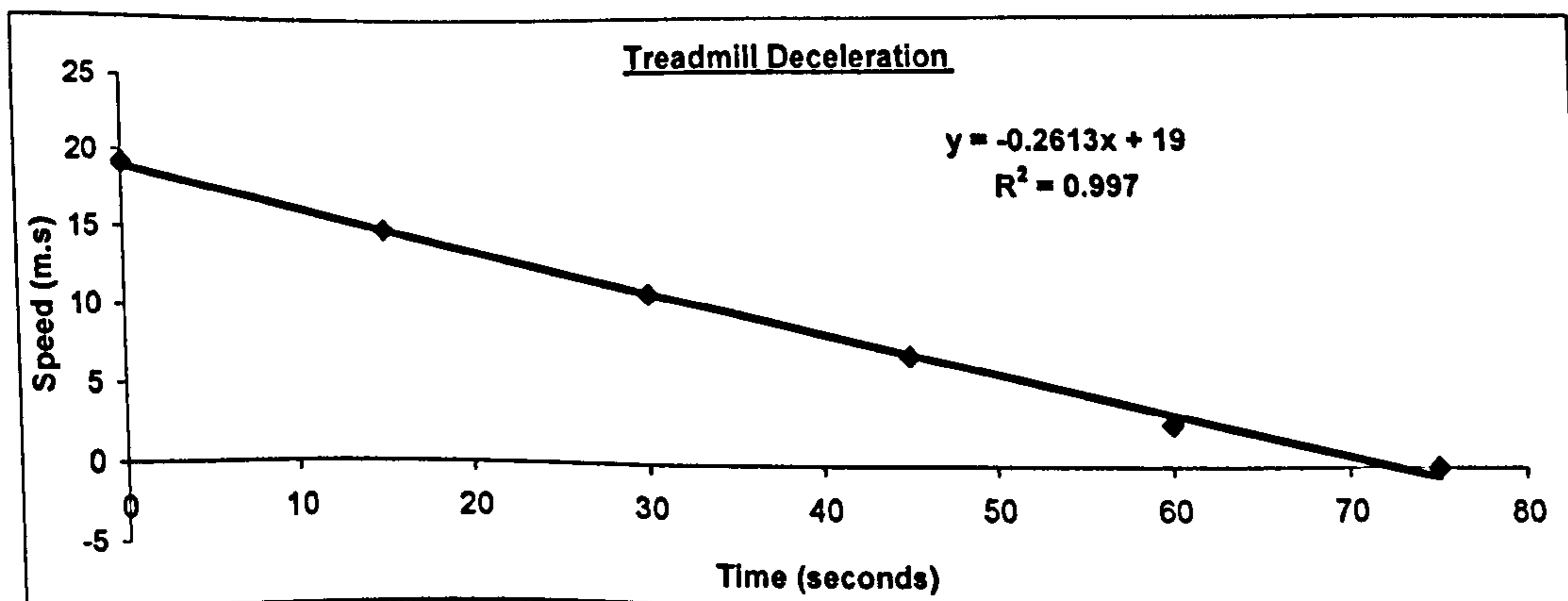
#### Acceleration

Time	km.hr	m.s	Distance (m)	Distance (m) /15 seconds	Acceleration m.s
0	0	0.0	0	10	0.6
15	2.3	0.6	10	11	0.8
30	5.0	1.4	21	11	0.8
45	7.7	2.1	32	11	0.8
60	10.4	2.9	43	12	0.8
75	13.2	3.7	55	11	0.8
90	15.9	4.4	66	11	0.8
105	18.6	5.2	78	3	0.2
120	19.2	5.3	80		



