Effects of Carbohydrate Intake on Metabolism During Exercise

by

Donald Peter Maurice MacLaren

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy (Liverpool John Moores University) following work undertaken at the Centre for Sport and Exercise Sciences, Liverpool John Moores University.

May 1995

ABSTRACT

The aims of this thesis were to assess the hormonal, metabolite, and metabolic responses to carbohydrate ingestion and to maintained hyperglycaemia using the glucose clamp technique in well-trained subjects. Carbohydrates were ingested before and during prolonged cycling in two forms i.e. glucose and maltodextrin. Performances were assessed as times to exhaustion. The hyperglycaemic clamp studies were employed on subjects under resting and exercise conditions. The effect of dietary status was investigated in the exercise study.

Ingestion of carbohydrate either before or during prolonged exercise produced consistent findings in terms of promoting carbohydrate oxidation ($P<0.01$). The resultant elevation of plasma glucose compared with placebo ingestion led to significant increases in plasma insulin but diminished concentrations of glucagon, the catecholamines, non-esterified fatty acids (NEFA), and B-hydroxybutyrate (B-OH). The consequences of the increased availability of carbohydrate were an increased time to exhaustion. No significant differences were observed between the forms of carbohydrate ingested for hormonal, metabolite, and metabolic measures, nor was there a significant difference in times to exhaustion.

The hyperglycaemic clamp technique proved reliable under resting and exercise conditions since plasma glucose concentrations of 12 mM were readily maintained. Under conditions of rest and exercise, maintained hyperglycaemia significantly impaired concentrations of plasma lipids, glucagon, the catecholamines, and cortisol, whereas plasma insulin was elevated. These changes resulted in increasing rates of glucose utilisation at rest, whilst during exercise maximal rates approaching 2 g min^{-1} were achieved. A small, but significant ($P<0.01$), muscle glycogen sparing was observed after exercise when hyperglycaemic.

The effects of muscle glycogen depletion resulted in a significant shift towards lipid metabolism when compared with muscle glycogen loading ($P<0.05$), which in turn inhibited lipid metabolism. The contribution of exogenous glucose to the total carbohydrate oxidation rate reached 100% when in the depleted state, but only reached 80% when glycogen loaded. The total rate of carbohydrate oxidation was found to be significantly elevated when loaded ($P<0.05$), and the plasma insulin concentrations were significantly elevated ($P<0.05$).

The effects of maintained hyperglycaemia during exercise on the plasma and red-cell amino acid concentrations caused a significant increase in alanine; a larger increase was evident in red-cell concentration than plasma concentration. Smaller or non-significant changes were found with the branched-chain amino acids.

The overall findings support the view that increases in supply of exogenous carbohydrates lead to an increase in the utilisation of carbohydrates as an energy source at the expense of lipids. This occurs due to the operation of the glucose-fatty acid cycle and to hormonal regulation. Maintaining hyperglycaemia at 12 mM does appear to compromise the glucose-alanine cycle and, when carbohydrate depleted, the glucose-fatty acid cycle.

ACKNOWLEDGEMENTS

The work presented in this thesis was performed over a period of many years, during which time numerous people have given me encouragement, advice, support, and technical help. I am deeply indebted to them all.

A special note of thanks must firstly go to all my subjects who generously volunteered to take part in the various experiments. They gave me their valuable time, their blood, and in some instances their muscle as well. Without them this work could not have taken place.

I am indebted to Prof. T. Reilly (School of Human Sciences, Liverpool JMU) as my supervisor and mentor. His support, advice, encouragement, and expertise cannot be understated. I really do owe him my most sincere thanks. Grateful thanks and appreciation is also extended to Dr. D. Billington (School of Biomolecular Sciences, Liverpool JMU) as my second supervisor.

Throughout the studies the help, advice and technical support of Dr. I. Campbell (Department of Anaesthesia, Withington Hospital, Manchester) has been incalculable. I treasured the opportunity to spend 12 months of a sabbatical period with him in 1987, when he was at the Royal Liverpool University Hospital. I am indebted to him for all the time given up to be present for blood sampling and for organising other medical help when required.

Sincere thanks are extended to Dr. C. Hopkins, Dr. K. Baldwin, and Dr.S. Foster (Royal Liverpool Hospital) who provided expert help in taking blood samples during the 'clamp' studies, and to Dr. J. Coakley (Department of Medicine, Liverpool University) who took the muscle biopsies.

Excellent biochemical technical assistance was given to me by Dr. M. Jackson (Department of Medicine, Liverpool University) for the muscle glycogen analyses, Dr. L. Hipkin, Dr N. Roberts, and Dr.J.Dutton (Department of Clinical Chemistry, Royal Liverpool Hospital) for the hormonal analyses, Ms. P. Maycock and Mrs. J. Fairhurst (M.R.C. Unit, Hope Hospital, Manchester) for the blood metabolite analyses, and Dr.D.Turner and Dr.J. Cooper (Department of Biochemistry, Coventry and Warwick Hospital) for the amino acid analyses.My sincerest thanks to you all.

The assistance, guidance and advice from Dr. K. Frayn (Sheikh Rashid Diabetes Unit, Radcliffe Infirmary, Oxford) was greatly appreciated, especially scince he 'brought' the hyperglycaemic glucose clamp technique to the U.K.

Heartfelt thanks go to all my family who were extremely supportive over the years and never wavered in their beliefs of my ability and capacity to complete the thesis.

The financial support of Liverpool JMU generally, and the Centre for Sport and Exercise in particular was appreciated.

Page

LIST OF FIGURES

Page

Page

Page

LIST OF TABLES

LIST OF PLATES

1. INTRODUCTION

1. INTRODUCTION

During exercise, the muscle cells provide a continual release of energy in the form of ATP to the contractile elements. There are several mechanisms for this production of ATP, all of which depend ultimately upon the uptake and oxidation of nutrients consumed and then stored in the body. Carbohydrates, fats and protein provide the necessary energy; in comparison to the fat and protein stores, the carbohydrates are severely limited. Approximately 400 g and 70 g of glycogen are stored in skeletal muscle and liver, respectively. Assuming a resting muscle glycogen concentration of 130 mM kg^{-1}, an active muscle mass of 22 kg while running at 70% maximal oxygen uptake (VO_2max), and a glycogenolytic rate of 1.4 mM kg^{-1} min^{-1}, it is estimated that depletion of muscle glycogen will occur after about 29 km of running (Sherman & Wimer,1991). Since carbohydrates are the preferred fuel for exercising muscle at intensities between 60 - 85% VO_2max despite the fact that muscle glycogen stores are limited, fatigue during prolonged exercise is related to muscle glycogen depletion (Ahlborg et al., 1967a; Bergstrom & Hultman, 1966; Vollestad & Blom, 1985). In this thesis, fatigue is defined as a failure to maintain the expected force or power output (Edwards,1981).

The marathon race (42.2 km) has provided a convenient focus for researchers concerned with metabolic factors in fatigue during prolonged exercise. Whilst the evidence from recent studies link fatigue during prolonged activities with muscle glycogen

depletion, early studies indicated hypoglycaemia as the factor determining fatigue during marathon running (Levine et al., 1924). A follow-up study by the same research group showed enhanced blood glucose levels and an improved performance by the marathon runners when they were supplied with carbohydrate after 24 km of the marathon (Gordon et al., 1925). Later studies yielded similar findings in dogs (Dill et al., 1932) and humans (Christensen & Hansen, 1939). Since hypoglycaemia is the result of an inability of the splanchnic glucose output to match tissue glucose uptake, the limited glycogen stores are ultimately responsible for this phenomenon.

The belief that muscle (and liver) glycogen depletion can explain fatigue during prolonged exercise at 60-85% VO_2max is further supported by the finding that dietary manipulations which increase pre-exercise muscle glycogen also improve performance. In contrast, procedures that reduce pre-exercise muscle glycogen levels impair performance (Ahlborg et al., 1967a; Bergstrom et al.,1967).

Carbohydrate ingestion during exercise has been repeatedly demonstrated to enhance endurance performance, primarily by maintaining the availability and oxidation of glucose (Coggan & Coyle, 1987;1988;1989). Evidence as to the preferred form of carbohydrate that should be ingested in order to extend performance is equivocal. Similar findings are obtained for glucose, maltose, sucrose, and maltodextrins with regard to performance and metabolic responses, but not for fructose

(Maughan, 1991).The problems with fructose ingestion are that it cannot be directly oxidised by muscle, and it has a slow rate of intestinal absorption.

Coyle et al. (1986) showed that cyclists were capable of exercising for an extra hour without any additional fall in muscle glycogen content when a glucose polymer was ingested. It appears that the prevention of hypoglycaemia and maintenance of an adequate rate of carbohydrate oxidation from blood glucose explain why the cyclists are able to continue exercising from 3 to 4 hours. The question remains why did these subjects stop exercising at 4 h when their rates of carbohydrate oxidation are high, their blood glucose concentration is normal, and their muscle glycogen levels are not depleted? In a further study, the same authors showed that even intravenous glucose infusion at a rate of 1.13 g min^{-1} after fatigue had occurred did not help subjects to sustain exercise at the chosen intensity for longer than 43 min (Coggan & Coyle, 1987).

Various methods have been employed to ascertain the contribution of carbohydrates as a fuel for muscles. Early studies relied exclusively on determining the respiratory exchange ratio (RER) of subjects whilst exercising. The RER is an indicator of the proportion of carbohydrate and fat being oxidised by the whole organism (this is different to the term RQ -respiratory quotient- which indicates the proportions at cellular or tissue level); a value of 1.00 indicates total dependance upon carbohydrates, whereas a value of 0.70 indicates a total dependance on fatty

acids. These values must be interpreted with caution due to (i) the uncertainty of the contribution of amino acid oxidation, (ii) the effect on V_{co_2} of respiratory compensation for metabolic acidosis, and (iii) the fact that the contribution of endogenous or exogenous carbohydrates cannot be distinguished. In spite of these limitations, the estimation of carbohydrate oxidation during steady state conditions is still obtained by employing the RER or the formula described by Consolazio et al. (1963).

More specific methods for determining the rate of oxidation of ingested (or infused) carbohydrates involve the use of naturally labelled [^{13}C] glucose (Massicote et al., 1986; 1989) or radiolabelled [^{14}C] carbohydrates (Hawley et al., 1992). The major advantage of the ^{13}C isotope approach is that subjects are not exposed to radioactivity. The fact that ^{13}C enrichment of carbohydrates is greater than fat results in claims that a 75% overestimation of the rate of oxidation of carbohydrates occurs (Peronnet et al., 1990). Comparisons using such data should, therefore, be considered as directional rather than absolute. Advantages of using ^{14}C allow for estimates of the rate of appearance of ^{14}C in blood as well as $^{14}CO_2$ exhaled. Some recent authors who used [^{14}C] glucose have suggested that this method may underestimate the extent to which blood glucose is oxidised during exercise because of the slow equilibrium of $^{14}CO_2$ with the bicarbonate pool (Coggan & Coyle, 1991).

The hyperglycaemic glucose clamp technique, which involves elevating blood glucose to a hyperglycaemic concentration and then 'clamping' the blood concentration at that value, has been employed to determine the rate of glucose utilization at rest (DeFronzo et al., 1979), under conditions of sepsis and trauma (White et al., 1987), and on patients suffering from colorectal cancers (Copeland et al., 1987). The technique proves useful in determining the disposal of glucose by the tissues of the body, but it is not possible by this method to provide information as to the rate of oxidation of the glucose infused.

Since carbohydrate stores within the body are limited and yet of great importance, there are mechanisms which regulate the use of muscle glycogen and blood glucose during prolonged exercise. The contribution of carbohydrates as an energy supply is reduced the longer the exercise duration, whilst the contribution of fats is reciprocally increased. This occurs in spite of a plentiful supply of carbohydrates and not as a consequence of depleted stores. The glucose-fatty acid cycle has been proposed to account for these changes in substrate utilization even when carbohydrates are ingested during exercise (Randle et al.,1963). The maintenance of blood glucose concentrations during prolonged exercise is nurtured by the splanchnic glucose output from gluconeogenesis. Significant contributions to gluconeogenesis arise from lactic acid and from the amino acid, alanine via the glucose-lactate cycle (Cori, 1931) and the glucose-alanine cycle (Felig,1975). The regulation of these substrates is controlled by circulating hormones, notably insulin, glucagon, the

catecholamines, cortisol, and growth hormone.

Recently, researchers have attempted to address the problems concerned with the ability of exercising muscle to utilize exogenous supplies of glucose at maximal rates but have invariably used ingested forms of carbohydrate. This thesis will examine both ingested and infused carbohydrates to determine the maximal rates of glucose utilisation by exercisng muscle, and to establish the hormonal changes that take place.

2. AIMS AND OBJECTIVES

2. AIMS AND OBJECTIVES

The aims of this thesis are to :

i) assess the metabolic and biochemical responses to prolonged exercise under conditions of carbohydrate ingestion and hyperglycaemia in trained subjects;

ii) develop the use of the hyperglycaemic glucose clamp technique in calculating the rate of glucose utilization during exercise after varying dietary regimens.

Accomplishment of these aims will enhance an understanding of the regulation of carbohydrate availability during prolonged exercise and in determining maximum rates of glucose utilization under such conditions.

These aims will be accomplished by means of the following objectives:-

1. to determine the effect of carbohydrate ingestion immediately prior to and/or during exercise on the hormonal, metabolite, and metabolic responses;

2. to appraise the effect of chain length of carbohydrate ingested prior to and/or during exercise on the hormonal, metabolite, and metabolic responses;

3. to investigate the hormonal, metabolite, and metabolic responses to maintained hyperglycaemia under conditions of rest, and exercise after varying dietary regimens;

4. to calculate the maximal rate of glucose utilization by exercising muscle using the hyperglycaemic glucose clamp technique;

5. to re-appraise the glucose-fatty acid cycle and the glucose-alanine cycle in the light of maintained hyperglycaemia;

6. to develop a model of the metabolic changes which take place under normal and hyperglycaemic conditions.

Achievement of these objectives requires the conduct of a series of experimental investigations.The results of these separate studies are synthesised to provide a better understanding of hormonal integration and metabolic regulation during exercise under normal and carbohydrate intake conditions. Since the hyperglycaemic clamp technique will be employed during exercise with the muscle carbohydrate stores varying in their pre-exercise glycogen concentration, it will be possible to affirm the maximal rates of carbohydrate utilization by exercising muscle. In doing so, the work will produce findings of relevance to enhancing the performance of endurance athletes.

3. REVIEW OF LITERATURE

3.1 INTRODUCTION

This chapter provides a background of research findings and concepts concerning aspects of carbohydrate metabolism in preparation for the topics considered in subsequent chapters. It also provides the basis for discussion of results reported in those chapters. It is necessary to be familiar with the methods employed in research on carbohydrate metabolism before reviewing the evidence for the importance of carbohydrates as an energy source during exercise. Attention will be drawn to the relevance of muscle and liver glycogen stores, and to the value (or otherwise) of carbohydrate ingestion and infusion on exercise performance. The contribution of endogenous and exogenous carbohydrates as energy sources can be realised on examination of their rates of oxidation. Rates of oxidation are examined in relation to the form of carbohydrate ingested.

The processes involved in carbohydrate metabolism are under neural and hormonal control. The interrelationships between insulin, glucagon, the catecholamines, and to a limited extent, cortisol and growth hormone in integrating fuel sources during exercise will be evaluated. Blood glucose is of significant importance in maintaining exercise at moderate-to-high intensities. An important feature is the transport of glucose across the plasma membrane in skeletal muscle and how the process may be activated during exercise. The role of insulin and muscle contraction in enhancing glucose uptake by skeletal muscle is appraised. Although the thesis is essentially concerned with

carbohydrate metabolism, the crucial involvement of lipids and, to some extent, amino acids cannot be neglected. The interplay between these energy sources needs to be understood. Finally, the the hyperglcaemic clamp technique, a method devised to quantify beta-cell sensitivity to glucose, is explained before some research findings concerning its application at rest and during exercise are reported.

3.2 CARBOHYDRATE METABOLISM: METHODS

At any given time, the plasma glucose concentration is a consequence of the sum of two opposite processes i.e. that of glucose delivery and that of tissue glucose utilisation. Hepatic glycogenolysis and gluconeogenesis are responsible for endogenous glucose delivery in the post-absorptive state, whilst glucose utilisation occurs in both insulin-sensitive and non-insulin-sensitive tissues and is partitioned into two major components - glucose oxidation and non-oxidative glucose disposal (i.e. the amount of glucose metabolised without being converted into carbon dioxide and water) (Gerich,1993).

Glycogen synthesis, *de novo* lipogenesis, synthesis of non-essential amino acids, and anaerobic glycolysis are pathways for non-oxidative glucose disposal, although in normal humans *de novo* lipogenesis and amino acid synthesis are considered to be quantitatively small (Hellerstein et al., 1991). Anaerobic glycolysis results in the formation of lactate/pyruvate, where a significant amount of pyruvate is not subsequently oxidised in the Krebs cycle and is released as lactate or alanine into the systemic circulation to be reconverted into glucose in the liver (Felig, 1973; Brooks, 1986). This cycling between glucose and three carbon compounds has been estimated to represent as much as 30% of total glucose metabolism in the post-absorptive state (Tappy et al., 1994).

Experimental determination of the various components of glucose metabolism has been the focus of many studies. Glucose delivery has been generally determined by arteriovenous differences and by glucose isotope dilution analysis during primed-continuous infusion of glucose labelled with radioactive or stable isotopes of carbon or hydrogen (Wolfe, 1992). Glucose oxidation has been estimated from respiratory gas exchange monitoring using indirect calorimetry (Frayn, 1983; Jequier & Felber, 1987) or from production of labelled CO_2 in breath during administration of carbon-labelled glucose (Robert et al., 1987; Wolfe, 1992). Indirect calorimetry, arteriovenous glucose differences, and isotope techniques differ markedly and provide qualitatively different information. These differences will be outlined in this section.

3.2.1 Arteriovenous Difference

Glucose metabolism during exercise has been studied traditionally by catheterisation of the arterial blood supply and venous drainage of a given tissue bed, thereby allowing measurement of the arteriovenous (a-v) glucose difference and the rate of blood flow. While the product of these does not indicate the ultimate fate or source of the glucose taken up or released, it does indicate the maximum possible contribution of glucose to the metabolic processes in the tissue bed under investigation. The a-v difference method provides the ability to examine glucose metabolism in specific tissue beds during exercise. This technique has been used to compare glucose uptake by active vs

non-active limbs (Ahlborg et al., 1975), and to help quantify the contribution of renal and hepatic tissue to overall glucose release (Wahren et al., 1971). The measurement of a-v differences has several disadvantages, the fact that it is invasive being the major one. Furthermore, simple measures of a-v difference do not provide an indication of the metabolic fate or source of the glucose that is exchanged during exercise. In addition, the a-v glucose differences across an exercising muscle are extremely small, being in the order of 0.1 to 0.4 mM (Ahlborg & Felig, 1982; Wahren et al., 1971), and therefore difficult to measure accurately. Variability in the determination of blood flow also adds to the variability of the calculated a-v glucose difference.

3.2.2 Indirect Calorimetry

Estimation of substrate oxidation rate from respiratory gas exchanges has been in use since the beginning of this century, and is based on the premise that the three major classes of substrate (carbohydrate, lipid, and protein), when oxidised, consume oxygen and release carbon dioxide in a ratio specific for each substrate. This respiratory exchange ratio (RER) is 1.00 for carbohydrates, 0.70 for lipids, and 0.84 for proteins (Livesey & Elia, 1988). Knowledge of the stoichiometry of substrate reaction with oxygen to produce carbon dioxide and water allows calculation of the substrate oxidation rate from respiratory oxygen and carbon dioxide exchanges (Jequier & Felber, 1987). This procedure provides estimates of net, not actual, substrate

oxidation. Net carbohydrate oxidation represents the sum of:-

a) oxidation to carbon dioxide and water of glucose from endogenous glycogen stores or exogenous carbohydrates,

b) glucose conversion to lipids in the process of *de novo* lipogenesis, and

c) glucose conversion to non-essential amino acids.

The importance of the latter two processes is considered to be quantitatively small. The same considerations hold true for estimates of net lipid oxidation. Hydrolysis of triglyceride yields three molecules of non-esterified fatty acids (NEFA) and one of glycerol. Glycerol cannot be reconverted to glycerol-3 phosphate in adipose tissue, but is converted to glucose in the liver via gluconeogenesis (Jahoor et al., 1990). Thus, oxidation of 1 mM tripalmitoyl-glycerol will correspond to the formation of 0.5 mM of glucose, and the concomitant oxidation of 0.5 mM glucose is included in the net oxidation of 1 mM Tripalmitoyl-glycerol calculated from indirect calorimetry. Theoretical validation of these calculations of net substrate oxidation rates has been performed (Frayn, 1983; Ferrannini, 1988).

Assuming complete conversion of glycerol released during lipolysis into glucose, net lipid oxidation includes actual oxidation of 0.5 mM glucose for each mole of triglyceride. Net carbohydrate oxidation therefore underestimates actual glucose oxidation due to this ongoing gluconeogenesis. Net substrate oxidation does nonetheless accurately reflect substrate balances in the basal state and in the post-prandial state, and the

standard calculations should not be changed. This rough estimation of gluconeogenesis accompanying lipid oxidation may be useful when comparing indirect calorimetry data with estimates from isotope techniques, although this estimate does not take into account Cori cycle activity, which may represent a significant portion of gluconeogenesis.

When steady-atate conditions are attained during exercise, gas exchange measurements can be used to estimate the total carbohydrate oxidised at any time, either by using the formula described by Consolazio et al. (1963) or by the table devised by Zuntz (1900).

3.2.3 Isotopic determinations: glucose delivery

Experimental calculations of systemic glucose delivery can be determined from analysis of the dilution in plasma glucose of an infused glucose tracer. These techniques have been in use for over 30 years (Steele, 1959). The rationale is that the ratio of labelled glucose to unlabelled glucose in arterialised plasma during constant intravenous infusion of a stable (^{13}C,) or radioisotope (^{14}C, ^{3}H) of glucose allows determination of the rate of systemic glucose delivery in steady state conditions:

$$\text{Glucose delivery} = \frac{\text{rate of tracer infused}}{\text{ratio of labelled to unlabelled glucose}}$$

During non-steady state conditions, corrections are used for

changes in the glucose pool size and in the tracer present in the glucose pool (Steele, 1959). The equations of Steele for steady-state or non-steady-state have been used widely, even though the one-compartment model which underlies these calculations has been recognised as being imperfect (Wolfe, 1992). In the post-absorptive state, glucose delivery corresponds essentially to hepatic glycogenolysis and gluconeogenesis of amino acids, glycerol, and lactate. In contrast to gluconeogenesis of amino acids or glycerol, which arise from catabolism of proteins and triglycerides, the whole-body lactate production originates largely from glucose and glycogen converted to lactate by anaerobic glycolysis in tissues including kidney, adipocytes, and skeletal muscle. Plasma glucose can also be extracted by liver cells, and undergoes the first steps of glycolysis prior to being reconverted into glucose. These so-called futile cycles, in which glucose is converted into glucose 6-phosphate and back to glucose, into fructose 1,6-bisphosphate and back to glucose, or into trioses and back to glucose, play an important role in the control of gluconeogenesis and glycogenolysis (Pilkis & Claus, 1991). These glucose cycles do not involve oxidation of glucose into carbon dioxide and water, and therefore represent pathways of non-oxidative glucose metabolism. However, ATP is used in these cycles and substrates must be oxidised to regenerate the ATP molecules. These glucose cycles will be measured as part of glucose delivery when labelled glucose loses its label on undergoing the cycle.

Systemic glucose delivery using isotope dilution analysis during infusion of various glucose tracers represent different pathways. Carbon-labelled tracers are reincorporated into glucose through all glucose cycles and therefore do not allow measurement of these cycles; they provide an estimate of glycogenolysis and of gluconeogenesis from amino acids and glycerol. Glucose labelled with hydrogen isotopes in position 6 (6-^{3}H) loses its label in the PEPCK cycle (pyruvate→oxaloacetate→phosphoenol pyruvate), and so includes the Cori and glucose-alanine cycles in the estimation. Glucose labelled with hydrogen in position 3 (3-^{3}H) loses its label at the reaction catalysed by the enzyme phosphofructoisomerase (fructose 1,6-bisphosphate→glucose), and so includes the cycling between these substrates. Glucose labelled with a hydrogen isotope in position 2 (2-^{3}H) loses its label at the hexokinase level, and so includes cycling between glucose and glucose-6-phosphate.

Glucose labelled with 2-^{3}H loses its tracer when it is transported to liver cells and enters the glucose-glucose-6-phosphate cycle; the newly formed glucose re-enters the systemic circulation as unlabelled glucose. This represents the sum of glucose arising from glycogenolysis, gluconeogenesis of amino acids, and all glucose cycles. Infusion with 3-^{3}H represents all the above (i.e. glycogenolysis, gluconeogenesis of amino acids and glycerol, and glucose cycles) with the exception of the glucose-glucose-6-phosphate cycle, whereas infusion with 6-^{3}H represents the above but excludes both the glucose-glucose-6-phosphate and fructose 1,6-bisphosphate-glucose

cycles. At the other extreme, glucose labelled with a carbon isotope retains its label through all glucose cycles, and so glucose delivery obtained using ^{14}C theoretically detects gluconeogenesis of amino acids and glycerol and glycogenolysis, but not from glucose cycles. This is not entirely true since significant amounts of the carbon label are lost through exchanges with Krebs cycle metabolites and with the CO_2 pool during gluconeogenesis from pyruvate (Hetenyi, 1982).

3.2.4 Isotopic determinations: glucose oxidation

Glucose oxidation can be measured in steady-state conditions during primed continuous infusion of carbon-labelled glucose by monitoring total carbon dioxide production, labelled to unlabelled CO_2 ratio, and labelled to unlabelled glucose ratio in arterialised plasma. The rationale is that the ratio of breath CO_2 to plasma glucose isotopic enrichment indicates the fraction of CO_2 produced by oxidation of glucose arising from the plasma pool (Robert et al., 1987):

$$\text{Glucose oxidation} = \frac{*CO_2/CO_2}{\text{plasma}*\text{glucose/glucose}} . VCO_2 . \frac{1}{R} . \frac{1}{0.134}$$

where $*CO_2$ is labelled CO_2, R is recovery of $*CO_2$ in breath, and 0.134 is the number of litres of CO_2 produced during oxidation of 1 mM of glucose. Thereby, this technique allows the determination of the oxidation rate of glucose which has passed through the systemic circulation, and so detects the oxidation of glucose

originating from hepatic glycogenolysis and gluconeogenesis. Indirect calorimetry does not distinguish between glycogenolysis and gluconeogenesis. It must be concluded, however, that hydrolysis of muscle glycogen and subsequent glucose oxidation cannot be detected by these methods.

Labelled CO_2 produced from oxidation of carbon-labelled glucose is known to be incompletely recovered in breath, even over prolonged periods of time. This is because labelled CO_2 is sequestered in slowly exchangeable sub-pools of CO_2 in the body (Wolfe, 1992). Due to the failure to account for the oxidation of muscle glycogen, the incomplete recovery of labelled CO_2 in breath, and the sequestration of labelled CO_2 in intermediary metabolites, the isotopic determination of glucose oxidation is likely to be underestimated. A recovery factor may be used to correct for latter, although Moodley et al. (1992) have suggested that as long as the breath samples are taken after 10 min, the lag in CO_2 expiration is negligible as is the underestimation resulting from slow equilibrium of the $^{14}CO_2$ with the bicarbonate pool.

3.2.5 Isotopic determinations: stable or radioactive labels

Stable isotopes involve naturally enriching the carbon atom using ^{13}C formed from C_4 plants such as sugar cane, corn or sorghum. Compared with ^{14}C isotope studies, the ^{13}C studies have the advantage that subjects are not exposed to radioactivity and so can be tested frequently. There are, however, several

disadvantages, which include the fact that the relatively low $^{13}C/^{12}C$ enrichment necessitates corrections for the ^{13}C that is present in all energy-yielding substrates (Schoeller et al., 1980). It is also worthy of note that the ^{13}C enrichment of carbohydrates is greater than that of lipids, and consequently any shift in the pattern of fuel utilisation will affect $^{13}CO_2$ production, and so complicate measures of carbohydrate oxidation (Barstow et al., 1989). Finally, the uncertainties concerning the degree to which carbohydrate utilisation shifts from endogenous to ingested carbohydrate during exercise also limits the accuracy of the correction of $^{13}CO_2$ measurements for ^{13}C background values (Peronnet et al., 1990). Investigations using naturally labelled ^{13}C glucose to determine the rate of oxidation of ingested carbohydrate during prolonged exercise have been undertaken (Massicotte et al., 1989,1990; Pallikarikas et al, 1986; Peronnet et al., 1990), with a suggestion that carbohydrate enriched with ^{13}C tends to overestimate the rate of oxidation by up to 75% (Perronet et al., 1990). It has been suggested that comparisons using such data should be regarded as directional rather than absolute (Hawley et al., 1992).

An alternative method for measuring the rate of ingested carbohydrate oxidation during exercise is to label the ingested carbohydrate with ^{14}C and use scintillation counting to monitor the appearance of ^{14}C in the expired CO_2. The advantage of using the ^{14}C isotope rather than the ^{13}C isotope is that there is virtually no naturally occurring background level of ^{14}C that must be accounted for when calculating exogenous carbohydrate

oxidation rate (Wolfe, 1992). The obvious disadvantage is that using ^{14}C exposes the subject to radioactivity. The amounts of radioactivity, however, are small; usually < 40 μCi l^{-1} is consumed (Hawley et al., 1992), which corresponds to a dose of approximately 0.02 rem (permissible radiation dose is 5 rem per annum).

3.2.6 Comparisons between the methods

Estimates of basal glucose oxidation in normal humans obtained from the production of labelled CO_2 during tracer infusion of carbon-labelled glucose have been reported to be lower than net carbohydrate oxidation using indirect calorimetry (McMahon et al., 1991). No such studies have been performed during exercise, nor have there been direct comparisons with a-v difference studies.

The apparent underestimations may be explained by oxidation of intramuscular glycogen stores and the sequestration of labelled CO_2 produced. During infusion of glucose or during hyperglycaemic clamps, glucose oxidations obtained with each technique become closer to each other due to suppression of muscle glycogenolysis and gluconeogenesis due to hyperinsulinaemia (McMahon et al., 1991). Glucose oxidation determined isotopically remains somewhat lower than net carbohydrate oxidation from indirect calorimetry (Tappy et al., 1995).

Isotope techniques, a-v difference studies, and indirect calorimetry differ in essence. Isotope techniques monitor fluxes of molecules irrespective of their origin. Glucose oxidation estimated from the production of labelled CO_2 of carbon-labelled glucose represents oxidation of plasma glucose originating from hepatic glycogen or hepatic gluconeogenesis, and underestimates values due to sequestration of labelled CO_2 in intermediary metabolite pools. Indirect calorimetry, on the other hand, provides net substrate oxidation rates, and reflects oxidation of glucose originating from endogenous glycogen or of exogenously administered glucose. Oxidation of glucose from gluconeogenesis is not reflected by net carbohydrate oxidation. Arterio-venous glucose differences purely reflect the uptake/release of glucose across a tissue bed without inference to the metabolic processes involved.

3.3 IMPORTANCE OF CARBOHYDRATES

During the past eighty years, it has been repeatedly demonstrated that exercise at an intensity above 50% VO_2 max cannot be maintained when carbohydrate stores in the body become depleted (Bergstrom et al., 1967; Christensen & Hansen, 1939; Coyle et al., 1983). Fatigue usually occurs when glycogen levels in the exercising muscle reach a critically low concentration (Bergstrom et al., 1967; Vollestad et al., 1984), or when hypoglycaemia is evident in some sensitive subjects (Coyle et al., 1983).

Since fatigue during exercise often results from carbohydrate depletion, there have been various studies concerned with the extent to which carbohydrate feedings may delay fatigue. This section will deal with some of the findings, particularly in relation to the effects on muscle and liver glycogenolysis, hepatic gluconeogenesis, glucose metabolism, and performance. The effects of exercise, with and without carbohydrate ingestion or infusion, on carbohydrate oxidation and the hormonal responses will be the subject of other sections in this review.

3.3.1 Carbohydrates and muscle glycogenolysis

The muscle glycogen content of an endurance trained athlete consuming a diet containing 50% energy from carbohydrate is approximately 130 mM kg^{-1} ww (Costill et al., 1981; Sherman et al., 1981). Bergstrom et al.(1972) were the first to show that muscle glycogen could be elevated significantly above normal

resting levels (approximately 204 mM kg^{-1} ww) by using two exhaustive bouts of exercise separated by 3 days on a low carbohydrate diet followed by 3 days on a high carbohydrate diet and rest. Earlier, studies from the same research group had clearly shown the relationship between diet, muscle glycogen concentrations, and time to exhaustion at 75% VO_2 max (Ahlborg et al., 1967; Bergstrom et al., 1967; Hermansen et al., 1967). More recently, it has been shown that the rate of muscle glycogen utilisation is most rapid during the early stages of exercise (Vollestad et al., 1984), and that it is exponentially related to exercise intensity (Vollestad and Blom, 1985). Furthermore, fatigue during such exercise occurs concomitantly with depletion of glycogen in specific fibres recruited during exercise (Vollestad et al., 1984). In addition, the pattern of muscle glycogen depletion is influenced by the mode of exercise; cycling results in almost total depletion of glycogen in the *vastus lateralis*, whereas running depletes the stores in the *gastrocnemius* and *soleus* muscles (although it has been difficult to show the same level of muscle glycogen depletion after fatigue due to running). The close relationship between muscle glycogen depletion and fatigue appears to be due to the inability of glycogen depleted muscle cells to maintain a sufficient rate of ATP resynthesis.

An important determinant of the rate of muscle glycogenolysis during exercise is training status. A major adaptation to endurance training is a reduction in utilisation of glycogen and production of lactate in contracting muscle (Hurley et al., 1986;

Jansson & Kaijser, 1987). This is related to the enhanced muscle oxidation capacity that results from such training (Gollnick & Saltin, 1982), and possibly to the lower levels of circulating catecholamines which have been shown to be related to muscle glycogen breakdown (Jannson et al., 1986).

Alterations in the pre-exercise diet can influence the rate of muscle glycogenolysis. A high fat intake reduces muscle glycogenolysis during exercise (Jannson & Kaijser, 1982), this is supported by studies displaying elevated plasma NEFA concentrations inhibiting muscle glycogenolysis (Costill et al., 1977; Rennie et al., 1976; Vukovich et al., 1993), probably by citrate inhibition of phosphofructokinase (PFK) activity. Recently, however, Hargreaves et al. (1991) observed little effect of elevated plasma NEFA on muscle glycogenolysis during knee-extension exercise. In contrast to enhanced availability of fats, a high carbohydrate diet resulting in elevated muscle glycogen levels has been shown to increase muscle glycogenolysis during exercise (Sherman et al., 1981).

If muscle carbohydrate status affects the rate of muscle glycogenolysis, would the ingestion or infusion of carbohydrate during exercise affect the rate of muscle glycogenolysis? This possibility was first raised when a series of experiments reported in the 1960's demonstrated that glucose infusion at rates of up to 3.5 g min^{-1} decreased net muscle glycogen breakdown during intermittent exercise by approximately 20% (Bergstrom & Hultman, 1967).

Similar results of lower muscle glycogen use as a consequence of glucose infusion (Bagby et al., 1978) and glucose feeding (Kuipers et al., 1986) were found in exercising rats, and led to the hypothesis that carbohydrate ingestion during exercise improves performance by slowing the rate of muscle glycogen degradation (Coyle & Coggan, 1984).

Studies which have used carbohydrate ingestion to elevate blood glucose during exercise in humans have yielded conflicting results with respect to muscle glycogen breakdown. Some studies have supported the original findings (Bjorkman et al., 1984; Brouns et al., 1989; Erickson et al., 1987; Hargreaves et al., 1984; Simard et al., 1988), whereas others have provided contrary results (Coyle et al., 1986; Fielding et al., 1985; Flynn et al., 1987; Hargreaves & Briggs, 1988; Mitchell et al., 1989; Noakes et al., 1988).

The first authors to examine the effects of carbohydrate ingestion on muscle glycogen use during exercise directly, reported that feeding subjects 43 g of sucrose every hour during 4 hours of intermittent cycling decreased glycogen concentration in the *vastus lateralis* by 20% less than during the placebo trial (Hargreaves et al., 1984). However, re-analysis of the data excluding four subjects who had significantly higher pre-exercise muscle glycogen contents for the placebo trial resulted in findings that support the view that there is no significant difference in muscle glycogenolytic rate (Coggan & Coyle, 1991). The apparent sparing of glycogen in the study appeared to be an

artifact of higher pre-exercise glycogen concentration in the placebo trial. It is of interest that the authors of the original study undertook a similar investigation later on, and found there was no significant difference in muscle glycogen breakdown with carbohydrate ingestion compared with placebo (Hargreaves & Briggs, 1988).

The results of a study by Bjorkman et al. (1984), showing greater muscle glycogen hydrolysis under glucose ingestion compared with placebo, can be explained by the fact that subjects exercised for longer under the glucose treatment; the actual rate of decrease in muscle glycogen between the trials was comparable. Similar conclusions were reached in a study on distance skated during a hockey match (Simard et al., 1988).

Definitive evidence of glycogen sparing necessitates demonstrating that when pre-exercise levels of muscle glycogen and the duration and intensity of exercise are the same, post-exercise muscle glycogen concentration should be significantly higher when fed carbohydrates. To date, at least six studies have been performed in which muscle glycogen concentrations have been measured before and after exercise of the same intensity and duration, performed with and without carbohydrate ingestion (Table 3.1). In five of these studies carbohydrate ingestion had no effect on the decrease in muscle glycogen during exercise. The contrary findings in one study (Erickson et al., 1987) may be due to the higher pre-exercise glycogen before the placebo trial (152.0 $\pm$ 19.5 mM kg^{-1} ww) as opposed to the glucose trial (138.2

± 13.6 mM kg^{-1} ww).

Three studies have shown that the type of carbohydrate ingested does not influence the rate of muscle glycogen utilisation (Erickson et al., 1987; Flynn et al., 1987; Noakes et al., 1988). Thus ingestion of glucose, fructose, or maltodextrins results in similar breakdown of glycogen.

Table 3.1 Changes in muscle glycogen level after exercise with and without carbohydrate ingestion.

Authors	**Exercise**		**Δ Muscle Glycogen (mM kg^{-1} ww)**	
	Duration	**Intensity**	**CHO**	**Placebo**
Coyle et al.(1986)	**105 min**	**70% VO_2 max**	**77.4**	**74.4**
Fielding et al.(1985)	**240 min**	**Intermittent (50% + 100% VO_2 max)**	**82.9**	**80.9**
Flynn et al.(1987)	**90 min**	**Total work(0.9 x10^{-1} Nm)**	**95.5**	**91.6**
Hargreaves & Briggs(1988)	**120 min**	**70% VO_2 max**	**62.8**	**56.9**
Mitchell et al.(1989)	**120 min** –	**70% VO_2 max for 105' + all-out for 15'**	**75.5**	**86.7**
Erickson et al.(1987)	**90 min**	**65-70% VO_2 max**	**61.8**	**91.4**

Although it appears that carbohydrate ingestion during exercise does not reduce the utilisation of muscle glycogen, ingesting carbohydrate in the hour prior to strenuous exercise (thereby elevating blood glucose) has been shown to increase muscle glycogen breakdown (Costill et al., 1977; Hargreaves et al., 1985). It is believed that the hyperinsulinaemia associated with glucose ingestion, together with the onset of muscle contraction, results in hypoglycaemia and an increased reliance on muscle glycogen. In addition, the anti-lipolytic effect of insulin results in lower NEFA levels during exercise which in turn increases glycogen use. The increase in glycogen utilisation is absent if blood glucose remains within the normal range (Hargreaves et al., 1987), or if the plasma insulin is low after fructose ingestion (Hargreaves et al., 1985), or if the exercise is of moderate intensity (Koivisto et al., 1985).

Some evidence from studies on infusion of glucose during exercise suggest that infusion may decrease the rate of muscle glycogenolysis (Bagby et al., 1978; Bergstrom & Hultman, 1967; Winder et al., 1988). Recently, Coyle et al. (1991) used the hyperglycaemic clamp technique to maintain a constant 10 mM blood glucose level in male subjects exercising at 70% VO_2 max, and found that muscle glycogen utilisation was similar to control trials. Hence, this provides further evidence that carbohydrate 'feeding' does not influence muscle glycogen breakdown during prolonged strenuous exercise. It may therefore be that improvements in endurance exercise performance are associated with maintenance of blood glucose levels and carbohydrate

oxidation rather than sparing muscle glycogen breakdown (Coggan & Coyle, 1987).

3.3.2 Carbohydrates, liver glycogenolysis and gluconeogenesis

During exercise, glucose uptake by contracting muscle can increase 20-30 fold depending on the exercise intensity and its duration (Katz et al., 1986; Wahren, 1977). It is generally believed that hepatic glucose output is closely matched to muscle glucose uptake with the resultant blood glucose concentration unchanged (Jenkins et al., 1985: Wahren, 1977). In contrast, recent studies have shown marked hyperglycaemia at the start of exercise (Hargreaves & Briggs, 1988; Hargreaves & Proietto, 1990; Kjaer et al., 1986), with the response being exaggerated in trained men. These findings suggest that hepatic glucose output is not always closely matched to peripheral glucose uptake, and may be subject to feed-forward regulation. According to this view, the increase in hepatic glucose production at the onset of exercise is a primary event related to activity in the motor neurons of the cerebral cortex. Thus, at the start of exercise, receptors in the working muscles and from the motor neurons in the brain elicit exercise-intensity dependent signals to areas in the central nervous system that influence glucose production (Galbo, 1983). Such regulation has been proposed in the control of the cardiovascular responses to exercise (Mitchell, 1990). Further evidence of feed-forward regulation of substrate mobilisation has been provided in subjects in whom the voluntary effort of exercise has been enhanced by partial neuromuscular

blockade. In these subjects, exercise results in a larger increase in hepatic glucose output than peripheral glucose uptake, and an exaggerated catecholamine response (Kjaer et al., 1987). It would appear that central neural drive increases autonomic neuroendocrine activity, resulting in substrate mobilisation (Kjaer et al., 1987). The increased sympathetic neural activity and/or adrenal medullary adrenaline secretion would stimulate liver glycogenolysis, resulting in enhanced hepatic glucose output. The regulation of hepatic glucose output during exercise involves a complex interaction between a number of neurohumoral mechanisms (Wasserman & Cherrington, 1991), and is the subject of discussion in another section (see section 3.5).

Whereas carbohydrate ingestion during exercise did not significantly affect muscle glycogen breakdown, hepatic carbohydrate metabolism appears to be affected by carbohydrate supplementation during exercise. Liver glycogenolysis has been shown to be reduced when exercising rats were infused with glucose during mild exercise (Bagby et al., 1978). The uptake of gluconeogenic precursors by the splanchnic bed has also been shown to be reduced significantly when male subjects ingested carbohydrate during low-intensity exercise (Ahlborg & Felig, 1976). These effects are mediated by the accompanying hormonal responses such as increased plasma insulin and depressed plasma glucagon and catecholamines. During moderate-intensity exercise, it appears that ingested carbohydrate can partially replace the liver as the source of glucose in circulation. For example, it

has been demonstrated that glucose ingested during exercise at 50-70% VO_2 max supplies up to 65% of the circulating glucose pool (Costill et al., 1973; Van Handel et al., 1980). The observations that relatively small increases in blood glucose concentration occur following carbohydrate ingestion during moderate-intensity exercise suggest that hepatic glycogenolysis or gluconeogenesis must be reduced. Similarly, glucose infusion during moderate-intensity exercise has been demonstrated to suppress glucose production partially in both rats (Winder et al., 1988) and humans (Jenkins et al., 1985). When hepatic glycogen stores become depleted during exercise, ingested carbohydrate represents almost the sole source of blood glucose supply because the rate of gluconeogenesis in exercising humans seems to be maximal at 0.2-0.4 g min^{-1} (Ahlborg et al., 1974; Ahlborg & Felig, 1982), and is suppressed by carbohydrate ingestion (Ahlborg & Felig, 1976).

A recent study of the effect of glucose ingestion on exercise at 69% peak pulmonary oxygen uptake led to the conclusion that carbohydrate ingestion during exercise suppresses hepatic glucose production to near basal levels (McConell et al., 1994), and that over the 2 h of exercise there was a 51% reduction in total hepatic glucose production. The authors of this investigation used labelled infused glucose which could not distinguish between liver glycogenolysis and gluconeogenesis; it is likely that both processes were reduced by carbohydrate ingestion. Certainly these observations are consistent with earlier studies which showed that hepatic glucose output is reduced when glucose is infused

during exercise (Felig & Wahren, 1979; Jenkins et al., 1985).

3.3.3 Muscle glucose uptake

Although muscle glycogen is the predominant carbohydrate energy source during the early stages of exercise, blood glucose becomes more important as exercise is continued (Wahren et al., 1971). During leg exercise, muscle glucose uptake can increase from a basal level of 0.1 mM min^{-1} to 3-4 mM min^{-1} depending on the exercise duration and intensity (Katz et al., 1986; Wahren et al., 1971). This increase in glucose uptake arises from an increase in muscle cell membrane permeability to glucose (Ploug et al., 1984; 1987), and to activation of the glycolytic pathway. Matching the augmented glucose uptake is an increase in hepatic glucose output so that blood glucose levels usually remain in the normal range (Jenkins et al., 1985). Initially this is due to hepatic glycogenolysis, but as exercise continues an increasing proportion arises from gluconeogenesis (Wahren et al., 1971).

Early research indicated that glucose uptake in muscle could be augmented only by the presence of small quantities of insulin (Berger et al., 1976; Vranic et al., 1976). More recently, this dogma has been seriously challenged. Various studies have shown that insulin and contractility act independently to stimulate glucose uptake; that contractile activity increases glucose uptake and insulin is not required (Ploug et al., 1984; Richter et al., 1984). Furthermore, the effects of insulin and contractile activity on glucose uptake have been shown to be

additive (Nesher et al., 1985; Wallberg-Henriksson, 1987). This additive effect of insulin and contractility suggests that independent mechanisms are involved in stimulating glucose transport.

The glucose uptake capacities of skeletal muscles differ widely according to muscle fibre types (Bonen et al., 1981; Richter et al., 1982). The insulin sensitivity and insulin responsiveness of slow oxidative (SO) fibres at rest , as in the soleus, is 2- and 8-fold greater than in glycolytic types such as in fast oxidative glycolytic (FOG) and fast glycolytic (FG) fibres, respectively (James et al., 1985). When muscle activity is increased, a greater absolute increase in glucose uptake is found in SO fibres than in FOG and FG fibres (Bonen et al., 1984). The reasons for these differences in glucose uptake are not clear, although they may be accounted for by differences in insulin binding between muscles and differences in glucose transporter availability (Bonen et al., 1986). Whatever the mechanisms involved, it is clear that exercise presents a very strong stimulus for taking up glucose into active muscle.

Studies in rats have shown that muscle glycogen loading reduces glucose uptake by exercising muscle (Richter & Galbo, 1986), and in humans there is a direct relationship between the number of glycogen-empty muscle cells and glucose uptake during exercise (Gollnick et al., 1981). Furthermore, Hargreaves (1990) observed an inverse relationship between muscle glycogen concentration in the *vastus lateralis* and leg glucose uptake during supine cycling

during supine cycling at 50% VO_2 max in untrained men. Although these results may not necessarily reflect cause and effect, the strong association suggests that muscle glycogen could play a role in the regulation of muscle glucose uptake. Such a regulatory mechanism would act to minimise the utilisation of blood glucose.

Glucose uptake in muscle is also influenced by the availability of blood-borne substrates. In the perfused rat hindlimb, glucose uptake is linearly related to the perfusate glucose concentration (Berger et al., 1975). Glucose uptake by leg muscles during low-intensity exercise in humans is enhanced when arterial blood glucose levels are increased by glucose ingestion (Ahlborg & Felig, 1976). Conversely, glucose uptake by resting muscle is reduced by 64% when plasma NEFA becomes elevated by Intralipid and heparin infusion, and by 33% during dynamic knee extensions (Hargreaves et al., 1993).

The mechanism underlying the decrease in glucose uptake by muscle as a result of higher levels of plasma NEFA may be due to citrate-mediated inhibition of PFK activity and accumulation of glucose-6-phosphate and glucose, thereby inhibiting glucose uptake. Furthermore, carbohydrate oxidation may be reduced because pyruvate dehydrogenase activity may be diminished by increased oxidation of NEFA and ketones (Randle et al., 1964). Hargreaves et al. (1993) have suggested a direct effect of NEFA on glucose transport.

3.3.4 Carbohydrate ingestion and performance

There have been numerous investigations into the effects of carbohydrate ingestion on endurance performance. These studies have examined the effects of supplementation either on time to exhaustion or on the time taken to achieve a set distance. Most of the studies have focused on the former, despite it being unrealistic of sporting events. Furthermore, studies have been divided into those where the carbohydrate has been ingested before the exercise, during the exercise, and both before and during exercise.

The first report on the beneficial effects of carbohydrate ingestion in humans was that by Gordon et al.(1925) who encouraged athletes to engage in a high carbohydrate diet 24 h before the Boston Marathon and to eat 'candies' from 24 km onwards. The runners' performances were enhanced, and their blood glucose levels post-race were elevated. Another early study showed that subjects who became exhausted during prolonged exercise recovered rapidly when fed carbohydrate and were able to continue exercising (Christensen & Hansen, 1939). No further studies were reported on carbohydrate ingestion and performance until the 1970's when five studies reported favourably on the effects of carbohydrate ingestion on performance (Brooke et al., 1975; Costill et al., 1973; Green & Bagley, 1972; Ivy et al., 1979; Muckle, 1973). All these studies showed the beneficial effects of carbohydrate ingestion on prolonged exercise, and related this fact to maintainance of blood glucose levels and

higher rates of carbohydrate oxidation. The studies of Coyle et al.(1983, 1986) and Coggan & Coyle (1987, 1989) added significantly to the understanding of how carbohydrate provision may be beneficial in prolonged activities. In the first study (Coyle et al., 1983) the subjects exercised at 70 % VO_2 max for 23 min longer after carbohydrate feedings compared with placebo; in the second study (Coyle et al., 1986) carbohydrate ingestion resulted in the subjects exercising for 60 min longer than when fed placebo; the third study (Coggan & Coyle, 1987) showed that when carbohydrate was ingested or infused to maintain euglycaemia at the point of fatigue, subjects exercised for 26 min and 43 min longer respectively than when placebo was ingested. The final study (Coggan & Coyle, 1989) was a repeat of the previous one except that subjects ingested a single large carbohydrate meal approximately 30 min before the point of fatigue, which resulted in prolonging the exercise by 36 min. In all these studies ingestion of carbohydrate promoted carbohydrate oxidation and elevated blood glucose concentrations.

Other investigations have demonstrated carbohydrate ingestion to increase exercise time to fatigue (Bjorkman et al., 1984; Wright et al., 1991), work output during exercise (Ivy et al., 1979; Mitchell et al., 1989; Williams et al., 1990), and improved sprint performance following prolonged exercise (Hargreaves et al., 1984). Although carbohydrate ingestion is able to delay fatigue, it cannot prevent it, and is of most benefit during prolonged exercise that is limited by carbohydrate availability. The beneficial effects of carbohydrate ingestion are related to

the maintenance of blood glucose levels and a high rate of carbohydrate oxidation at a time when muscle glycogen levels are low (Coggan & Coyle, 1987). At this time, blood glucose is the major source of carbohydrate for contracting skeletal muscle, which is capable of utilising glucose at a rate of 1 to 1.5 g min^{-1} (Coggan & Coyle, 1987). Despite adequate blood glucose availability, exercise is eventually terminated. This illustrates the complexity of the fatigue process, and suggests that factors other than carbohydrate availability may be involved. Nevertheless, the critical importance of carbohydrate for endurance exercise cannot be ignored.

Evidence relating to the effectiveness of the type of carbohydrate on performance has been the subject of many investigations (Bjorkman et al., 1984; Flynn et al., 1987; Massicotte et al., 1989; Murray et al., 1989; Noakes, 1990; Owen et al., 1986). In studies in which the effects of ingesting glucose have been compared directly to maltodextrins or sucrose during exercise, either alone or in combination, little difference in their ability to improve performance has been found (Flynn et al., 1987; Massicotte et al., 1989 Murray et al., 1989; Owen et al., 1986), although Noakes (1990) has recently suggested that long chain glucose polymers are more readily used by muscles during exercise than glucose or fructose solutions. The only carbohydrate solutions which have generally not led to improvements in performance are fructose solutions (Bjorkman et al., 1984; Murray et al., 1989).

If carbohydrate feeding during exercise enhances performance, what effect does ingestion of carbohydrate before exercise have on subsequent performance? Some early studies on glucose ingestion in the hour immediately prior to exercise have shown that this leads to an elevation of glucose and insulin at the onset of exercise, and has a subsequent adverse effect on metabolism during the early stages of moderate to high-intensity exercise (Costill et al., 1977; Foster et al., 1979; Koivisto et al., 1981). Under these conditions, where the percentage glucose solution ingested was high (25% solution), blood glucose declines rapidly during exercise due to the high concentrations of insulin. Fat oxidation is also depressed and so the exercising muscle relies more heavily on endogenous glycogen stores than on exogenous provision.

Some studies have provided an indeterminate response to carbohydrate ingestion before exercise (Devlin et al., 1986; Hargreaves et al., 1987; Koivisto et al., 1981) whereas in contrast there have been a number of studies reporting beneficial effects of ingesting carbohydrate prior to exercise (Gleeson et al., 1986; Neufer et al., 1987; Peden et al., 1989). Variations in the timing, the dose, the type of carbohydrate, and the ensuing exercise intensity make it difficult to arrive at firm conclusions. It is suggested that if carbohydrate is ingested before exercise, any possible 'rebound hypoglycaemia' may be offset by ingesting carbohydrates during exercise.

3.4 CARBOHYDRATE OXIDATION

Over the past decade much of the research on carbohydrate metabolism has focused on the uptake and utilisation of glucose by skeletal muscle at rest and during exercise. Advances have arisen primarily because researchers have adopted more sophisticated experimental techniques. In particular, the use of glucose tracers have aided the experimental control necessary to begin to interpret glucose metabolism by muscle. In humans the studies of ^{13}C-glucose have helped thoroughly revise knowledge about the fate of glucose. This section will briefly review some of these findings with respect to glucose oxidation rates.

3.4.1 Oxidation of ingested glucose during exercise

Costill et al. (1973) were the first to investigate the contribution of ingested glucose to the energy demands of exercise in humans. Glucose oxidation was studied in subjects who ingested 32 g of ^{14}C-labelled glucose and then ran at 65% VO_2 max for 60 min. The initial appearance of ^{14}C in the carbon dioxide occurred 5 to 7 minutes after the ingestion, although the levels of radioactivity remained low for the rest of the bout. Calculated rates of exogenous carbohydrate oxidation accounted for only 5% of the total carbohydrate oxidation during 60 min of exercise. The conclusions drawn were that while glucose feedings during prolonged exercise may conserve hepatic glycogen, they were of limited importance for muscle metabolism. Similar results of 10% oxidation of ingested ^{14}C-glucose were obtained when

subjects exercised for 60 min at 50% VO_2 max (Van Handel et al., 1980). The rates of oxidation of the exogenous sources from these studies was 1.6 g h^{-1} (0.027 g min^{-1}) and 4.2 g h^{-1} (0.07 g min^{-1}) for Costill et al. (1973) and Van Handel et al. (1980) respectively.

These low rates of oxidation of exogenous carbohydrate are in contrast with other investigations which have found much higher oxidation rates (Decombaz et al., 1985; Guezennec et al., 1989; Hawley et al., 1992; Massicotte et al., 1986, 1989, 1990; Pallikarakis et al., 1986; Pirnay et al., 1977). All these studies used ^{13}C-glucose and found rates of carbohydrate oxidation of 0.5 to 0.9 g min^{-1}; ten fold higher than previously obtained.

Pirnay et al. (1977) found that ingestion of 100 g of glucose resulted in almost complete oxidation (95 g) after 225 min of exercise at 50% VO_2 max, and that this represented approximately 40% of the total carbohydrate oxidised. After 120 min of this exercise 57 g had been oxidised, and this represented nearly 45% of total carbohydrate oxidation. Interestingly, when 100 g glucose were ingested 3 h before exercise (45% VO_2 max), only 11% was metabolised in the 3 h rest period, but in the first 30 min of exercise 19% of the glucose was recovered as $^{13}CO_2$, and over 4 h of exercise 68% of the glucose was recovered (Jandrain et al., 1984). The mean rate of carbohydrate oxidation from these two studies was 0.65 g min^{-1}. These findings were confirmed by other groups in which the carbohydrate was ingested as one bolus

(Guezennec et al., 1989; Krzentowski et al., 1984).

Studies using multiple feedings of labelled glucose have demonstrated similar exogenous glucose oxidation rates to those studies in which the glucose was given as one bolus i.e. 0.5 to 0.9 g min^{-1} (Hawley et al., 1992; Massicotte et al., 1986, 1989, 1990; Moodley et al., 1992; Rehrer et al., 1992). One study in which the subjects were given 400 g of glucose did result in a peak glucose oxidation rate of 1.16 g min^{-1} (Pallikarakis et al., 1986). Although repetitive feedings would be expected to accelerate the delivery of glucose from the stomach to the duodenum (Noakes et al., 1991), the fact that similar peak rates of glucose oxidation were found after single and multiple feedings suggest that exogenous carbohydrate may not be limited by gastric emptying. Instead, it has been suggested that oxidation in the early stages of exercise is limited by either the rate of absorption from the intestine into the blood, or the demand for glucose by muscles (Moodley et al., 1992; Rehrer et al., 1992).

Moodley et al. (1992) gave their subjects 67.5 g, 90 g, and 135 g of glucose in 9 x 100 ml feedings while cycling at 70 % VO_2 max for 90 min. They found that 35% of the glucose delivered to the intestine was oxidised, representing approximately 17 g. Slightly higher oxidation rates were found in the study by Rehrer et al. (1992) in which subjects were given 58 g of glucose whilst exercising at 70% VO_2 max for 80 min. They found that 55 g of glucose was emptied from the stomach and that 31.5 g were

oxidised, representing 57% oxidation of ingested glucose.

Are the low rates of oxidation of exogenous glucose due to time taken for absorption or due to lack of muscle demand? If exercise intensity is increased, then the demand for glucose by the working muscle is elevated, and so the rate of exogenous glucose oxidation should be increased. When exercise is performed at intensities of 22%, 39%, 51%, and 64% VO_2 max, glucose oxidation increases so that after 90 min of exercise 16%, 33%, 41%, and 44% of the glucose were recovered respectively as carbon dioxide (Pirnay et al., 1982). This suggests a plateau in glucose oxidation is attained at about 50-64% VO_2 max, and that glucose delivery to the blood had become limiting. Support for this conclusion comes from a study in which the rate of glucose oxidation from subjects under control and glycogen-depleted conditions was examined (Ravussin et al., 1979). No significant differences were noted in the rate of glucose oxidation, although the percentage contribution to total carbohydrate oxidation was 62% for depleted subjects and 29% under normal dietary conditions.

3.4.2 Oxidation of ingested carbohydrates other than glucose

Numerous studies have been performed examining the oxidation rates of carbohydrate sources other than glucose, and include fructose, maltose, sucrose, maltodextrins, and starch (Decombaz et al., 1985; Hawley et al., 1992; Massicotte et al., 1986, 1989; Moodley et al., 1992; Rehrer et al., 1992; Saris et al., 1993).

With the exception of fructose ingested in the fed state (Massicotte et al., 1986,1989) and insoluble starch (Guezennec et al., 1989; Saris et al., 1993), there have been no differences observed between the type of carbohydrates ingested. Moodley et al. (1992) examined the rates of glucose oxidation for 7.5%, 10%, and 15% solutions of glucose, sucrose, and two glucose polymers of differing chain length. Total exogenous carbohydrate oxidised in 90 min of exercise at 70% VO_2 max was 16 g, 16g, 21 g, 24 g, for glucose, sucrose, glucose polymer (11 chain), and glucose polymer (22 chain) respectively. These amounts represented mean oxidation rates of between 0.45 to 0.80 g min^{-1}, which were confounded by the carbohydrates being ingested at three different concentrations. Nevertheless, no significant differences were apparent unless the carbohydrates were ingested at 15% solutions when there was a clear advantage of the glucose polymers over the glucose and sucrose.

Contradictory findings have been reported concerning the exogenous carbohydrate oxidation rates for starch. Guezennec et al. (1989), using naturally enriched ^{13}C-carbohydrates reported a lower oxidation rate for starch than for glucose or glucose polymers in solution. Recently, however, Hawley et al. (1991) using a ^{14}C-glucose or starch label, reported that insoluble starch could be oxidised at a rate of 1.8 g min^{-1} after 90 min of exercise. This rate was twice that found for a glucose polymer solution of the same concentration in the same study. In addressing this apparent contradiction, Saris et al. (1993) examined the rates of oxidation of soluble and insoluble starch

using ^{13}C label instead of the ^{14}C label used by Hawley et al. (1991), because they felt that the ^{14}C-glucose label may have been preferentially taken up and subsequently oxidised at a different rate depending on its solubility characteristics. The soluble starch did indeed exhibit significantly higher peak and mean oxidation rates (1.10 g min^{-1} and 0.84 g min^{-1}) than did the insoluble starch (0.81 g min^{-1} and 0.50 g min^{-1}). Furthermore, these values were significantly lower than those reported by Hawley et al. (1991). Their conclusions were that the physical characteristics of a carbohydrate solution in terms of solubility, influences the exogenous carbohydrate oxidation rate. The soluble carbohydrate, being oxidised at a higher rate during exercise than an isocaloric insoluble carbohydrate, should be a preferred energy source for endurance athletes since the ingested soluble carbohydrate represents a larger proportion of the total carbohydrate utilised during exercise, thus saving endogenous stores.

3.4.3 Oxidation of infused glucose

The idea that gastric emptying may limit the ultimate rates of ingested carbohydrate oxidation during the early stages of exercise have been clearly refuted by the results of investigations which have shown that the amount of carbohydrate leaving the stomach after repeated ingestion of solutions containing carbohydrates is more than double the amount oxidised (Hawley et al., 1992; Moodley et al., 1992; Rehrer et al., 1992). Peak rates of exogenous carbohydrate oxidation rise to nearly 1

g min^{-1} during the later stages of exercise. The question arises as to what could be the maximal rates of exogenous glucose oxidation. In attempting to answer this question, it would not be appropriate to ingest the carbohydrate due to absorption characteristics. Clearly it is advantageous to use glucose infusion and thereby bye-pass intestinal absorption and liver glucose uptake. Two studies to date have examined the oxidation of infused glucose during exercise, and both used the hyperglycaemic clamp technique devised by DeFronzo et al. (1979). Coyle et al. (1991) maintained hyperglycaemia at 10 mM using the clamp technique while subjects exercised for 120 min at 73% VO_2 max and concluded that an oxidation rate of 2.6 g min^{-1} in the last hour was possible. Without the use of labelled tracers it was not possible for Coyle et al.(1991) to determine the proportion of infused glucose that was oxidised. Hawley et al.(1994) addressed this problem in an investigation which employed euglycaemic and hyperglycaemic clamping to measure glucose infusion rates but also included ^{14}C-glucose labelling. Almost identical rates of glucose infusion were found under conditions of maintained hyperglycaemia to the study by Coyle et al.(i.e. 2.9 g min^{-1} in the last 20 min), but interestingly the maximum rate of glucose oxidation was 1.8 g min^{-1}. The excess glucose infused did not spare muscle glycogen (a result similarly obtained by Coyle et al., 1991), and was suggested to have been incorporated into non-exercising muscle. Euglycaemia resulted in a glucose oxidation rate of approximately 1 g min^{-1} in the last 20 min of exercise. This restriction of glucose oxidation rate under euglycaemic infusion suggests that glucose oxidation by

skeletal muscle is precisely regulated by the plasma glucose concentrations which, in turn, regulate hepatic glucose uptake and release (Hawley et al., 1994).

3.5 GLUCOSE TRANSPORT

Glucose uptake by exercising muscles is sustained because of the interaction of several physiological systems. An increase in hepatic glucose production, increased cardiac output, and decreased vascular resistance to muscle ensure that the glucose supply to the working muscle is adequate, while insulin (Berger et al., 1975; Vranic et al., 19; Wasserman et al., 1992) and contraction (James et al., 1985; Richter et al., 1985; Wallberg-Henriksson & Holloszy, 1984) act to maintain the efficiency of glucose extraction sufficiently high. Other factors such as activation of the glycolytic and oxidative enzymes responsible for glucose disposal and enhanced membrane glucose transport increase glucose uptake. This section examines insulin binding and the effects of muscle contractility on glucose uptake during exercise, and how these are related to the role of glucose transporters.

3.5.1 Insulin binding

It is well accepted that the action of insulin is initiated by the binding of the hormone to its receptor followed by a cascade of responses beyond the receptor. The first action beyond insulin binding seems to be the activation of the protein tyrosine kinase of the β subunit of the receptor, followed by mobilisation of the glucose transporter. The insulin receptor is a tetramer with two extracellular subunits containing the hormone binding sites and

two β-subunits which exhibit ATP binding and tyrosine kinase activity (Czech, 1985).

The hypothesis that augmented glucose uptake in exercised muscles is attributable to increased insulin binding has not been borne out. Although glucose uptake and rates of glycolysis and glycogenesis are increased after non-exhaustive exercise in the *soleus* and EDL muscles of mice, insulin binding is not altered (Bonen et al., 1984). Similar results have been obtained with insulin binding in humans after intense exercise (Bonen et al., 1986). Conversely, glucose uptake can be reduced in the presence of corticosterone while insulin binding remains unaltered (Tan & Bonen, 1985). Collectively these data suggest that acute alterations in glucose uptake in exercising skeletal muscles cannot be accounted for by changes in insulin binding to its receptor.

3.5.2 Contractile activity

Insulin is not required to support increments in glucose transport during muscular activity (Nesher et al., 1985; Ploug et al., 1984; Richter et al., 1985). When blood flow is controlled, contractile activity still increases glucose transport, suggesting a contractile-related effect on muscle membrane (Richter et al., 1985). The work by Nesher et al. (1985) strongly indicates that glucose uptake and metabolism are increased in response to greater contractile activity *in vitro*. The addition of insulin further stimulates glucose uptake, with

the effects of insulin and contractility being additive (Henriksen et al.,1990; Ploug et al., 1992). This suggests that insulin and contractions stimulate glucose uptake by different mechanisms, or mobilise different pools of glucose transporters (Douen et al., 1990).

A recent study by Zinker et al.(1993) used dogs which were infused with somatostatin to suppress insulin release, and then infused with glucose and insulin:-

i) during rest and treadmill exercise at rates that recreated limb glucose and insulin levels previously evident during exercise,

ii) at rest to normalize limb glucose to rates evident with exercise and insulin to basal levels,

iii) at rest to normalise limb glucose and insulin to rates evident with exercise.

Measures of limb glucose uptake and limb glucose oxidation were assessed using tracers. The authors concluded that glucose and insulin infusion at rest accounted for 28% of the limb glucose uptake found during exercise, and so 72% must be related to contractile-related events, and that only 7% of limb glucose oxidation could be ascribed to insulin and glucose alone (i.e. 82% being related to contraction). Therefore, changes in insulin and glucose loads, in and of themselves, are only minor contributors to the exercise-induced increase in glucose uptake and play virtually no part in controlling the exercise-induced increase in glucose oxidation.

What could the exercise-induced contractile factors be? The local muscle factors could be subdivided into effects related to the muscle microvasculature and to intrinsic muscle changes. It is possible that increased capillary recruitment may increase glucose uptake by increasing capillary surface area and exposure to glucose transporters or insulin receptors on the membrane of the muscle. Exercise can increase conductance through arterioles that perfuse muscle by changes in adenosine, temperature, and pH. Changes in the metabolic status within the muscle due to enhanced glycolysis and oxidation will stimulate glucose uptake, as will exercise-induced translocation of glucose transporters.

3.5.3 Glucose transporters

The ability to transport glucose across the plasma membrane is a feature common to nearly all cells, from the simple bacterium to highly specialised mammalian cells. The glucose transporters are a facilitated diffusion transport system which can only transport down a concentration gradient. Such a passive transport system is most effective when the cell is exposed to a fairly constant level of glucose. The primary function of the facilitative glucose carrier is to mediate the exchange of glucose between the blood and the cytoplasm of the cell. This may involve a net uptake or output of glucose from the cell, depending on the type of cell, its metabolic state, and the metabolic state of the organism. For example the muscle cell lacks significant levels of glucose-6-phosphatase and is therefore incapable of producing free glucose, so it is only

concerned with glucose uptake and metabolism. The liver cell, on the other hand, is a net producer of glucose in the post-absorptive state. The glucose transporters form a selective pathway between three major pools of glucose, i.e. the blood, the extracellular fluids, and the cytoplasm of cells.

Five glucose transporter isoforms have been identified so far , and have been designated GLUT 1 to 5 based on the chronological order of cloning of their genes (Silverman, 1991). The gene that codes for the red cell carrier is GLUT 1; GLUT 2 codes for the glucose transporters in hepatocytes and the β-cells of the pancreas; GLUT 3 codes for widely distributed transporters; GLUT 4 codes for the insulin-dependent carriers in adipocytes, skeletal muscle and heart; and GLUT 5 codes for carriers in the intestine and brain. This section will deal almost exclusively with GLUT 4, the glucose transporter associated with muscle and adipocytes (James et al., 1989).

GLUT 4 transporters are the 'insulin-sensitive' cell transporters because they respond to insulin with a rapid and reversible increase in glucose transport. These transporters are expressed at highest levels in brown fat, then heart, followed by slow oxidative fibres, fast glycolytic fibres, and finally white fat (James et al., 1989). GLUT 4 is responsible for insulin-stimulated transport because it is by far the most abundant isoform in fat and muscle, and its level of expression in various muscle and fat types generally corresponds to the magnitude of insulin-stimulated glucose disposal in that cell type (James et

al., 1989).

Glucose transport in the insulin-sensitive tissues has received considerable attention because of the importance of this process in the maintenance of whole body glucose homeostasis. The transport step is rate-limiting for glucose uptake into muscle under most conditions (Ziel et al., 1989), and so glucose transport would appear to be an important step in whole-body glucose disposal. This hypothesis is supported by recent glucose clamp experiments in humans that indicate a direct correlation between muscle GLUT 4 levels and the rate of whole-body glucose disposal (Eriksson et al., 1992).

Evidence was first presented 15 years ago that insulin augments glucose transport in isolated rat adipocytes via the redistribution of transporter molecules (Cushman & Wardzala, 1980). Since then, the translocation phenomenon has been established as the major mechanism by which insulin acutely stimulates glucose transport in muscle cells (James et al., 1989; Kern et al., 1990). Other factors known to increase transport via translocation include cAMP (Kelada et al., 1992) and muscle activity (Douen et al., 1990; Hirshman et al., 1988).

Endurance training produces up to twofold increases in the concentration of GLUT 4 protein in rat skeletal muscle (Neufer et al., 1992; Rodnick et al., 1992), whereas acute exercise does not (Neufer et al., 1992). In humans, muscle GLUT 4 protein is elevated in endurance trained men compared with weight-matched

sedentary men (McCoy et al., 1994), and after 12-14 weeks of endurance training in sedentary, middle-aged, and older insulin-resistance men (Houmard et al., 1993; Hughes et al., 1993). Improvements in insulin action were also observed in these studies. The improvements in GLUT 4 protein levels in rats after endurance training correspond with an increase in contraction and insulin-stimulated glucose uptake (Rodnick et al., 1992).

Because skeletal muscle is responsible for up to 75% of insulin-stimulated glucose uptake and glucose transport is the rate-limiting step in skeletal muscle glucose metabolism (Yki-Jarvinen et al., 1987), the level of GLUT 4 protein in skeletal muscle may be an important determinant of whole body insulin sensitivity. A strong correlation has been found between GLUT 4 and glucose disposal during euglycaemic hyperinsulinaemia in athletes and sedentary control subjects (Ebeling et al., 1993). Furthermore, training-induced increases in GLUT 4 are matched to insulin-stimulated glucose uptake in humans (Dela et al., 1993). Changes in GLUT 4 have also paralled those in the activity of oxidative enzymes in skeletal muscle after endurance training ((Neufer et al., 1992; Rodnick et al., 1992) and detraining (McCoy et al., 1994; Neufer et al., 1992).

Insulin-stimulated glucose uptake has been found to be related to GLUT 4 protein levels in some studies (Dela et al., 1993; Ebeling et al., 1993) but not in others (Andersen et al., 1993; Hughes et al., 1993). In rats, insulin-stimulated glucose uptake is directly related to skeletal muscle GLUT 4 (Kern et al., 1990;

Megeney et al., 1993). McConell et al. (1994) found an inverse relationship between total GLUT 4 protein and glucose uptake during 40 min of cycling at 70% VO_2 max, and also found no change in GLUT 4 as a result of the exercise. These results suggest that skeletal muscle with an apparent enhanced capacity for glucose transport and oxidation may exhibit lower exercise-stimulated glucose uptake. Although at first this may seem surprising, it should be remembered that endurance training increases oxidative capacity in muscle and consequently results in lower glucose turnover and oxidation during exercise (Coggan et al., 1992).

It may be concluded that the GLUT 4 proteins are involved in regulation of glucose uptake during exercise. In turn, these transporters are influenced by insulin concentrations, training status, and contractility of muscle.

3.6 HORMONAL REGULATION OF CARBOHYDRATE METABOLISM

In order to meet the increased energy demands during exercise, the energy stores in liver, muscle, and adipose tissue must be mobilised and regulated. The nervous and endocrine systems are involved in this regulation in a coordinated manner. Although fat represents potentially a much larger energy store than carbohydrates, there is a tighter control of glucose homeostasis than of fatty acid homeostasis (Vranic & Lickley, 1990). The reason for this is that neuroendocrine factors control both glucose production by the liver and glucose uptake by muscle, whereas neuroendocrine control of fatty acid fluxes is limited to fatty acid production by the adipose tissue. The control of these processes by the hormones insulin, glucagon, adrenaline, noradrenaline, cortisol, and growth hormone are reviewed in this section.

3.6.1 Hepatic glucose production

The regulation of hepatic glucose output during exercise involves a complex interaction between a number of neurohormonal mechanisms (Wasserman & Cherrington, 1991). Factors which play a role include insulin, glucagon, catecholamines, sympathetic neural activity and blood glucose levels. At the start of exercise, signals from the central area of the brain, which activate the motor cortex to result in muscle contraction, also activate higher endocrine centres to cause the release of hormones. This has been referred to as 'central command' (Kjaer

et al., 1987) and serves as a feed-forward contol of hormonal and metabolic change similar to that for regulation of circulation (Mitchell, 1990). Experiments in humans have shown that during the early stages of exercise in well-trained men, hepatic glucose output exceeds peripheral glucose uptake, resulting in a significant hyperglycaemia (Hargreaves & Proietto, 1994). Since hepatic glucose output is not always matched to peripheral glucose uptake, a feed-forward mechanism has been proposed. Further evidence of feed-forward regulation has been provided in subjects in whom the voluntary effort of exercise was enhanced by partial neuromuscular blockade. In these subjects, exercise resulted in a larger increase in hepatic glucose output than peripheral glucose uptake and an exaggerated catecholamine response (Kjaer et al., 1987). Thus, it appears that increased sympathetic activity and increased adrenal medullary adrenaline secretion stimulate hepatic glycogenolysis which results in enhanced liver glucose mobilisation. This is further supported by an investigation showing that addition of arm exercise to leg exercise increases sympathoadrenal activity, resulting in a significantly elevated hepatic glucose output and blood glucose concentration (Kjaer et al., 1991). In the study by Hargreaves and Proietto (1994), the abolition of hyperglycaemia by β-adrenergic blockade clearly implicates adrenaline activity in the stimulation of hepatic glucose output during exercise.

Recently the role of the sympathetic nerves in stimulating hepatic glucose output has been brought into question. In healthy subjects the coeliac ganglion (innervating the liver) was blocked

with local anaesthesia to investigate the role of the sympathetic liver nerves for the production of glucose during exercise (Kjaer et al., 1990). Hepatic glucose output increased during exercise despite blockade. Furthermore, when high doses of adrenaline were infused to mimic physiological conditions, the rate of liver glucose output increased significantly. These findings indicate that adrenaline can enhance hepatic glucose output during exercise in humans, and that the sympathetic nervous stimulation is not necessary. Further support to this conclusion was obtained in liver-transplant patients, who were capable of matching glucose uptake by exercising muscle with glucose output by the liver (Kjaer et al., 1992). Clearly, hepatic nerves are not essential for the exercise-induced rise in mobilisation of glucose from the liver in humans.

The mismatch between hepatic glucose production and peripheral glucose utilisation is most pronounced during intense exercise, and is larger in endurance trained subjects than controls (Kjaer et al., 1986). This difference may be due to the possibility that endurance trained subjects have elevated liver glycogen stores, or that they have a larger increase in plasma concentrations of adrenaline (Kjaer et al., 1986). The traditional view that endurance training reduces the levels of plasma adrenaline during exercise (Winder et al., 1978) only holds true at the same absolute work-rate. Exercise at the same relative intensity results in increased secretion of adrenaline by trained athletes (Kjaer et al., 1986). The higher plasma concentration of adrenaline in trained compared with untrained subjects at high

exercise intensities, combined with the trained subjects' higher rate of hepatic glucose production during exercise suggests that adrenaline enhances hepatic glucose output during exercise.

Other hormones have been implicated in the regulation of hepatic glucose production during exercise. Studies in dogs have emphasised the importance of changes in the insulin/glucagon ratio (Issekutz & Vranic, 1980; Wasserman et al., 1989a,b). In these studies, when insulin and glucagon secretion is blocked by somatostatin and then the levels controlled by infusion, impaired hepatic glucose output results when either the insulin concentration is decreased and glucagon kept depressed or when glucagon concentrations are increased but insulin held constant (Wasserman et al., 1989a,b). The normal exercise response of a decrease in insulin and an elevation in glucagon resulting in enhanced glucose output by the liver has also been shown to occur in exercising humans (Wolfe et al., 1986; Hirsch et al., 1991). The study of Hirsch et al.(1991) used the islet clamp technique (somatostatin infusion with glucagon and insulin infusion at fixed rates) show that both decrements in insulin and increments in glucagon play important roles in the prevention of hypoglycaemia during exercise, and do so by signalling increases in hepatic glucose production.

The tenet that glucagon secretion increases during exercise is well supported by plasma glucagon measurements in humans (Hoelzer et al., 1986; Tuttle et al., 1988; Wolfe et al., 1986). Furthermore, the powerful inhibitory role of insulin on hepatic

glucose production at rest (Rizza et al., 1981) would suggest that the decrease in plasma insulin concentration seen in exercise above 50% VO_2 max (Galbo, 1983) also plays a role. Thus, changes in insulin and glucagon concentration during exercise appear to be important in stimulating hepatic glucose production during exercise.

In summary, according to recent findings hepatic glucose output during exercise is elevated due to an increase in plasma adrenaline and glucagon, and to a diminution of plasma insulin. The human liver is richly innervated with sympathetic nerves, and although sympathetic stimulation may play a secondary role, the action of catecholamines is also considered to be important.

3.6.2 Muscle glycogenolysis

The increase in muscle glycogenolysis that occurs during exercise results from the interplay between local and hormonal regulatory mechanisms. These include calcium and cyclic-AMP (c-AMP) mediated transformation of phosphorylase from a relatively inactive *b* form to the active *a* form, allosteric activation of phosphorylase by exercise-induced alterations in muscle metabolites, and changes in substrate levels (Chasiotis et al., 1982). The increase in sarcoplasmic calcium that occurs during excitation-contraction coupling activates phosphorylase by binding to the calmodulin subunit of phosphorylase kinase and to troponin (Picton et al., 1981). In addition to calcium, phosphorylase transformation is under hormonal control, and adrenaline-induced increases in c-AMP

are responsible for activation of phosphorylase (Richter et al., 1982). Removal of the adrenal medulla from rats results in a reduction in muscle glycogenolysis during exercise at the same exercise intensity, although the effect is reversed when adrenaline levels are restored to normal (Richter, 1984). Furthermore, adrenaline infusion has been shown to enhance muscle glycogen breakdown after electrical stimulation (Spriet et al., 1988) and during exercise in humans (Jansson et al., 1986). Experiments in which beta-adrenergic receptors were blocked by propanolol during exercise have resulted in reduced muscle glycogenolysis (Galbo et al., 1976; Chasiotis, 1983).

Insulin is a powerful stimulus for muscle glycogen synthesis (Richter et al., 1984). Consequently, it would be expected that a decrease in plasma insulin concentration during exercise would facilitate muscle glycogenolysis. Elevated levels of plasma insulin have been shown to decrease breakdown of muscle glycogen during muscle contractions (Berger et al., 1976), although the converse has not been reported.

Clearly, an exercise-induced elevation of adrenaline and inhibition of insulin will lead to the increase in c-AMP within exercising skeletal muscle, thereby promoting activation of phosphorylase and enhancing glycogenolysis. Stimulation of phosphorylase activity has also been demonstrated by metabolic changes during exercise which allosterically affect it.

3.7 LIPID METABOLISM

Non-esterified fatty acids (NEFA) have been recognised as one of the main energy sources during rest as well as during exercise (Bulow, 1988). In males, the adipose tissue comprises approximately 10-20% of total body mass (20-30% in females), and is the major energy store of the body. About 50% is located subcutaneously, while the rest is located around intra-abdominal organs, the heart, between and within skeletal muscle, and along blood vessels in the extremeties. In the adult male, about 400 MJ are stored in the adipose tissue compared with 4-5 MJ of stored carbohydrate in the liver and muscle. During exercise at an intensity greater than 70% VO_2 max, carbohydrate is a major energy source initially. In contrast, during prolonged exercise of moderate intensity (50-60% VO_2 max) there is a progressive change from carbohydrate metabolism to lipid metabolism. Initially, carbohydrate contributes about 80% of the oxidative metabolism while later in exercise lipid contributes up to 90% (Bulow, 1981). Clearly there is an association between carbohydrate and lipid sources of energy; the integration being hormonally mediated. This section examines both the role and regulation of fatty acids as energy sources during exercise.

3.7.1 NEFA metabolism and exercise

Non-esterified fatty acid (NEFA) are derived from three main sources. The first is adipose tissue, from which they are mobilised as albumin-bound NEFA. The second source is the

intramuscular triglycerides, and the third source is the circulating triglycerides bound to lipoproteins. In exercising humans it has been estimated that about 60% of the NEFA metabolised during prolonged exercise is derived from extramuscular depots (Havel et al., 1967). The intramuscular triglyceride stores have been found to contribute significantly to metabolism during prolonged running. A decrease of 30-50% in the triglyceride content of muscle has been found after such exercise (Essen et al., 1977; Froberg & Mossfeldt, 1971). It has also been proposed that circulating triglycerides may contribute to muscle lipid metabolism during exercise (Terjung et al., 1982). In spite of the evidence that energy contributing to lipid oxidation can be derived from intramuscular triglyceride stores and from circulating triglycerides, there are more research findings relating to the contribution of NEFA.

During exercise NEFA mobilisation from triglyceride stores has characteristics in common with glucose production in so far as mobilisation may exceed tissue needs and thereby result in plasma accumulation (Galbo, 1983). The fact that NEFA metabolism is less well understood than glucose metabolism is due to the fact that glucose is produced almost entirely in one organ, the liver, whereas NEFAs are produced during lipolysis in many tissues. Furthermore, pure fat tissue is difficult to study *in vivo*, as selective catheterisation has proved to be difficult. Recently, a microdialysis techique has been used to study adipose tissue lipolysis *in situ* (Arner et al., 1990), as has a method for sampling the venous blood draining the subcutaneous adipose

tissue of the anterior abdominal wall in humans (Coppack et al., 1989; Frayn et al., 1989).

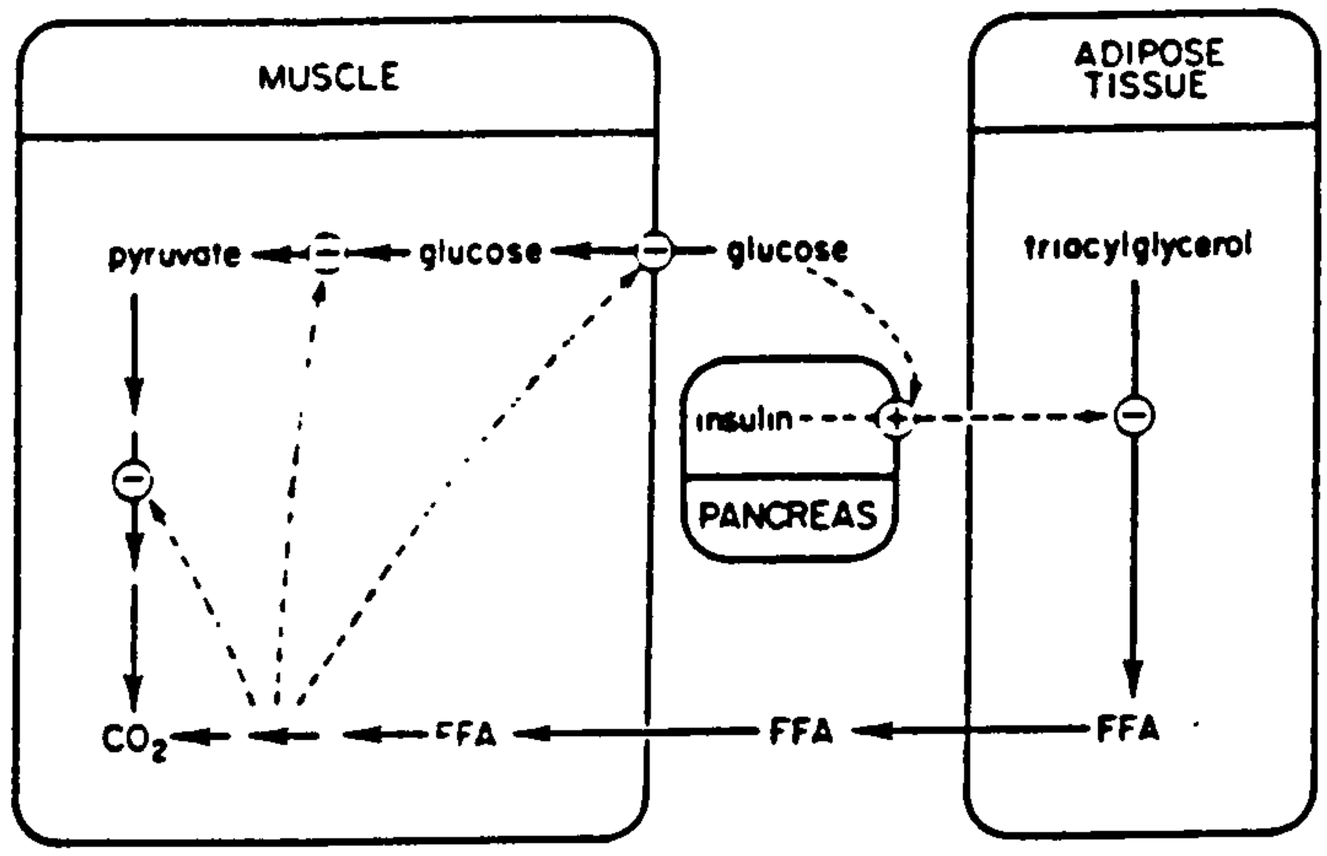
Complete hydrolysis of one triglyceride molecule gives rise to three NEFA molecules and one glycerol molecule, and so the rate of lipolysis can be evaluated from measures of plasma NEFA and glycerol. These concentrations increase with time during exercise, and a direct linear relationship between the plasma concentration and turnover of extracellular glycerol and NEFA has been found over a range of concentrations at rest and during exercise (Shaw et al., 1975). It has also been shown that plasma NEFA concentrations correlate with NEFA uptake in the exercising leg (Hagenfeldt, 1979). In agreement with a cause and effect relationship between plasma NEFA level and lipid oxidation in muscle the experimental decrease or increase in plasma NEFA levels may be accompanied by parallel changes in fat oxidation, with the opposite affect on carbohydrate oxidation (Bergstrom et al., 1969; Costill et al., 1977).

Plasma concentrations of NEFA and glycerol cannot be used unequivocally to determine the rates of turnover of NEFA and glycerol since at a given plasma NEFA concentration the NEFA turnover increases with metabolic rate (Shaw et al., 1975). Hence, meaningful comparisons of NEFA levels between differing exercise intensities are difficult. Furthermore, the plasma concentrations of NEFA turnover do not accurately reflect lipolysis. During prolonged running, a ratio of NEFA to glycerol turnover of 2-2.5 has been found in dogs (Shaw et al., 1975).

This is lower than the theoretical value of 3 for complete lipolysis, and indicates that some of the NEFAs released from triglycerides are re-esterified - an intracellular cycling of NEFA. The latter has been confirmed in humans exercising at 40% VO_2 max (Wolfe et al., 1990) and 50-70% VO_2 max (Hodgetts et al., 1991), although the actual rate of re-esterification decreased in the later stages of exercise in both studies.

The rate of lipolysis in adipose tissue can be estimated from the glycerol produced, since glycerol formed by lipolysis cannot be re-utilised in this tissue due to low concentrations of α-glycerokinase (Bulow, 1988). Re-esterification occurs using NEFA and glycerol-3-phosphate from glucose, and is stimulated by insulin. Adipose tissue lipolysis increases during exercise of moderate intensity. In dogs, the rate of appearance of glycerol increases fourfold during 3 h of exercise (Shaw et al., 1975). Similarly, a fourfold increase in a-v difference of plasma glycerol has been determined over the inguinal, subcutaneous depot in exercising dogs (Bulow, 1982). This rate of lipolysis, 2-4 $\mu M\ g^{-1}\ h^{-1}$, corresponds to 40-50% of the maximal rate obtained during *in vitro* studies (Despres et al., 1984). The rate of lipolysis increases the greatest extent after the onset of exercise, whereas the increase later on during the exercise bout is modest in comparison ((Bulow, 1982). This indicates that lipolysis in adipose tissue is almost constantly stimulated during exercise.

Considerable interest has focused on the interaction between carbohydrate and lipid metabolism since the proposal of the glucose-fatty acid cycle (Randle et al., 1963, 1964). As first demonstrated by these authors, oxidation of fatty acids inhibits glucose uptake, glycolysis, glycogenolysis, and pyruvate oxidation in cardiac muscle. These effects appear to be mediated by the accumulation of citrate, which inhibits PFK activity, which in turn results in glucose-6-phosphate accumulation, which inhibits hexokinase activity, which results in reduced glucose uptake (see Fig 3.1). Furthermore, there is an inhibition of pyruvate dehydrogenase activity as a result of elevated acetyl CoA concentrations due to enhanced β-oxidation of fatty acids. Randle et al. (1963,1964) proposed that this phenomenon also occurred in skeletal muscle, and played an important role in the regulation of whole-body substrate metabolism.



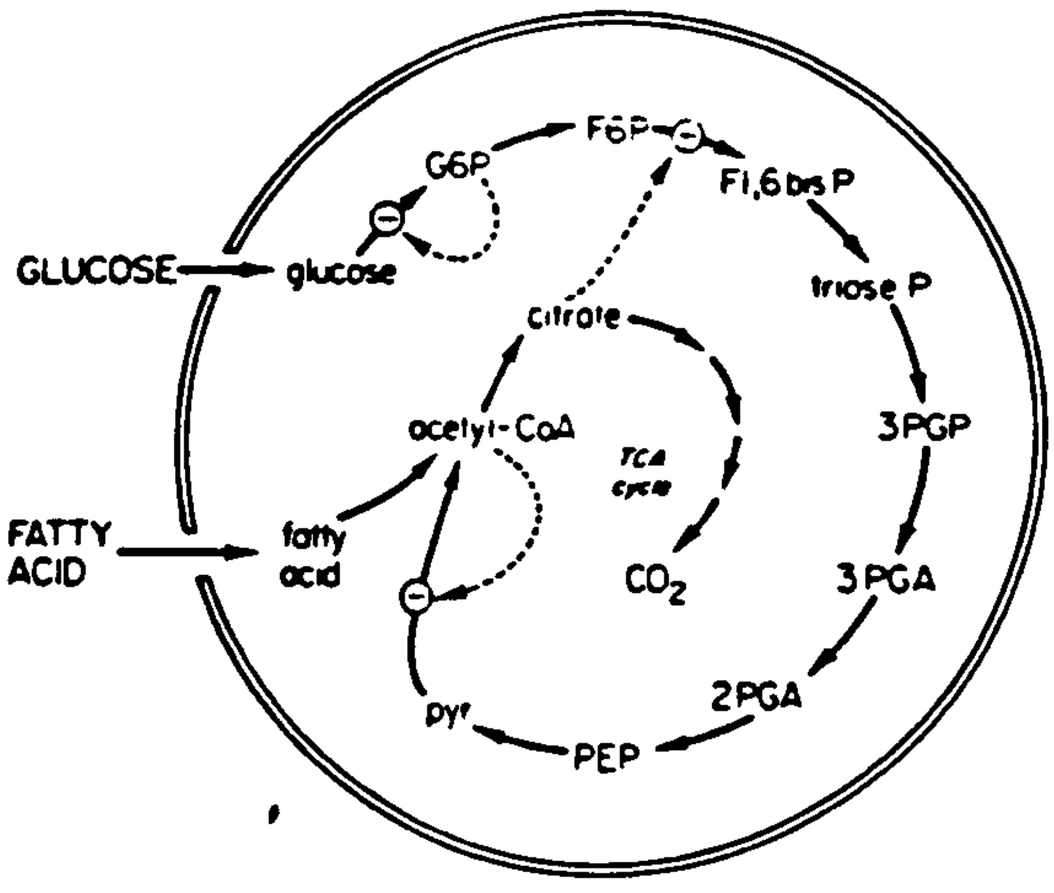


Diagram showing the mechanisms by which increased fatty acid oxidation inhibits glucose utilization and oxidation

Figure 3.1 The glucose-fatty acid cycle.

Doubt persists as to whether this cycle operates in human skeletal muscle at rest and during exercise. Elevation of plasma NEFA at rest has been shown to inhibit glucose utilisation (Ferrannini et al., 1983), although the physiological significance of plasma NEFA in the control of glucose oxidation has been questioned (Wolfe et al., 1988). During exercise, elevations of plasma NEFA have been shown to decrease muscle glycogen use during treadmill running (Costill et al., 1977), whereas inhibition of NEFA mobilisation by nicotinic acid increases muscle glycogen use (Bergstrom et al., 1969). In contrast, carbohydrate oxidation during prolonged moderate-intensity exercise remains unaffected by elevation of plasma NEFA (Hargreaves et al., 1991; Ravussin et al., 1986). The study by Hargreaves et al. (1991), although demonstrating that carbohydrate oxidation is unaffected by intralipid and heparin infusion, did show a significant 33% reduction in leg glucose uptake.

The effect of diet on fuel selection during exercise can be attributed to the size of the glycogen stores. When the glycogen stores are full, the oxidation of NEFA in muscle is inhibited compared to situations when the muscles are depleted (Gollnick et al., 1981). The increase in plasma NEFA concentration during exercise is attenuated after a high-carbohydrate diet than after a high-fat diet (Galbo et al., 1979), and glucose infusion during exercise produces a similar attenuating response on both plasma NEFA and glycerol (Bulow, 1981). Similarly, ingestion of glucose during prolonged exercise diminishes lipolysis (Ahlborg et al.,

1976). These results clearly demonstrate that the glucose-fatty acid cycle functions in skeletal as well as cardiac muscle.

3.7.2 Regulation of lipid mobilisation

Enhanced sympathoadrenal activity and a depressed concentration of circulating insulin are the main stimuli for lipolysis in humans during exercise (Galbo,1983). Acute β-adrenergic blockade during exercise results in a reduced secretion of both glycerol and NEFA, although increased concentrations are found during α-blockade (McLeod et al., 1984). When exercising dogs are infused with propanolol a strong inhibition of glycerol mobilisation takes place (Issekutz, 1978; Wolfe et al., 1981). Endurance is impaired by β-adrenergic blockade in man (Galbo et al., 1976). In dogs, the impaired exercise capacity can be restored by infusion of intralipid and heparin to elevate NEFA levels to those normally associated with exercise without blockade (Nazar et al., 1975). Contrary to these findings in man and dog, the sympathoadrenal system does not seem to play an essential role in the lipolytic response to exercise in the rat (Federspil et al., 1975).

Increases in cortisol and growth hormone are both longer-term activators of lipolysis (Frayn,1989). Growth hormone exerts its action on fat mobilisation after 60 min of exercise (Moller et al., 1990), and so its reinforcement of lipolysis is of significance during sustained exercise.