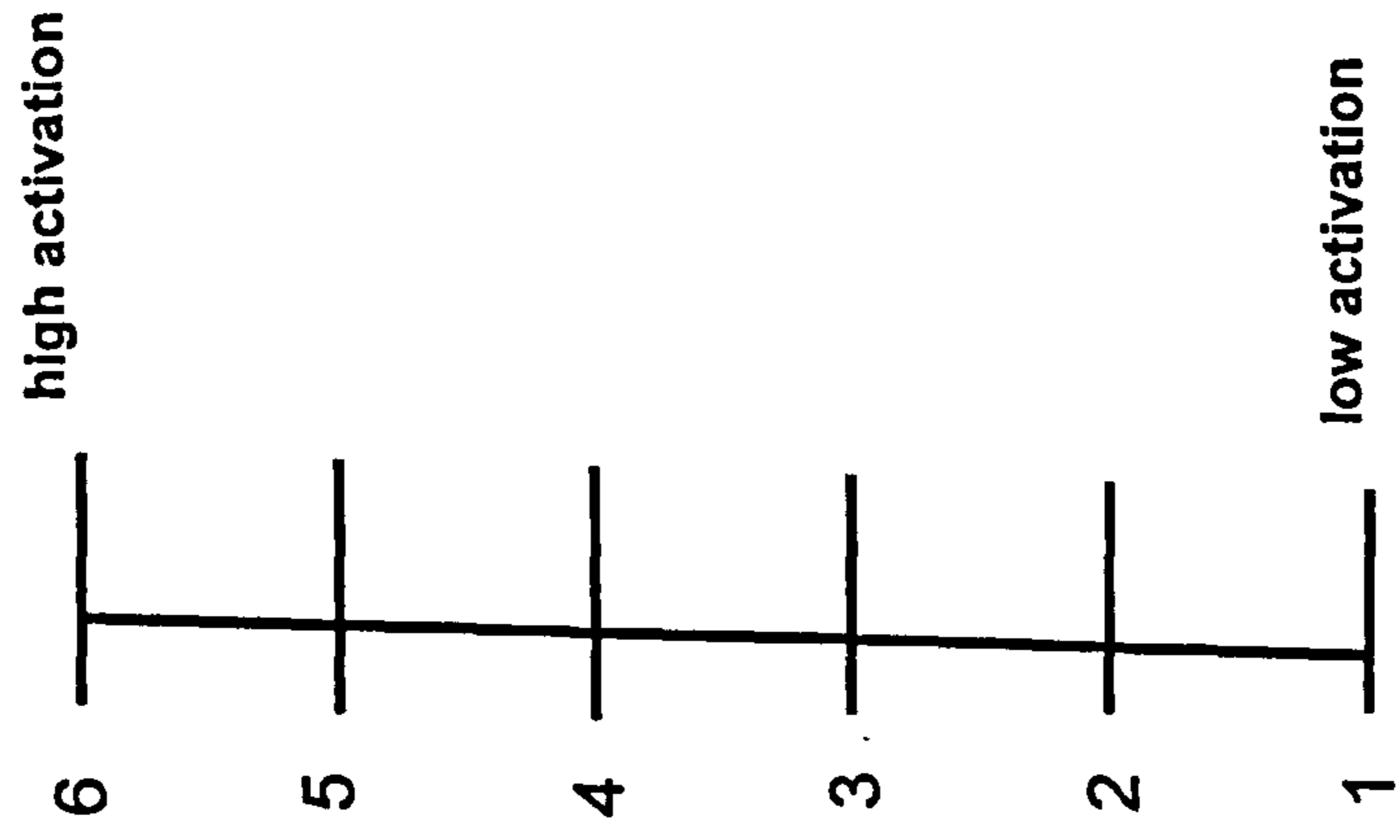
#### Deceleration

Time	km.hr	m.s	distance m	Distance (m) /15 seconds	Deceleration m.s
0	19.2	5.3	80	17	1.1
15	15.1	4.2	63	17	1.1
30	11.1	3.1	46	17	1.1
45	7.1	2.0	30	18	1.2
60	2.7	0.8	11	11	0.8
75	0	0.0	0	0	0.0