Depression of insulin levels during exercise is a further hormonal mechanism of importance for the increased lipolytic rate during exercise. The concentrations of circulating NEFA and glycerol during exercise are higher in the fasted and fat-fed subjects than in normal control subjects, while insulin concentration is lower (Galbo et al., 1979). Insulin stimulates lipogenesis, and a common effect of insulin is an enhanced sensitivity of adipocytes to the lipogenic effect of insulin (Savard et al., 1987). This is likely to be in conjunction with elevated rates of glucose uptake by adipocytes (Craig et al., 1981). Since enhanced lipogenesis will lead to re-esterification of NEFAs, an increase in insulin level during exercise will clearly inhibit lipolysis. Such an event is only likely to occur when carbohydrates are ingested during exercise.

During incremental exercise, lipolysis and changes in insulin and catecholamine concentrations are directly related to exercise intensity (Galbo, 1992). Increases in adrenaline concentration inhibit the release of insulin from the pancreas, and a combination of elevated adrenaline and reduced insulin stimulates lipolysis. These relationships are the focus of the glucose-fatty acid cycle.

The amount of NEFA mobilised from adipose tissue depends on the rate of lipolysis and on the competition between the NEFA-carrying capacity of the perfusing blood, and the process of re-esterification in the adipocytes. Whereas the rates of lipolysis and re-esterification are hormonally mediated, the NEFA-carrying

capacity is determined by the albumin concentration of the blood, by the blood flow, and by the number of NEFA-binding sites on the albumin already occupied. Each albumin molecule can only bind a finite number of NEFA molecules, and does so with decreasing affinity (Ashbrook et al., 1975). An increase in the NEFA-albumin ratio will lead to re-esterification (Madsen et al., 1986) and so decrease the release of NEFA. A futile cycle involving lipolysis and re-esterification, controlled to some extent by the NEFA-albumin ratio appears to govern the release of NEFA. Adipose tissue blood flow increases during exercise (Bulow, 1983), and this increase in blood flow has been demonstrated to enhance NEFA mobilisation during exercise (Bulow & Tondevold, 1982). The increase in blood flow has been proposed to be secondary to metabolic events connected to lipolysis (Bulow, 1983), and has been found to be mediated by adenosine (Martin & Bockman, 1986).

High NEFA-albumin ratios in arterial blood have been shown to increase the vascular resistance in adipose tissue (Bulow et al., 1985). The effect is apparent at a ratio of approximately 3:1, and is increased significantly with this ratio. It has been suggested that this mechanism will inhibit the removal of NEFA from adipose tissue (Madsen et al., 1986). Recently, Hodgetts et al.(1991) showed that NEFA release occurred despite a NEFA-albumin ratio >6:1 in some subjects.

Nevertheless, the results from various studies suggests that the during exercise, lipolysis increases rapidly to a constant level due to the stimulation by sympathoadrenal factors and a

decreasing insulin concentration. Secondary to this, adipose tissue blood flow increases to promote the removal of NEFA from the adipocytes. The rate of lipolysis is stimulated in excess of the need of exercising tissue since about 65% of the liberated NEFAs are re-esterified (Bulow, 1982). The amount of NEFA mobilised from adipose tissue is also in excess of the utilisation, leading to an increase in the NEFA-albumin ratio. As the NEFA-albumin ratio increases, there is an opposition to excessive NEFA mobilisation due to increased re-esterification and to an increase in vasconstriction. Figure 3.2 diagrammatically illustrates this concept.

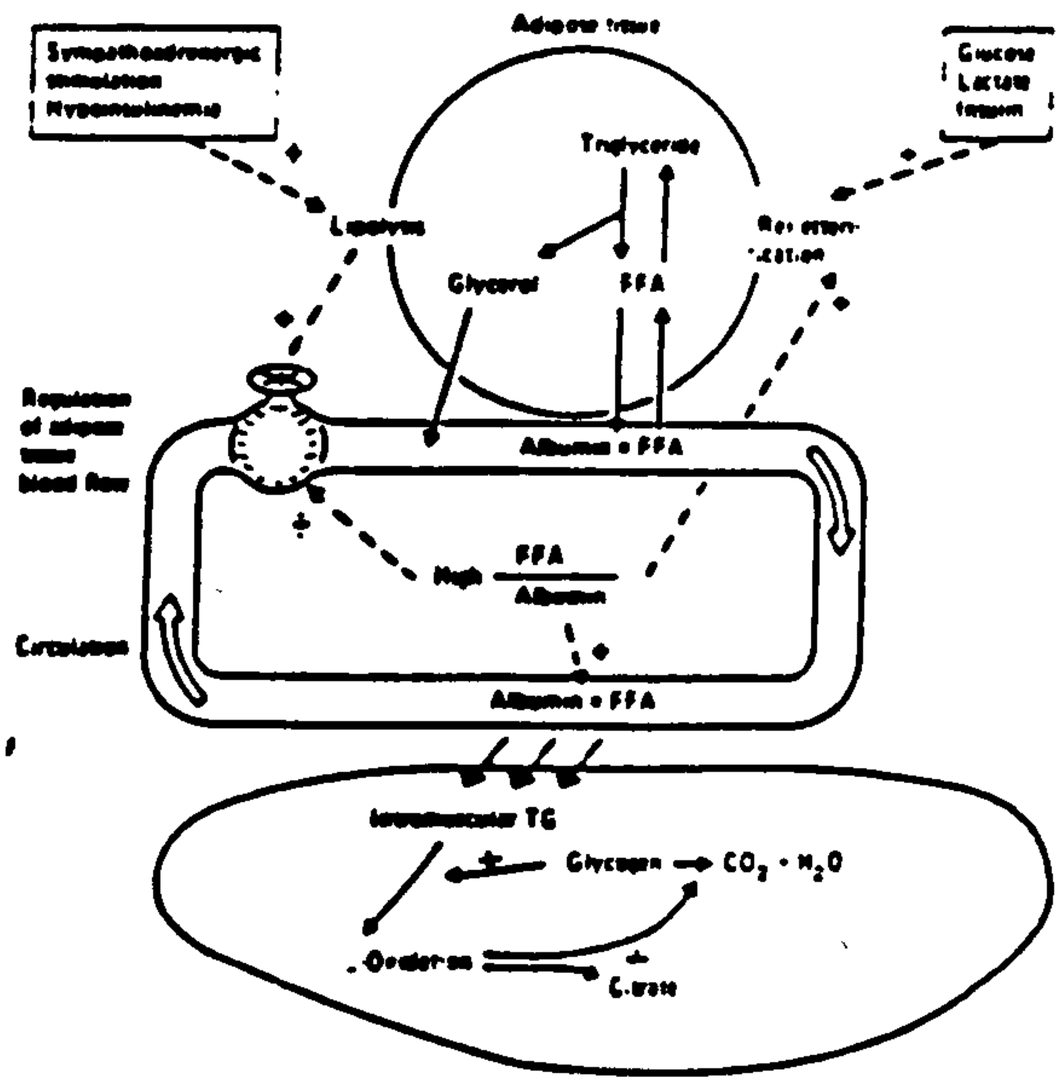


Fig 3.2 Regulating mechanisms of lipid mobilisation and use in adipose tissue and skeletal muscle during exercise (after Bulow, 1988).

3.8 AMINO ACID METABOLISM

Although protein synthesis is suppressed during exercise (Rennie et al., 1980), a release of amino acids from skeletal muscle normally occurs (Ahlborg & Felig, 1976; Felig & Wahren, 1971). At rest, the net release of alanine and glutamine exceeds that of all the other amino acids, accounting for over 50% of the total amino acids released (Felig & Wahren, 1974). During exercise alanine is the major amino acid released from muscle (Felig & Wahren, 1971;1974). The increase in alanine release is dependent both on exercise intensity and exercise duration (Viru, 1987). As a consequence of this, Felig and Wahren (1971) proposed a glucose-alanine cycle to account for the large efflux of alanine from muscle, and its use as a gluconeogenic substrate by the liver. In this scheme pyruvate is provided from glycolysis, while glutamate is the immediate amine donor in a reaction catalysed by alanine transaminase, an enzyme which is abundant in skeletal muscle (Mole et al., 1973). The amine group arises from multiple transamination reactions, especially those involving the branched-chain amino acids, which channel the nitrogen to glutamate (Fig 3.3).

The branched-chain amino acids appear to play a pivotal role in alanine formation in skeletal muscle (Haymond & Miles, 1982; Odessey et al., 1974). In prolonged exercise, branched-chain amino acids are released from the liver and taken up by muscle (Felig, 1977; Wahren et al., 1975). Skeletal muscle contains the largest fraction of branched-chain amino acid transaminase in the

body (Mole et al., 1973), and during exercise the transamination of leucine is markedly augmented (Hood & Terjung, 1987), giving rise to a two-fold increase in the rate of transfer of leucine nitrogen to alanine (Wolfe et al., 1984). An elevation of plasma alanine is instrumental in the maintenance of a high gluconeogenic rate (Mallette et al., 1969). This is considered important since gluconeogenesis maintains blood glucose levels and prolongs submaximal exercise (John-Alder et al., 1986).

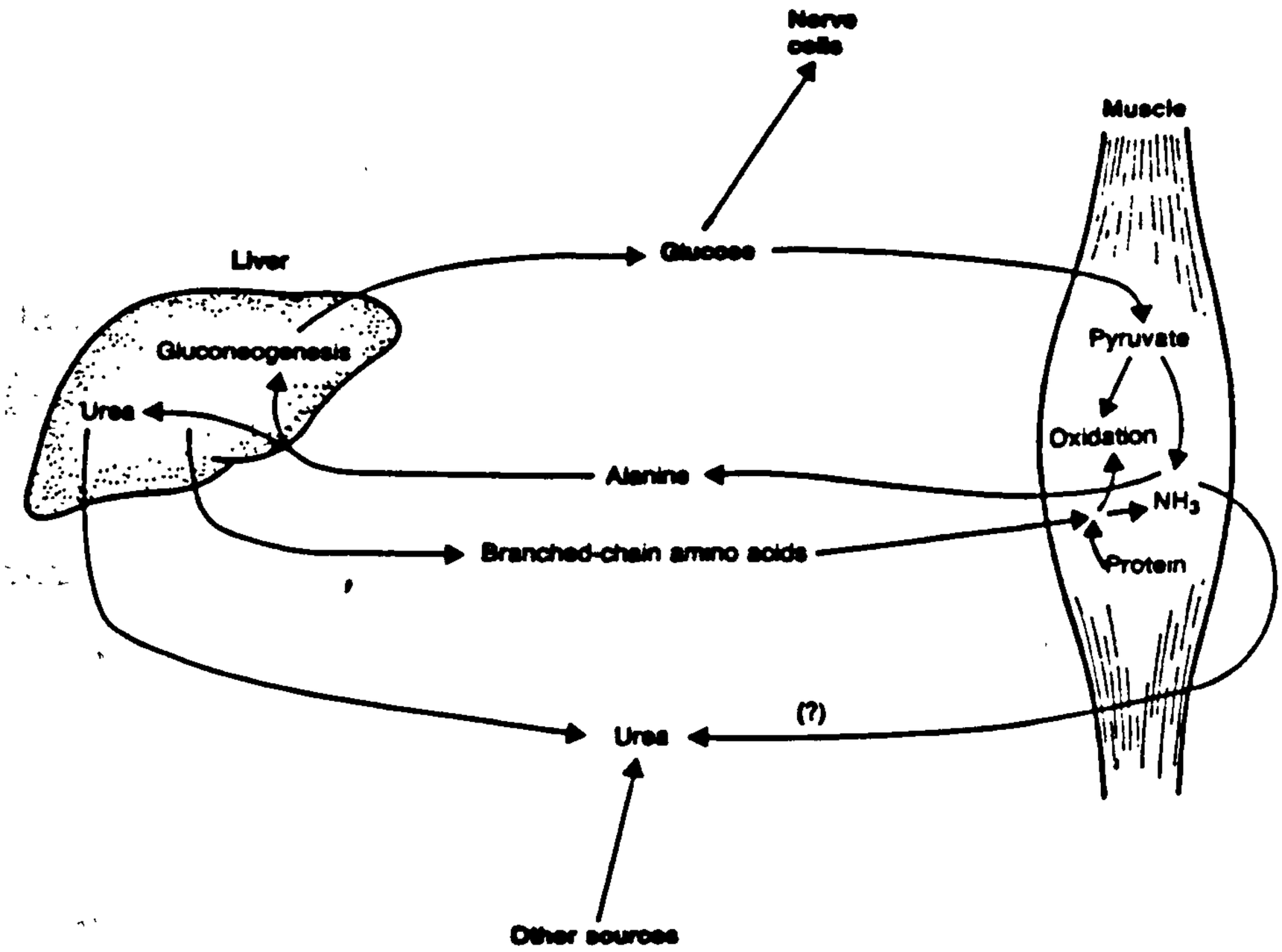


Fig 3.3 The glucose-alanine cycle (after Viru, 1987).

One of the important functions that amino acids may play during prolonged exercise are as substrates for glucose synthesis. Prevention of hypoglycaemia during exercise is especially important because glucose is not only used by the contracting muscle but is also the major fuel for the central nervous system. During the first 60 min of endurance exercise liver glycogen is the primary source of blood glucose, but as the exercise duration increases then gluconeogenesis plays a more significant role. Ahlborg et al.(1974) measured the splanchnic uptake of glucose during exercise in humans and found that gluconeogenic rate was elevated ten-fold. During the third hour at 60% VO_2 max gluconeogenic substrates accounted for 60% of the total splanchnic glucose output. It must be remembered that alanine is only one of the substrates, the others being lactate and glycerol.

The hormonal control of gluconeogenesis involves glucagon, cortisol, and adrenaline (Kraus-Friedman, 1984), all of which become elevated during prolonged exercise (Galbo,1983), and is consistent with increased gluconeogenesis. The action of glucagon and adrenaline is mediated through increased cell concentrations of cAMP. The resultant is that the activity of the key regulatory enzymes pyruvate kinase, PEP carboxykinase, and 1,6-bisphosphatase is promoted (Dohm et al., 1985). The role of cortisol in enhancing gluconeogenesis is concerned with stimulation of hepatic alanine aminotransferase activity (Viru, 1987). Inhibition of these hormones by glucose ingestion (Galbo, 1983) or glucose infusion (Coyle et al., 1991) results in

impaired gluconeogenesis.

The branched-chain amino acids and alanine are capable of being oxidised directly by exercising muscle through their respective ketoacid dehydrogenases (Hood & Terjung, 1990). The activities of the branched-chain ketoacid dehydrogenases have been most studied, and have been found to increase significantly during exercise (Wagenmakers et al., 1984). It is likely that the maximum energy supplied via direct oxidation is no more than 3% (Hood & Terjung, 1990), and that the overall energy supply during the later stages of prolonged exercise from amino acids is unlikely to exceed 10% (approximately 7% coming from the glucose-alanine cycle).

3.9 THE HYPERGLYCAEMIC CLAMP TECHNIQUE

Two glucose clamp techniques have been devised in order to quantify beta-cell sensitivity to glucose and tissue sensitivity to insulin (DeFronzo et al., 1979). The hyperglycaemic clamp assesses the former, whereas the euglycaemic clamp is used to assess the latter. In the hyperglycaemic glucose clamp technique, the plasma glucose concentration is elevated significantly above basal levels by a priming infusion of glucose and then maintained at that concentration by adjusting the rate of glucose infused. Because the plasma glucose concentration is kept constant, the rate of glucose infused is an index of glucose metabolism. The euglycaemic insulin clamp technique however, involves raising the insulin concentration and maintaining it at approximately 100 μU ml^{-1} by a prime continuous infusion of insulin. The plasma glucose concentration is then held constant at basal levels by variable glucose infusion. The glucose infusion rate in this instance relates to the uptake of glucose by all the tissues in the body, and is an index of tissue sensitivity to exogenous insulin. A requirement for both techniques is access to equipment for analysing glucose concentrations rapidly.

The aim of the hyperglycaemic clamp is to raise the plasma glucose concentration acutely to a fixed hyperglycaemic plateau, and then to maintain it at that level for the duration of the test (normally 2 h). This is accomplished by an intravenous glucose infusion consisting of two phases; a 15 min priming phase to raise the plasma glucose concentration to the plateau, and a

maintenance phase wherein the glucose infusion is computed at 5 min intervals based on the plasma glucose concentration. The prime dose necessary to raise the plasma glucose concentration to hyperglycaemic levels is approximately 240 mg kg^{-1} body mass or 36 mg kg^{-1} body mass for each 1 mM increase in plasma glucose concentration.

The hyperglycaemic clamp technique has been employed to determine glucose metabolism in sepsis (White et al., 1987), in patients with colorectal cancer (Copeland et al., 1987, 1988), in patients with multiple organ failure (Green, 1990), and in exercising subjects (Coyle et al., 1991; Hawley et al., 1994). Determination of the glucose utilisation rates from these studies can be seen in Table 3.2. Clearly there is an increase in glucose disposal/utilisation as a consequence of increased activity, and an attenuated response due to cancer, sepsis and trauma.

3.9.1 Hyperglycaemic clamp and rest

The four studies reported above which have involved maintained hyperglcaemia at rest, kept the blood glucose clamped at 12 mM (Copeland et al., 1987,1988; Green, 1990; White et al., 1987). The control subjects in each of the studies showed a slow but continuous increase in glucose disposal during the 'clamp'. What is quite clear on examination of the data is that age impairs glucose disposal. The study by Green (1990), produced glucose disposal rates greater than the other studies, and this was followed by the study of White et al.(1987), and then that of

Copeland et al.(1987,1988). The control subjects in the latter study were 69 and 72 years of age, whereas those for White et al. (1987) were 36 years, and those for Green (1990) were 30.5 years old. The results also show that there is impaired glucose disposal as a result of illness, sepsis and trauma.

Not surprisingly, the insulin concentrations became elevated during the 'clamps',with an initial peak occurring after the prime infusion followed by a steady rise. Although the control subjects produced a greater response, the pattern was similar in all subjects. Responses of the counter-regulatory hormones showed either no change or a slight decrease. Thus, the release of the catecholamines, glucagon, growth hormone, and cortisol were suppressed. The effects of the attenuated counterregulatory hormonal response and the significant increase in insulin resulted in plasma levels of NEFA and glycerol being drastically reduced. This is clearly in agreement with the concept of a glucose-fatty acid cycle operating.

3.9.2 Hyperglycaemic clamp and exercise

Only two studies to date have employed the hyperglycaemic clamp technique during exercise in humans (Coyle et al.,1991; Hawley et al., 1994). These studies raised the blood glucose concentration to 10 mM before 'clamping' for 2 hours.

Coyle et al. (1991) examined the effects of hyperglycaemia on muscle glycogen use and carbohydrate metabolism using 8 well

-trained cyclists. During 2 h of exercise at 70% VO_2 max the glucose disposal rate increased from 1.6 g min^{-1} to 2.6 g min^{-1}. This was accompanied by an elevation in the total rate of carbohydrate oxidation significantly above control values and accounted for all the glucose infused. Plasma insulin concentration was elevated significantly only after 40 min, and it appears that this could have been responsible for the marked increase in carbohydrate metabolism in the last 60 min of exercise. In spite of this, no significant differences were apparent in muscle glycogen use during the hyperglycaemic trial and the control. The authors calculated that an additional 160 g of the glucose infused could not be accounted for by oxidation, and suggested that this may have been disposed of by hepatic glycogen synthesis, synthesis of glycogen in inactive muscle, and storage in adipose tissue. Certainly, fat oxidation was significantly diminished since a difference of approximately 0.4 g min^{-1} between control and experimental conditions was evident after 2 h. Likewise, NEFA levels were also significantly lower. The contribution of energy from fats was approximately 30% at the end of the exercise period.

Hawley et al. (1994) found glucose disposal rates almost identical to those of Coyle et al. (1991), and confirmed the exceptional ability of well-trained subjects to dispose of large amounts of infused glucose. Associated with the elevation of plasma glucose was a significant hyperinsulinaemia. In this study, Hawley et al.(1994) showed that hepatic glucose production was totally supressed, and that fat oxidation only accounted for

between 12 and 18% of the total oxidation rate. Also as in the study by Coyle et al.(1991), no muscle glycogen sparing was observed.

These studies using the hyperglycaemic clamp technique clearly support the concept of a glucose-fatty acid cycle existing during exercise, clearly confirm the research finding that carbohydrate 'feeding' does not spare muscle glycogen, and suggest that a maximum rate of glucose uptake and oxidation by muscle is approximately 2.5 g min^{-1}.

Table 3.2 Glucose disposal/utilisation rates (g min^{-1}) calculated from various studies.

Author	Subjects	Level of Activity	Glucose Disposal (g min^{-1})
White et.al. (1987)	Surgical (n=12)	Rest	0.30
	Control (n=11)	Rest	0.52
Copeland et al.(1987)	Cancer (n=20)	Rest	0.26
	Control (n=15)	Rest	0.30
Copeland et al. (1987)	Cancer (n=18)	Rest	0.22
	Control (n=13)	Rest	0.38
Green (1990)	Patient (n=14)	Rest	0.38
	Control (n=10)	Rest	0.79
Coyle et al. (1991)	Athlete (n=8) 70% VO_2 max	20 min	1.6
		120 min	2.6
Hawley et al. (1994)	Athlete (n=12) 70% VO_2 max	5 min	0.4
		120 min	2.0

3.10 SUMMARY

Carbohydrates are clearly an important source of energy for prolonged exercise, particularly at moderate-to-high intensities. The depletion of muscle glycogen stores has been associated with fatigue, and is specific to the muscle fibre type being recruited. Increasing the glycogen stores by dietary intake prior to exercise leads to an enhanced performance, although generally no sparing of glycogen is observed when carbohydrates are ingested during the exercise. Improvements in performance due to ingestion of carbohydrates during exercise are probably due to an increased availability of blood glucose for muscle uptake and oxidation.

High carbohydrate diets and carbohydrate ingestion during exercise lead to increased rates of carbohydrate oxidation and attenuated fat oxidation, whereas the response without carbohydrate ingestion entails a progressive increase in fat oxidation. This is as a consequence of the glucose-fatty acid cycle operating. Maximal rates of glucose uptake by muscle during prolonged exercise using the hyperglycaemic clamp technique reveal that 2.5 g min^{-1} is possible, and that this occurs in spite of muscle glycogen depletion. In the later phases of exercise, infused glucose accounts for the total carbohydrate oxidation. Ingested carbohydrate is incapable of supplying the total carbohydrate needs of the exercising athlete, accounting for a maximal reported oxidation rate of 1.0 g min^{-1}. The form of carbohydrate ingested does not influence the rate of carbohydrate

oxidised nor significantly affect performance, unless high levels of fructose are used whereby resultant gastrointestinal discomfort may contribute to impaired performance. The solubility of complex carbohydrates ingested can however influence the rate of exogenous carbohydrate oxidation, with insoluble starches producing a lower rate.

The control of carbohydrate metabolism is hormonally mediated, although there is a crucial input from the nervous system *vis a vis* glucose production by the liver during the early stages of exercise and involves a feed-forward mechanism. Muscle contractility is also involved in activating glucose transporters for muscle glucose uptake during exercise in spite of a decline in circulating insulin. The normal exercise response of a decrease in plasma insulin and increases in plasma catecholamines, glucagon, growth hormone, and cortisol, is attenuated on ingestion of a carbohydrate. The hormonal response to glucose infusion during exercise has not been reported except for insulin.

Although carbohydrates are generally considered to be of significant importance for exercise performance, the role of lipids and amino acids should not be underestimated. Lipid oxidation may account for as much as 80% of the total oxidative processes during the later stages of moderate exercise. The major source of lipids are the NEFAs from adipose tissue, although intramuscular and plasma triglycerides are important. The glucose-fatty acid cycle ensures that there is reciprocal use of

lipids and carbohydrates as energy sources. Carbohydrate ingestion during exercise favours carbohydrate oxidation and utilisation, whereas fat infusion inhibits muscle glucose uptake.

Amino acids, particularly alanine and the branched-chain amino acids, play a small yet significant role in energy metabolism during prolonged exercise. The increase in plasma glucagon after 30-40 min of exercise stimulates hepatic gluconeogenesis. The release of cortisol supports the process by ensuring that gluconeogenic substrates are available. The glucose-alanine response occurs maximally after 60 min of exercise to enable hepatic glucose output to be maintained. The role of branched-chain amino acids in stimulating alanine synthesis and release by active muscle accounts for the observations of net release of alanine from muscle. A combination of involvement in hepatic glucose production and direct oxidation by exercising muscles results in the suggestion that amino acids may contribute as much as 10% of the total energy provision during the later phases of exercise.

The hyperglycaemic glucose clamp technique was originally devised to quantify beta-cell sensitivity to glucose, and was therefore used to examine rates of glucose disposal in cancer patients, those patients with multiple organ failure, and cases of sepsis and trauma. Impaired rates of glucose disposal found in these subjects clearly relate to the construction of appropriate feeding strategies in hospitals. More recently, the clamp technique has been employed on subjects whilst exercising.

Maximal rates of glucose disposal under conditions of exercise at 70% VO_2 max are significantly greater than those found at rest in control subjects and those with cancer, trauma, sepsis, and multiple organ failure. Despite high rates of glucose disposal, muscle glycogen is significantly depleted during exercise. After 120 min of exercise with maintained hyperglcaemia, exogenous glucose may account for the total carbohydrate oxidised.

This thesis will now seek to undertake the objectives laid down in Chapter 2, drawing on information presented in this section.

4. HORMONAL AND METABOLIC RESPONSES TO GLUCOSE AND MALTODEXTRIN INGESTION PRIOR TO EXERCISE WITH OR WITHOUT GUAR GUM

(Aspects of this work were published in the International Journal of Sports Medicine, 1994, vol 15, No 8, pp 466-471, a copy of which can be found in Appendix B).

4. HORMONAL AND METABOLIC RESPONSES TO GLUCOSE AND MALTODEXTRIN INGESTION PRIOR TO EXERCISE WITH OR WITHOUT GUAR GUM

4.1 INTRODUCTION

Carbohydrate ingestion during exercise results in prolonging the activity whereas studies of ingestion before exercise have produced equivocal results. The ingestion of carbohydrate in the hour before exercise may cause a decline in blood glucose concentration, an increase in muscle glycogen utilization, and a greater carbohydrate oxidation in the first 20 min of exercise (Costill et al., 1977; Hargreaves et al., 1985).

Some studies have not reported an increase in muscle glycogen use following such pre-exercise feedings (Gleeson et al., 1986; Hargreaves et al., 1987; Koivisto et al., 1985). This may be due to the fact that hypoglycaemia was not as pronounced in the latter studies. Equivocal findings have also resulted when assessing endurance performance following carbohydrate ingestion in the hour before exercise. Some studies have showed negative effects (Costill et al., 1977) or no significant effect (Foster et al., 1979; McMurray et al., 1983; Hargreaves et al., 1987), whilst others have shown an improvement in performance (Gleeson et al., 1986; Peden et al., 1989).

Carbohydrate ingestion immediately before exercise would not be expected to cause rebound hypoglycaemia due to the inhibitory effects of elevated catecholamines on insulin during the exercise. Results would be expected to be similar to studies

is ingested during the exercise. In these studies, the exogenous carbohydrate appears to supplement liver glycogen stores and thereby maintain plasma glucose levels (Coyle et al.,1986), although conflicting results have been obtained regarding sparing of muscle glycogen (Hargreaves & Briggs, 1988). Furthermore, exogenous carbohydrate has been shown to provide substrate for exercising muscle, contributing up to 68% of the glucose oxidized during prolonged exercise (Hawley et al., 1992). These provide the basis for the following hypotheses:-

Hypothesis 1: *Time to fatigue increases under conditions of carbohydrate ingestion.*

Hypothesis 2: *An enhanced carbohydrate oxidation rate and reduced fat oxidation rate are evident under conditions of carbohydrate ingestion.*

The enhanced carbohydrate oxidation and diminished fat oxidation with ingestion of carbohydrate results from an increase in circulating blood glucose and insulin affecting the glucose-fatty acid cycle (Ahlborg & Felig, 1976). Consequently:-

Hypothesis 3: *Plasma glucose levels remain elevated whilst there is an attenuation of the NEFA concentrations after carbohydrate ingestion.*

Hypothesis 4: *Plasma insulin concentrations are elevated whereas plasma glucagon and plasma catecholamine levels are lowered in response to carbohydrate ingestion.*

During the last decade much attention has been focused on the optimum type of carbohydrate to ingest in order to prolong physical performance. Various studies have been reported on the efficacy of glucose, fructose, maltose, sucrose, and maltodextrins (Murray, 1987). Solutions of maltodextrins comprised of polymerised glucose units of varying chain lengths exert less osmotic pressure than equimolar solutions of glucose or other simple sugars. This characteristic is considered to be advantageous in the light of research indicating the role played by osmolality in determining gatric emptying rates, and may well lead to an enhanced uptake of maltodextrin compared with glucose. It follows therefore that:-

Hypothesis 5: *Maltodextrin ingestion produces an enhanced carbohydrate oxidation, higher plasma glucose and reduced plasma NEFA concentrations when compared with glucose ingestion, and that this results in elevated plasma insulin and lower plasma glucagon and catecholamine levels.*

The addition of guar gum, an endosperm extract of the Indian Cluster bean, to carbohydrate meals and drinks attenuates post

Preliminary testing. Maximal oxygen uptake (VO_2max) was determined on a Monark 911 cycle ergometer using a continous, incremental test to volitional exhaustion. Pedal frequency was maintained at 60 rev min^{-1} throughout the test, with 30 watt increments every 2 min until the subject could no longer maintain the cadence. Oxygen consumption (VO_2) was monitored continuously with subjects breathing through a low resistance valve (Jakeman & Davies, 1979) to an automated gas analysis system (P.K.Morgan, Rainham, UK). The latter incorporated a Fleisch No 3 pneumotachograph for measuring ventilation, a paramagnetic oxygen analyser, and an infra-red carbon dioxide analyser. Calibration of the analysers was achieved by using certified gases of known concentration (British Oxygen Corporation, UK) prior to each test. Values for VO_2 were obtained at 1 min intervals up to the point of volitional exhaustion. Results from the VO_2max test were used to establish the exercise intensity that corresponded to each subject's 65% and 75% value in the subsequent trials.

Experimental design. Subjects visited the laboratory on five occasions, with each visit separated by no more than 3 weeks. Immediately prior to each cycle ride at 65% VO_2max for 90 min, the subjects consumed 400 ml of either water (Pl), or 1 g kg^{-1} body mass of glucose (Gl) or maltodextrin (Md), or 1 g kg^{-1} body mass of glucose (Gl+Gu) or maltodextrin (Md+Gu) with 8% guar gum. All drinks were orange flavoured, and the order of trials was counterbalanced. The carbohydrate ingestion resulted in an energy intake of 1253.3 $\pm$ 149 5 kJ, with the concentration of the drinks being 18.65 $\pm$ 2.2%. The choice of concentration for the guar gum

was reached on examination of the findings of Jenkins et al. (1977) and on the viscosity encountered.

After an overnight fast of approximately 12 hours, each subject arrived at the laboratory, voided urine and changed into appropriate clothing. A 16 guage cannula was then inserted into an antecubital vein under local anaesthesia before the subject rested for 30 min. Following this period of time, two 10 ml samples of blood were withdrawn in lithium-heparin tubes (one containing Trasylol/Aprotinin for later analysis of glucagon). Subjects then ingested the trial drink over a 2-3 min period before cycling at an exercise intensity corresponding to 65% VO_2max. After 15, 30, 45, 60, 75, and 90 min of exercise, measures of VO_2 and respiratory exchange ratio (RER) were made over a 5 min period. Further duplicate 10 ml blood samples were taken at 15, 30, 60, and 90 min of exercise. Following 90 min of exercise, the subjects were allowed to rest for 10 min before a timed cycle to exhaustion was undertaken at an exercise intensity corresponding to 75% VO_2max. A final set of blood samples was taken at the point of exhaustion (a time point when the subject could no longer keep pedalling).

Analyses. Venous blood samples were collected in lithium-heparin tubes and used immediately for the determination of haemoglobin by the cyanmethhaemoglobin method, and for packed cell volume using a micro-haematocrit centrifuge (Hawksley Ltd., UK). Changes in plasma volume were estimated according to the method of Dill and Costill (1974). The remaining blood was centrifuged at a

speed of 3,000 rev min^{-1}, aliquoted, and then stored at -20°C before being analysed for glucose, lactate, non-esterified fatty acids (NEFA), insulin, and glucagon. Plasma samples for the determination of catecholamines were stored at -70°C.

Plasma glucose concentrations were analysed using the GOD-PERID enzymatic spectrophotometric method (Bergmeyer & Bernt, 1974) with kits supplied from Boehringer-Mannheim, UK and on a Cobas-Bio Centrifugal analyser (Roche Products Ltd, Welwyn Garden City, Herts, UK). The coefficient of variation (CV) at concentrations of 4-6 mM was 3.5%, and the within-batch precision of repeated pooled samples was less than 4%.

Plasma NEFA was assayed using an enzymatic spectrophotometric method (NEFA kit from WAKO Chemicals, Germany supplied by Alpha Laboratories, London) in conjunction with the Cobas-Bio centrifugal analyser. The CV at a concentration of 1.5 mM was 4.9% (Precinorm, Boehringer-Mannheim, UK).

Perchloric acid extracts of plasma lactate were also analysed on the centrifugal analyser using the enzymatic method of Gutman and Wahlefeld (1974). The CV of plasma samples between 2-4 mM was 2.0%.

Plasma insulin was determined using radio-immunoassay (RIA) with an Insulin RIA kit (IM.78) from Amersham International, Amersham, UK. Within-assay CV quoted by the manufacturers of the kit was 12% at 5uIU ml^{-1} and less than 5% at insulin concentrations of

between 15 and 100 uIU ml^{-1}.

Plasma glucagon was measured using a commercially available ^{125}I-glucagon RIA kit (IDS Ltd, Bournemouth, UK). Between-batch precision quoted by the manufacturer was less than 15% at concentrations between 140-900 pg ml^{-1}.

Plasma catecholamines were first concentrated and partially purified using a two-tiered technique for plasma preparation (Frayn & Maycock, 1983). This involved an initial clean-up using Bond Elut cation exchange columns (AnalytiChem International, Harbor City, CA), followed by adsorption of the buffered eluate on "in-house" prepared alumina columns. Catecholamine concentrations were then measured by reverse-phase high pressure liquid chromatography (HPLC) and electro-chemical detection (LDC Analytical, Stone, Staffs, UK). Catecholamine concentrations were obtained by calculating the areas under the curve from the chromatograms produced. The percentage recovery of standards was 74% (±12.0%) for noradrenaline, and 71% (±9.7%) for adrenaline. Between-batch precision was less than 10%.

Calculations. Carbohydrate oxidation rates were calculated from the non-protein RER values and VO_2, using Zuntz's (1900) table (Appendix A).

Statistics. Results from the plasma and respiratory analyses were subjected to analysis of variance (ANOVA) with repeated measures. Where significant differences were found, a post-hoc HSD test was

employed. Results for the times to exhaustion were examined using paired t-tests. Significance was accepted at the 0.05 level of probability.

4.3 RESULTS

The times to exhaustion at 75% VO_2max (Table 4.1) showed significant differences between the carbohydrate trials and the placebo trial ($P< 0.05$). Paired t-tests highlighted these improvements, and it can be seen that they reflect a 128%, 166%, 176%, and 196% increase in performance for Gl, Gl+Gu, Md, and Md+Gu respectively. No significant differences were apparent between the carbohydrate trials except for Gl and Md+Gu.

Table 4.1 Times to exhaustion and 't'-values.

Trial	Time to Exhaustion (s)	Pl	Gl	Gl+Gu	Md	Md+Gu
Pl	163 ± 53	-				
Gl	372 ± 155	3.9*	-			
Gl+Gu	423 ± 156	4.1*	2.0	-		
Md	450 ± 186	4.5*	1.4	0.7	-	
Md+Gu	483 ± 167	4.7**	2.9*	1.3	0.5	-

Significant differences were found for RER and for VO_2 between the placebo and carbohydrate trials according to ANOVA ($F_{4,16}$=20.6; P<0.01 and $F_{4,16}$=5.2; P<0.05), with the latter providing higher values than the placebo. These findings resulted in significantly reduced rates of carbohydrate oxidation for the placebo trial compared to the carbohydrate trials ($F_{4,16}$=26.2; P<0.01). Tables 4.2-4.4 present the mean data for RER, VO_2, and carbohydrate oxidation rate respectively.

Table 4.2 Mean (±SD) respiratory exchange ratios during the five trials.

Time (min)	Pl	Gl	Gl+Gu	Md	Md+Gu
15	0.86±0.015	0.86±0.016	0.86±0.018	0.87±0.047	0.86±0.017
30	0.84±0.005	0.88±0.026	0.88±0.020	0.89±0.012	0.88±0.020
45	0.81±0.013	0.90±0.019	0.88±0.016	0.90±0.015	0.88±0.015
60	0.80±0.008	0.89±0.013	0.89±0.019	0.89±0.020	0.85±0.021
75	0.79±0.011	0.89±0.037	0.88±0.024	0.89±0.022	0.85±0.021
90	0.79±0.008	0.87±0.028	0.86±0.023	0.87±0.013	0.83±0.027

Table 4.3 Mean (±SD) oxygen consumption (ml min^{-1}) for the five trials.

Time (min)	Pl	Gl	Gl+Gu	Md	Md+Gu
15	2198±435	2368±572	2310±469	2393±523	2205±443
30	2374±660	2614±852	2476±673	2581±690	2530±595
45	2413±726	2569±763	2551±721	2593±740	2599±659
60	2430±697	2628±853	2598±708	2642±798	2688±658
75	2588±667	2576±800	2684±816	2670±741	2647±692
90	2612±650	2634±610	2710±746	2678±695	2686±714

The total amount of carbohydrate oxidized was estimated by averaging the carbohydrate oxidation rates for each trial and multiplying by the time to exhaustion. The placebo trial produced a total amount of carbohydrate oxidized of 93 g, whereas the carbohydrate trials gave estimated values of 163 g, 158 g, 168 g, and 143 g for Gl, Gl+Gu, Md, and Md+Gu, respectively. Subtraction of the placebo value from the carbohydrate values give an indication of the amount of glucose oxidized by the muscle from that which is ingested, assuming that the endogenous stores are utilized to the same extent. This would point to the possibility that 85-100% of the ingested carbohydrate is oxidised

in the exercise period.

Table 4.4 Mean (±SD) carbohydrate oxidation rates (g min^{-1}) for the five trials.

Time (min)	Pl	Gl	Gl+Gu	Md	Md+Gu
15	1.36±0.36	1.50±0.52	1.43±0.44	1.49±0.44	1.50±0.33
30	1.24±0.34	1.85±0.59	1.75±0.57	1.93±0.63	1.82±0.51
45	0.96±0.26	2.04±0.68	1.80±0.63	2.03±0.69	1.91±0.55
60	0.91±0.30	1.92±0.64	1.95±0.66	1.93±0.67	1.89±0.60
75	0.89±0.30	1.81±0.44	1.89±0.61	1.99±0.59	1.80±0.49
90	0.85±0.27	1.71±0.48	1.74±0.55	1.79±0.46	1.69±0.42

Mean plasma glucose concentrations were similar for all conditions at rest. Levels fell slowly during exercise under Pl, although ingestion of carbohydrate maintained the plasma glucose levels above resting values throughout the exercise period (Fig 4.1). Significant differences between the treatments were evident ($F_{4,16}$=6.2; P<0.01), with the post-hoc tests highlighting the differences between the placebo and the carbohydrate trials (P<0.05). No significant differences were found between the carbohydrate trials.

The placebo trial resulted in a gradual and significant increase in NEFA concentration during exercise ($F_{5,20}$=12.08; P<0.01), whereas ingestion of carbohydrate attenuated this response (Fig 4.2). There were significant differences in NEFA concentrations between the trials ($F_{4,16}$=6.2; P<0.01) with the post-hoc tests emphasising the differences between Pl and the carbohydrate trials (P<0.05). The differences between the carbohydrate trials were not significant (P>0.05).

Lactate concentrations increased significantly from rest (Fig 4.3), and were notably higher after the ride to exhaustion at 75% VO_2max compared to the ride at 65% VO_2max ($F_{5,20}$=39.8; P<0.01). There were significant differences between Pl and the carbohydrate trials ($F_{4,16}$=9.1; P<0.01), with Pl producing significantly lower values (P<0.01).

The plasma insulin concentrations decreased during exercise in the placebo trial (Fig 4.4), although all carbohydrate trials resulted in significant increases in plasma insulin concentration within the first 30 min (P<0.01) before decreasing. The effects of the carbohydrate trials were significantly different ($F_{4,16}$=4.3; P<0.05), but only between Md and Pl (P<0.05). No significant differences were apparent between the carbohydrate trials.

The results for plasma glucagon showed a significant rise during exercise ($F_{5,20}$=10.0; P<0.01), but no significant differences were found between the trials ($F_{4,16}$=0.5 P>0.05). Figure 4.5

illustrates the mean glucagon concentrations during the trials.

Significant differences in plasma adrenaline concentration were found between the drinks ingested ($F_{4,16}$=3.6; P<0.05).The post-hoc tests established that the adrenaline concentrations for Pl were significantly higher than for Md (P<0.05). No significant differences were found between the carbohydrate trials (Fig 4.6).

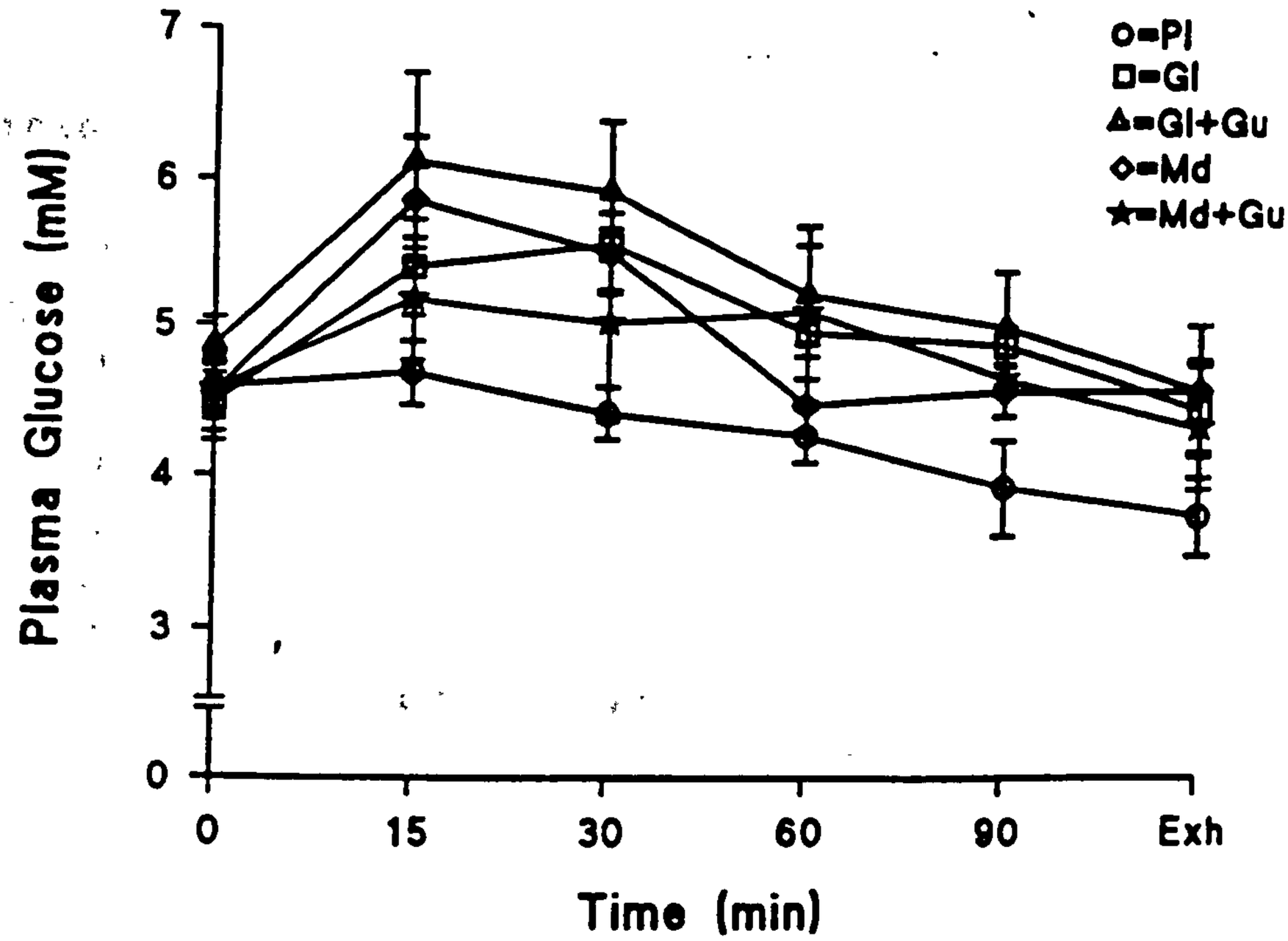


Fig 4.1 Mean (±SEM) plasma glucose concentrations (mM) during the five trials.

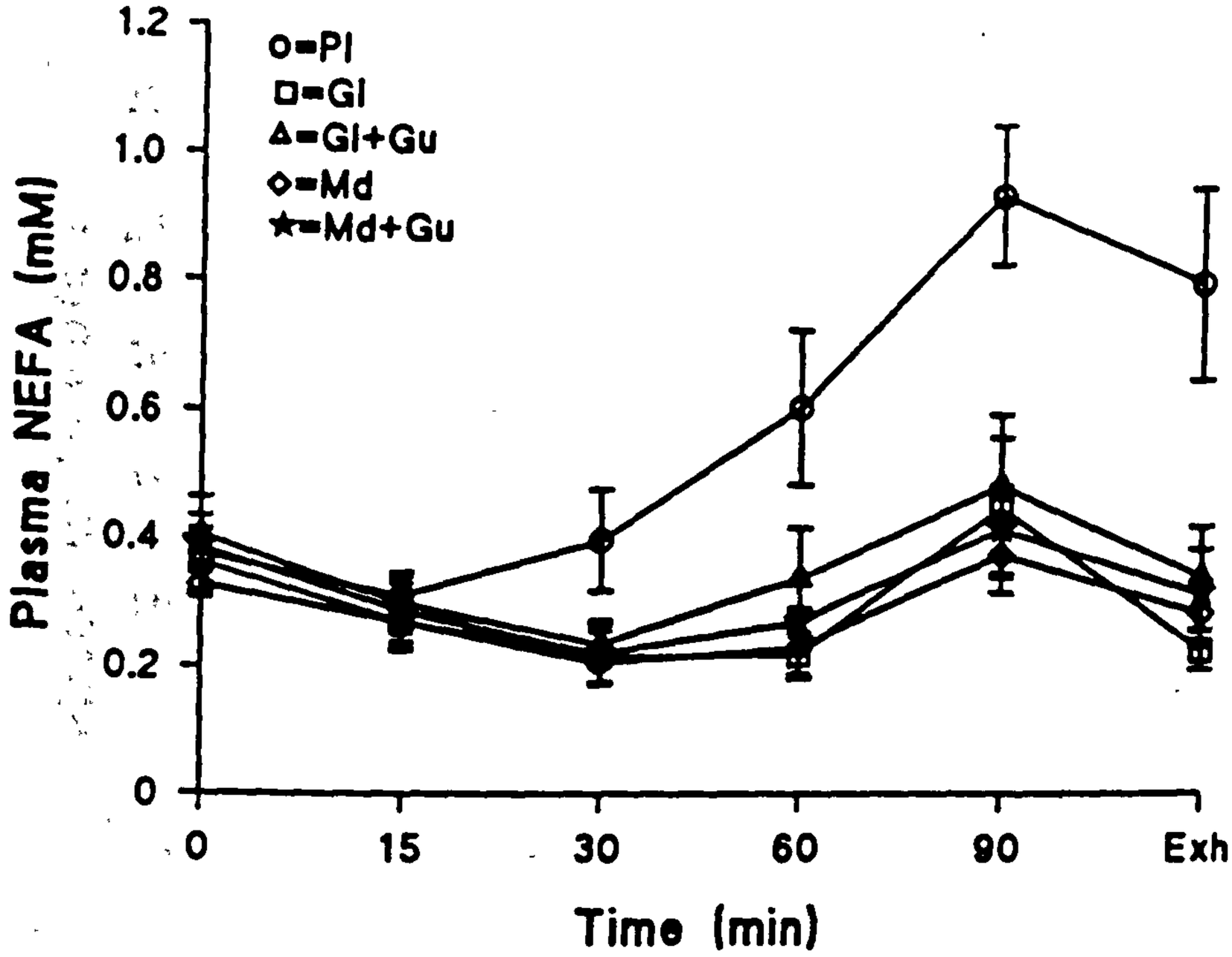


Fig 4.2 Mean (±SEM) plasma NEFA concentrations (mM) during the five trials.

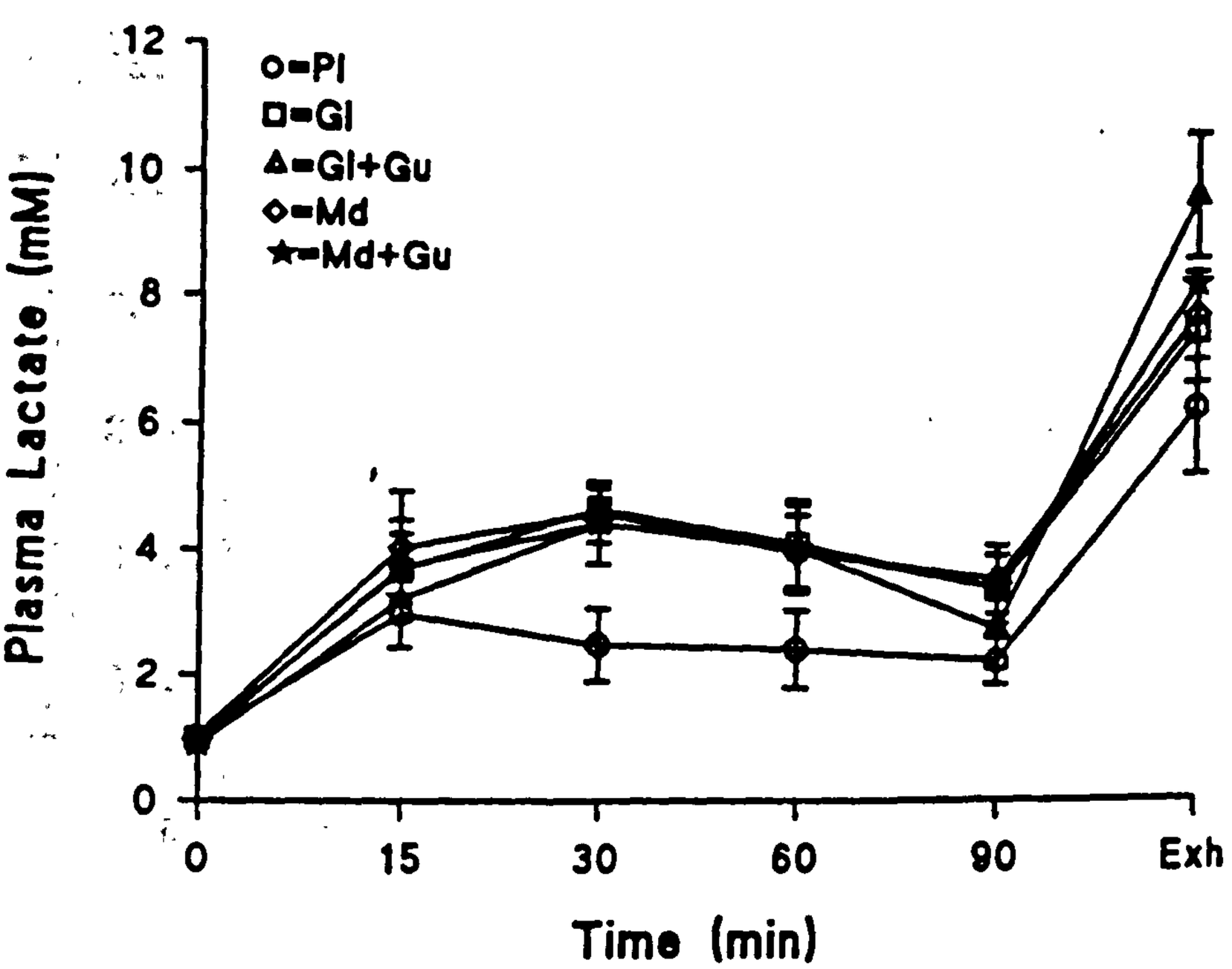


Fig 4.3 Mean (±SEM) plasma lactate concentrations (mM) during the five trials.

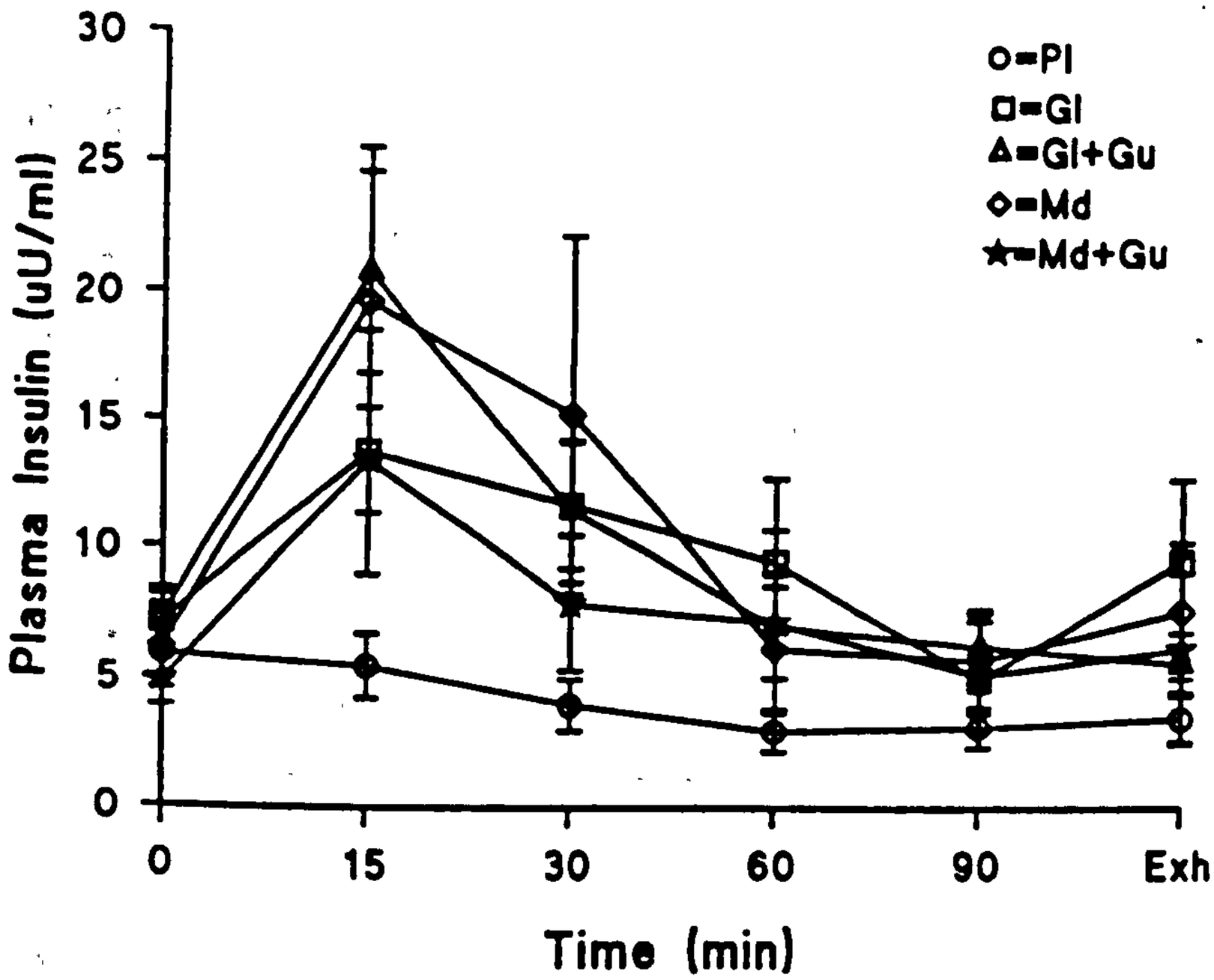


Fig 4.4 Mean (±SEM) plasma insulin concentrations (μU ml^{-1}) during the five trials.

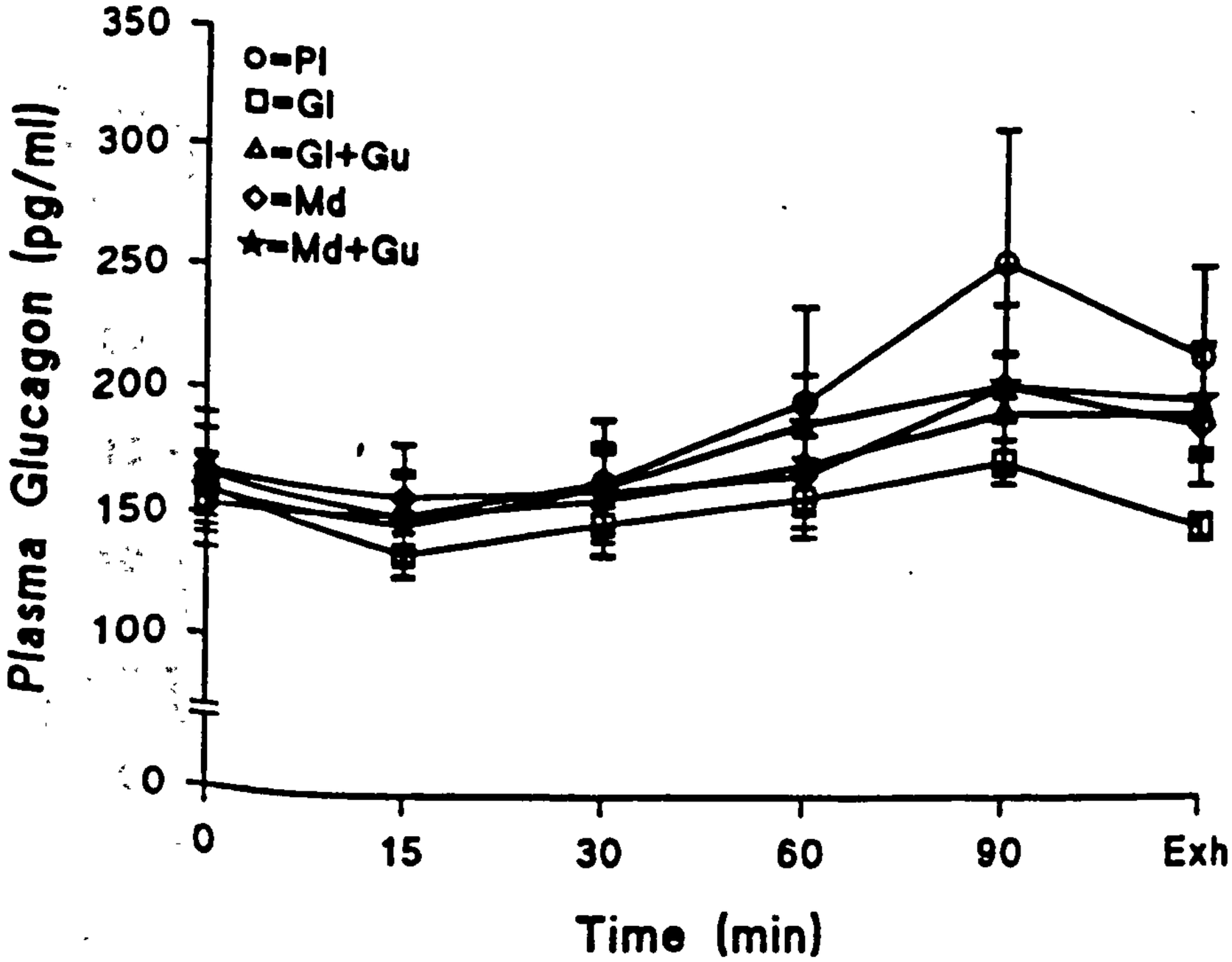


Fig 4.5 Mean (±SEM) plasma glucagon concentrations (pg ml^{-1}) during the five trials.

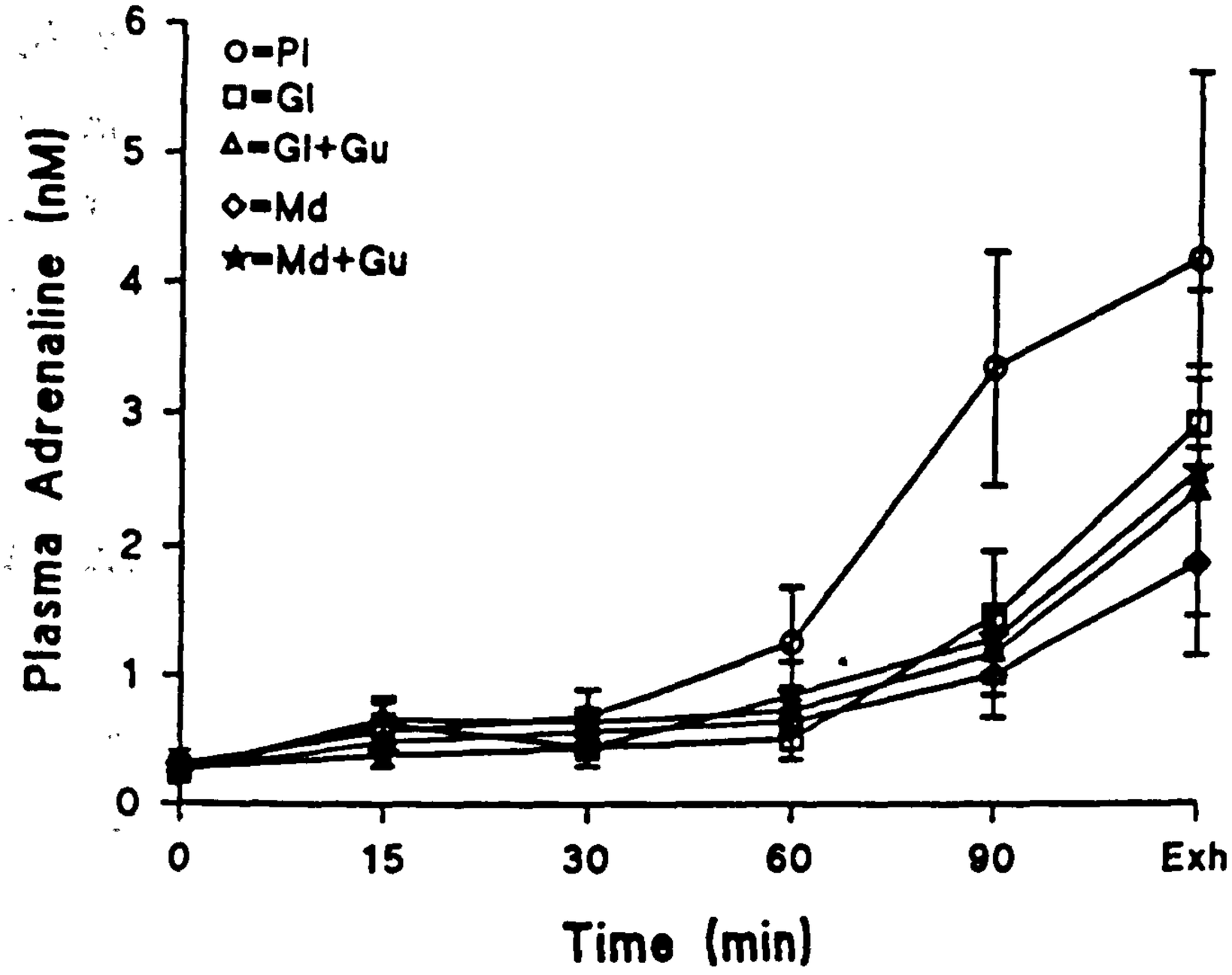


Fig 4.6 Mean (±SEM) plasma adrenaline concentrations (nM) during the five trials.

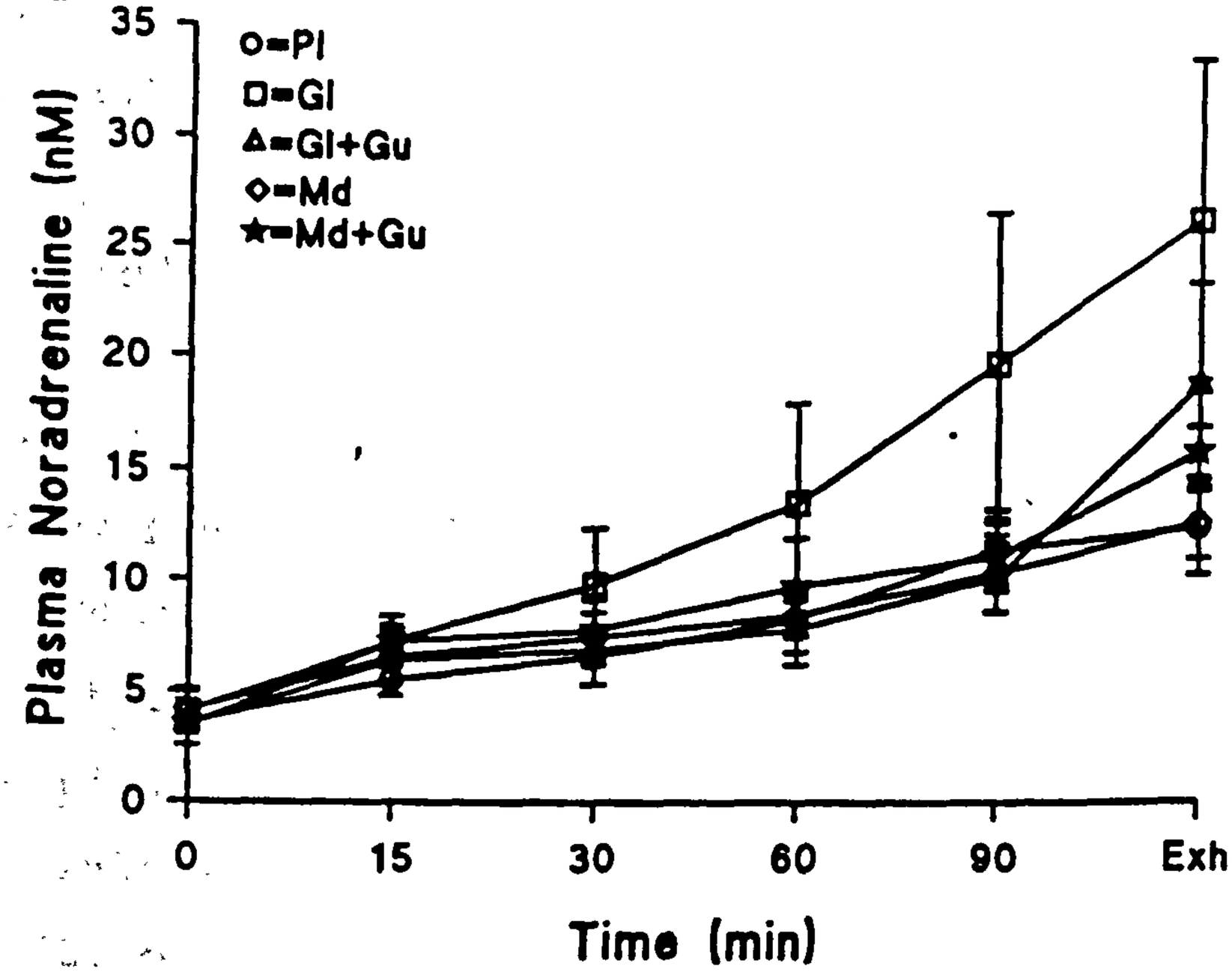


Fig 4.7 Mean (±SEM) plasma noradrenaline concentrations (nM) during the five trials.

Mean plasma noradrenaline concentrations (Fig 4.7) increased significantly during the course of the exercise irrespective of the trial ($F_{5,20}$=16.4; P<0.01). The ANOVA yielded no significant main effect between the trials ($F_{4,16}$=1.2; P>0.05).

4.4 DISCUSSION

Carbohydrate ingestion has been associated with enhanced exercise performance, and exogenous carbohydrate may serve as a supplementary fuel at a time when muscle glycogen stores are compromised (Coyle et al, 1986). Reductions in blood glucose have been shown to result in lowered muscle glucose uptake, a decreased rate of carbohydrate oxidation, and an impaired performance (Coyle et al., 1986; Coggan & Coyle, 1987; Neufer et al., 1987).

Due to the reduced availability of NEFA following carbohydrate ingestion, it would be expected that a greater proportion of energy is derived from carbohydrate stores. The significantly elevated plasma glucose levels and reduced plasma NEFA levels after carbohydrate ingestion points to the possibility of more energy being derived from carbohydrates under such conditions. Estimation of carbohydrate oxidation from VO_2 and RER confirmed this. Indeed it would appear from our calculations that most, if not all, of the carbohydrate ingested immediately prior to the exercise may have been oxidised i.e. approximately 75 g in 90 min. This is close to the rates of 50-70 g in 120 min found by

Massiccote et al. (1989).

The results of this study are clearly in accord with the observations of others that carbohydrate ingestion promotes plasma glucose concentrations, enhanced carbohydrate oxidation, and elevated RER values. As a consequence, there was a significant increase in time to exhaustion. Although muscle glycogen concentrations were not determined, the enhanced performance was likely to result either from a muscle glycogen sparing and/or an enhanced availability of exogenous glucose to the active muscles. The enhanced performance at 75% VO_2max after 90 min of steady-state exercise as observed in the carbohydrate trials may have resulted from an enhanced availability, uptake, and utilization of plasma glucose.

Concentrations of adrenaline and noradrenaline were found to increase significantly with the duration of exercise, the noradrenaline concentration being consistently higher than the adrenaline. The values in this study reflect the stress of exercise duration and intensity on the subjects. Carbohydrate ingestion was found to attenuate the rise in adrenaline (particularly Md), although no significant effect was obtained for noradrenaline. Changes in the availability of glucose alter the activity of sympathetic centres in the hypothalamus (Galbo, 1983). Glucose infusion during exercise causes a marked decrease in plasma adrenaline levels (Galbo et al., 1979). Since adrenaline stimulates adipose tissue lipolysis and inhibits insulin secretion, its decrease in plasma would result in lowered

fatty acid metabolism. The present results highlight the inhibitory effect of carbohydrate ingestion on plasma NEFA. This is further amplified by the elevation of plasma insulin during the carbohydrate trials. Insulin is a potent inhibitor of lipolysis in adipose tissue, and together with its stimulating effect on glucose uptake by exercising muscle, leads to the increased use of carbohydrates and reduced reliance of fats in these trials.

The form of carbohydrate and the addition of guar gum did not significantly affect either carbohydrate availablilty or performance. This is not entirely surprising in so far as the same total amount of carbohydrate and fluid was ingested in all carbohydrate trials. Although the osmolalities of the drinks would have varied, the caloric content and fluid volume are the most important considerations for gastric emptying of beverages whilst exercising (Murray, 1987). The delay in the elevation of plasma glucose and the expected impaired insulin response with guar gum did not materialise. It is possible that this finding was due to a combination of exercise and the concentration of guar gum used.

4.5 CONCLUSIONS

This study showed that ingestion of Gl, Gl+Gu, Md, and Md+Gu resulted in similar hormonal, metabolic, and performance responses to exercise. When compared to a placebo trial, however, there were significantly elevated concentrations of

plasma glucose and lactate, but significantly reduced levels of NEFA (thus supporting *hypothesis 3*). Elevated plasma glucose resulted in stimulation of insulin with concomitant inhibition of glucagon and the catecholamines (thus supporting *hypothesis 4*). The consequences of these changes on metabolism were an enhanced oxidation of carbohydrate and an improved performance (thus supporting *hypotheses 1 and 2*). No advantages were apparent when maltodextrin was ingested compared with glucose ingestion, nor were there any additional advantages when guar gum was added to the carbohydrates (thus refuting *hypotheses 5 and 6*).

5. HORMONAL AND METABOLIC RESPONSES TO GLUCOSE AND MALTODEXTRIN INGESTION DURING EXERCISE

5. HORMONAL AND METABOLIC RESPONSES TO GLUCOSE AND MALTODEXTRIN INGESTION DURING EXERCISE

5.1 INTRODUCTION

Carbohydrate ingestion during exercise has been shown to enhance performance of prolonged exercise (Coggan & Coyle, 1987; Coyle et al., 1983; Neufer et al.,1987). This improvement in performance may not necessarily be due to a sparing of muscle glycogen use during exercise, but it is more likely that the exogenous carbohydrate maintains elevated blood glucose levels at a time when the exercising muscles rely upon blood glucose for energy (Coyle,1991).

Whereas the previous experiment was concerned with the effect of carbohydrate ingestion immediately prior to exercise, the present experiment is concerned with the effects of carbohydrate ingestion administered prior to and during exercise. Since it has been established that carbohydrate ingestion results in elevated plasma glucose concentrations, enhanced carbohydrate oxidation, elevated insulin levels , and enhanced performance irespective of the form of carbohydrate, this study used a model in which the subjects were pre-stressed by undertaking a VO_2max test before steady state exercise to exhaustion began. Carbohydrate or placebo was ingested after the VO_2max test and during the subsequent exercise. Such an experiment should further emphasise the tendency towards using carbohydrate as an energy source, and the resultant changes in the hormonal mileu. Thus:-

Hypothesis 7: *Carbohydrate ingestion results in enhanced carbohydrate oxidation, reduced fat oxidation, elevated plasma glucose, lactate and insulin concentrations, and reduced plasma NEFA, glucagon, and catecholamine concentrations compared with placebo.*

Furthermore, since carbohydrates are being provided before and during exercise, a greater carbohydrate oxidation rate than in the previous experiment is anticipated, and that any potential beneficial effect of maltodextrin compared with glucose is likely to be realised. Hence:-

Hypothesis 8: *The difference in the mean rate of carbohydrate oxidation between the carbohydrate ingestion trials and the placebo trial is greater than the 0.83 g min^{-1} obtained in the previous experiment.*

Hypothesis 9: *Maltodextrin ingestion results in an enhanced carbohydrate oxidation, higher plasma glucose and reduced plasma NEFA concentrations when compared to glucose ingestion, and that this results in elevated plasma insulin and lower plasma glucagon and catecholamine levels.*

Hypothesis 10: *Maltodextrin ingestion results in a significantly longer time to exhaustion than is the case with glucose ingestion.*

4.2 METHODS

Subjects. Six male subjects who regularly participated in road cycling gave informed consent to take part in this study. The mean (±SD) age, body mass, and VO_2max were 27.3 (±4.3) years, 74.5 (±8.3) kg, and 4243 (±613) ml min^{-1} respectively.

Experimental design. Subjects visited the laboratory on three separate occasions, with a gap of no more than 3 weeks between each visit to eliminate any possible training effect. During each visit , which took place at 09:00 h after an overnight fast, subjects had an indwelling cannula placed into the back of their non-dominant hand 20 min before one 10 ml and one 5 ml resting blood sample were taken. A VO_2max test was then undertaken on an electronically braked cycle ergometer in the same manner as described in the previous chapter. Following the VO_2max assessment, there was a 20 min recovery period during which the cycle ergometer was pedalled at a 60 watt power output for 10 min. Half-way through the recovery period a 200 ml portion of either water as placebo (Pl), 10% glucose (Gl), or 10% maltodextrin (Md) was ingested; all drinks were flavoured with diabetic orange cordial so as to make them indistinguishable in taste. At the end of the recovery period further blood samples

were obtained before exercise began at a exercise intensity corresponding to 65% of the VO_2max which had just previously been measured. Subjects exercised to fatigue (the point at which the desired exercise intensity could not be maintained); the time to fatigue was recorded to the nearest minute. Blood samples were taken at 15, 30, 60 min, and at the point of fatigue, as were measures of VO_2 and RER for the determination of carbohydrate oxidation rate. At 20, 40, and 60 min intervals further 200 ml portions of the test drink were administered. Testing was performed in a balanced order.

Analyses. Blood samples were immediately centrifuged at 3000 rev min^{-1}. The plasma was then removed and frozen for later analysis for glucose, NEFA, lactate, insulin, glucagon, adrenaline, and noradrenaline using the same procedures as described previously.

Statistics. Time to exhaustion was treated by using paired 't'-tests. The plasma data and those of carbohydrate oxidation rates were analysed by ANOVA followed by Newman-Keuls post-hoc test if the F-value was significant at the 5% level of probability.

5.3 RESULTS

Table 5.1 illustrates the times to exhaustion for each of the trials together with the calculated 't'-values. No significant differences were apparent between the trials.

Significant differences between the trials were noted for the oxidation of carbohydrates ($F_{2,10}$=196.2;P<0.001), with the post-hoc test highlighting the significant differences between the two

Table 5.1 Times to exhaustion (min) and 't'-values for the three trials

Trial	Time to Exhaustion (min)	Pl	Gl	Md
Pl	104.7±12.1	-		
Gl	108.8±14.4	1.40	-	
Md	118.0±21.1	2.34	2.02	

carbohydrate treatments and the placebo (P<0.01). No significant differences were found between Md and Gl (P>0.05).

An increase in the rate of carbohydrate oxidation was observed during the exercise period ($F_{3,15}$=12.85;P<0.01), which was exclusively due to the carbohydrate trials (P<0.05). A reciprocal response was observed under conditions of placebo ingestion (Fig 5.1).

Mean plasma glucose concentrations were similar at rest for all conditions. Figure 5.2 reveals that the placebo trial resulted in plasma glucose concentrations which were consistently lower than the carbohydrate treatments throughout exercise. At the point of fatigue hypoglycaemia was not evident in any trial, although the placebo trial exhibited significantly lower concentrations than the others ($P<0.01$). Significant differences between the trials were confirmed using ANOVA ($F_{2,10}=23.87; P<0.01$), with the placebo trial producing significantly lower concentrations than the carbohydrate trials ($P<0.05$). No significant differences were apparent between the Gl and Md trials. Analysis of variance also revealed a significant change with time ($F_{6,30}=8.35; P<0.01$), with the Gl and Md treatments producing elevations in glucose concentrations throughout exercise ($P<0.05$).

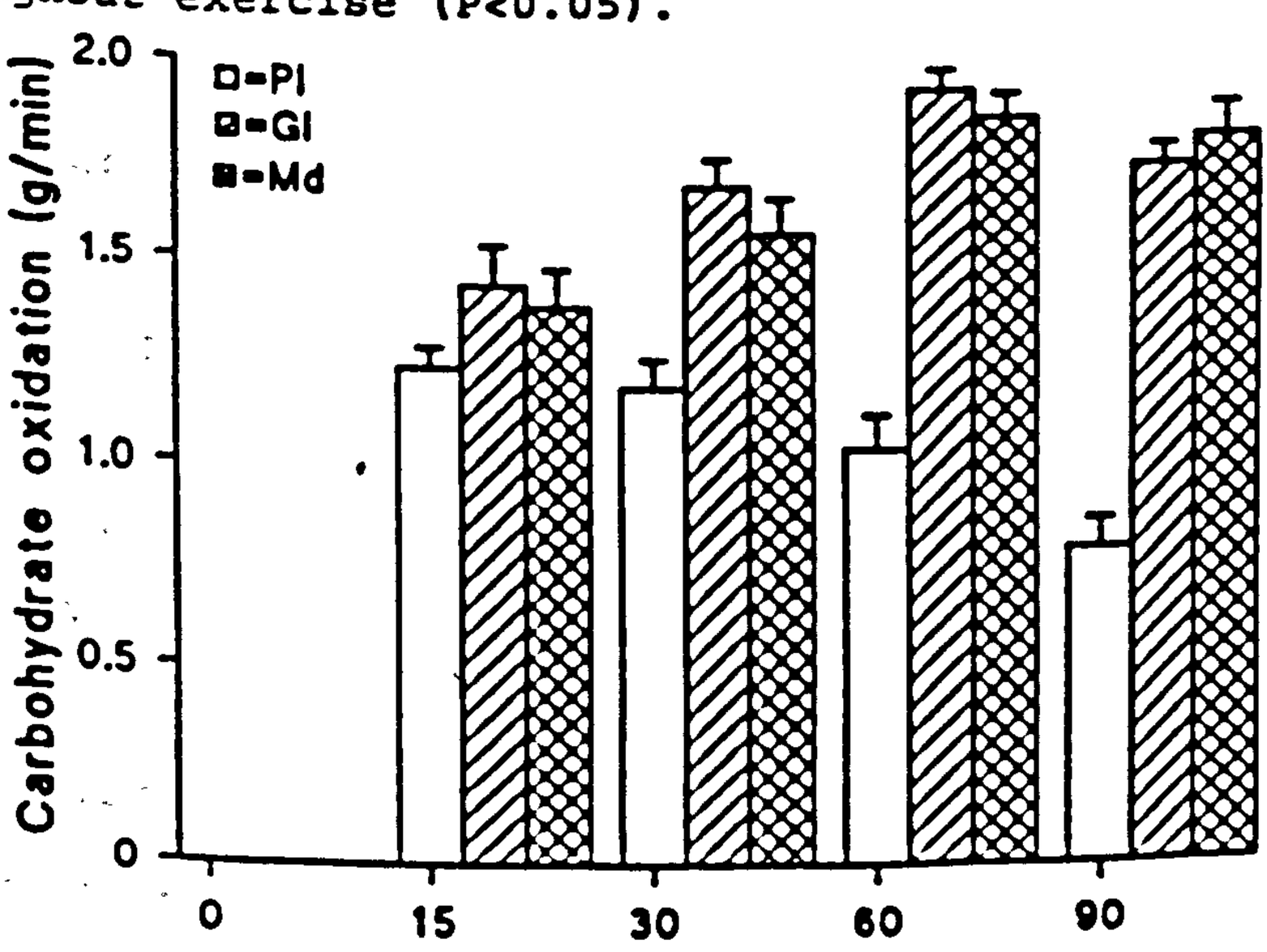


Fig 5.1 Mean (±SEM) rates of carbohydrate oxidation (g min^{-1}) for the three trials (□ = Pl, ▨ = Gl, ▩ = Md).

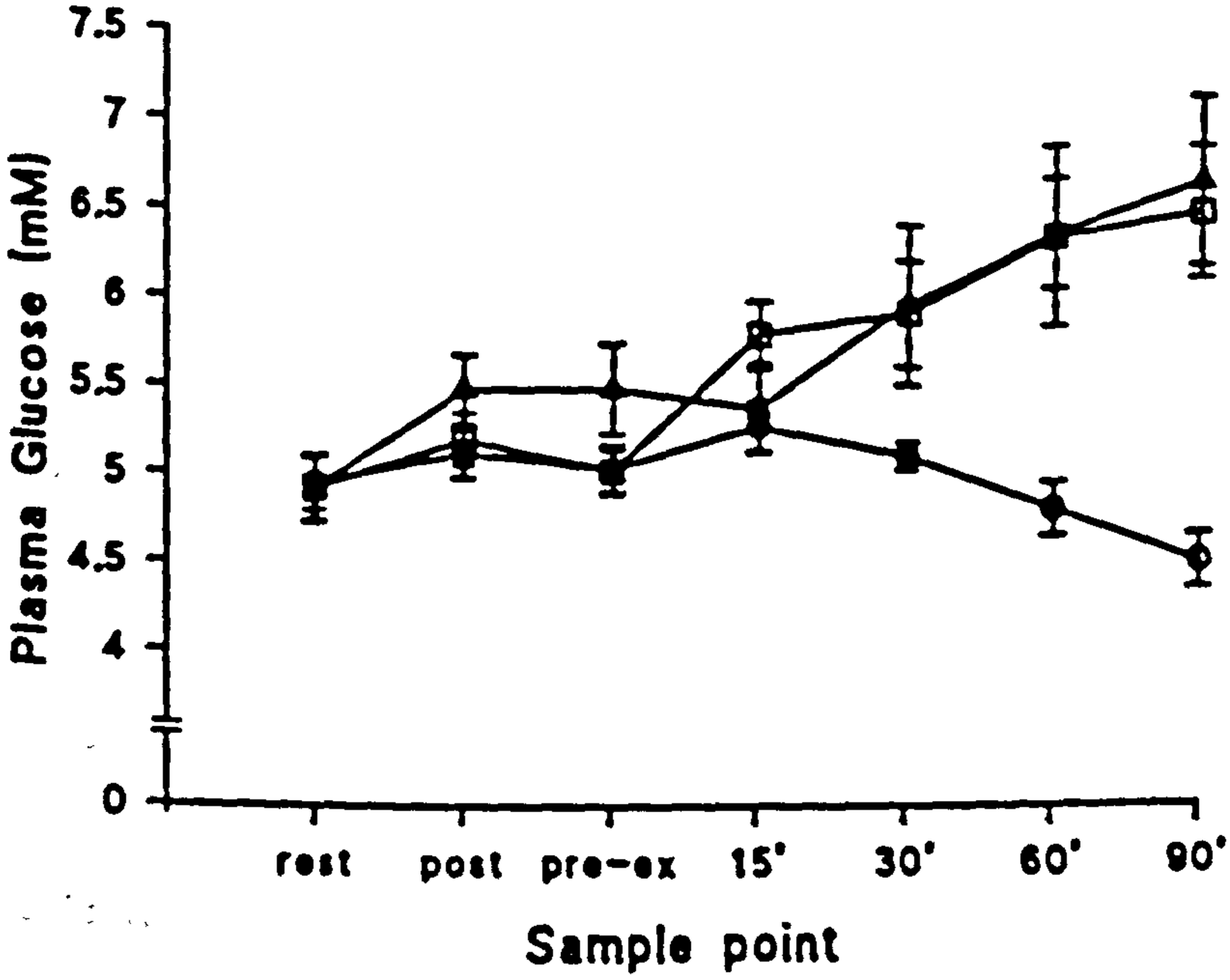


Fig 5.2 Mean (±SEM) plasma glucose concentrations (mM) for the three trials (O = Pl, □ = Gl, ▲ = Md).

The placebo trial produced a typical NEFA response during exercise in that plasma concentrations became elevated as the exercise progressed; the final mean concentration was nearly fivefold higher than the pre-exercise concentration. Attenuated responses were obtained under carbohydrate ingestion resulting in a significant time effect ($F_{6,30}$=144.13;P<0.01). Differences between the trials can clearly be seen (Fig 5.3), and this was reinforced by ANOVA ($F_{2,10}$=31.15;P<0.01). Post-hoc analyses revealed that these differences were between the carbohydrate trials and the placebo (P<0.01), but not between Gl and Md.

Lactate concentrations were significantly elevated after the

VO_2max test ($P<0.01$) to values averaging 11.4 mM; this represents an 800% increase from rest, and is in keeping with the criteria for subjects having reached maximum oxygen uptake. During the ensuing exercise period lactate concentrations decreased, but not to resting levels. Carbohydrate ingestion resulted in the plasma levels being consistently higher than for the placebo (Fig 5.4). A significant time effect ($F_{6,30}=107.57;P<0.01$) and significant effect of carbohydrate intake ($F_{2,10}=6.21;P<0.05$) on plasma lactate was highlighted by ANOVA.

Plasma insulin concentrations showed a similar reponse to the previous experiment during exercise under placebo, in that the levels decreased during exercise. The GL and Md treatments resulted in a trend towards a decrease during exercise (Fig 5.5) although they produced significantly higher concentrations ($F_{2,10}=7.36;P<0.05$) than the placebo.

Plasma glucagon rose significantly during exercise ($F_{5,25}=17.14;P<0.01$) and differed between the trials ($F_{2,10}=9.04;P<0.01$); glucagon concentrations were significantly higher for placebo compared with Gl and with Md ($P<0.05$). Figure 5.6 illustrates the mean plasma glucagon concentrations during the trials.

A significant effect of time with regard to plasma adrenaline concentrations was revealed ($F_{5,25}=123.55;P<0.01$), with the levels increasing during the exercise period (Fig 5.7). An attenuation

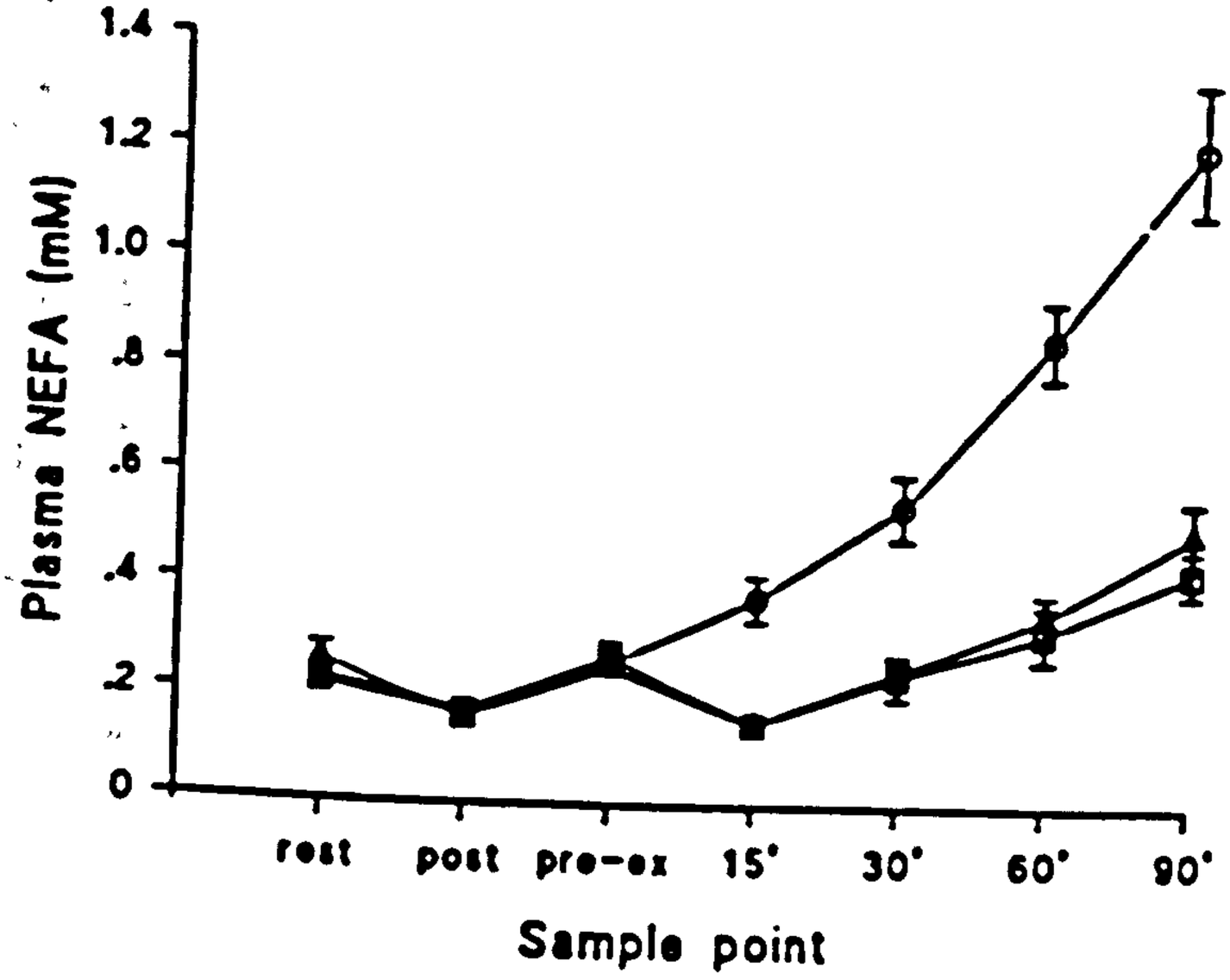


Fig 5.3 Mean (±SEM) plasma NEFA concentrations (mM) for the three trials (O = Pl, □ = Gl, Δ = Md)

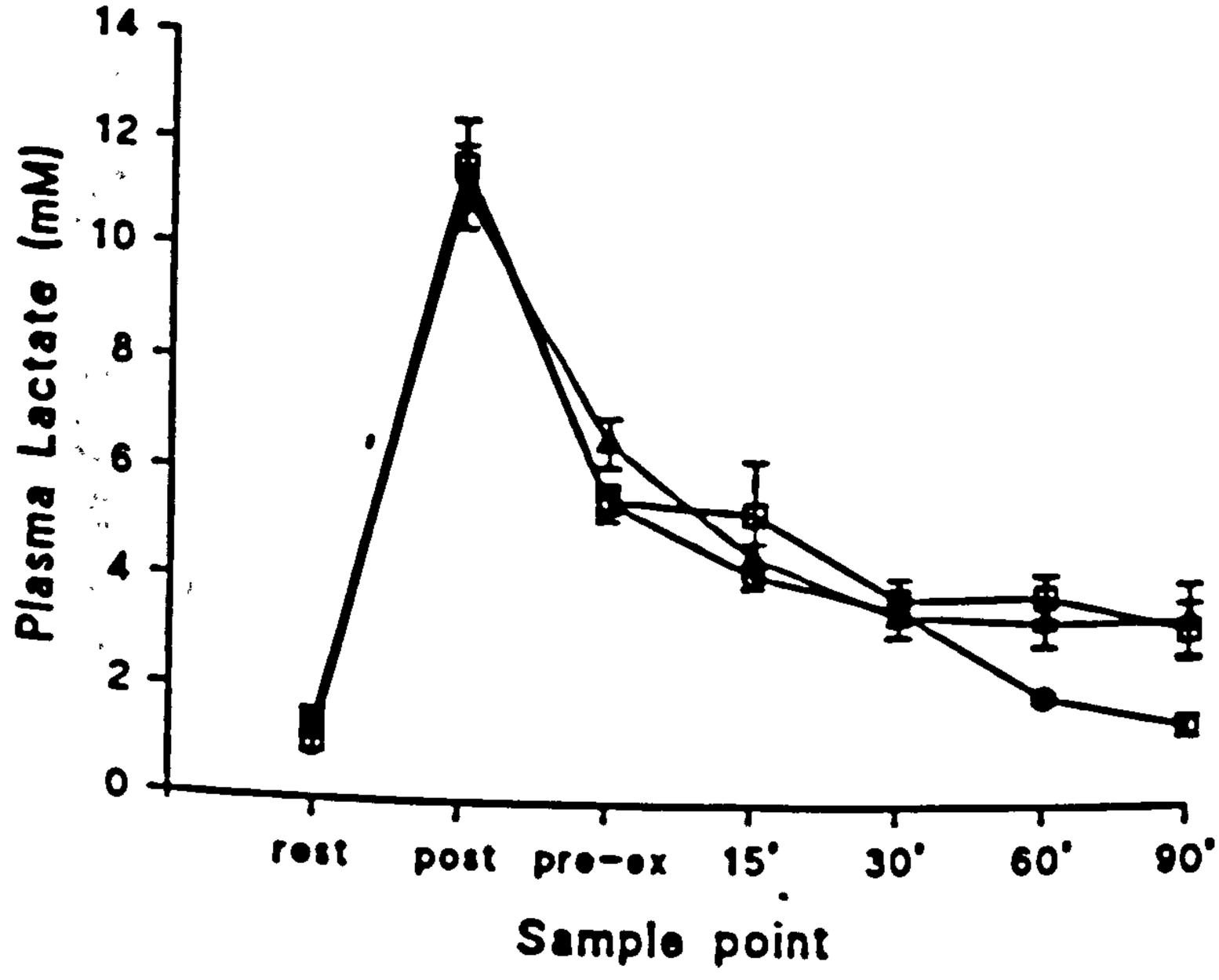


Fig 5.4 Mean (±SEM) plasma lactate concentrations (mM) for the three trials (O = Pl, □ = Gl, Δ = Md).