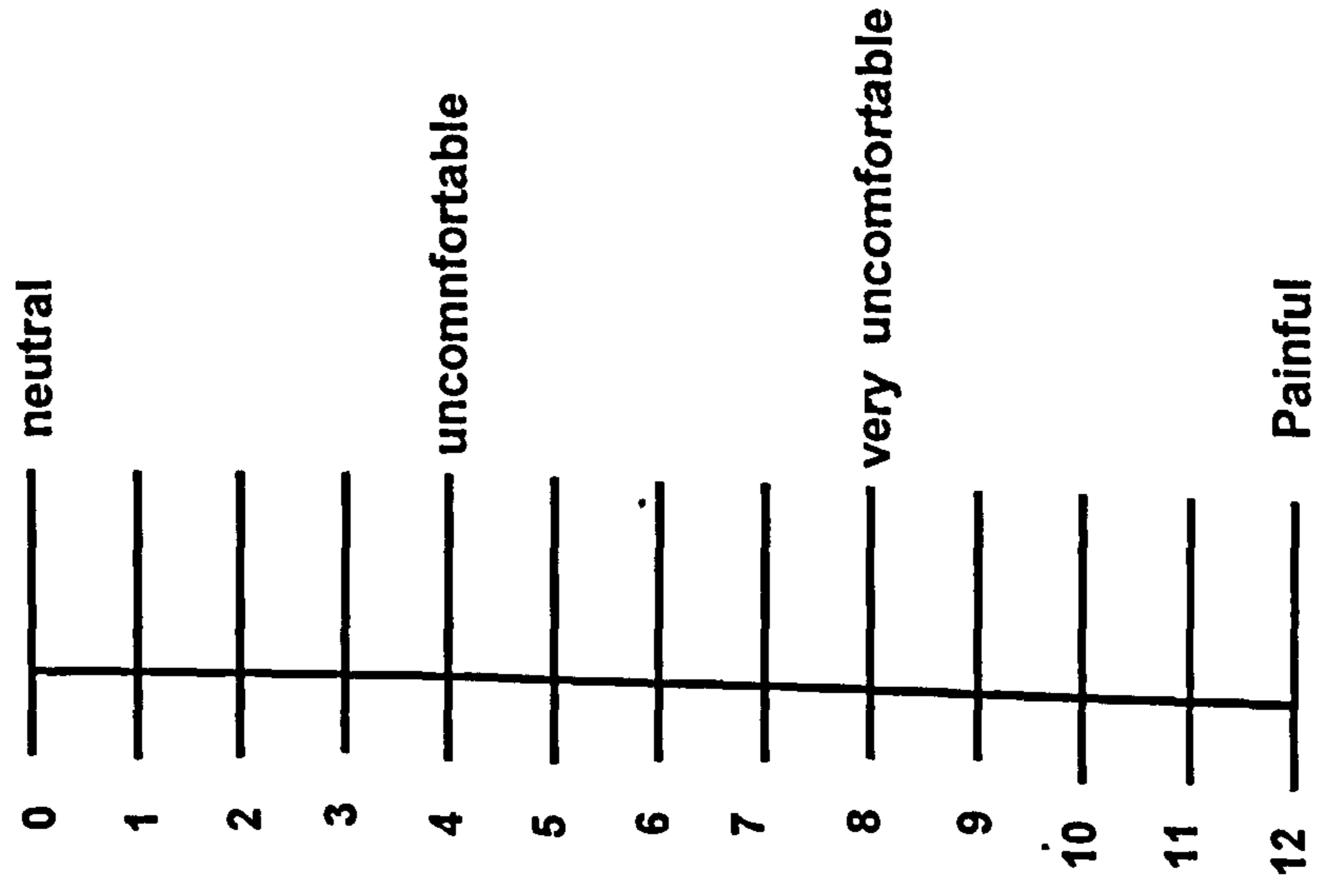


# APPENDIX E

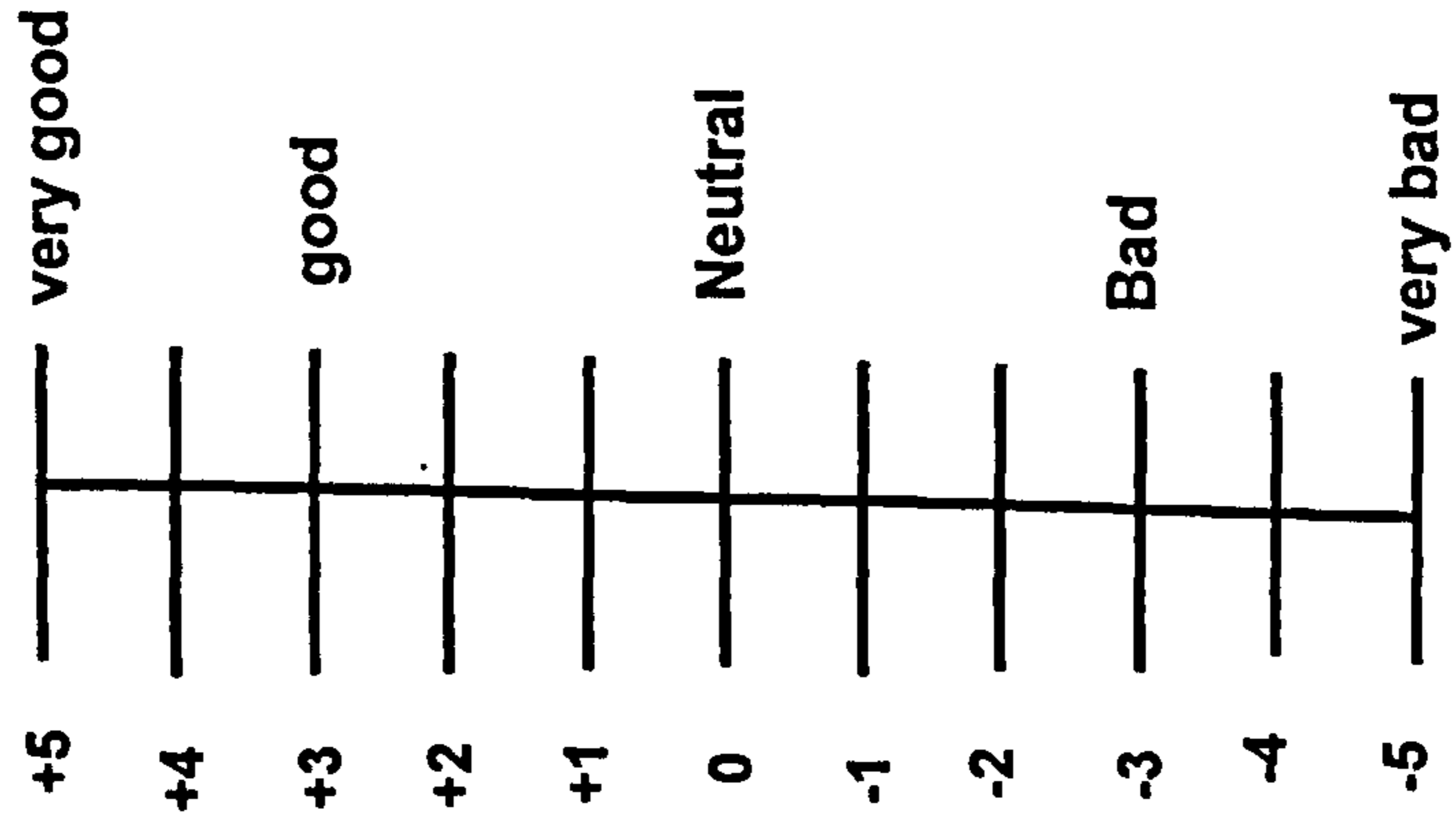
**Felt Arousal Scale**



**GI Comfort scale**

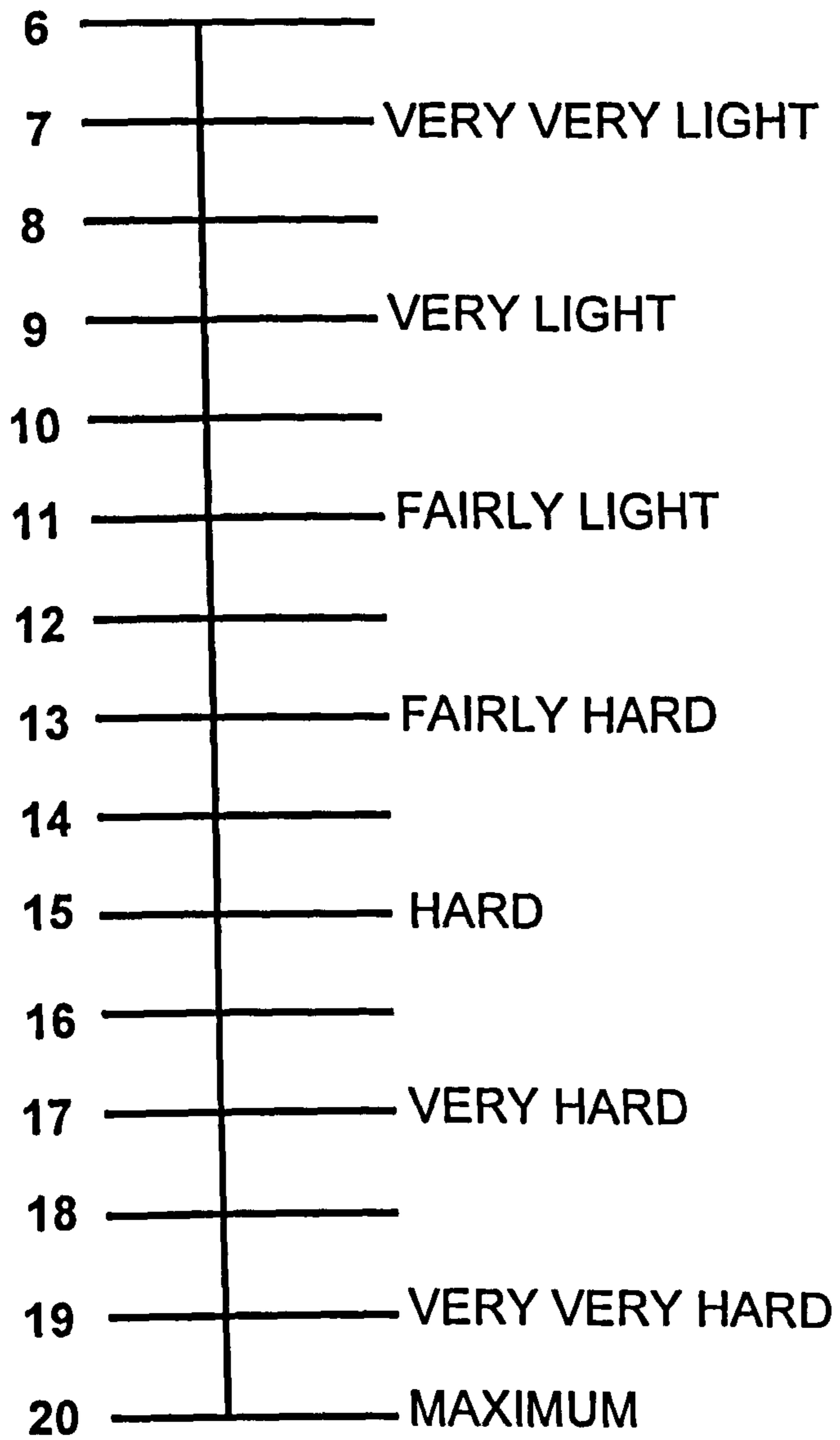


**Feeling scale**



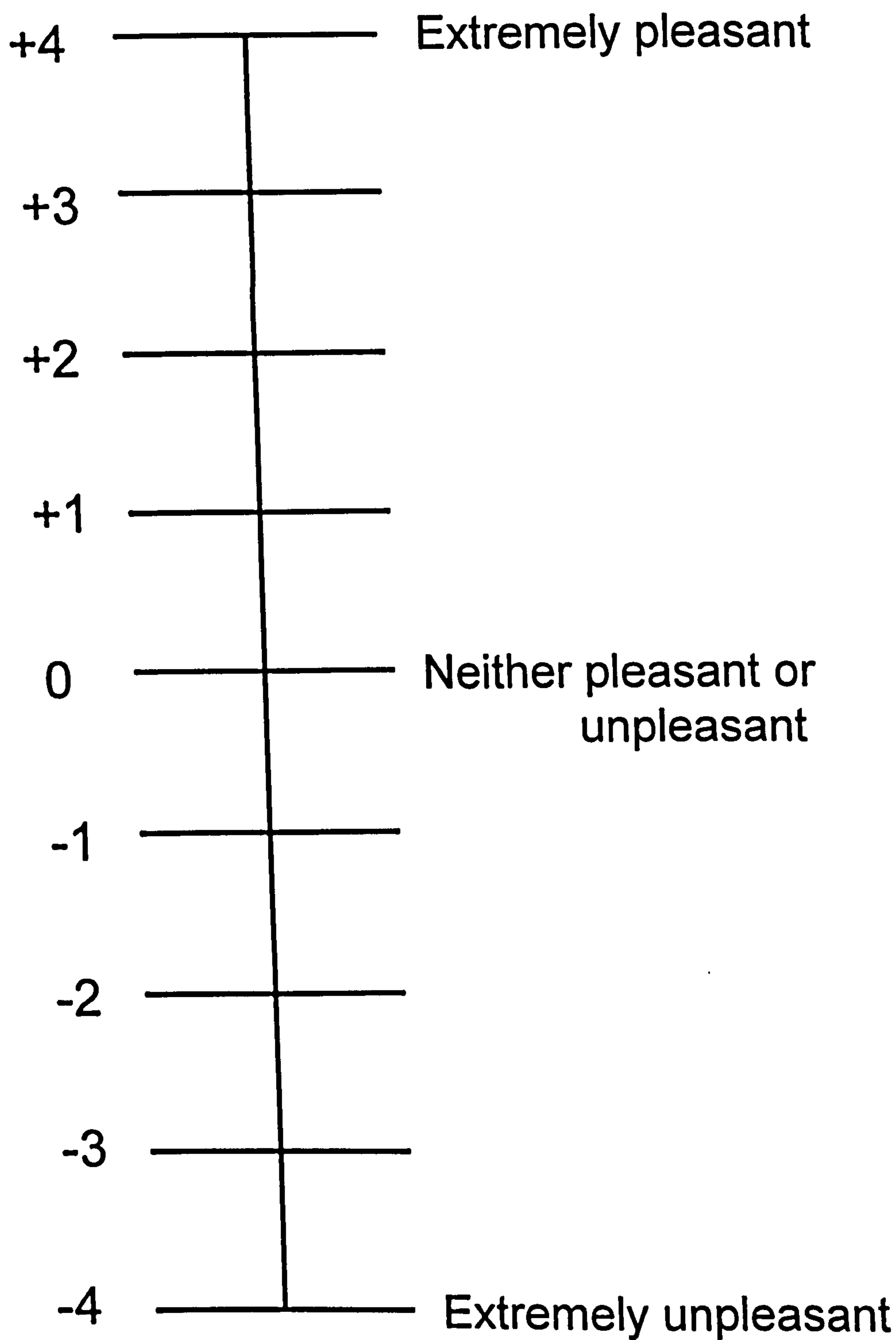
## APPENDIX F

### Rating of Perceived Exertion (RPE) Scale



**APPENDIX G**

**Pleasure - displeasure**



## APPENDIX H

Composition of pre-exercise meal provided to each participant.

Participant No.	White bread (g)	Jam (g)	Corn flakes (g)	Skimmed Milk (g)	Orange squash (ml)	Water (ml)