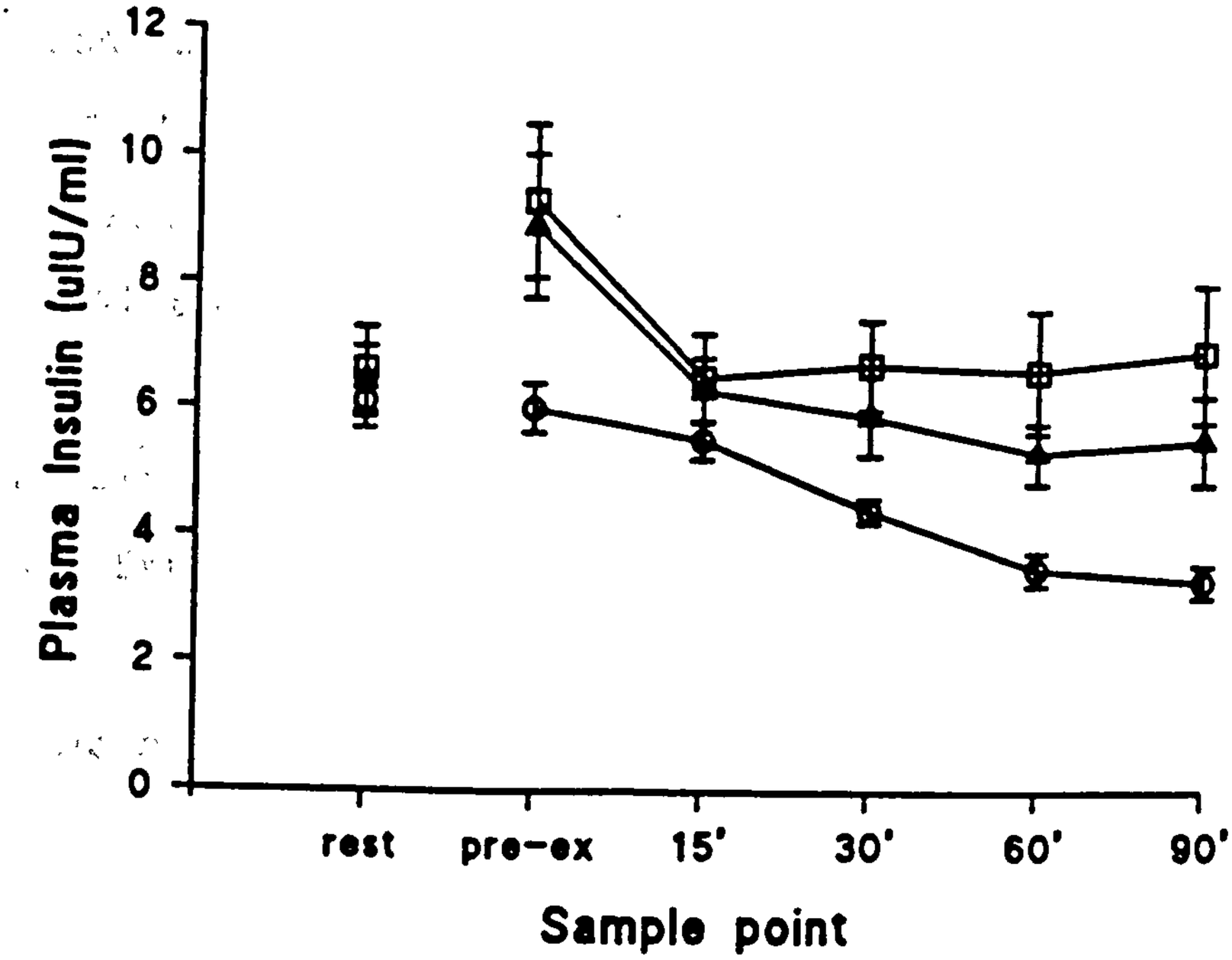


Fig 5.5 Mean (±SEM) plasma insulin concentrations ($\mu U\ ml^{-1}$) for the three trials (O = Pl, □ = Gl, Δ = Md)

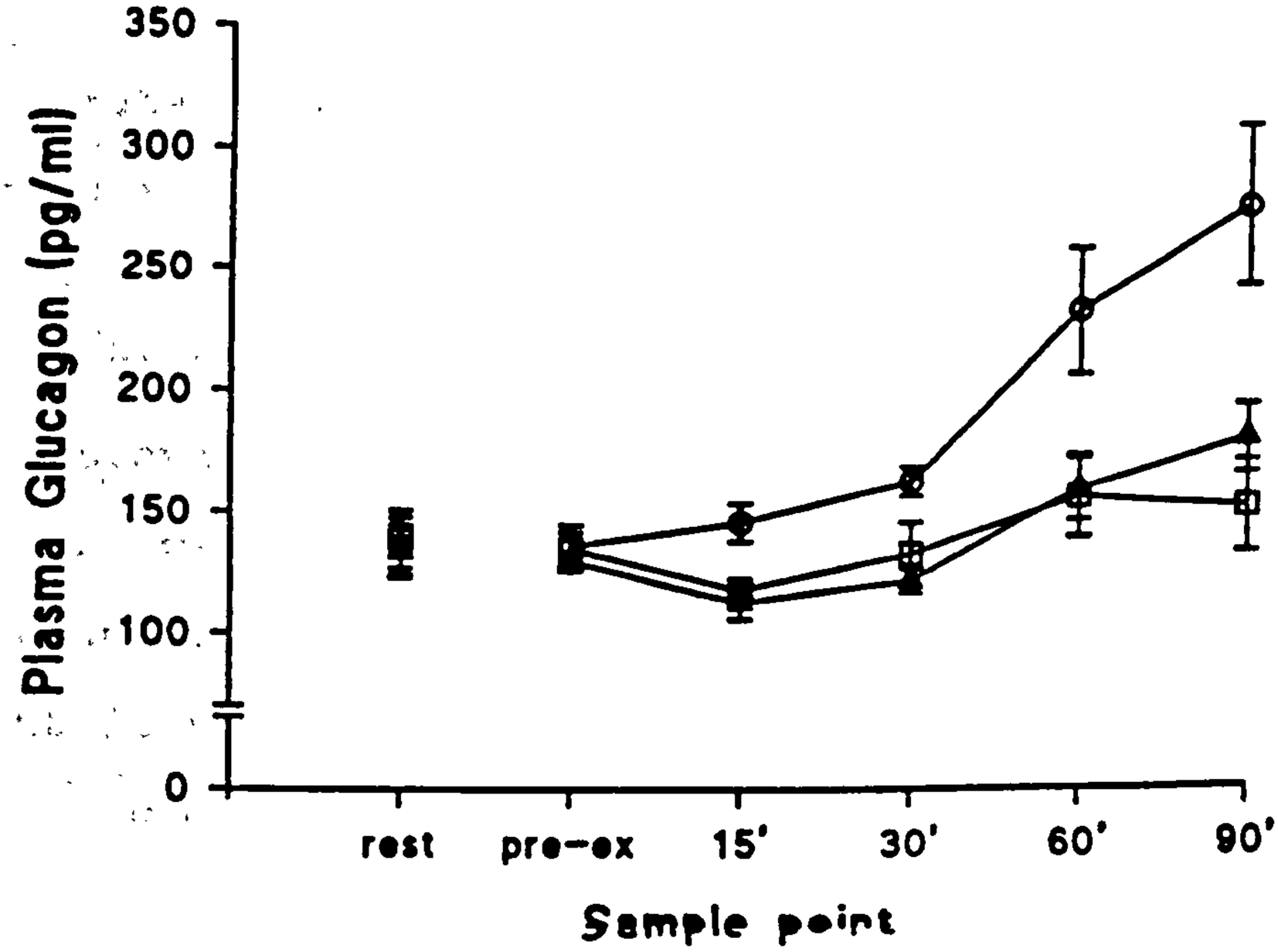


Fig 5.6 Mean (±SEM) plasma glucagon concentrations ($pg\ ml^{-1}$) for the three trials (O = Pl, □ = Gl, Δ = Md).

of responses was evident when comparing placebo with Gl and Md ($F_{2,10}$=4.80;P<0.05), although the only difference was between the placebo and Gl (P<0.05). There were no significant differences between Gl and Md.

Mean plasma noradrenaline concentrations (Fig 5.8) increased by 126% at fatigue compared with the pre-exercise levels. This change was significant according to ANOVA ($F_{5,25}$=71.76;P<0.01). Differences between the trials was,likewise, significant ($F_{2,10}$=5.05;P<0.05); ingestion of Gl and Md evoked an attenuated response (P<0.05).

5.4 DISCUSSION

Carbohydrate ingestion during exercise has been shown to promote performance and to delay fatigue (Coggan & Coyle, 1984). Although the differences between Pl and the carbohydrate trials were not statistically significant in this study, the 3.9% and 12.7% improvements in time to exhaustion are evident, and also in keeping with other studies which have revealed that time to exhaustion can be improved by 10% (Neufer et al.,1981). Also apparent is the 8.5% increase in time to exhaustion for the Md treatment compared with the Gl treatment. This response is not as great as the 20%, but non-significant, finding between the two treatments in the previous experiment, but nonetheless reflects a trend in favour of Md as an ergogenic aid.

As in the previous experiment, the ingestion of carbohydrate resulted in an enhanced plasma glucose response and an attenuated plasma NEFA response. Consequently there was a significant oxidation of carbohydrate when compared to the Pl trial. The greater availability of glucose from blood during the later stages of exercise has been proposed as a reason for improving endurance performance (Coyle, 1991). Estimated carbohydrate oxidation rates after 60 min of exercise were 0.9 g min^{-1}, 1.84 g min^{-1}, and 1.83 g min^{-1} for placebo, Gl, and Md respectively. Although it is not possible to determine how much of this oxidation is due to exogenous sources, clearly there appears to be a significant rate of carbohydrate being used when fatigue develops. The elevated plasma glucose concentrations at the time of fatigue would suggest that either the glucose cannot be taken up at a sufficiently high rate for use by skeletal muscle, or that other fatigue factors are involved. Since muscle glycogen stores were not examined during this study, it is not possible to quantify the relations between exogenous and endogenous carbohydrate useage.

The total carbohydrate oxidation rates estimated from this study were almost identical with those obtained from the previous investigation; mean rate of 1.08 g min^{-1} (total of 97 g over 90 min) for Pl, 1.81 g min^{-1} (total of 163 g over 90 min) for Gl, and 1.65 g min^{-1} (total of 149 g over 90 min) for Md. Similar grams of carbohydrate were consumed in both the previous investigation and this one (i.e. 80 g), although the concentration and timing of the drinks varied. The differences

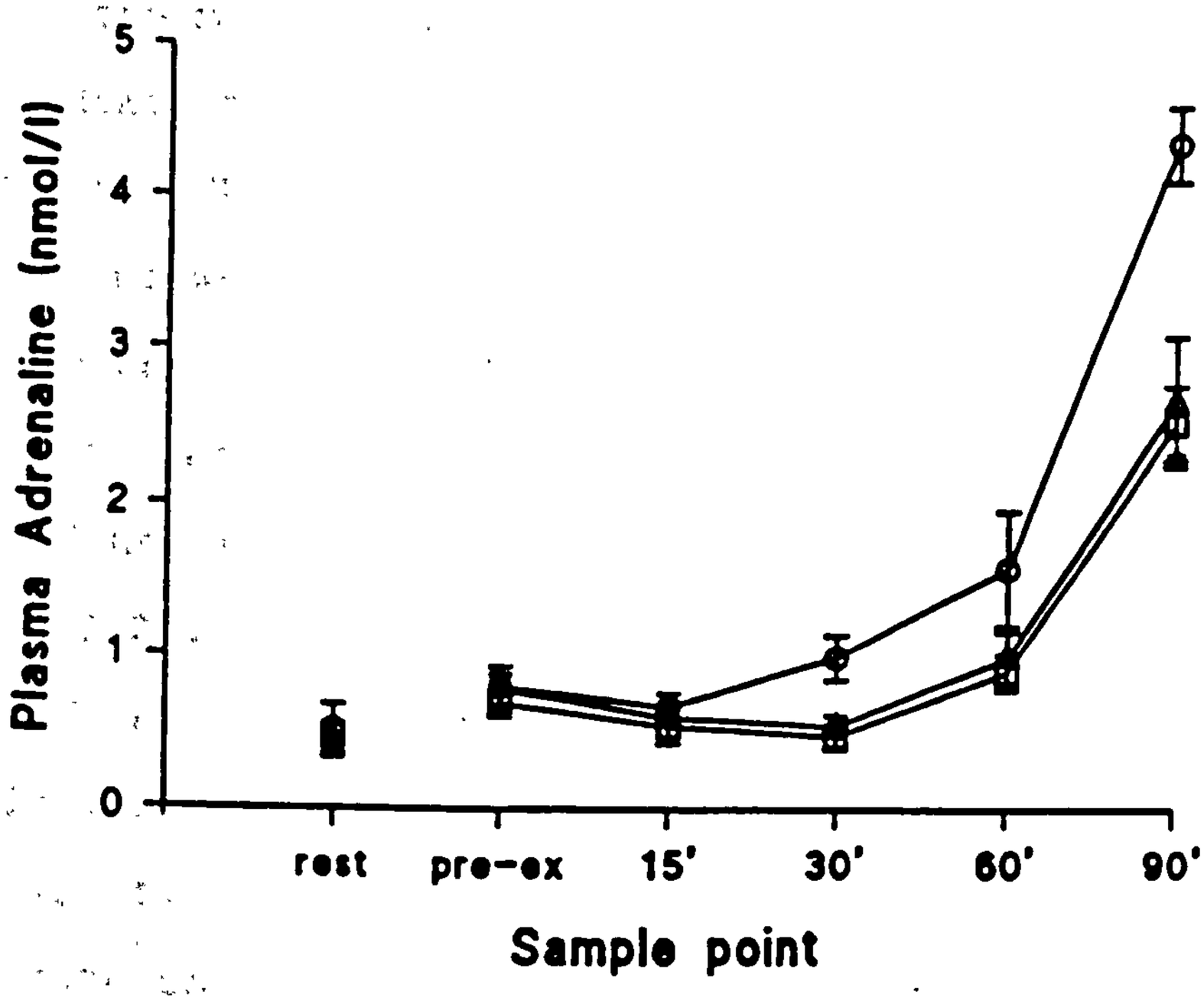


Fig 5.7 Mean (±SEM) plasma adrenaline concentrations (nmol l^{-1}) for the three trials (O = Pl, □ = Gl, ▵ = Md).

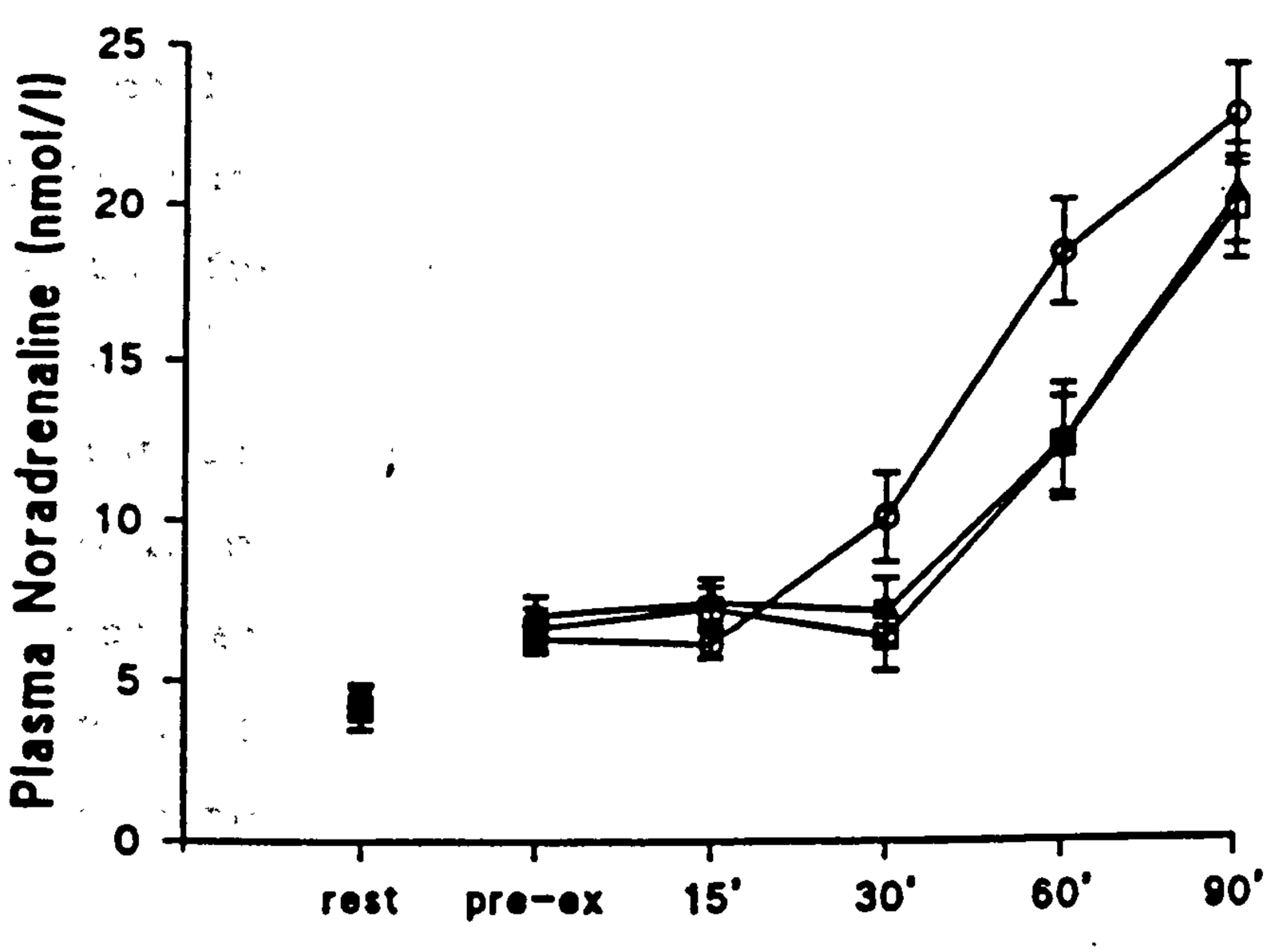


Fig 5.8 Mean (±SEM) plasma noradrenaline concentrations (nmol l^{-1}) for the three trials (O = Pl, □ = Gl, ▵ = Md).

between the mean total carbohydrate oxidation rates for the carbohydrate trials and the Pl were 0.58 g min^{-1} and 0.73 g min^{-1} for Md and Gl respectively. This was lower than the values reported between Pl and Md (0.84 g min^{-1}), and Pl and Gl (0.78 g min^{-1}) in the previous study. It appears that total carbohydrate oxidation rate is independent of the form and timing of carbohydrate ingested. This is supported by results from Moodley et al (1992). Their values of 148 to 164 g of total carbohydrate oxidised in 90 min of cycling at 70% VO_2 max were almost identical with the findings from this study. Furthermore, it is probable that 20-40% of the exogenous carbohydrate will contribute to the total oxidation rate of carbohydrates, as has been demonstrated by tracer studies (Massicotte et al.,1989; Moodley et al.,1992; Saris et al.,1993).

The hormonal responses determined in this experiment have modulated the substrate availability and resultant oxidation of metabolic fuels for the exercising muscles to function. Decreasing concentrations of insulin and increasing concentrations of glucagon and the catecholamines have invariably resulted in enhanced availability of NEFA as an energy source, thereby conserving muscle glycogen. Confirmation of the increased reliance on fats as exercise progressed can be seen with the decrease in total carbohydrate oxidation rate. On the other hand, ingestion of carbohydrate increased plasma insulin and reduced the availability of NEFA, leading to an increased reliance on carbohydrates.

The stimulation of insulin secretion by carbohydrates was balanced by inhibition of glucagon, an insulin antagonist, as well as an attenuated catecholamine response. The exact mechanism of the interrelationship between glucose and catecholamine is not understood. As the exercise proceeded, the increase in catecholamine secretion ensured that insulin concentrations did not rise appreciably, although they were higher than for the Pl trial. The fact that the insulin concentration did not rise above 6-7 uU ml^{-1} even when carbohydrate drinks were given at regular intervals throughout the trials demonstrates the inhibitory effect of elevated adrenaline concentrations prior to the steady-state exercise. Adrenaline concentrations have been shown to return to near pre-exercise levels within 20 min (Galbo, 1983), but clearly the plasma values were still elevated 20 min after the VO_2max test.

5.5 CONCLUSIONS

The results from this study were consistent with the previous investigation in that carbohydrate ingestion promoted carbohydrate oxidation, elevated plasma glucose and insulin concentrations, but diminished lipid oxidation and the plasma FFA, glucagon, and catecholamine responses (thus supporting *hypothesis 7*). Consistent findings were also evident between this and the previous study on examination of the differences between the placebo trial and carbohydrate ingestion trial for the mean rates of carbohydrate oxidation (thus refuting *hypothesis 8*).

Maltodextrin ingestion during exercise failed to enhance carbohydrate oxidation rates, plasma glucose and insulin concentrations significantly, or diminish significantly the plasma NEFA, glucagon, and catecholamine concentrations compared to the glucose ingestion trial (thus refuting *hypothesis 9*). The result of similar hormonal, metabolite, and metabolic responses between the two carbohydrate trials resulted in no significant effect on time to exhaustion (thus refuting *hypothesis 10*). Clearly carbohydrate ingestion favours utilisation of carbohydrate as a metabolic fuel, although the form and timing of carbohydrate ingested is not of significance.

6. HORMONAL AND METABOLIC RESPONSES TO MAINTAINED HYPERGLYCAEMIA AT REST

6. HORMONAL AND METABOLIC RESPONSES TO MAINTAINED HYPERGLYCAEMIA AT REST

6.1 INTRODUCTION

It is generally accepted that the glucose-fatty acid cycle operates in the heart and in skeletal muscle under resting conditions (Randle et al., 1963, 1964). Oxidation of fatty acids inhibits glucose uptake, glycolysis, glycogenolysis, and pyruvate oxidation, whilst ingestion of carbohydrates or infusion of glucose attenuates this response. Non-esterified fatty acids (NEFA) have been recognised as one of the main energy sources during rest as well as during exercise. Bulow (1981) reported that 46% of the energy at rest is derived from fats. The rate of mobilisation of NEFA shows an inverse relationship to the amount of carbohydrate being used in the body. Studies have shown that increasing quantities leave adipose tissue and enter the blood during fasting, whereas there is a drop in their release and a fall in blood levels following a meal or glucose infusion (Dole, 1956). These responses would not be reflected to the same degree if high levels of blood glucose can be maintained. Thus:-

Hypothesis 11 *Maintained hyperglycaemia results in enhanced carbohydrate oxidation, diminished fat oxidation, and decreases in plasma NEFA and glycerol.*

Variations in circulating hormones control the release of fatty

response remains unchanged in normal subjects (White et al., 1987). Since it has been demonstrated that insulin secretion is stimulated by increased levels of blood glucose and that there is a concomitant inhibition of glucagon secretion, it is hypothesised that:-

Hypothesis 12 *Maintained hyperglycaemia results in stimulation of insulin secretion with a concomitant inhibition of glucagon production. Catecholamine and cortisol concentrations are unaffected.*

Studies which have used control subjects under conditions of maintained hyperglycaemia have shown that glucose utilisation rate is steadily increased during the infusion (DeFronzo et al., 1979; Copeland et al., 1987; White et al., 1987). Hence:-

Hypothesis 13 *Maintained hyperglycaemia results in a steady increase in glucose utilisation.*

The use of the hyperglycaemic glucose clamp technique and subsequent measures of VO_2, RER, hormones, and metabolites should enable useful information to be gleaned concerning the regulation of metabolism at rest. Furthermore, this study provides a useful base from which to extend the technique to conditions of exercise.

6.2 METHODS

Subjects. Five healthy subjects, 4 male and 1 female, volunteered to take part in this study, and gave their informed consent in accordance with the ethics committees of the Regional Health Authority and of Liverpool Polytechnic (now Liverpool John Moores University). The mean (±SD) age, height, and body mass of the subjects were 32.2 ± 9.3 years, 1.80 ± 0.08 m, 78.8 ± 11.5 kg, respectively.

Experimental design. On the day of the trial, subjects arrived in the laboratory at 08:00 hours after a 12-hour overnight fast. Urine was voided before two 16 guage IV cannulae (H.G.Wallace, Colchester, UK) were inserted under local anaesthesia. One was placed into a forearm vein of the left arm for infusion of 20% dextrose; the other hand was placed in a hot-box heated to 60°C which served to 'arterialise' the blood (Abumrad et al., 1981) before the cannula was inserted retrogradely in a dorsal hand vein. Slow infusion of 0.9% saline (Galen Research Laboratories Ltd, Antrim, N.Ireland) was used to maintain patency. An IMED 928 volumetric infusion pump (IMED Ltd, Abingdon, UK) was used to infuse the glucose.

Following insertion of the lines, the subjects rested for 20 min. The trial commenced with a 30 min baseline period during which mean blood glucose concentrations were determined, and blood samples were taken for later analysis of metabolites and hormones. Following this time, a priming infusion of the 20%

dextrose was administered over 20 min , according to the algorithm of DeFronzo et al. (1979), in order to raise blood glucose accutely to 12 mM. The trial continued for a further 2 hours and 40 min whilst the blood glucose was 'clamped' at 12 mM. This was achieved by analysis of blood glucose every 5 min, and the rate of infusion of dextrose altered within 90 seconds using a computer program written for a Sharp MZ80B computer.

Oxygen uptake (VO_2) and VCO_2 were measured over the 30 min baseline period and throughout the glucose infusion period continuously. A mobile metabolic cart and ventilated hood system were used based on that described by Fellows and Macdonald (1985). Subjects rested supine on a bed with a ripple mattress (Talley Ripplebed Model D70, Talley Medical Equipment Ltd, Borehamwood, UK).

Blood samples were taken during the baseline period and every 20 min during the clamp for subsequent analyses for insulin, cortisol, NEFA, and glycerol. Samples taken at the start and at 1,2,3 hours were analysed for adrenaline and noradrenaline, and samples taken at baseline and 3 hours for glucagon. Blood samples for insulin, cortisol, catecholamines, NEFA and glycerol were taken into 10 ml lithium-heparin tubes, whereas blood for glucagon analysis was taken into the same tubes but with 1 ml Trasylol (Bayer Ltd, Newbury, UK) added. Samples were centrifuged immediately at 3,000 rev min^{-1}, the plasma removed and stored on ice until the end of the trial. Thereafter, samples for catecholamines were stored at -70°C, and all other samples at

-20°C.

Urine was collected at the end of the clamp for analysis of glucose, and for determination of volume. Consequently it was possible to estimate the urinary glucose losses.

Calculations. The glucose utilisation rate was calculated as the amount required to maintain a constant blood concentration of 12 mM, after correction for urinary losses (DeFronzo et al.,1979). These rates of whole body glucose utilisation were calculated for successive 20 min periods throughout the clamp, ignoring the 'prime' infusion. No estimate was made of hepatic glucose production (see Appendix C).

The carbohydrate oxidation rates were calculated via indirect calorimetry. This incorporated VO_2 and RER measurements and used the table of Zuntz (Appendix A).

Analyses.Whole blood glucose was analysed using a glucose oxidase technique incorporating an oxygen electrode YSI Model 23A glucose analyser (Yellow Springs Instruments Inc, Yellow Springs, Ohio). Within-batch precision of repeated pooled assays during hyperglycaemic clamping was less than 3.4%; between-batch precision was less than 2.8%.

Perchloric acid extracts of plasma were assayed for glycerol using a fluorimetric technique on the Cobas-Bio centrifugal analyser according to the method of Harrison et al (1988).

Reagents were supplied by Boehringer-Mannheim, UK Ltd. Between-rotor precision of repeated pooled plasma assay was less than 3.5% for a mean glycerol concentration of 0.363 mM.

Plasma analyses for insulin was measured using a Coat-A-Count solid phase ^{125}I-insulin RIA kit (Diagnostic Products Ltd, Abingdon, UK).Between-batch precision quoted by the manufacturer of the kit was less than 10% at insulin concentrations between 10 and 95 uU ml^{-1}.

Statistics. Paired 't'-tests were undertaken to examine differences between the time points for the variables measured. Pearson's product moment correlations were performed between NEFA, glycerol, insulin, carbohydrate and fat oxidation rates in order to determine any possible relations. Significance was accepted when $P<0.05$.

6.3 RESULTS

Glucose utilisation rate increased steadily throughout the clamp (Fig 6.1), starting with a value of 32.3 uM kg^{-1} BWt min after 20-40 min to a value of 81.1 uM kg^{-1} BWtmin after 180 min; this represented a 151% increase. T-tests revealed that significant differences from the first 20 min period commenced between 60-80 min ($P<0.05$), and were further elevated during the final 20 min ($P<0.01$).

The increase in glucose utilisation rate was matched by a similar steady increase in the rate of carbohydrate oxidation (Fig 6.2), together with a reciprocal fall in lipid oxidation (Fig 6.3). The change in carbohydrate and lipid oxidation represented a 231% increase and a 72% decrease from resting levels respectively; the change from the post-prime values were a 197% increase and a 72% decrease respectively. These differences were highlighted by 't'-tests ($P<0.01$). Indeed, the significant differences were established after 20 min ($P<0.01$). There were significant increases in carbohydrate oxidation between 60 and 120 min ($P<0.01$) but not after that time; identical results were found for the decrease in lipid oxidation. Differences emerged on examination of the data for rate of total carbohydrate oxidation and the rate of glucose infused (rate of glucose utilisation), where it can be clearly established that the rate of infusion was greater than the rate of oxidation (Fig 6.4).

Figure 6.5 shows that hyperglycaemia was maintained at 12 mM. The deviation from the mean was small, indicating the success of the 'clamp'.

Plasma concentrations of NEFA and glycerol were depressed by hyperglycaemia (Figs 6.6 and 6.7). The concentrations decreased by 96% and 75% for NEFA and glycerol respectively from rest to the end of the clamp; these percentage changes are almost identical to the percentage decrease in fat oxidation. Examination of Table 6.1 emphasises the significant correlations between the changes in plasma fat metabolites and fat oxidation,

as well as the inverse relationships with plasma insulin and carbohydrate oxidation.

Plasma insulin levels increased significantly during the clamp (Fig 6.8). The concentration after the prime infusion was significantly higher than the resting value ($P<0.05$), with this increase being steadily maintained to 60 and 120 min ($P<0.01$). No significant differences were found after 120 min.

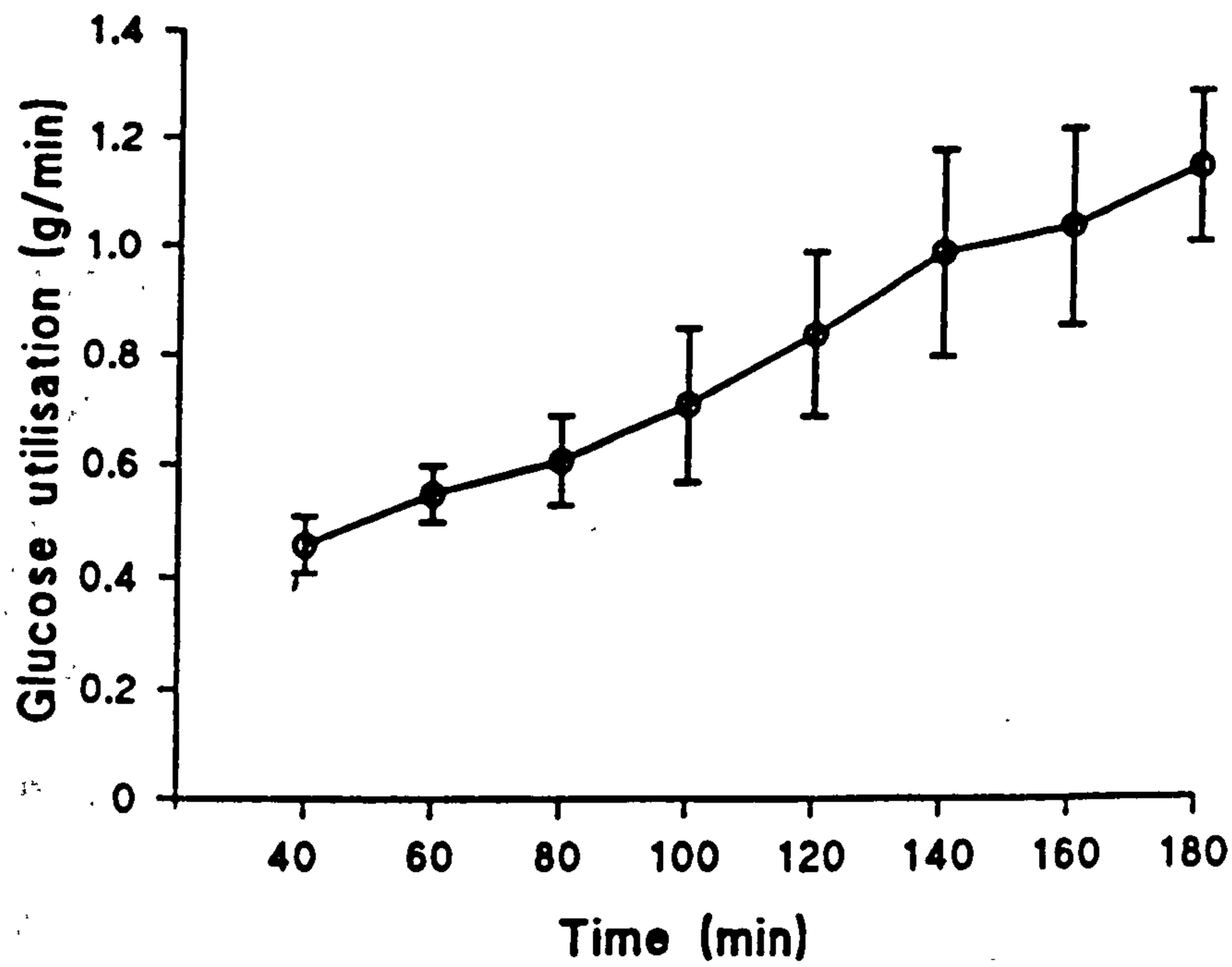


Fig 6.1 Mean (±SEM) rate of glucose utilisation (g min^{-1}) during 180 min of bed-rest when hyperglycaemic clamped.

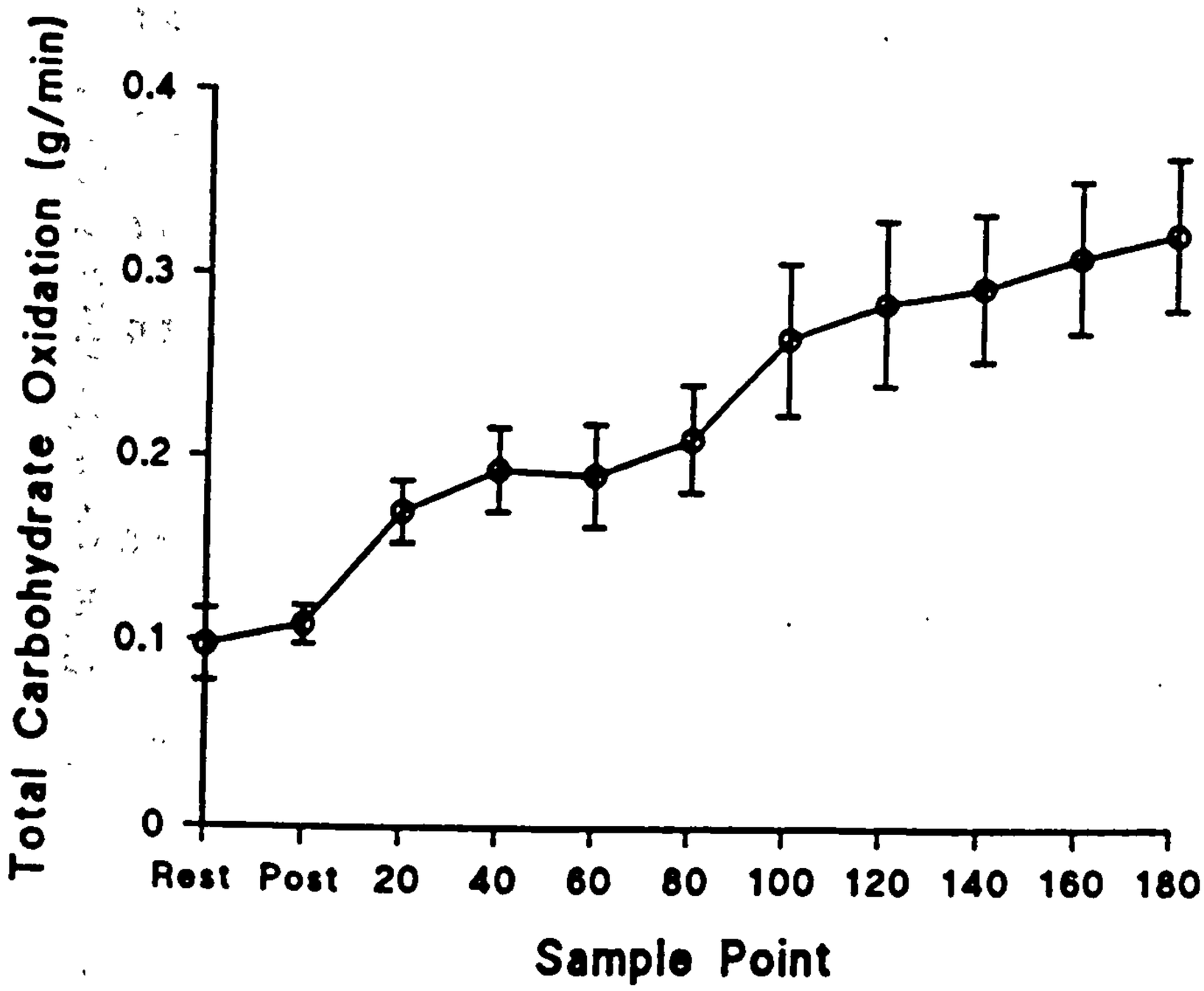


Fig 6.2 Mean (±SEM) rate of total carbohydrate oxidation (g min^{-1}) during 180 min of bed-rest when hyperglycaemic clamped.

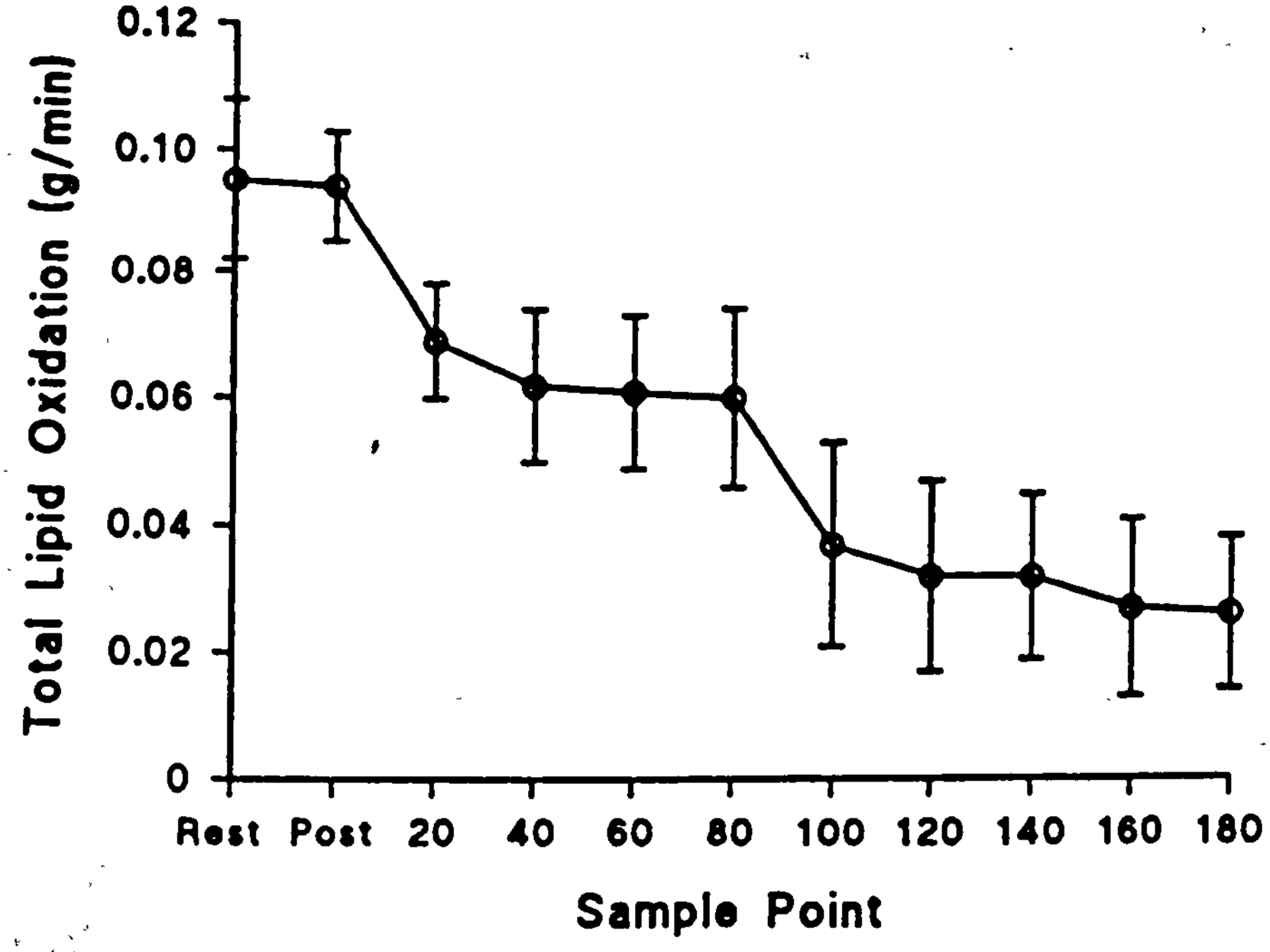


Fig 6.3 Mean(±SEM) rate of total lipid oxidation (g min^{-1}) during 180 min of bed-rest when hyperglycaemic clamped.

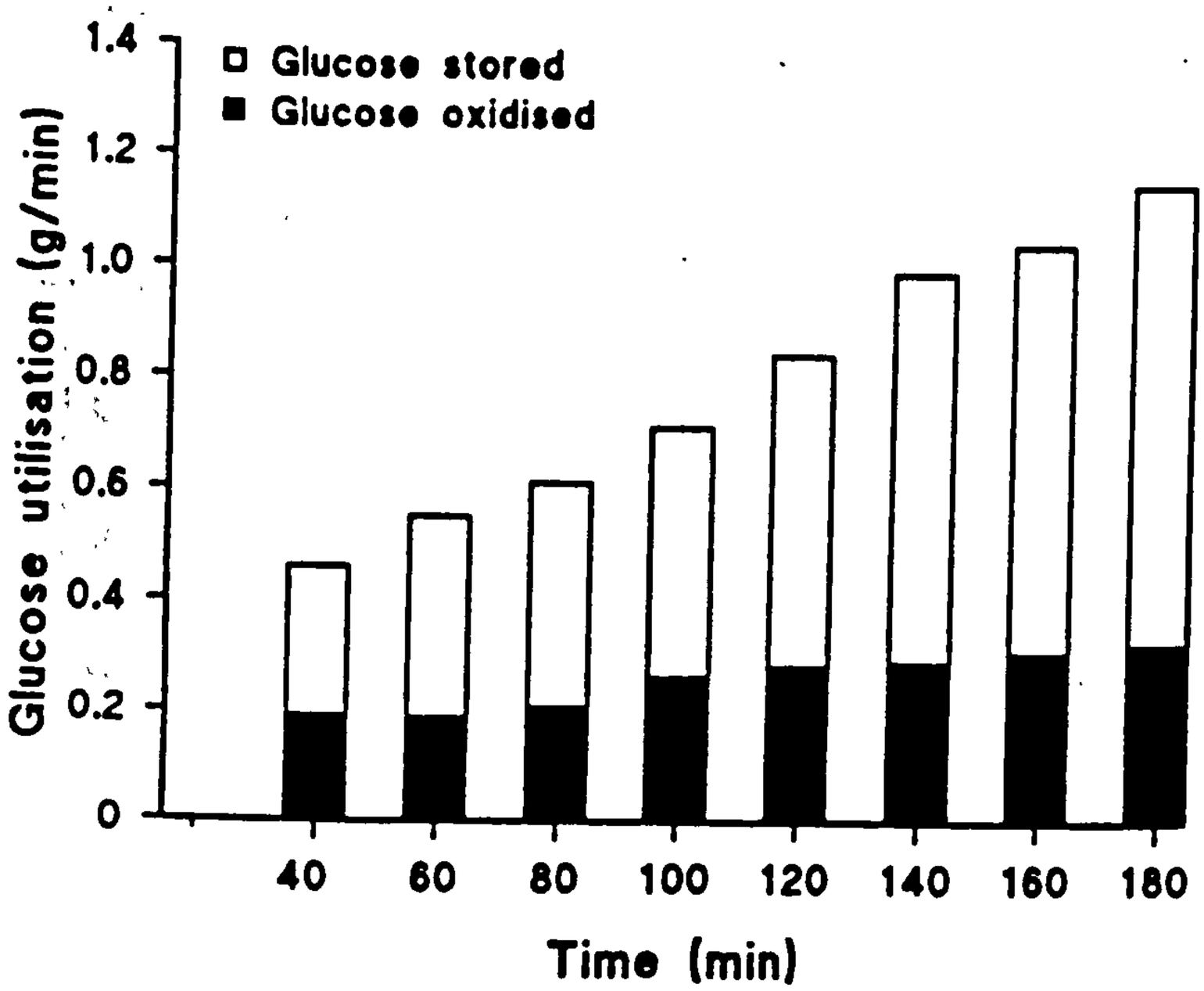


Fig 6.4 Differences in glucose utilisation between the amount oxidised (■) and the amount infused, resulting in the storage rate (□).

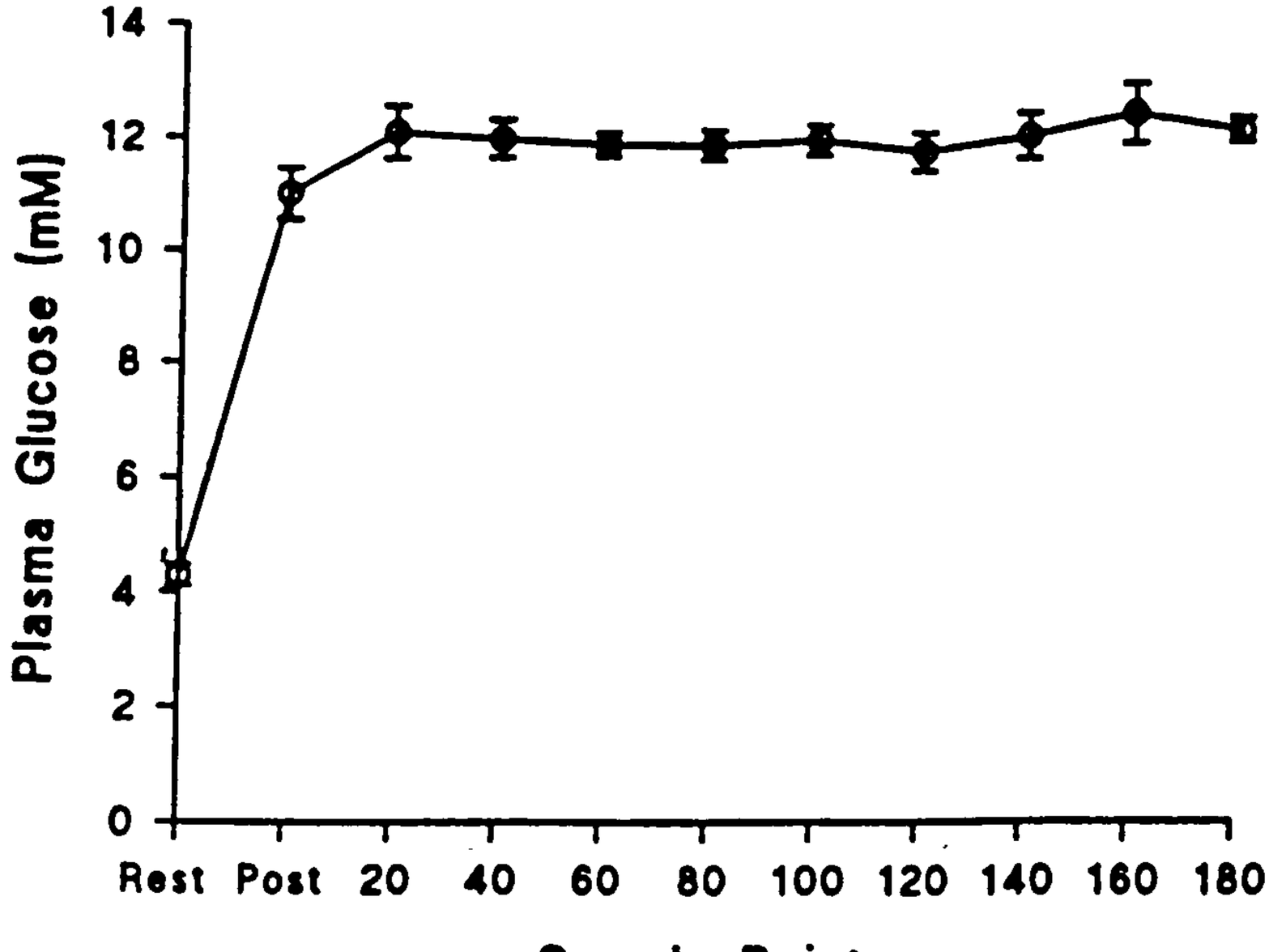


Fig 6.5 Mean (±SEM) plasma glucose concentrations (mM) at rest, after prime infusion of glucose, and during the 180 min of bed-rest when hyperglycaemic clamped.

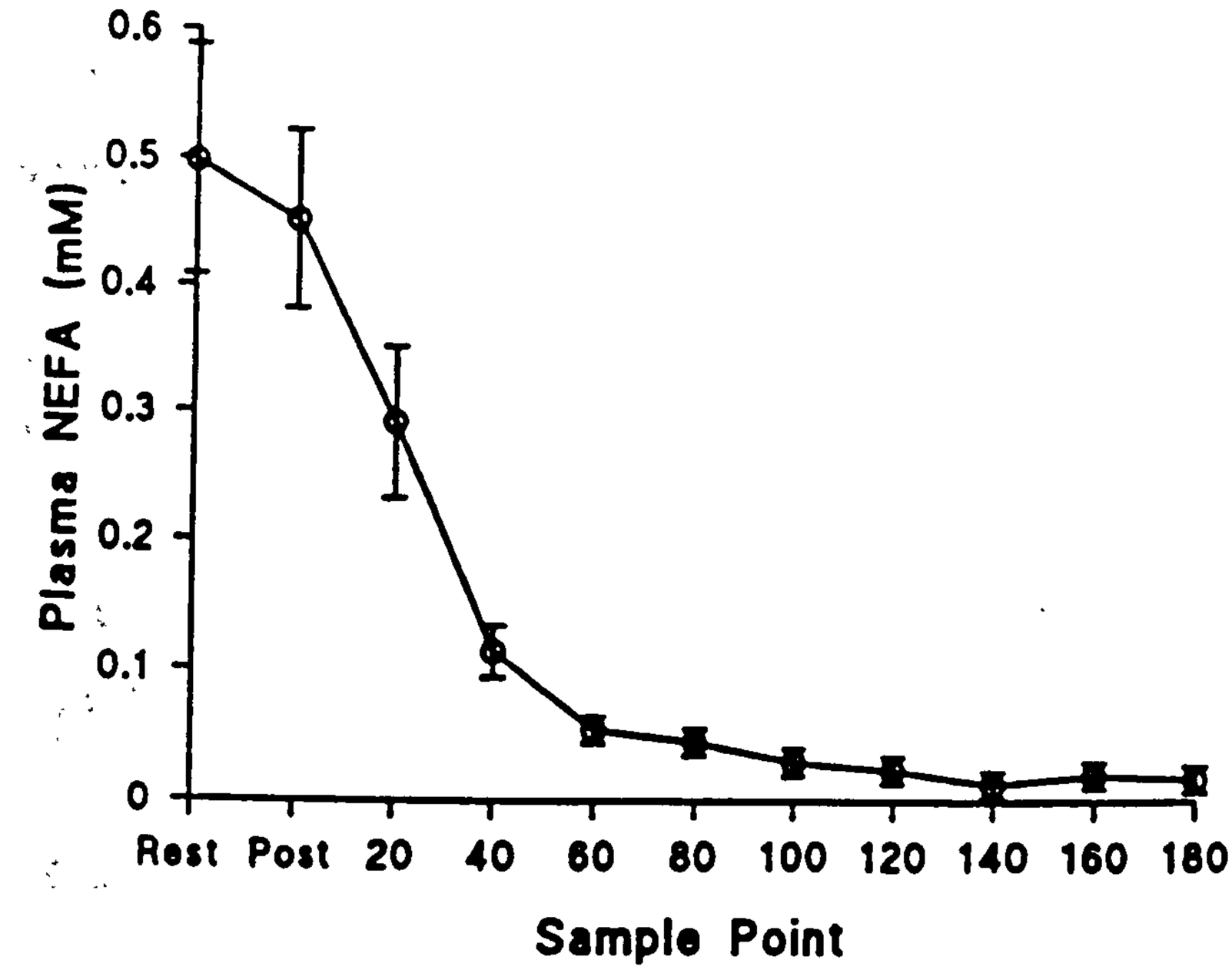


Fig 6.6 Mean (±SEM) plasma NEFA concentrations (mM) at rest, after prime infusion of glucose, and during the 180 min of bed-rest when hyperglycaemic clamped.

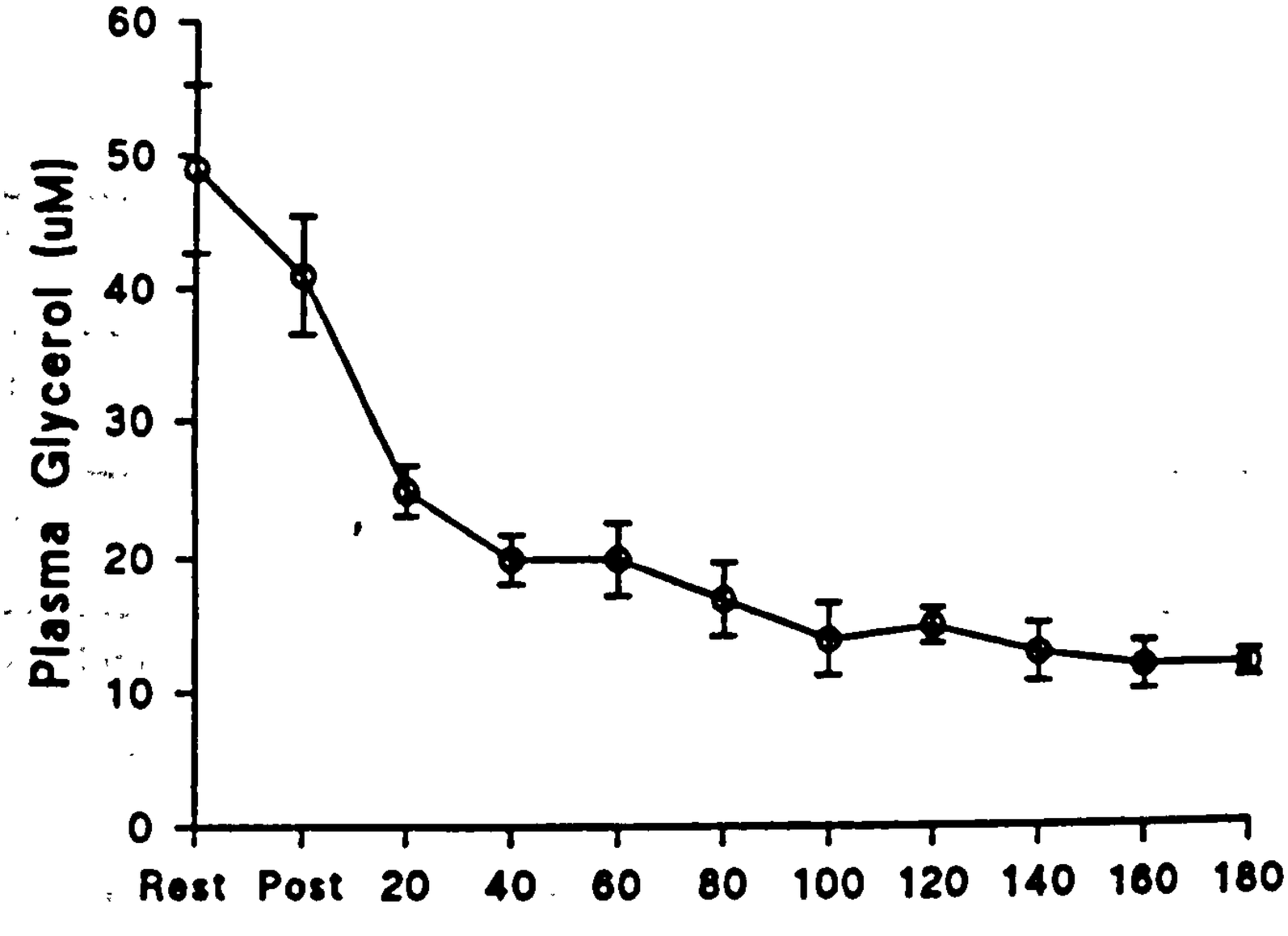


Fig 6.7 Mean (±SEM) plasma glycerol concentrations (μM) at rest, after prime infusion of glucose, and during the 180 min of bed-rest when hyperglycaemic clamped.

A paired 't'- test revealed a significant drop in plasma glucagon concentration from rest (98.2 ± 39.4 pM) to the end (58.0 ± 32.7 pM) of the clamp ($P<0.05$). This represented a 41% decrease.

Results for the hormones cortisol (Fig 6.9), adrenaline (Fig 6.10), and noradrenaline (Fig 6.11) showed no significant changes during the clamp, although there was an upward trend in noradrenaline concentration.

Table 6.1 Correlation matrix of plasma concentrations of NEFA, glycerol and insulin, and rates of carbohydrate (CHO) and lipid oxidation

	NEFA	Glycerol	Insulin	CHO Oxidation
NEFA	-			
Glycerol	0.97 *	-		
Insulin	-0.86 *	-0.87 *	-	
CHO oxidation	-0.88 *	-0.86 *	0.97 *	-
Lipid oxidation	0.90 *	0.92 *	-0.95 *	-0.96 *

* $P<0.01$

6.4 DISCUSSION

The hyperglycaemic clamp procedure provides an index of glucose metabolism since the plasma glucose concentration is held constant and the rate of glucose infusion is a measure of the total amount of glucose metabolised. The technique is a means of quantifying the amount of glucose metabolised by the body and of assessing beta-cell activity to glucose (DeFronzo et al., 1979).

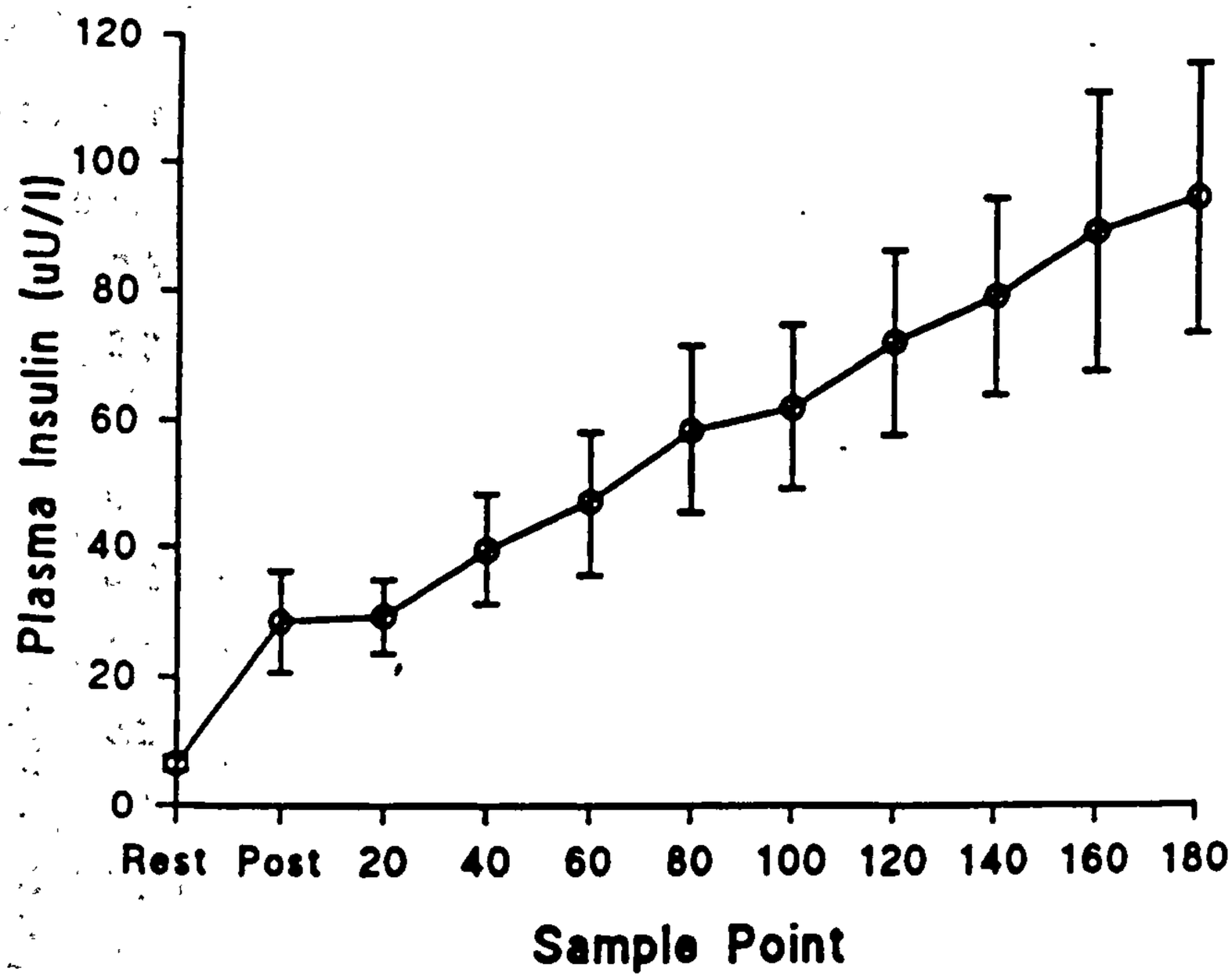


Fig 6.8 Mean (±SEM) plasma insulin concentrations ($\mu U\ ml^{-1}$) at rest, after prime infusion of glucose, and during the 180 min of bed-rest when hyperglycaemic clamped.

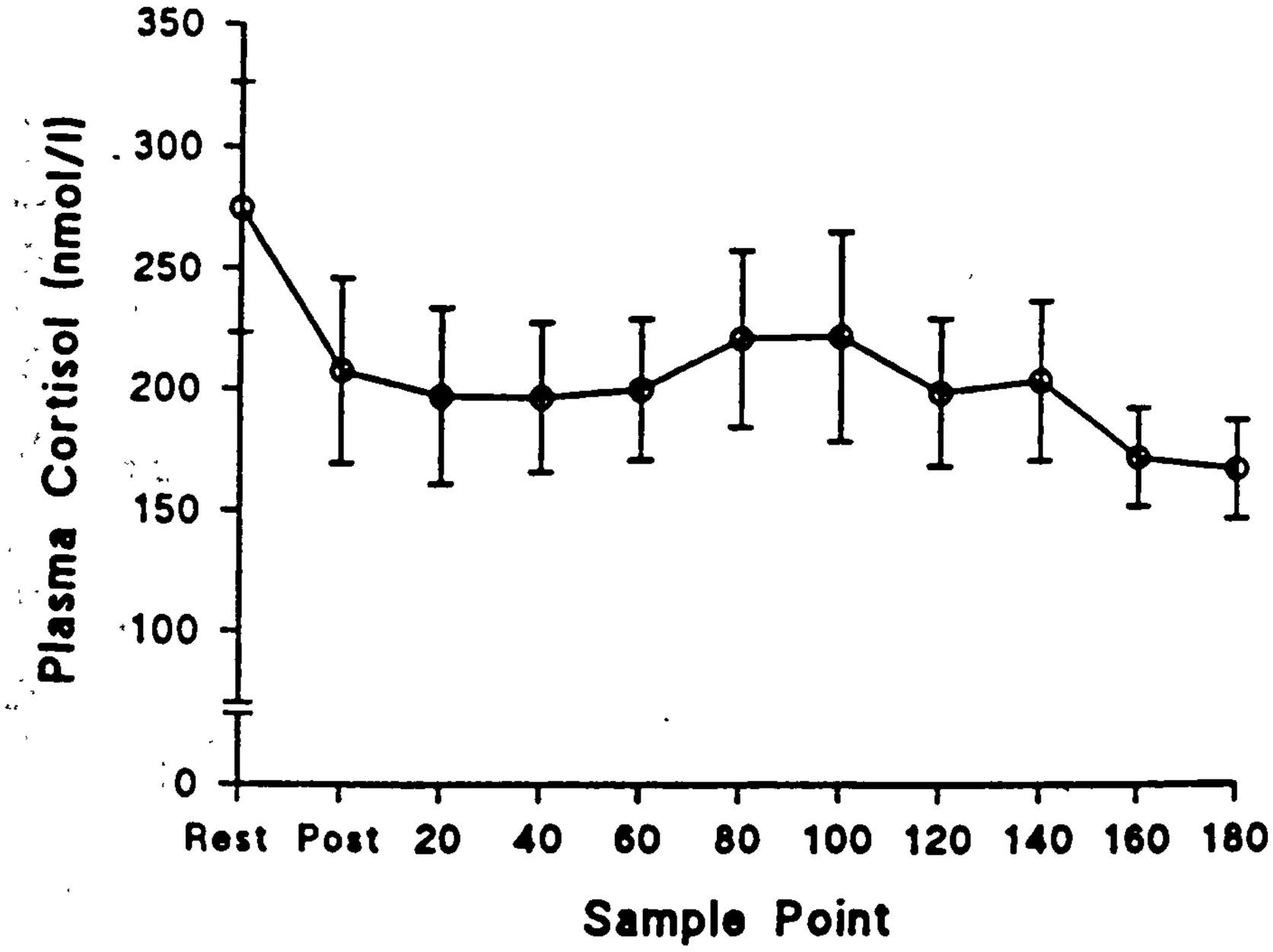


Fig 6.9 Mean (±SEM) plasma cortisol concentrations (nM) at rest, after prime infusion of glucose, and during the 180 min of bed-rest when hyperglycaemic clamped.

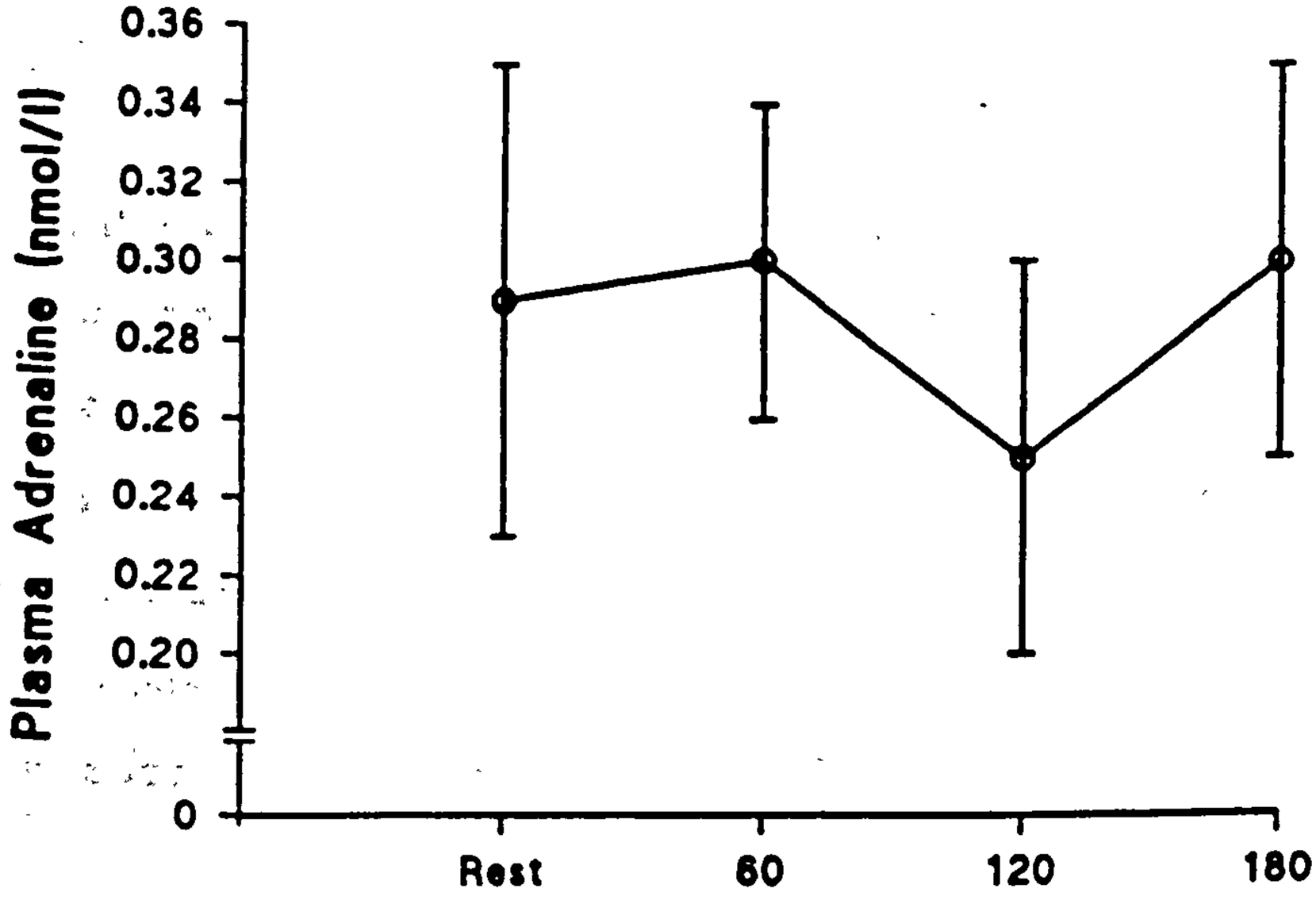


Fig 6.10 Mean (±SEM) plasma adrenaline concentrations (nM) at rest and during 180 min of bed-rest when hyperglycaemic clamped.

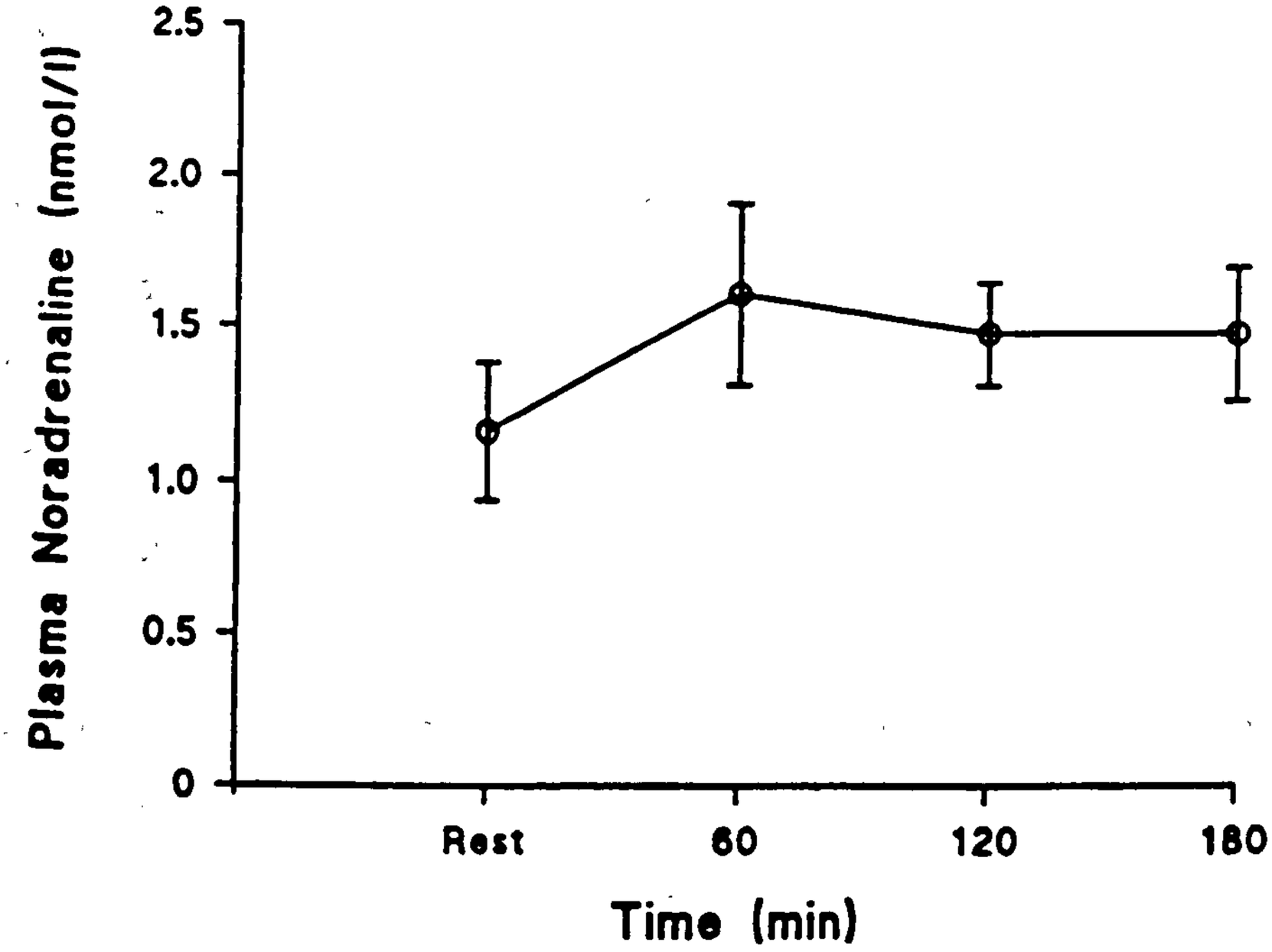


Fig 6.11 Mean (±SEM) plasma noradrenaline concentrations (nM) at rest and during 180 min of bed-rest when hyperglycaemic clamped.

The rate of glucose utilisation was similar to that found by others, both in terms of the values obtained and in the pattern of change (Copeland et al., 1987; DeFronzo et al., 1979; White et al., 1987). The calculation of glucose utilisation rate does not take into account hepatic glucose output. However, hepatic gluconeogenesis is known to be suppressed during glucose infusion at a rate greater than 5.6 uM kg^{-1} min (Wolfe at al., 1979). Consequently, the glucose utilisation rates of 32.3-81.1 uM kg^{1-} min encountered in this study together with the diminished glucagon levels would indicate repression of glucose output from the liver. The glucose utilisation rate therefore reflects total

glucose metabolism in the body.

A value of 32.3 uM kg^{-1}.min meant that an estimated 0.462 g min^{-1} of glucose was being metabolised; this is based on the subjects' mean body mass of 79.4 kg and the gram molecular weight of glucose of 180. At a glucose utilisation rate of 81.1 uM kg^{-1} min the mean estimated glucose being metabolised was 1.16 g min^{-1}. Since the estimated carbohydrate oxidation rates were 0.190 g min^{-1} and 0.324 g min^{-1} at these time points, the surplus glucose was probably being stored as muscle and liver glycogen, and being converted to triglycerides in adipose tissue (Fig 6.4).

The increase in RER suggest a shift in fuel utilisation from fat to glucose; this was reinforced on examination of the oxidation rates. Further support may be gleaned by the observed decrease in plasma NEFA and glycerol. It is suggested that plasma glycerol levels may reflect the rate of lipolysis more accurately than plasma NEFA, as once the glycerol is released the adipocytes are unable to reuse it (Bulow & Madsen, 1986). The higher correlation between plasma glycerol concentration with fat oxidation (r=0.92) than for plasma NEFA with fat oxidation (r=0.90) would appear to support the above, as would the similarity in percentage change in plasma glycerol concentration (-75%) and percentage change in fat oxidation (-75%) compared with percentage change in NEFA (-95%).

Insulin is a potent inhibitor of lipolysis, and is secreted in response to elevations of blood glucose. It is not surprising

therefore to notice the significant increase in plasma insulin concentrations as a consequence of hyperglycaemia. DeFronzo et al. (1979) have stated that the normal insulin response is biphasic, with an immediate rapid rise in the first 6 min followed by a progressive increase. The results of this study show a similar pattern (Fig 6.8). The consequences of the elevation in insulin are realised with the decreases in glycerol and NEFA, and hence the significant negative correlation obtained. Insulin enhances the entry of glucose into adipocytes and then promotes the conversion of glucose into fatty acids (Goldrick et al., 1974). Insulin also inhibits lipolysis by binding to insulin receptors; only 2-5% of the receptor sites have to be occupied in order to have a maximal inhibitory effect (Gammeltoft, 1984).

The hormonal concentrations of the catecholamines and cortisol reinforce the consideration of impaired fat mobilisation and oxidation, for these hormones are responsible for promoting lipolysis and thereby stimulating fat utilisation. No significant changes were apparent in their plasma concentrations, although the slight (27%) increase in noradrenaline levels is consistent with an increase in sympathetic activity under conditions of hyperglycaemia (Young et al., 1980).

6.5 CONCLUSIONS

The results from this experiment demonstrated that maintained hyperglycaemia during rest enhanced carbohydrate oxidation, reduced fat oxidation, decreased plasma NEFA, glycerol, and glucagon, increased insulin concentration and glucose utilisation, and produced no significant changes in catecholamines and cortisol (thus supporting *hypotheses 11 and 12*). Furthermore, the rate of glucose utilisation steadily increased during the trial (thus supporting *hypothesis 13*).

7. HORMONAL AND METABOLIC RESPONSES TO MAINTAINED HYPERGLYCAEMIA DURING EXERCISE

7. HORMONAL AND METABOLIC RESPONSES TO MAINTAINED HYPERGLYCAEMIA DURING EXERCISE

7.1 INTRODUCTION

Hormonal and metabolic changes during exercise have been described in Chapters 4 and 5. Prolonged, intense exercise resulted in increased plasma levels of catecholamines and glucagon, and a concomitant reduction in insulin concentration. These changes favoured the release of fatty acids from triglyceride stores and their subsequent use as an energy source. The effect of carbohydrate ingestion prior to or during exercise, on the other hand, resulted in an attenuation of the responses of catecholamines and glucagon, and an elevation of plasma insulin concentration. The consequences of these changes were seen in maintainance of hyperglycaemia (certainly of euglycaemia), a diminished fatty acid and glycerol plasma concentration, and greater oxidation of carbohydrate.

Few studies have been performed whilst glucose infusion was administered to exercising subjects (Coyle et al., 1991; Hawley et al., 1994). These studies showed that glucose infusion producing hyperglycaemia resulted in an elevation of plasma insulin, a high rate of carbohydrate oxidation, and a concomitant decrease in plasma NEFA and fat oxidation. The authors did not examine other metabolites or counter-regulatory hormones, and infused the glucose only whilst exercising. Furthermore, the plasma glucose concentrations were maintained at 10 mM throughout

these trials. This experiment was performed to examine the hormonal and metabolic responses to maintained hyperglycaemia at 12 mM, using the glucose clamp technique, whilst subjects exercised for 120 min at 70% VO_2max. The 'prime' infusion was, however, started 30 min before exercise began so that carbohydrate metabolism was stimulated prior to exercise. Thus insulin concentration was significantly elevated at the start of exercise.

Hypothesis 14 *The maintenance of hyperglycaemia during exercise causes a continual rise in insulin levels, a suppression of catecholamines, glucagon, cortisol, growth hormone, and lipids.*

Hypothesis 15 *Glucose utilisation rate increases steadily throughout exercise when hyperglycaemia is maintained, and this is matched by an equivalent increase in carbohydrate oxidation rate.*

Because the energy demands for exercise are greater than that at rest, an infusion of glucose is likely to result in an enhanced uptake and greater use of the glucose during exercise. Therefore it is hypothesised that:-

Hypothesis 16 *Glucose utilisation rates and carbohydrate oxidation rates are higher throughout exercise than those exhibited under resting conditions.*

Studies examining the effect of carbohydrate feeding on muscle glycogen use have reported glycogen sparing (Bjorkman et al., 1984; Brouns et al., 1989; Hargreaves et al., 1984),or no glycogen sparing (Ahlborg & Bjorkman.,1987; Coyle et al., 1986; Hargreaves et al.,1987). Because hyperglycaemia will be maintained throughout this experiment, and the uptake of glucose may reach saturation kinetics, the result is likely to be a reduced need for muscle glycogen. Hence:-

Hypothesis 17 *Hyperglycaemia results in significant muscle glycogen sparing.*

The amino acid responses during exercise have been investigated less frequently than other metabolites. In fact there has been no study to date which has examined the changes in red blood cell and plasma amino acids during exercise; plasma amino acids only being examined. These studies (Felig & Wahren, 1971) have shown that plasma alanine and glutamine concentrations become elevated during exercise, with little significant change in the other amino acids. A study in which the red cell amino acid changes are also measured may determine whether the erythrocyte is relatively inert with regard to amino acid transport, or is a carrier of amino acids, or a store of amino acids. Furthermore under

conditions of hyperglycaemia, it may be possible to examine how the glucose -alanine cycle is affected. Therefore it is hypothesised that:-

Hypothesis 18 *Exercise results in an elevation of red cell and plasma alanine concentrations, and unchanged levels of the branched chain amino acids.*

Hypothesis 19 *Hyperglycaemia results in an attenuated response of plasma alanine and branched chain amino acids.*

7.2 METHODS

Subjects. Eight healthy, well trained, male subjects gave their informed consent in accordance with the procedures approved by the Ethics Committees of the Royal Liverpool University Hospital, and of Liverpool John Moores University. Mean age, body mass, and VO_2max were, 36.6 ± 11.6 years, 68.9 ± 6.7 kg, and 3994 ± 540 ml min^{-1}.

Experimental design. Maximal oxygen uptake (VO_2max) was detremined using a continuous incremental loading test to volitional exhaustion on an electrically-braked cycle ergometer. Procedures and the on-line gas analysis system were identical to those described in Chapter 5.

On the day of the trial, subjects arrived in the laboratory at 08:00 hours after an overnight fast, and without having engaged in physical activity during the previous 24 hours. Subjects voided urine before an indwelling 16 gauge IV cannula was inserted under local anaesthesia into a forearm vein of the left arm. At the same time, the right hand was placed in a hot box heated to 60°C, which served to 'arterialize' the blood (Abumrad et al., 1981). After 20 min, a further cannula was inserted retrogradely into a dorsal vein of the right hand for later blood sampling. Slow infusion of 0.9% saline was used to maintain patency. A volumetric pump was used to infuse glucose into the left arm. Following insertion of the lines, subjects rested for 20 min before a resting blood sample was taken. A primary infusion of 20% dextrose was then started according to the method of DeFronzo et al. (1979) in order to raise the plasma glucose concentration to 12 mM. The prime infusion period of 30 minutes was followed by the withdrawal of a further blood sample before a 2 hour bout of exercise at 70% VO_2max began. During the prime infusion and the exercise bout, plasma glucose concentration was maintained close to 12 mM by varying the rate of infusion every 5 min according to the arterialised plasma glucose concentration using an Analox GM7 analyser (Analox Instruments, London,UK). Changes in infusion rate were calculated using a Sharp MZ 80B computer. Two 10 ml blood samples were taken at rest, after the prime infusion (0 min), and at 20,40,60,80,100, and 120 min of exercise for the determination of plasma insulin, glucagon, adrenaline, noradrenaline, cortisol, growth hormone (GH), NEFA, lactate, glycerol, B-hydroxybutyrate, and amino acids. The red

blood cells used for amino acid determination were washed twice with isotonic saline and centrifuged each time at 3000 rev min^{-1} before being finally separated and stored at -40°C for analysis of amino acids. Measurements of VO_2 and RER were also undertaken over a 5 min period at 15,30,45,60 90, and 120 min of exercise, as well as at rest and the last 5 min of the prime infusion. Plates 1-4 illustrate the equipment used.

Immediately after exercise, subjects were requested to lie down whilst the infusion rate was slowly decreased so as to prevent 'rebound' hypoglycaemia. Furthermore, two muscle biopsy samples were taken from the anterior quadriceps (Plates 5 & 6) using the conchotome method (Edwards et al., 1980) after administration of a local anaesthetic and following an incision of the skin and muscle fascia. Each muscle sample was placed in a sterile Eppendorf tube before being plunged into liquid nitrogen. The samples were then stored at -70°C until analysis for glycogen content.

Three weeks after the first trial, subjects repeated the process using a 0.9% saline infusion instead of the dextrose. The rate of infusion of the saline was identical to that determined in the hyperglycaemic trial.

'Resting' muscle biopsy samples were taken three weeks after the second trial. Subjects were asked to conform to their previous normal eating and exercise patterns for 48 hours prior to the sample being taken.

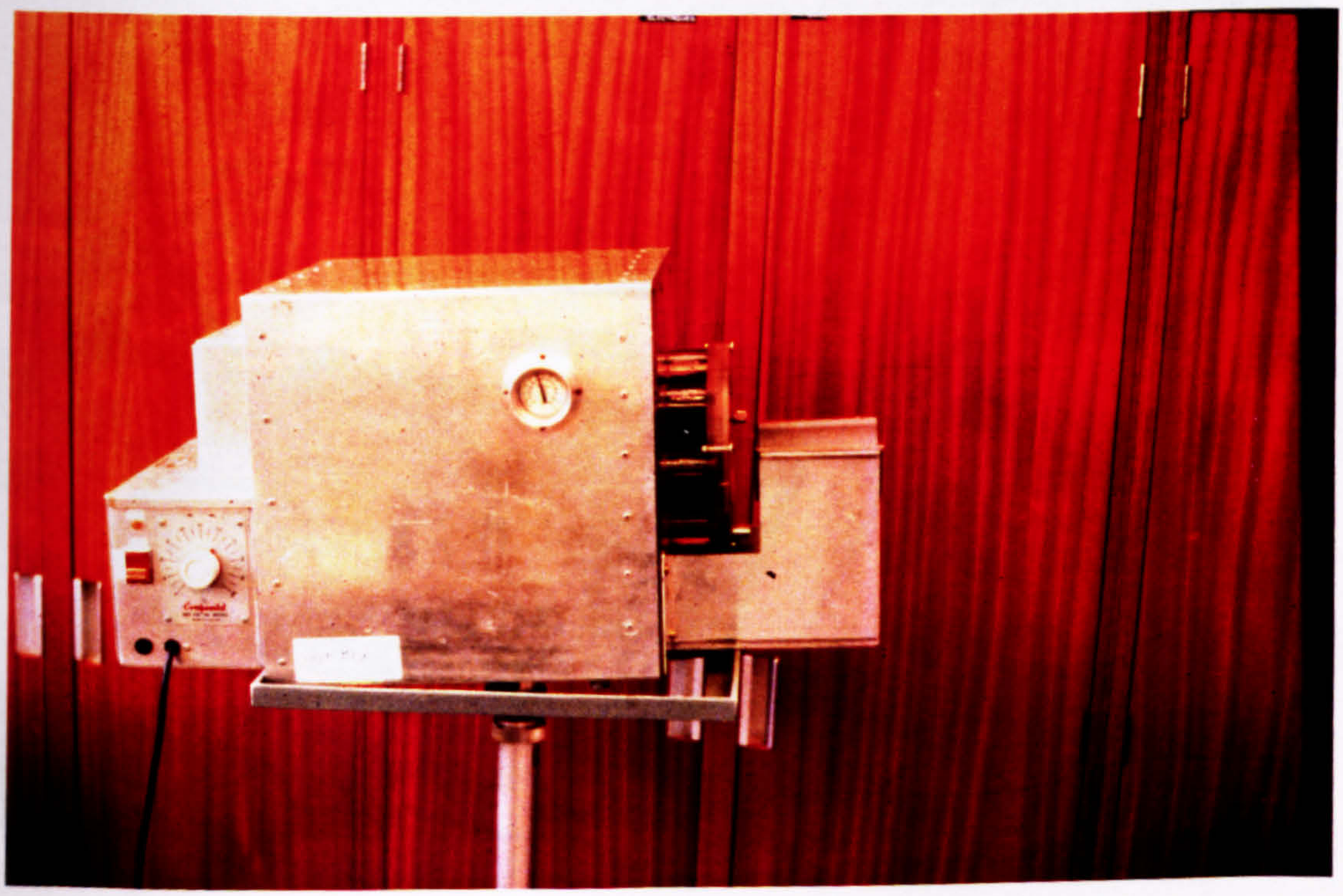


Plate 1 The 'hot-box' for heating superficial hand veins and thus arterialising blood.

Plate 2 The Analox analyser for fast determination of plasma glucose concentration.

Plate 3 Arterialised blood sample being withdrawn from a heated hand placed in the hot-box.

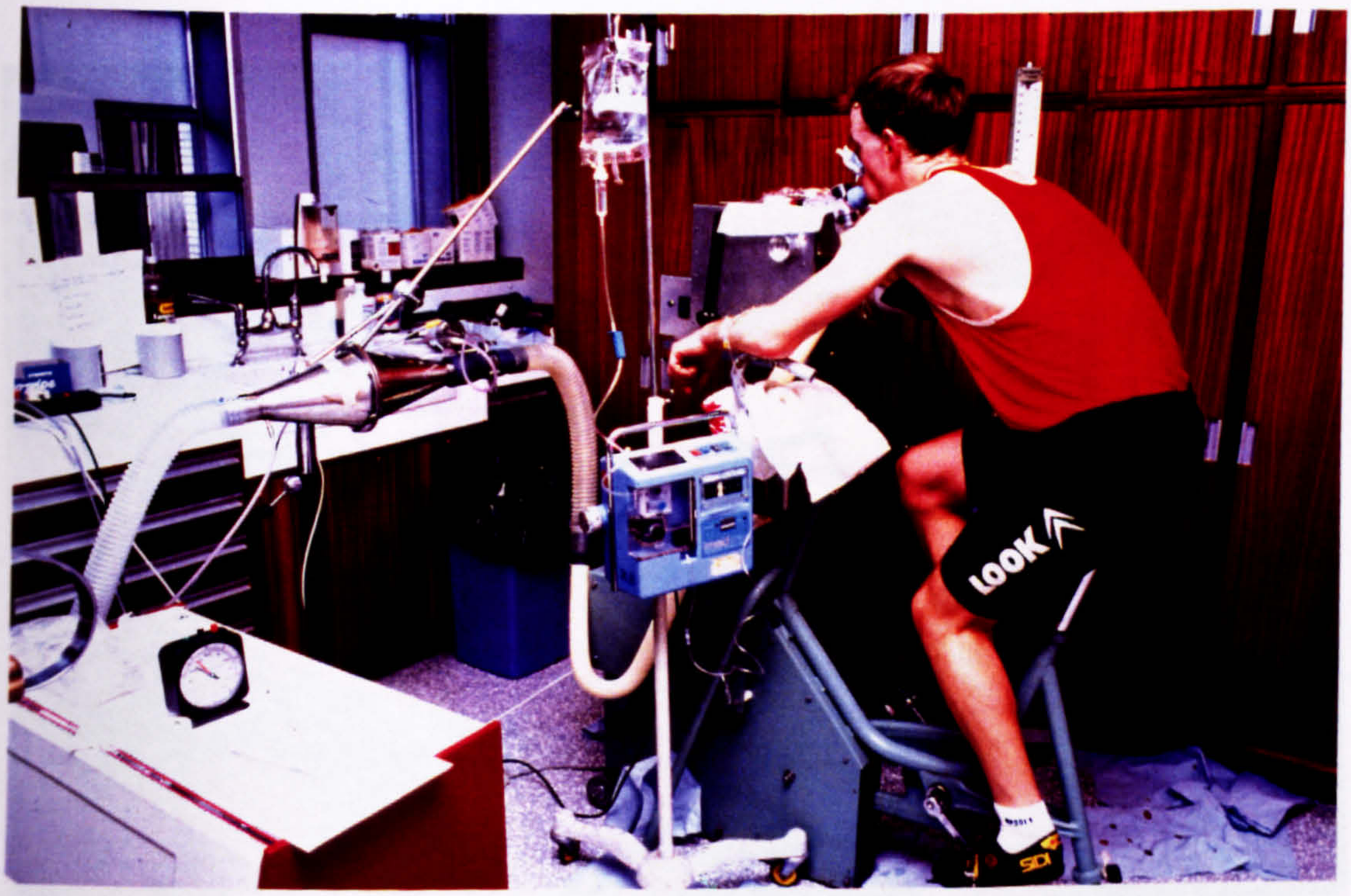


Plate 4 The experimental 'set-up'.

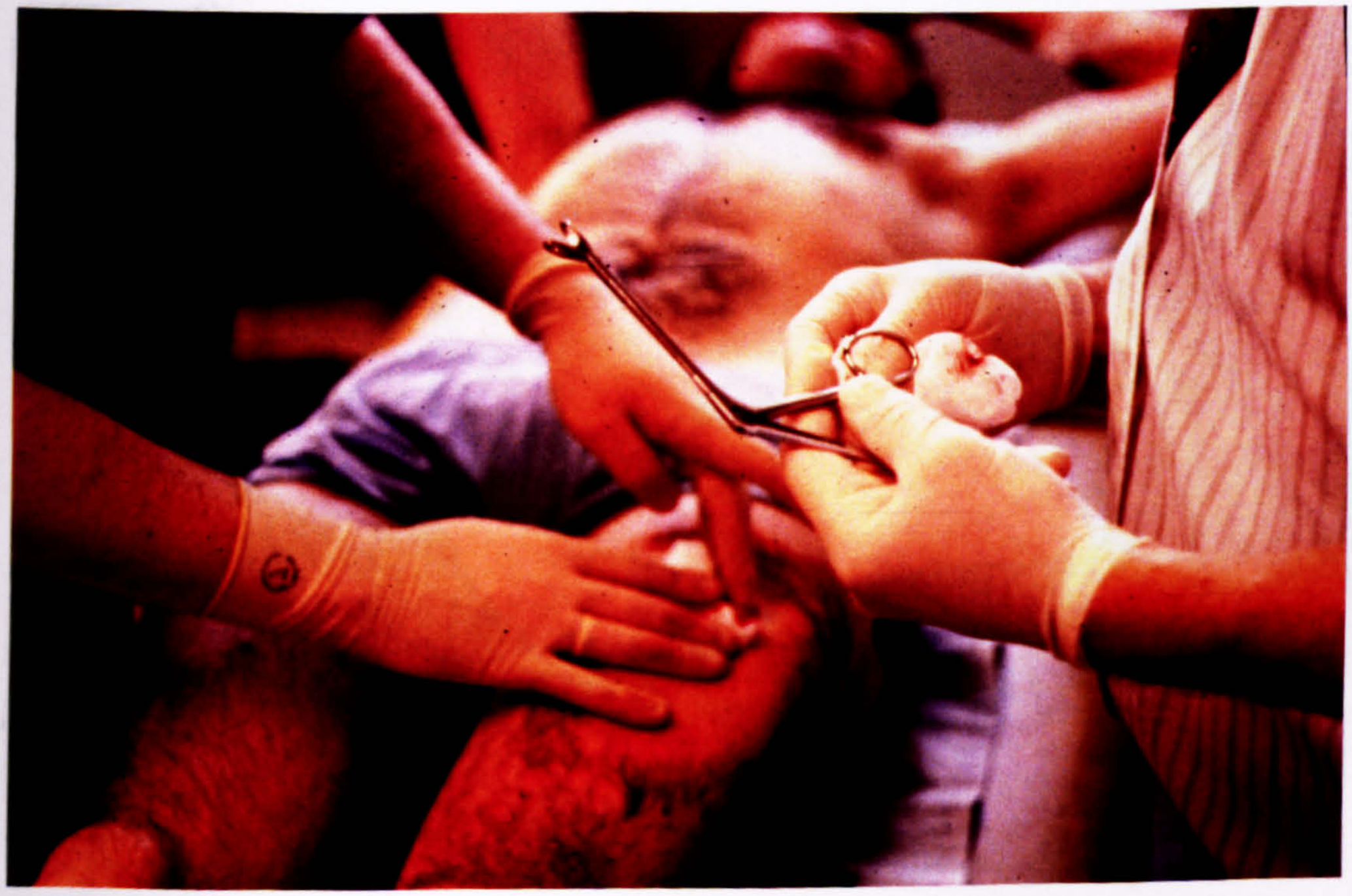


Plate 5 Subject recumbent and prepared for a muscle biopsy post-exercise. Note the conchotome.

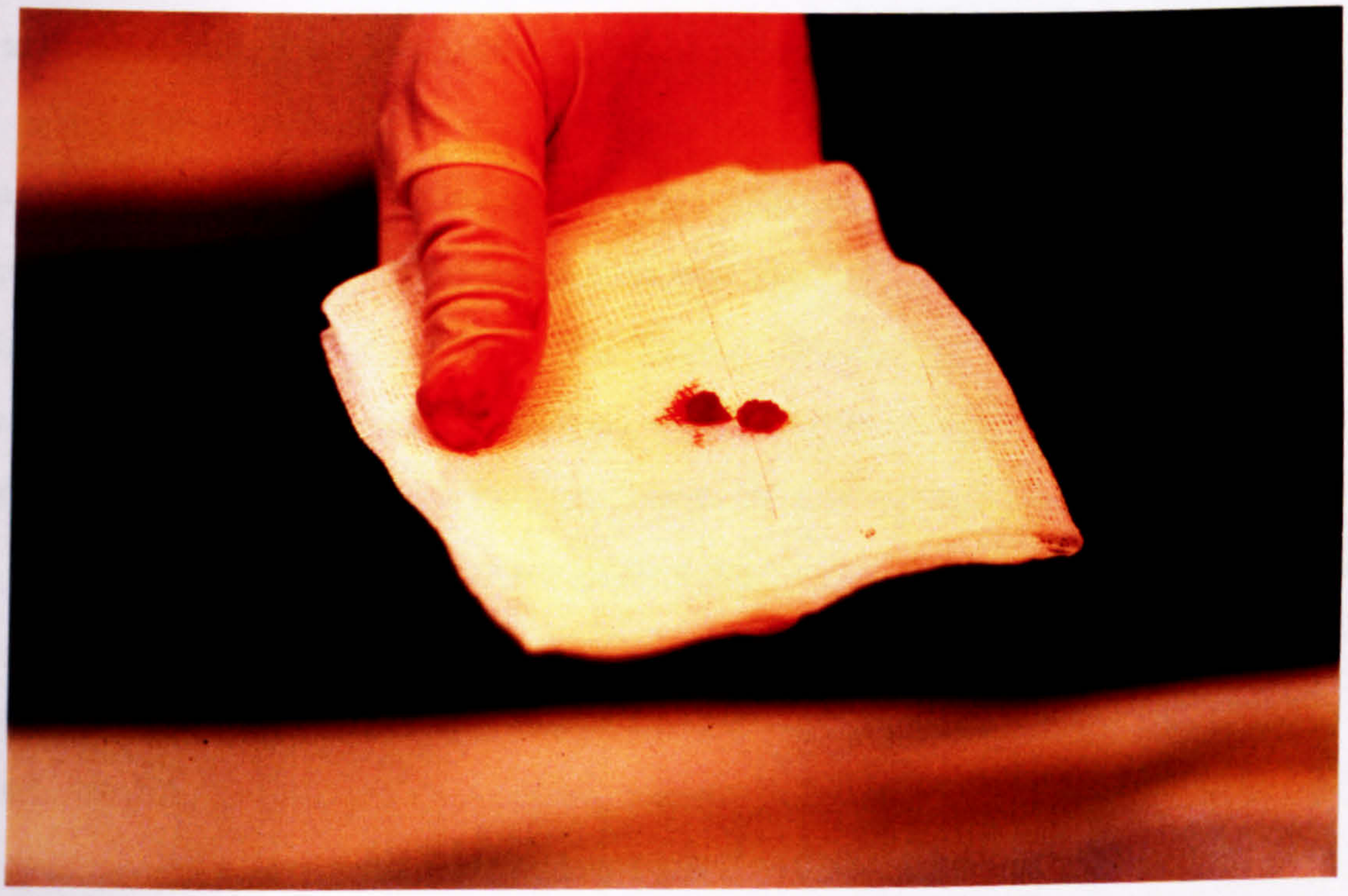


Plate 6 Muscle samples removed from the subject's leg taken by the conchotome.