1	125	80	70	300	200	315
2	110	70	56	280	147	315
3	120	80	65	300	150	315
4	130	80	65	350	200	315
5	110	74	56	280	147	315
6	110	74	56	280	147	315
7	110	74	56	280	147	315
8	130	80	65	350	200	315
9	130	80	65	350	200	315
10	130	80	65	350	200	315
<b>Mean</b>	121	77.2	62	312	174	315
<b>SD</b>	10	4	5	34	28	0

Mean nutritional composition of the pre-exercise meal (g).

	White bread (g)	Jam (g)	Corn flakes (g)	Skimmed Milk (g)	Orange squash (ml)	Water (ml)
CHO	56	53	56	14	15	nil
Protein	10	0.5	5	11	1	nil
Fat	2	nil	0.5	0.6	0.2	nil
Energy (kcal)	265	201	233	100	63	nil

Total macronutrient content of pre-exercise meal

	g
CHO	194
Protein	28
Fat	3
Energy (kcal)	862

## APPENDIX I

### Estimation of carbohydrate and fat oxidation using indirect calorimetry

The proportion 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 is negligible to energy metabolism. The following method for calculating CHO and fat oxidation by indirect calorimetry is adapted from Peronnet & Massicotte, 1991).

The oxidation of 1.0 g of CHO uses 0.828 l of oxygen (O<sub>2</sub>) and produces 0.828 l of carbon dioxide (CO<sub>2</sub>).

The oxidation of fat uses 1.989 l of oxygen (O<sub>2</sub>) and produces 1.419 l of carbon dioxide (CO<sub>2</sub>).

The whole body oxygen consumption ( $\dot{V}O_2$ ) and carbon dioxide production ( $\dot{V}CO_2$ ) is calculated from expired air gases.

$$\text{CHO oxidation (g}\cdot\text{min}^{-1}) = 4.585 * \dot{V}CO_2 - 3.225 * \dot{V}O_2$$

$$\text{Fat oxidation (g}\cdot\text{min}^{-1}) = 1.695 * \dot{V}O_2 - 1.701 * \dot{V}CO_2$$

#### Reference:

(Peronnet et al., 1991)