Analyses. Analytical procedures for the catecholamines, insulin, glucagon, NEFA, and glycerol were as described in Chapters 4-6. Plasma cortisol was measured using an 'in-house' RIA with reagents supplied by Bioanalysis Ltd (Cardiff, UK). An ^{125}I-labelled antigen was used as tracer and separation of the bound and free fractions was achieved using a second antibody raised against the anti-cortisol antiserum. Between-batch precision was 15% at cortisol concentrations of 175-920 nmol l^{-1}. The assay shows a bias of less than 10% on the National External Quality Assessment Scheme (NEQAS).

Plasma GH was measured using an 'in-house' RIA with reagents supplied by the Supra Regional Assay Laboratory (Royal Infirmary, Edinburgh, UK), and an ^{125}I-label supplied by NETRIA (St. Bartholomew's Hospital, London, UK). Between-batch precision was less than 10% at GH concentrations of between 1 mu l^{-1} and 80 mu l^{-1}. Bias on the NEQAS was less than 10%.

Plasma amino acid profiles were quantified as the OPA derivatives after separation on reversed phase HPLC incorporated into the automated ASTED procedure (Anachem, Luton, UK). The red cell amino acid concentrations were measured on red blood cell haemolysate solutions. Samples of whole blood were centrifuged at 3,000 rev min^{-1} for 15 min, the plasma used as above, and the packed cells washed twice with isotonic saline. The washed packed cells (PVC approximately 90-95%) were stored at -40°C to assist in haemolysis. Fifty microlitres of thawed packed cells were subsequently diluted with 100 ul of deionised water, and amino

acid analysis carried out as the OPA derivatives on ASTED. The amino acid concentrations were expressed as uM l^{-1} for plasma, and uM l^{-1} of packed red blood cells for the erythrocyte content.

Freeze-dried muscle biopsy samples were prepared and analysed for glycogen concentration according to the method of Edwards et al. (1975). The authors reported the variation between analyses of a pooled muscle powder varied 6.1% for acid-insoluble glycogen.

Statistics. Analysis of variance with repeated measures was used to determine whether there were any significant differences between the trials and the various time points for the plasma metabolites and plasma hormones. Significant F-values were followed up using a Tukey post-hoc test. The carbohydrate oxidation rates were subjected to the determination of the areas under the curve (Matthews et al., 1990) before a 't'-test was applied to determine any significant differences. The glucose utilisation rates were analysed by employing a one-way ANOVA in order to determine if significant differences accrued with time. Related 't'-tests were performed on the resting levels of plasma and erythrocyte amino acid concentrations, and on the muscle glycogen concentrations. Significance was accepted at $P<0.05$.

7.3 RESULTS

Glucose infusion maintained plasma glucose concentrations at approximately 12 mM whilst saline infusion resulted in a relatively constant value of 5 mM being achieved (Fig 7.1). During the exercise period plasma glucose levels under glucose infusion ranged from 11.5 $\pm$ 1.0mM to 12.6 $\pm$ 0.6mM, thus reflecting the suitability of the 'clamp' procedure during exercise. Hypoglycaemia was not exhibited by any of the subjects under saline infusion where plasma glucose concentrations at 100 min and 120 min were 5.1 $\pm$ 0.4 mM and 4.7 $\pm$ 0.7 mM respectively. Analysis of variance reinforced the significant differences between the trials ($F_{1,7}$=1178.6; P<0.01). Differences were

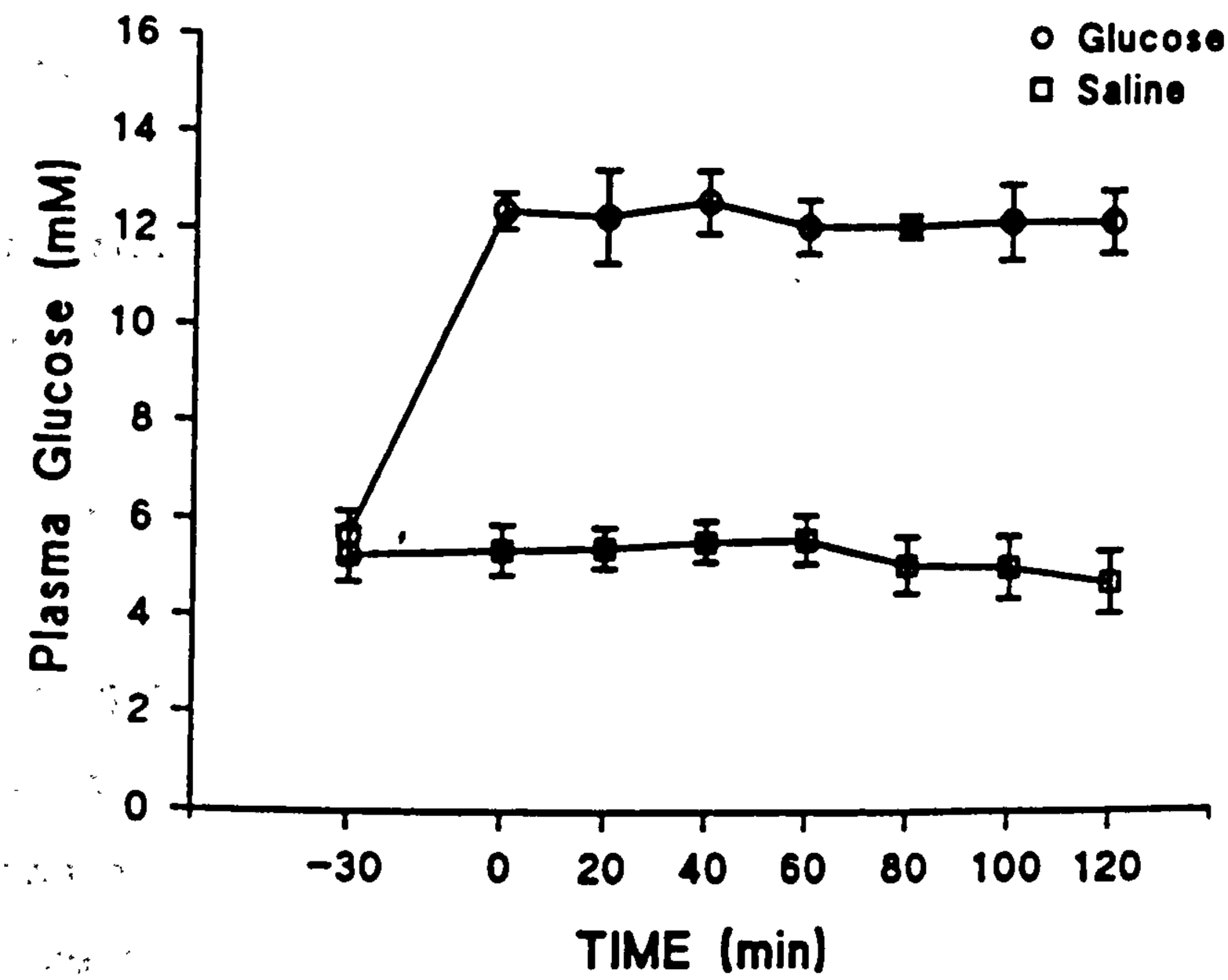


Fig 7.1 Mean ($\pm$SEM) plasma glucose concentrations (mM) at rest (-30 min), after prime infusion of glucose (0 min), and during 120 min of exercise.

apparent with respect to time ($F_{7,49}$=95.1; P>0.01), although this was mainly due to the changes in concentration from rest to post-prime glucose infusion (P<0.01).

Elevated plasma insulin concentrations resulting from glucose infusion were apparent (Fig 7.2). Mean values rose from 7.0 ± 2.5 uU ml^{-1} at rest to 25.9 ± 6.3 uU ml^{-1} after prime infusion. During subsequent exercise the insulin concentrations became elevated up to 60 min (33.7 ± 15.9 uIU ml^{-1}) before falling to 20.9 ± 6.1 uIU ml^{-1} at 120 min. Although ANOVA revealed there was a significant difference with respect to time ($F_{7,49}$=5.24; P<0.05), the differences were only apparent between the resting levels and those under hyperglycaemia (P<0.05). Exercise under conditions of saline infusion showed a typical exercise response, with plasma insulin levels falling from 7.2 ± 2.4 uU ml^{-1} at the start to 3.3 ± 0.5 uU ml^{-1} after 120 min. These changes were significant ($F_{7,49}$=17.15; P<0.01), with a continuous decrease during exercise up to 80 min (P<0.05). Furthermore, ANOVA revealed significantly higher plasma insulin concentrations under glucose infusion ($F_{1,7}$=34.8; P<0.01).

Plasma glucagon concentrations were significantly depressed under glucose infusion ($F_{1,7}$=7.61; P<0.05). Values remained unchanged during exercise; mean concentrations ranged from 368 ± 116 pg ml^{-1} at rest to a level of 401 ± 176 pg ml^{-1} at 100 min, to a final concentration of 347 ± 128 pg ml^{-1} (Fig 7.3). Under saline infusion however, the concentrations increased significantly during exercise from a pre-exercise concentration of 371 ± 101

pg ml^{-1} to a final concentration of 571 ± 179 pg ml^{-1} ($F_{7,49}$=6.8; P<0.05). Significant changes from pre-exercise levels were apparent only after 60 min (P<0.05).

Plasma adrenaline concentrations increased significantly during exercise under both treatments ($F_{5,35}$=129.64; P<0.01), although the response under glucose infusion was attenuated (Fig 7.4). Differences were found between the trials, with the saline infusion resulting in significantly higher concentrations than the glucose infusion ($F_{1,7}$=25.03; P<0.01).

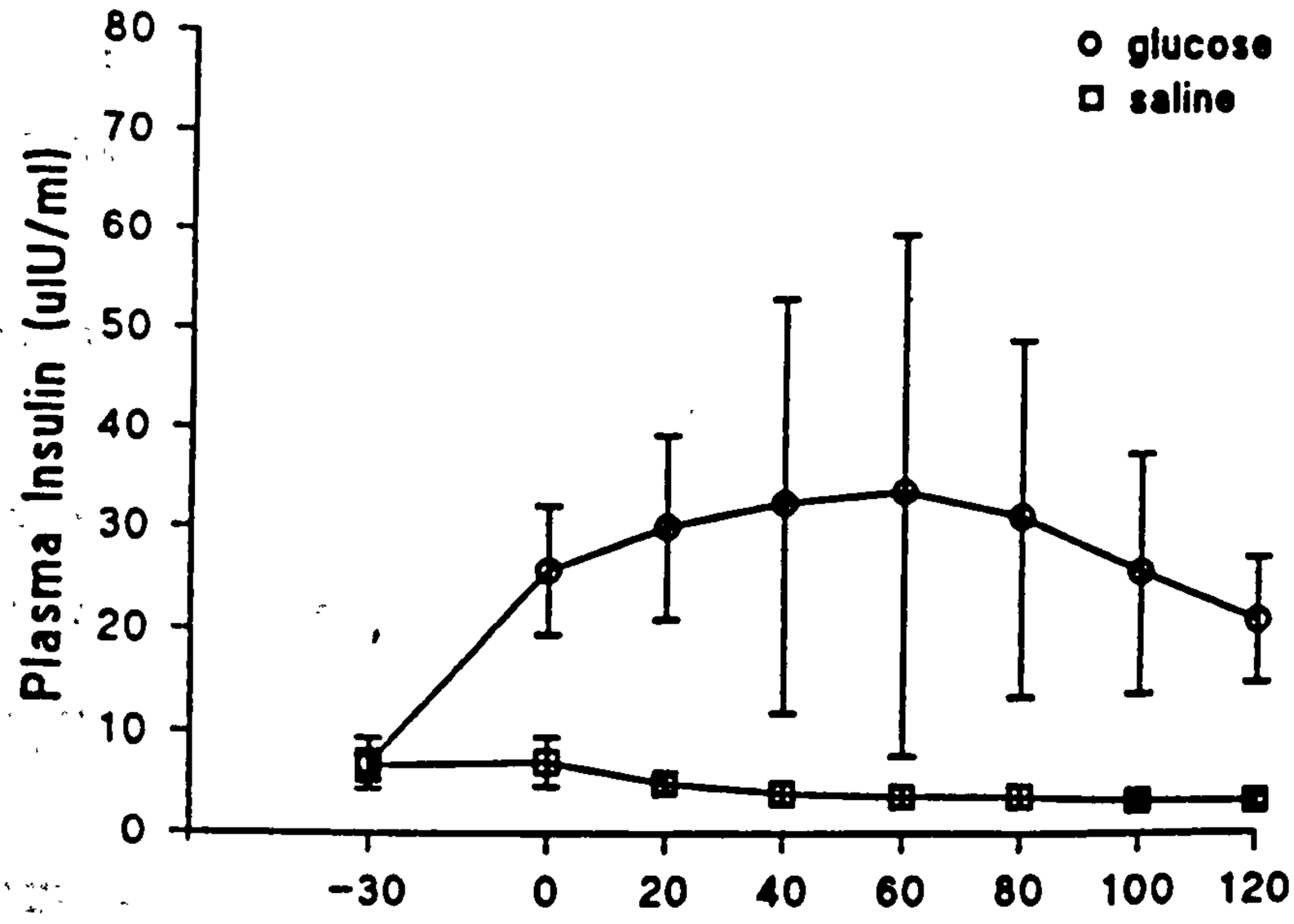


Fig 7.2 Mean (±SEM) plasma insulin concentrations ($\mu U\ ml^{-1}$) at rest (-30 min), after prime infusion (0 min), and during 120 min of exercise.

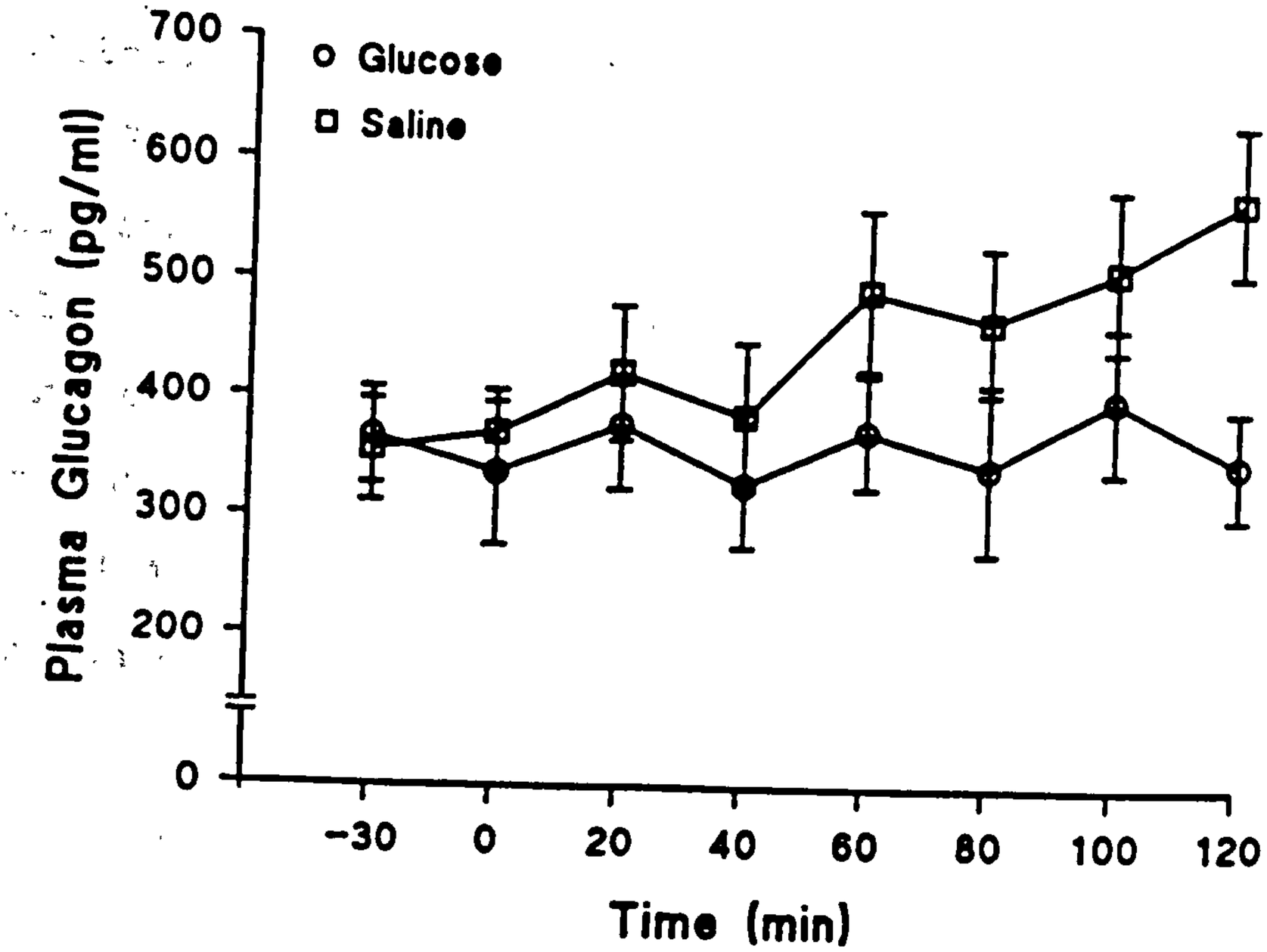


Fig 7.3 Mean (±SEM) plasma glucagon concentrations (pg ml^{-1}) at rest (-30 min), after prime infusion (0 min), and during 120 min of exercise.

Plasma noradrenaline concentrations exhibited similar patterns to those of adrenaline (Fig 7.5), in that there was a significant increase with duration of exercise ($F_{5,35}=28.4$; $P<0.01$) and a significant attenuation of concentrations with the glucose infusion compared to saline infusion ($F_{1,7}=11.1$; $P<0.01$).

No significant differences were found for plasma cortisol concentrations between the trials ($F_{1,7}=4.47$; $P>0.05$), but a significant increase was found with the duration of exercise ($F_{7,49}=3.28$; $P<0.05$). The latter was due to significant increases in concentration under the saline treatment after 100 and 120 min ($P<0.05$). Figure 7.6 illustrates these differences.

Significant differences were apparent between the trials for plasma GH (Fig 7.7), with the glucose infusion trial producing significantly lower concentrations ($F_{1,7}$=8.2; P<0.05). Plasma GH levels increased significantly during exercise, particularly under saline infusion ($F_{7,49}$=6.47; P<0.01). Highest mean concentrations were obtained after 60 min in the glucose infusion trial (14.5 $\pm$ 11.2 mu l^{-1}), and after 80 min in the saline infusion trial (24.6 $\pm$ 12.9 mu l^{-1}).

Plasma lactate concentrations became elevated during exercise in both trials (Fig 7.8). These values remained significantly elevated throughout the exercise period ($F_{7,49}$=10.7; P<0.01), although glucose infusion produced a significantly higher response than saline infusion ($F_{1,7}$=6.3; P<0.05).

The plasma NEFA, glycerol, and B-hydroxybutyrate (B-OH) responses to saline and glucose infusion were similar. Significant differences were found between the trials for NEFA ($F_{1,7}$=26.7; P<0.01), glycerol ($F_{1,7}$=51.1; P<0.01), and for B-OH ($F_{1,7}$=29.9; P<0.01); the glucose infusion resulted in impaired concentrations compared with saline infusion. During exercise under saline infusion, concentrations of these metabolites increased significantly (Figs 7.9, 7.10, 7.11); analysis of variance revealed the following for NEFA ($F_{7,49}$= 11.7; P<0.01), glycerol ($F_{7,49}$= 78.5; P<0.01), and for B-OH ($F_{7,49}$= 20.8; P<0.01). The concentrations under glucose infusion remained depressed for NEFA and for B-OH, although plasma glycerol concentrations increased significantly throughout exercise.

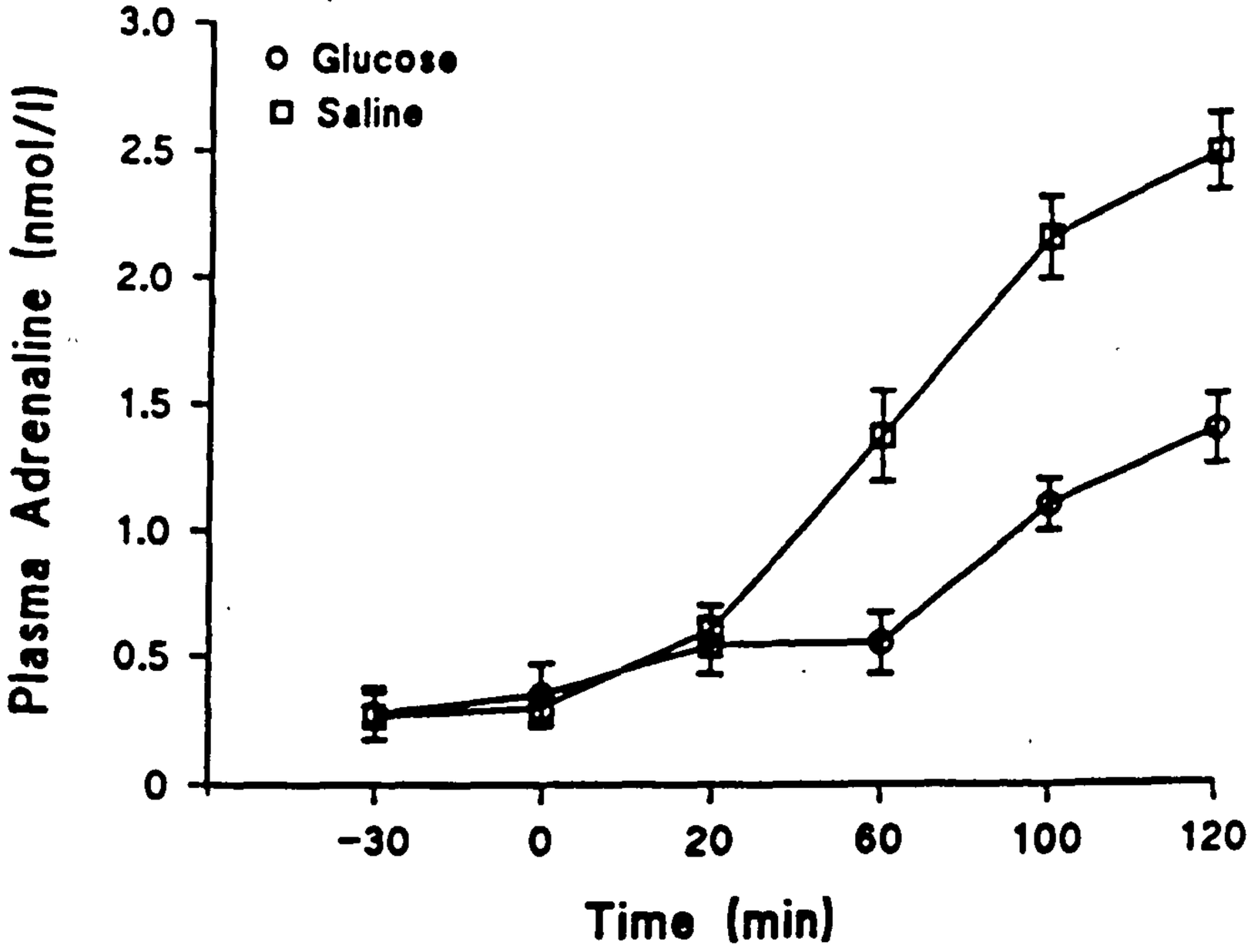


Fig 7.4 Mean (±SEM) plasma adrenaline concentrations (nM) at rest (-30 min), after prime infusion (0 min), and during 120 min of exercise.

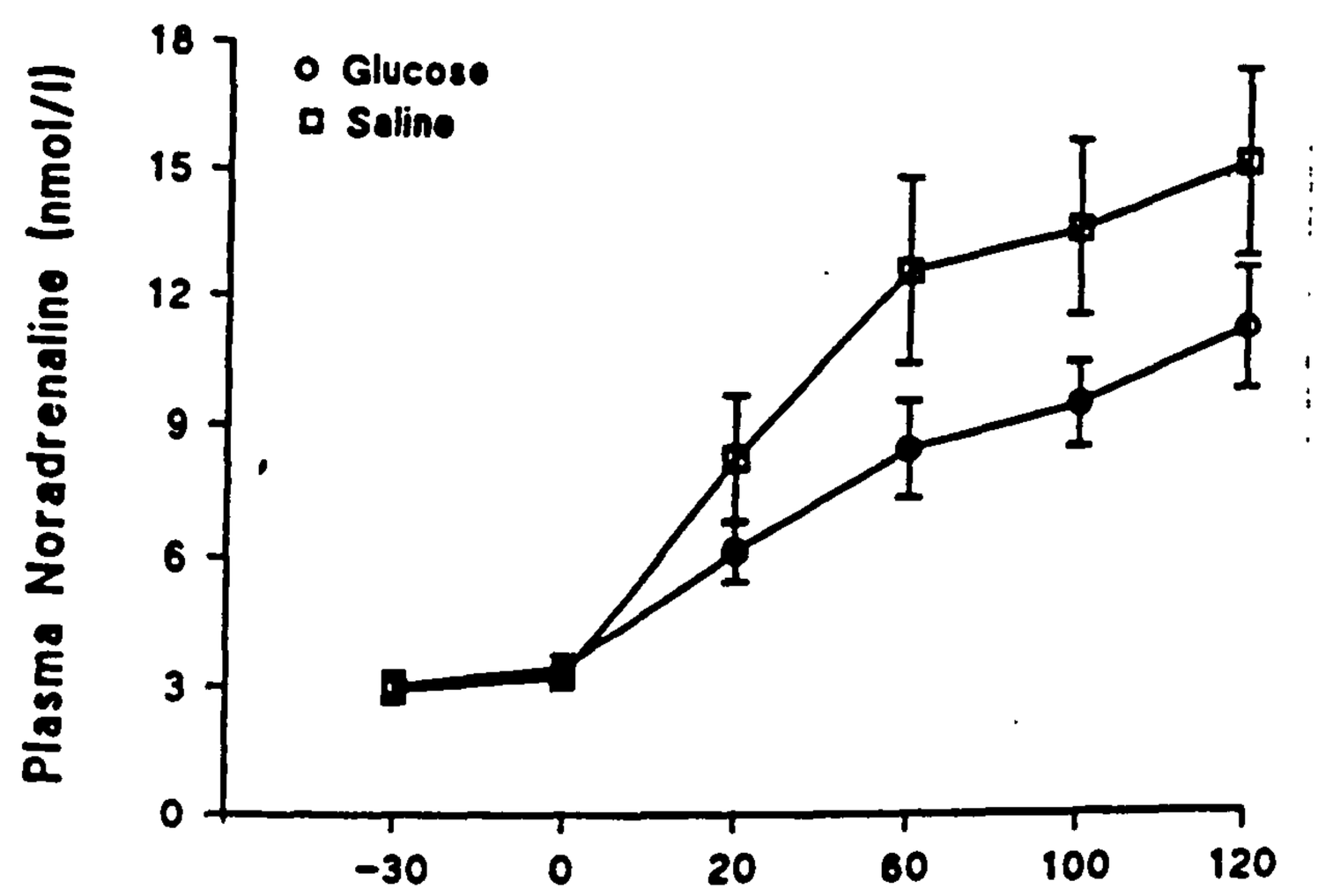


Fig 7.5 Mean (±SEM) plasma noradrenaline concentrations (nM) at rest (-30 min), after prime infusion (0 min), and during 120 min of exercise.

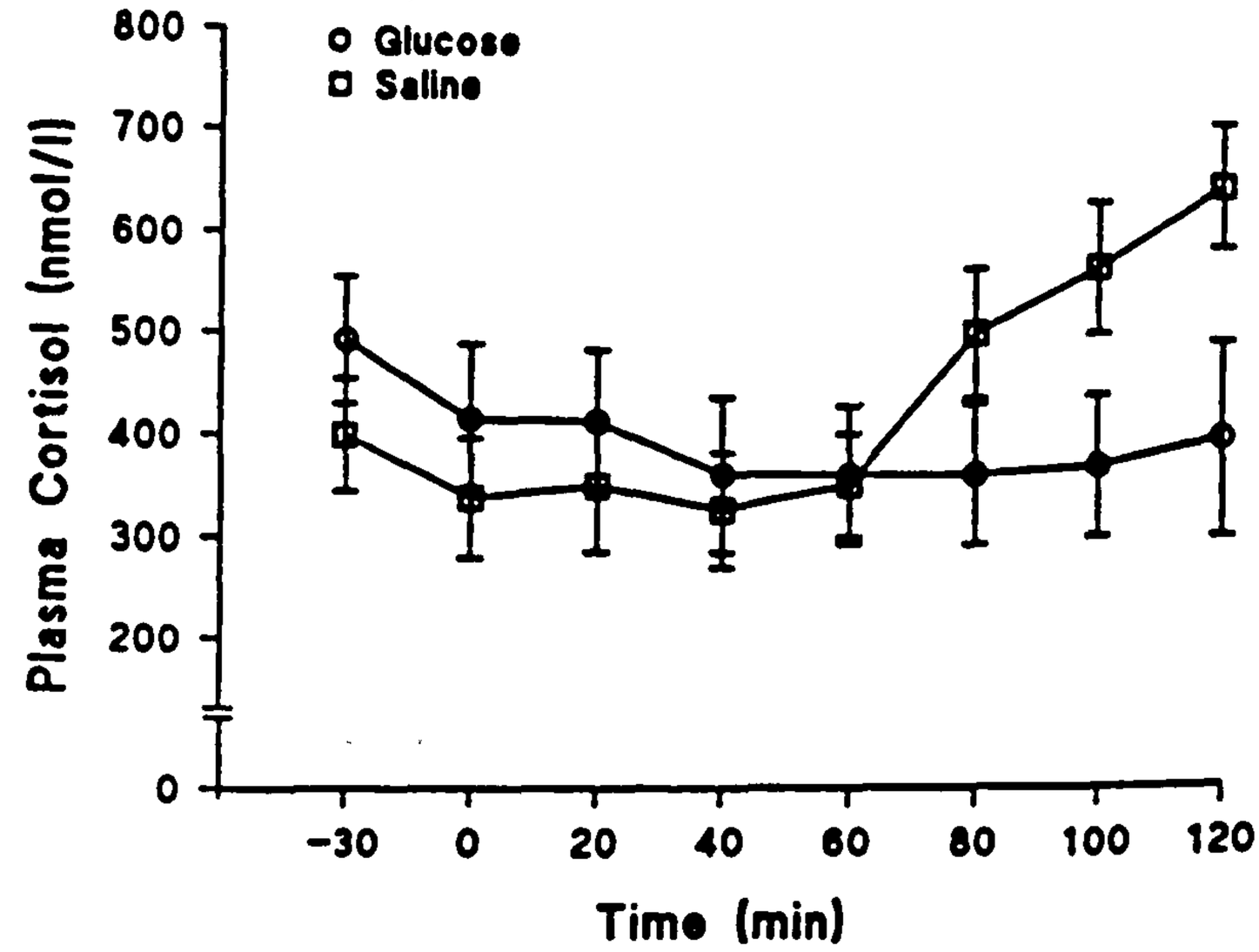


Fig 7.6 Mean (±SEM) plasma cortisol concentrations (nM) at rest (-30 min), after prime infusion (0 min), and during 120 min of exercise.

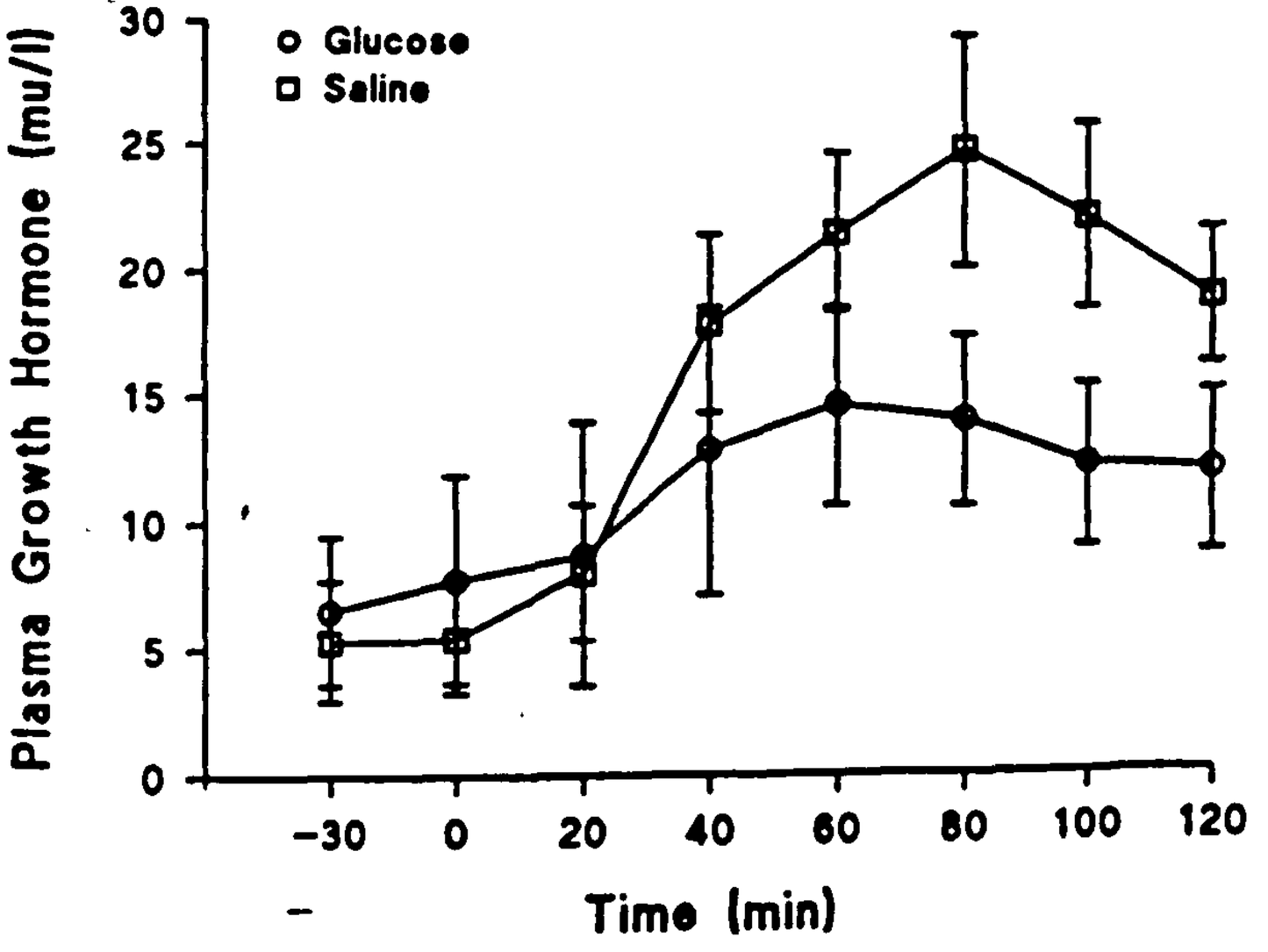


Fig 7.7 Mean (±SEM) plasma growth hormone concentrations ($m\mu l^{-1}$) at rest (-30 min), after prime infusion (0 min), and during 120 min of exercise.

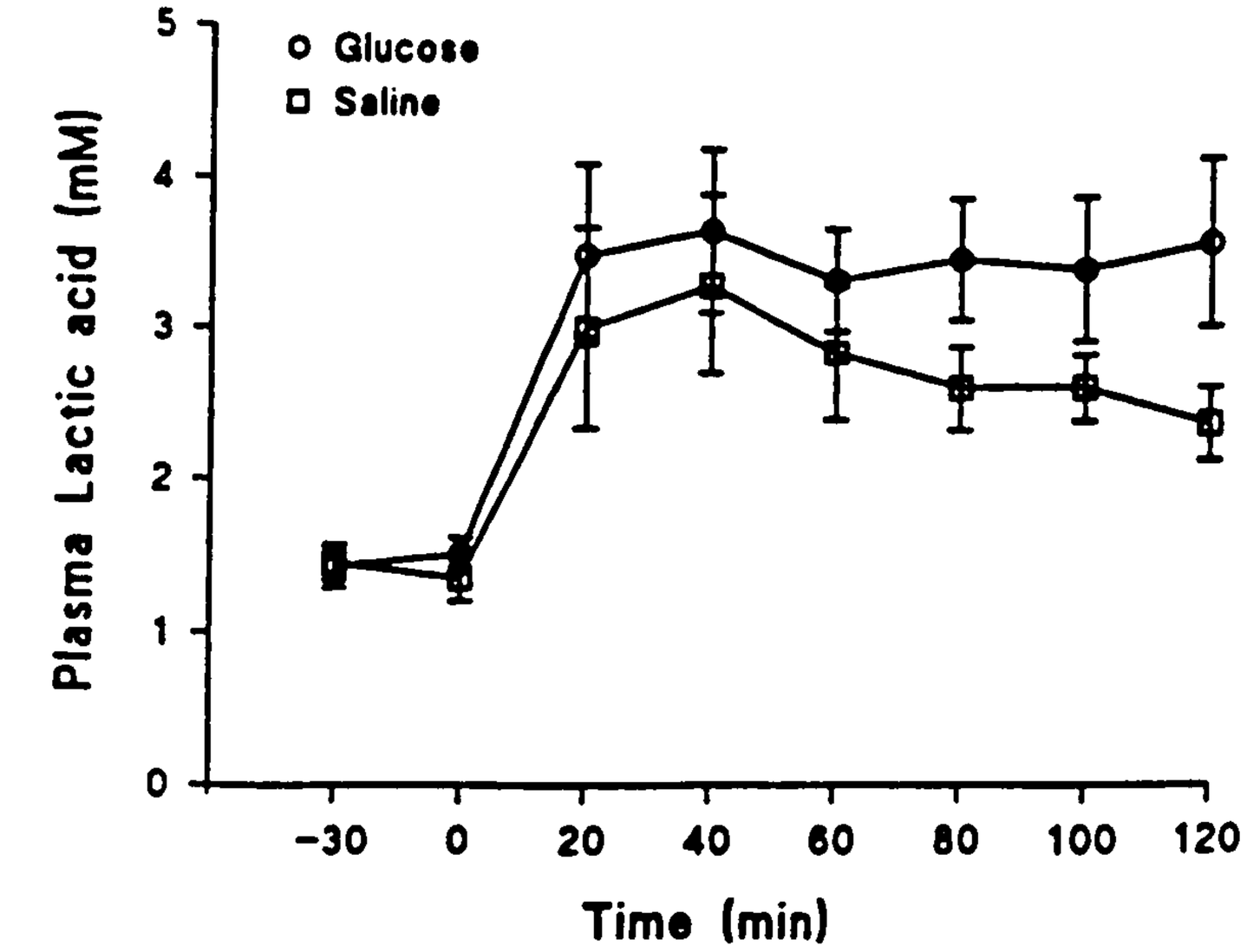


Fig 7.8 Mean (±SEM) plasma lactate concentrations (mM) at rest (-30 min), after prime infusion (0 min), and during 120 min of exercise.

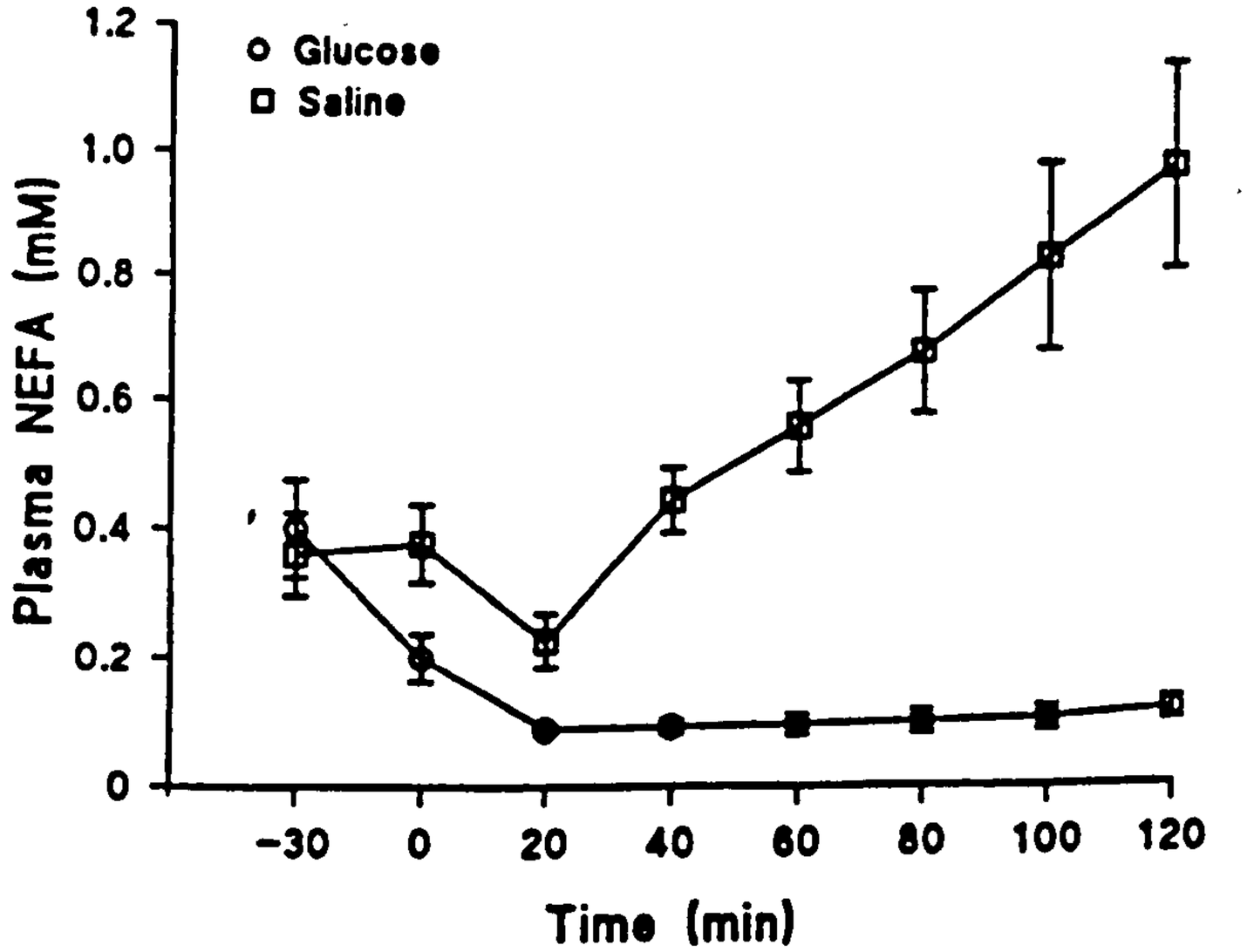


Fig 7.9 Mean (±SEM) plasma NEFA concentrations (mM) at rest (-30 min), after prime infusion (0 min), and during 120 min of exercise.

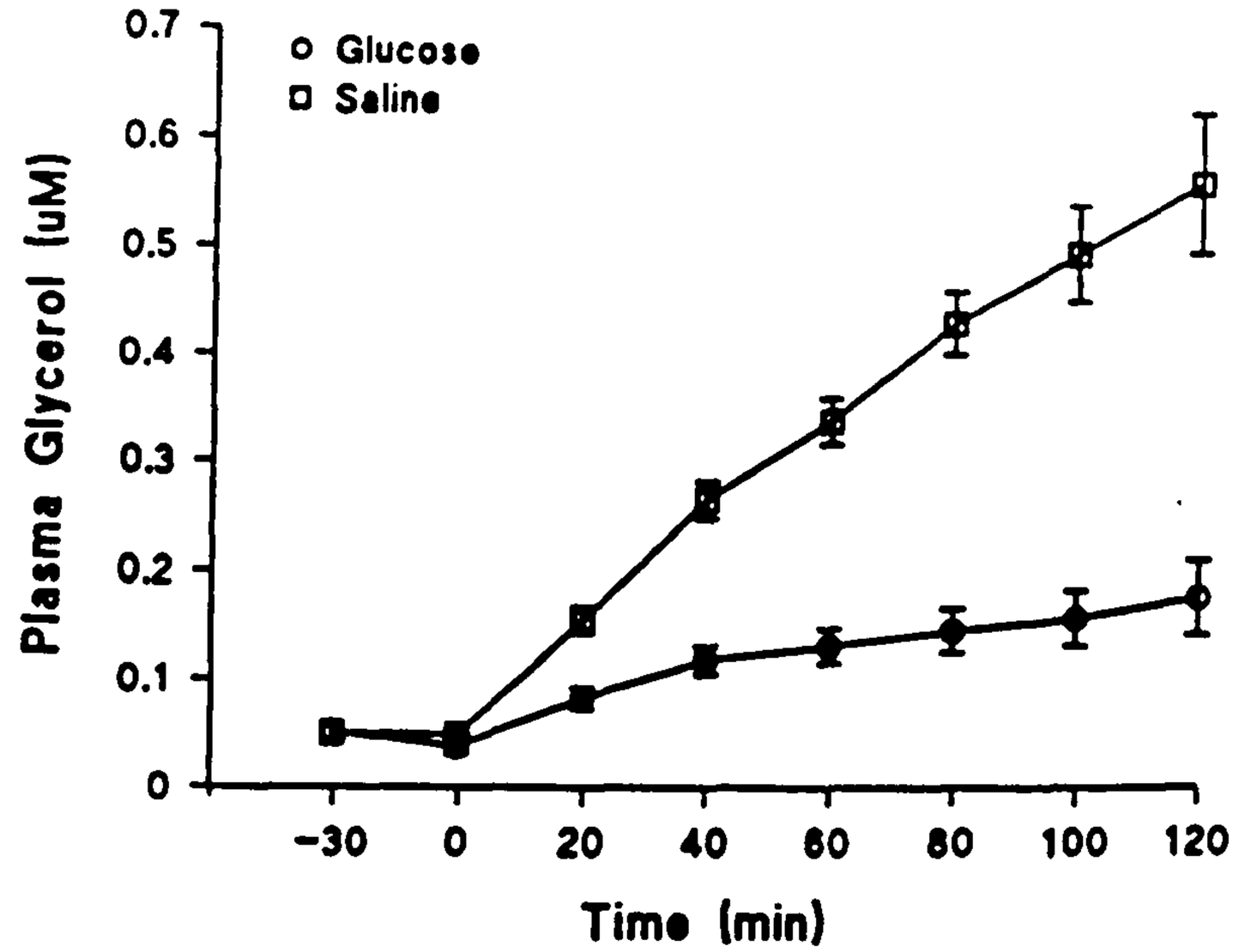


Fig 7.10 Mean (±SEM) plasma glycerol concentrations (μM) at rest (-30 min), after prime infusion (0 min), and during 120 min of exercise.

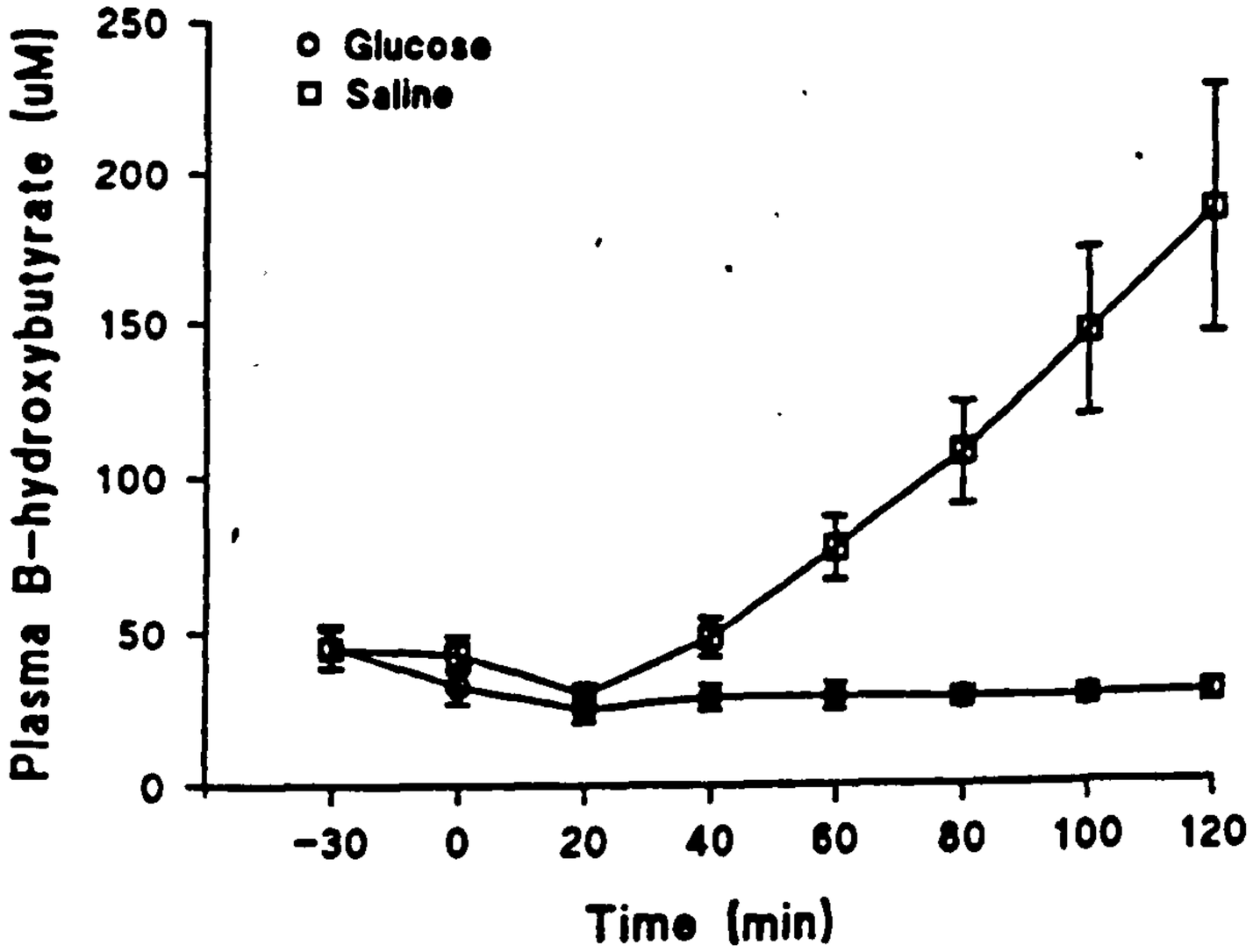


Fig 7.11 Mean (±SEM) plasma B-OH concentrations (μM) at rest (-30 min), after prime infusion (0 min), and during 120 min of exercise.

The data collected on all the 20 amino acids determined at rest and during exercise under both infusion conditions can be seen in Appendix D. Only the results for alanine, glutamine, and the branched-chain amino acids will be dealt with in greater detail here. The reason being concerned with the roles played by these amino acids in the glucose-alanine cycle.

The plasma and erythrocyte amino acid concentrations observed during the basal period can be seen in Table 7.1. The resting amino acid concentrations in the erythrocytes were significantly higher than in plasma with regard to alanine, isoleucine, leucine, and valine, and significantly lower for glutamine.

Table 7.1 Mean (±SEM) plasma and red-cell amino acid concentrations at rest.

Amino Acid	Plasma (μM)	Erythrocyte (μM)	t-value	P-value
Alanine	389.8±32.7	1304.6±180.7	5.98	0.001
Glutamine	466.1±46.9	119.1±17.8	8.02	0.001
Isoleucine	56.9±5.9	155.9±24.9	4.32	0.01
Leucine	122.4±10.0	401.8±21.6	14.18	0.001
Valine	220.3±27.1	540.9±94.8	3.33	0.05

The 30 min 'prime' infusion resulted in a significant decrease in plasma concentration for isoleucine ($P<0.05$) after glucose infusion (Table 7.2). Significant decreases in plasma concentration were also evident for alanine and isoleucine ($P<0.05$) after saline infusion, although a significant increase was found for red cell alanine after glucose infusion ($P<0.05$). The percentage changes in concentration of the amino acids after the prime infusion can be seen in Table 7.3.

Table 7.2 Mean (±SEM) plasma and red cell amino acid concentrations (μM) under glucose and saline infusion at rest and after 30 min prime infusion.

Amino Acid	Infusion	Plasma		Red Cell	
		Rest	Post-prime	Rest	Post-prime
Alanine	Glucose	418±48	379±48	1422±308	1665±304
	Saline	361±46	327±46	1187±220	1136±220
Glutamine	Glucose	483±57	495±74	127±21	215±84
	Saline	449±82	424±76	141±26	142±29
Isoleucine	Glucose	57±7	48±6	169±49	257±113
	Saline	57±10	50±9	143±21	138±38
Leucine	Glucose	114±14	97±15	402±42	476±36
	Saline	131±15	118±15	402±21	454±62
Valine	Glucose	197±13	186±17	614±175	935±424
	Saline	243±54	224±47	468±88	411±83

Table 7.3 Percentage changes in amino acid concentration after the 30 min prime infusion.

Amino Acid	Plasma		Red Cell	
	Glucose	Saline	Glucose	Saline
Alanine	-9%	-9%	+17%	-4%
Glutamine	+2%	-6%	+122%	0
Isoleucine	-16%	-12%	+52%	-3%
Leucine	-15%	-10%	+18%	+13%
Valine	-6%	-8%	+52%	-12%

During exercise the plasma amino acid concentrations showed a tendency to rise under both conditions (Figs 7.12-7.21). Significant increases were seen during glucose infusion for plasma concentrations of alanine ($P<0.05$). Saline infusion, on the other hand, resulted in significant increases in plasma levels of alanine and glutamine ($P<0.05$), whereas significant increases in red cell levels were found for alanine, isoleucine and leucine ($P<0.05$).

Significant differences were apparent between the glucose and saline infusion trials during exercise ($P<0.05$) for plasma concentrations of leucine at 60 and 90 min. Similarly, significant differences were found between the trials for red cell concentrations of alanine at 30, 60 and 90 min ($P<0.01$) and

120 min ($P<0.05$), isoleucine at 30 min ($P<0.05$), and valine at all time points during exercise ($P<0.05$).

The rates of plasma amino acid changes during exercise were significantly lower for glutamine ($P<0.05$) during glucose infusion compared with saline infusion. Rates of red cell amino acid changes showed significant increases in rates of uptake for alanine ($P<0.001$) during glucose infusion (Table 7.4).

Calculations of the carbohydrate oxidation rates from the RER and VO_2 data resulted in the findings displayed in figure 7.22. Determination of the mean area under the curves for each subject, followed by a paired t-test revealed significantly higher ($t=8.47$; $df=7$; $P<0.01$) carbohydrate oxidation rates for glucose infusion (320.8 $\pm$ 56.3 g in 120 min) compared with saline infusion (229.7 $\pm$ 46.1 g in 120 min). A one-way analysis of variance for each trial showed there was no significant difference in carbohydrate oxidation with time under glucose infusion ($F_{4,28}= 0.6$; $P>0.05$), but a significant decrease under saline infusion ($F_{4,28}= 17.7$; $P<0.01$). The carbohydrate oxidation rate at 120 min of exercise is eight times greater than at 120 min of rest from the previous study i.e. 2.6 g min^{-1} as compared with 0.3 g min^{-1}.

Table 7.4 Rates of uptake/release (μM min^{-1}) of amino acids during exercise under glucose and saline infusion. (↑ = glucose trial > saline trial; ↓ = glucose trial < saline trial).

Amino Acid	Plasma			Red Cell		
	Glucose	Saline		Glucose	Saline	
Alanine	2.1 ***	1.33 **	↑	20.45 ***	5.03 **	↑ ***
Glutamine	0.22	1.77 **	↓ *	0.37	0.39	↓
Isoleucine	0.02	0.02	-	0.61	0.97 *	↓
Leucine	0.08	0.06	↑	3.93 ***	2.45 *	↑
Valine	0.16	0.12	↑	3.10	0.49	↑

(* P<0.05, ** P<0.01, ***P<0.001)

The data on lipid oxidation rates were an inverse of that for carbohydrate oxidation (Fig 7.23). Significant differences were apparent between the trials ($F_{1,7}$=61.98; P<0.01), with the saline trial producing higher rates of oxidation than under glucose infusion. As the exercise period progressed there was a significant shift towards lipid oxidation under saline infusion ($F_{4,28}$= 9.22; P<0.01).

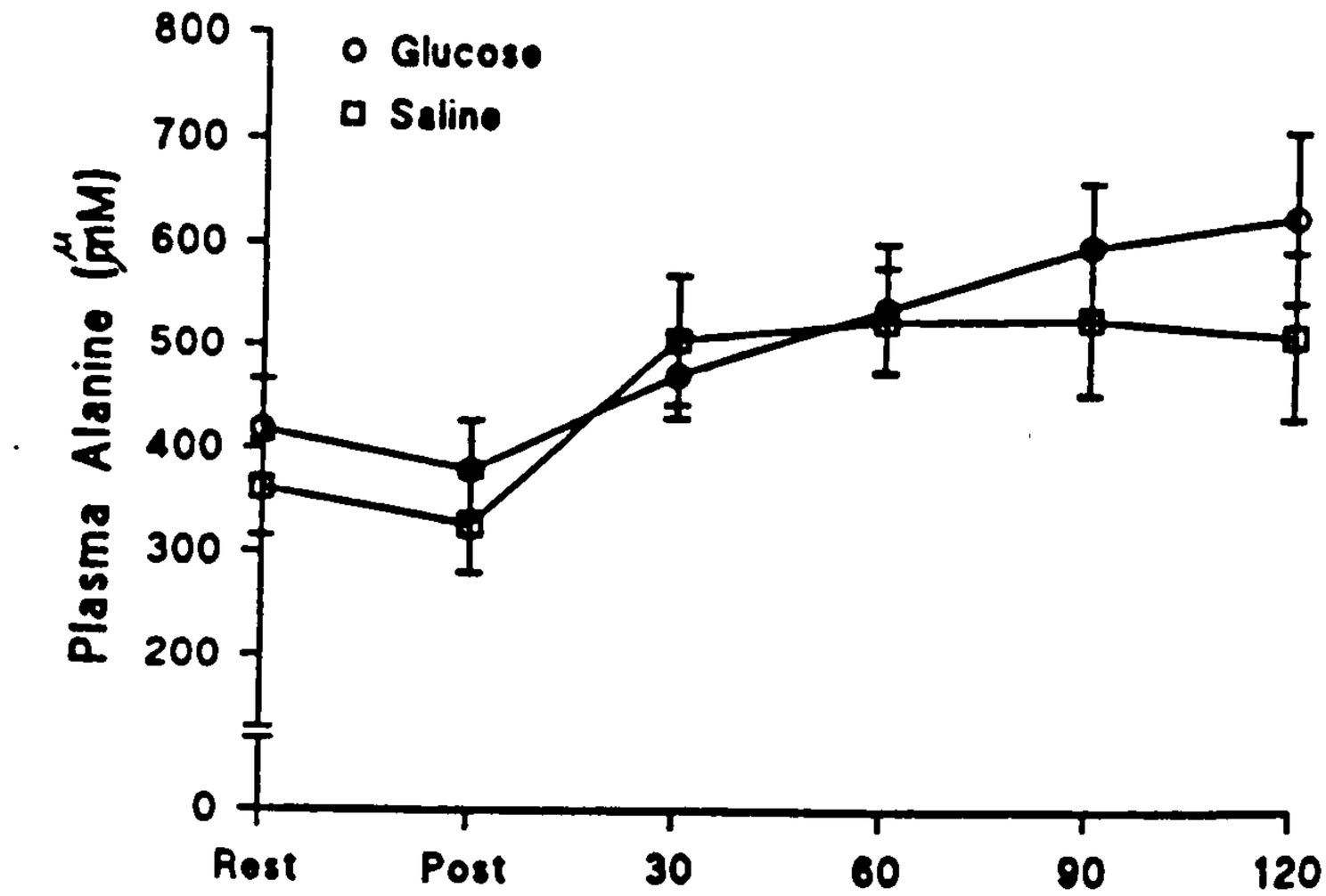


Fig 7.12 Mean (±SEM) plasma alanine concentrations (μM) at rest, after prime infusion (post), and during 120 min of exercise.

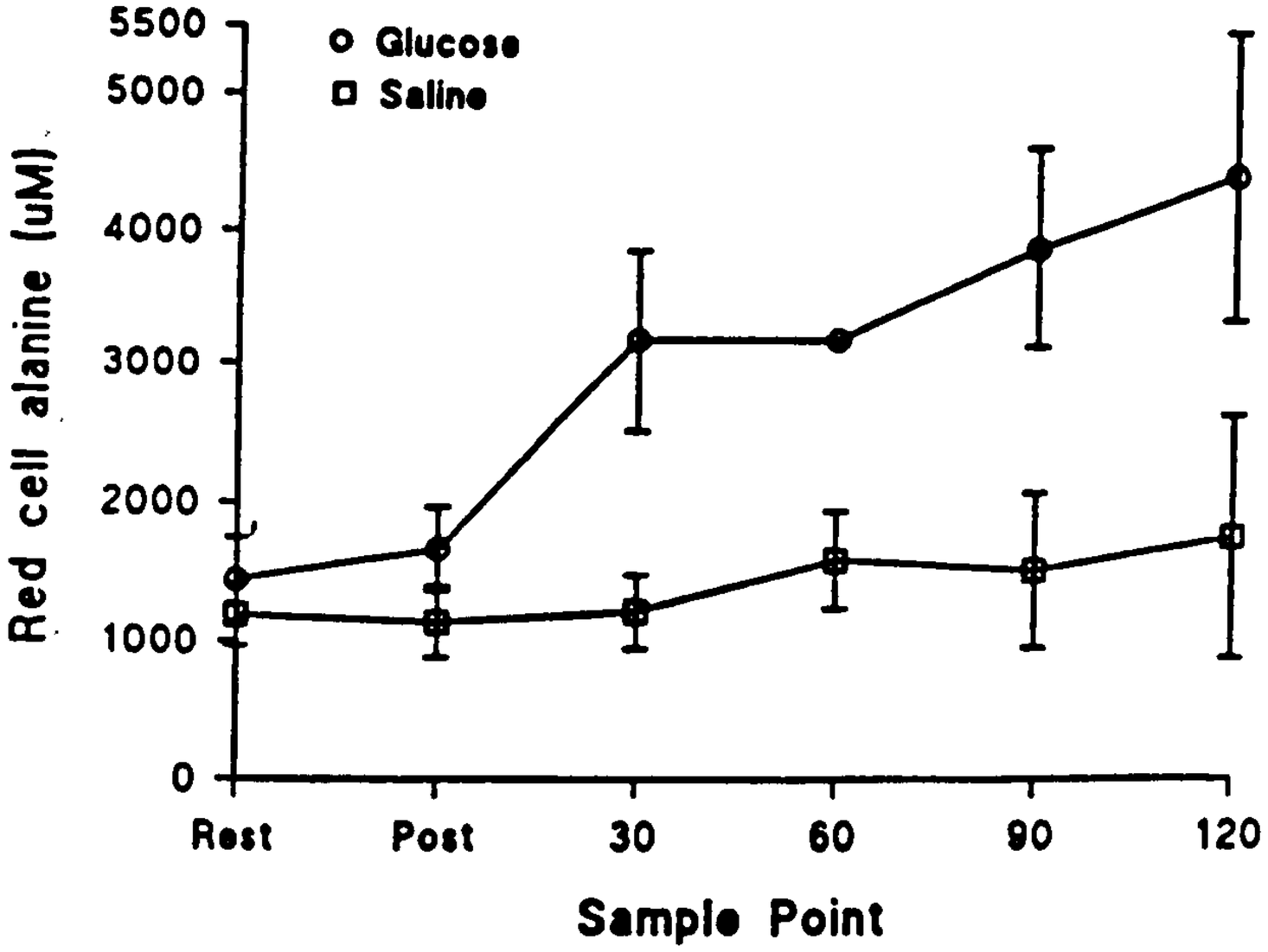


Fig 7.13 Mean (±SEM) red cell alanine concentrations (μM) at rest, after prime infusion (post), and during 120 min of exercise.

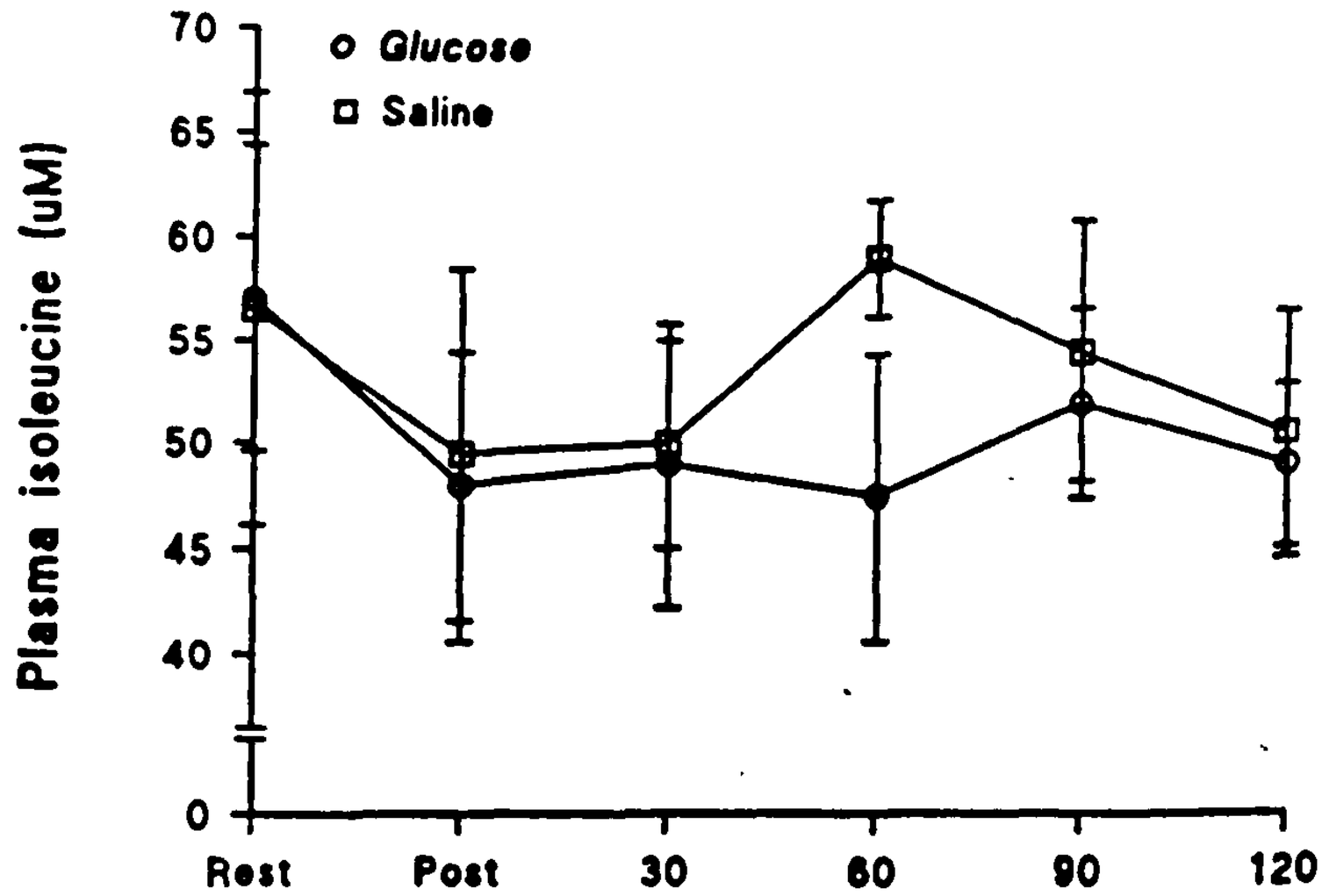


Fig 7.14 Mean (±SEM) plasma isoleucine concentrations (μM) at rest, after prime infusion (post), and during 120 min of exercise.

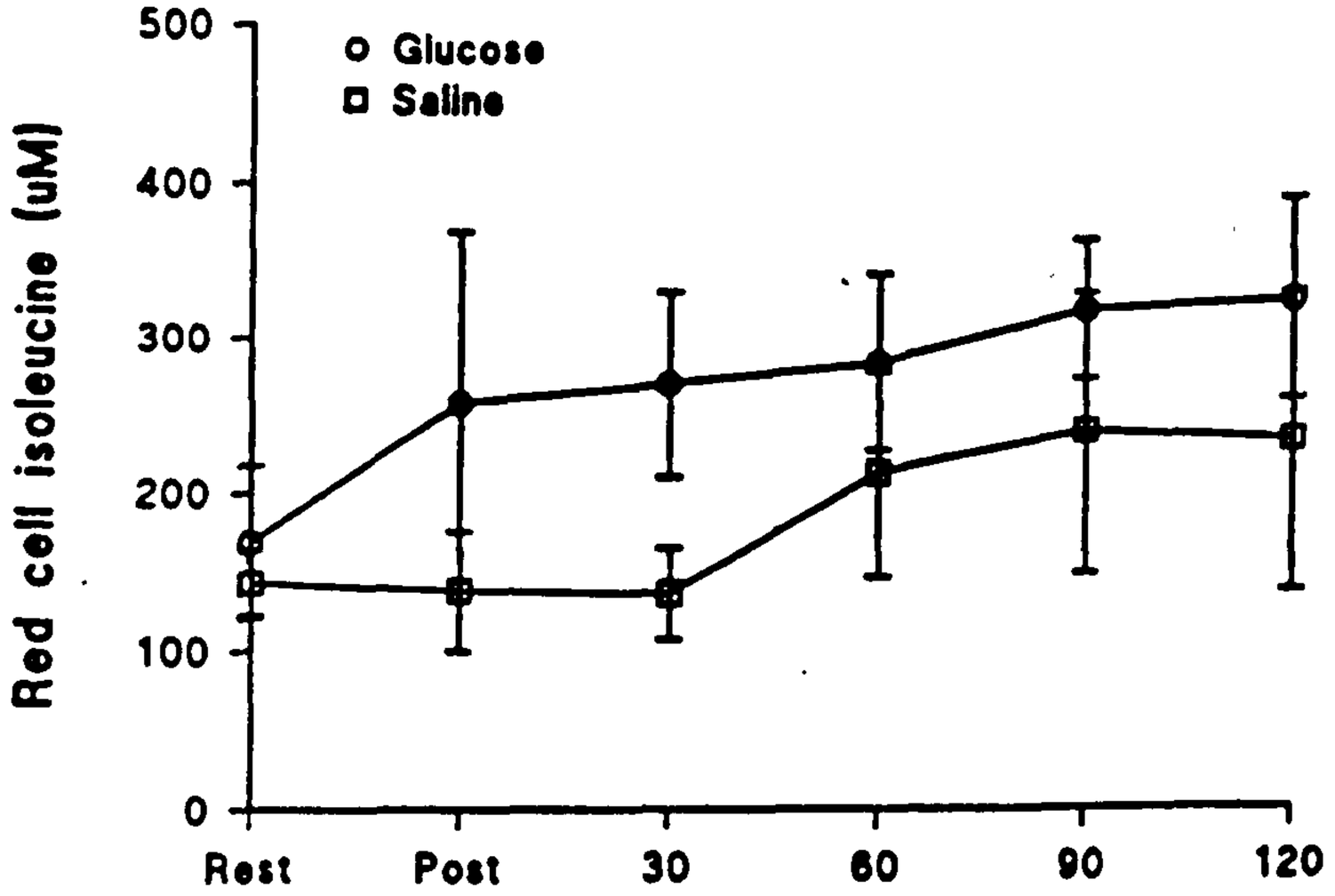


Fig 7.15 Mean (±SEM) red cell isoleucine concentrations (μM) at rest, after prime infusion (post), and during 120 min of exercise.

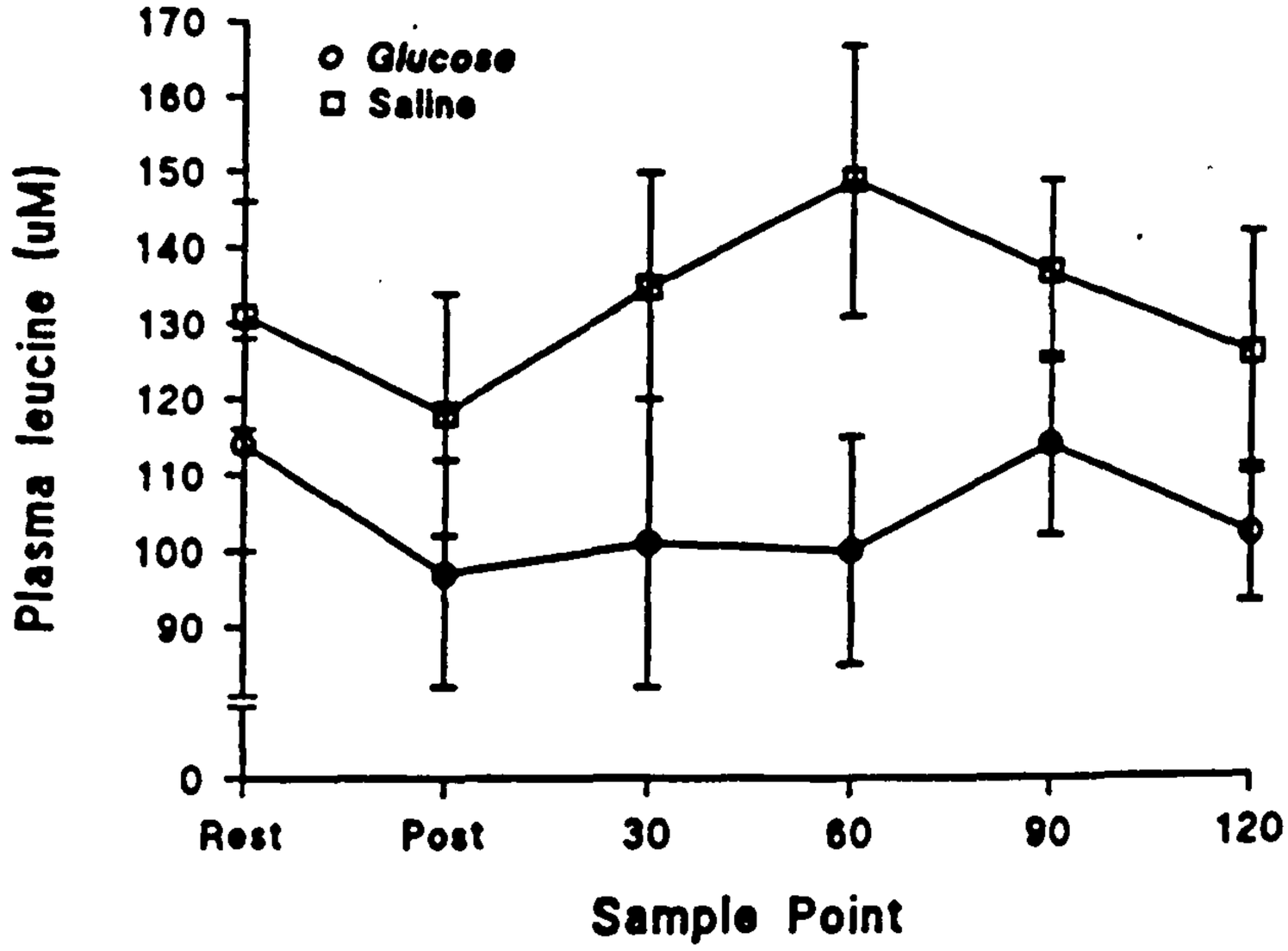


Fig 7.16 Mean (±SEM) plasma leucine concentrations (μM) at rest, after prime infusion (post), and during 120 min of exercise.

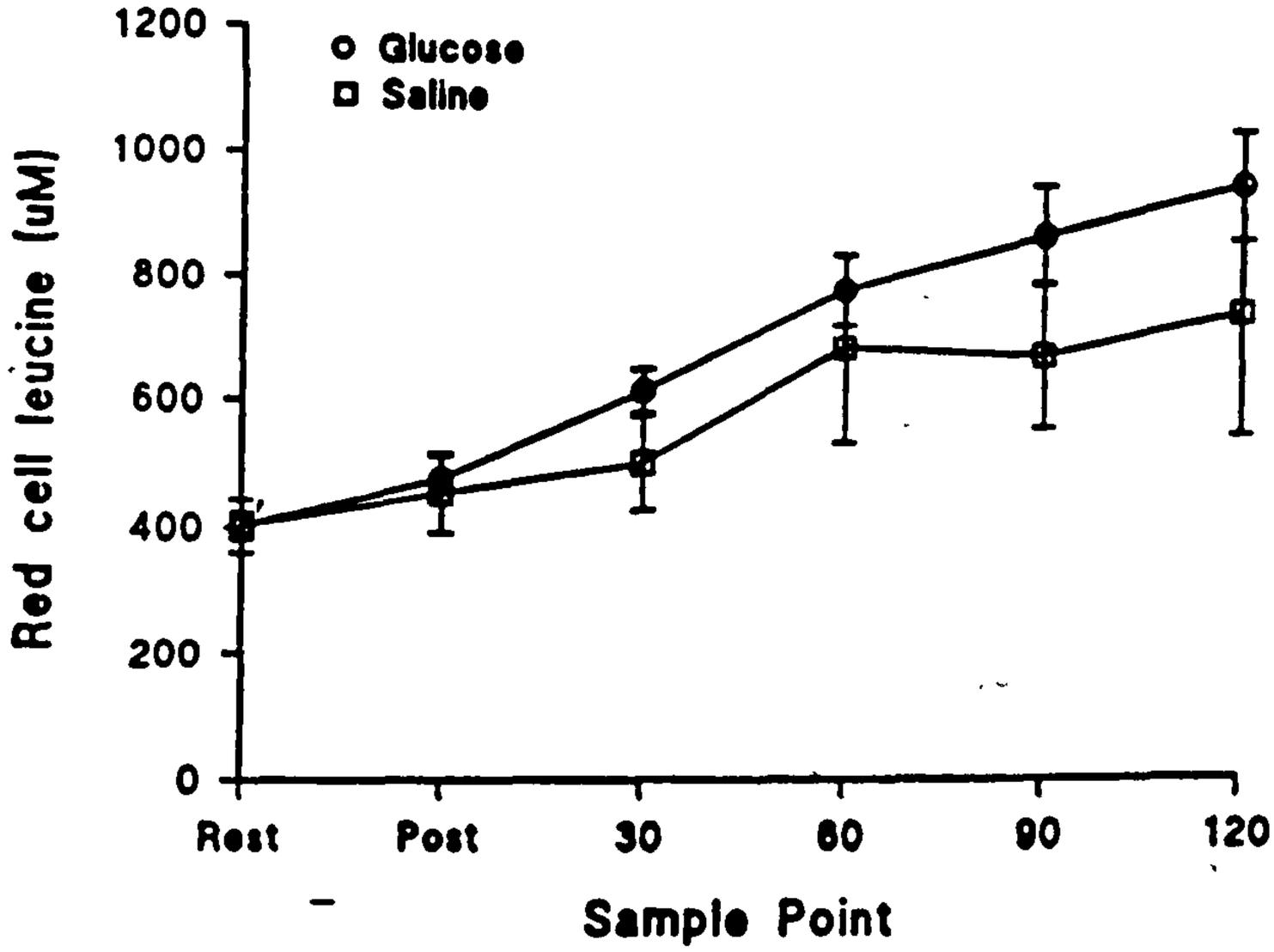


Fig 7.17 Mean (±SEM) red cell leucine concentrations (μM) at rest, after prime infusion (post), and during 120 min of exercise.

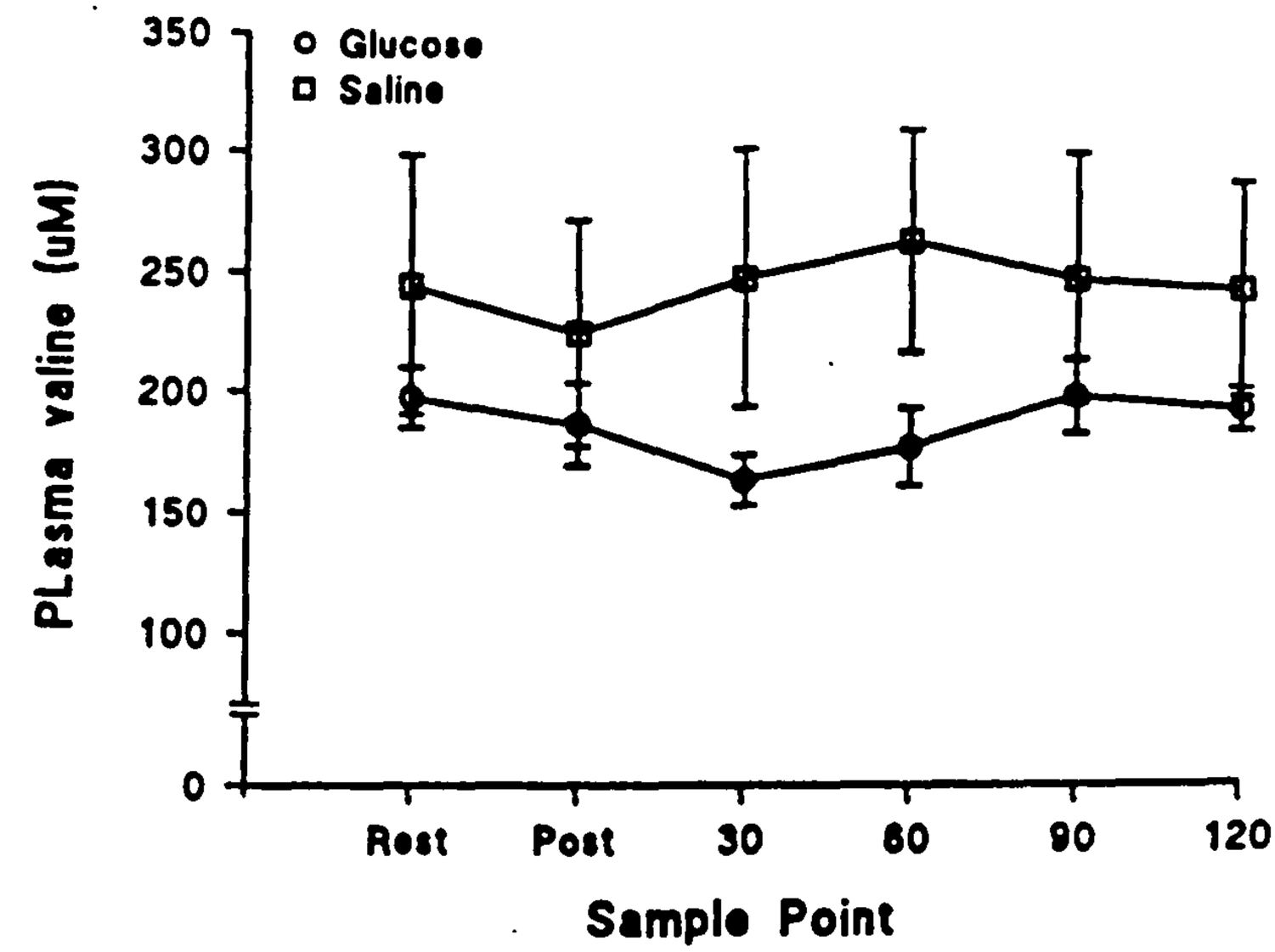


Fig 7.18 Mean (±SEM) plasma valine concentrations (μM) at rest, after prime infusion (post), and during 120 min of exercise.

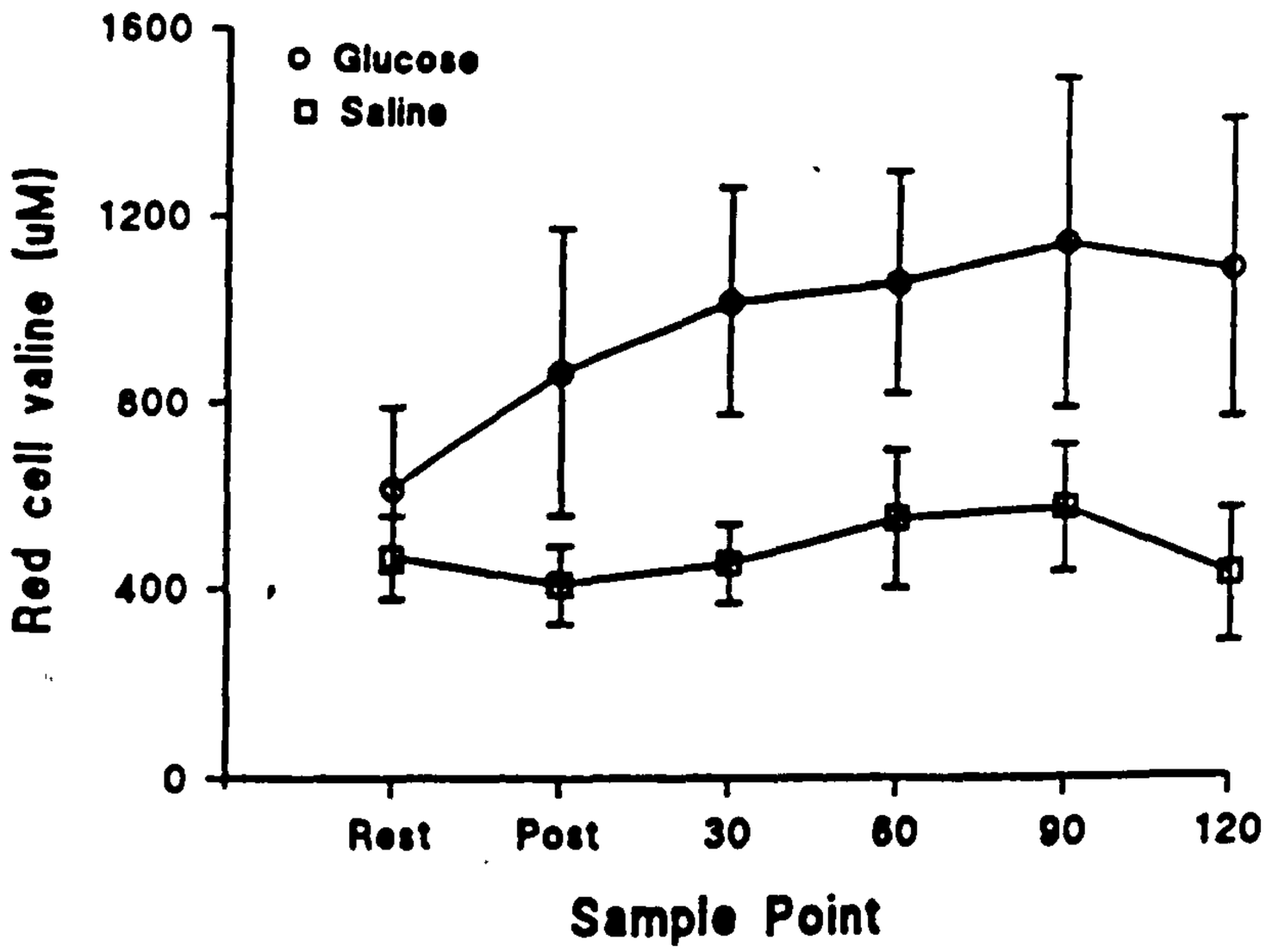


Fig 7.19 Mean (±SEM) red cell valine concentrations (μM) at rest, after prime infusion (post), and during 120 min of exercise.

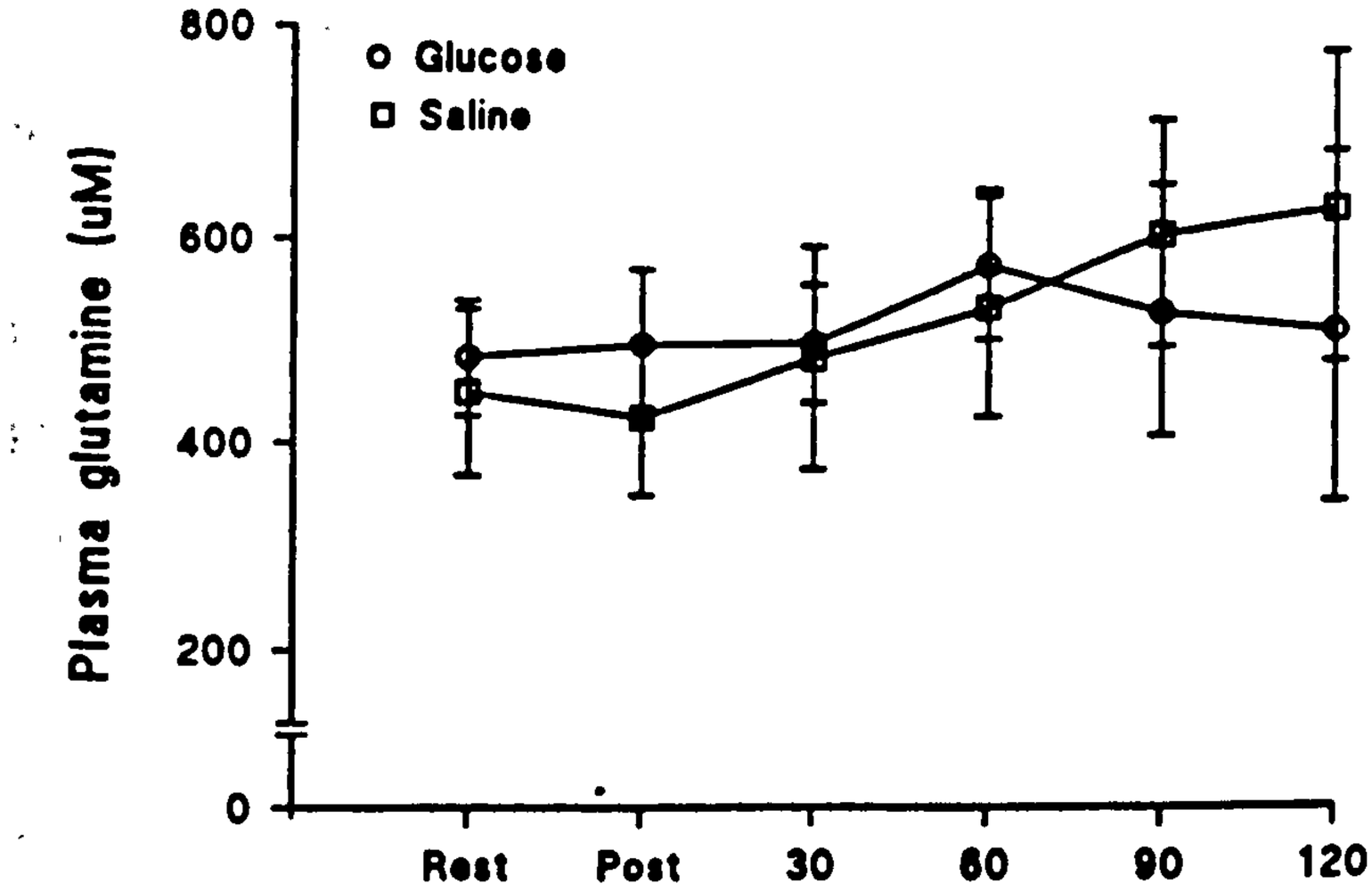


Fig 7.20 Mean (±SEM) plasma glutamine concentrations (μM) at rest, after prime infusion (post), and during 120 min of exercise.

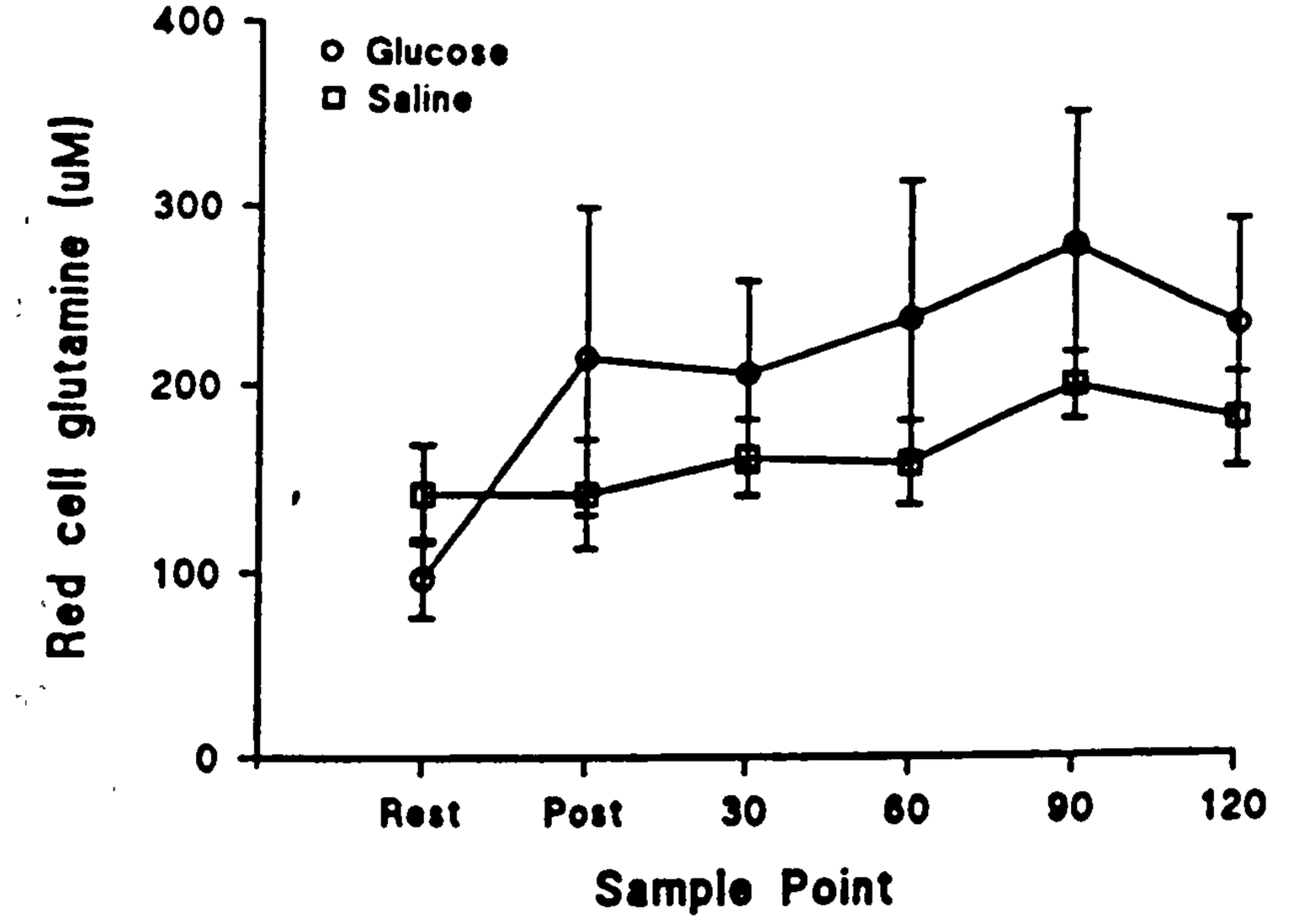


Fig 7.21 Mean (±SEM) red cell glutamine concentrations (μM) at rest, after prime infusion (post), and during 120 min of exercise.

The rate of glucose utilisation, as measured by the glucose clamp procedure, increased significantly during exercise ($F_{5,35}$=9.75; P<0.01). Figure 7.24 illustrates that this increase occurred up to 80-100 min before a plateau was reached. The value of 1.8 g min^{-1} (146 μm kg^{-1} min^{-1}) is a 61% increase from the start of the exercise period, and a 125% increase compared to the bed-rest values at 120 min in the previous experiment.

Figure 7.25 examines the relationship between the rate of carbohydrate utilisation and the rate of carbohydrate oxidation. When expressed as a percentage of the mean total carbohydrate oxidation rate, the mean glucose utilisation rate was 40.8%, 53.7%, 57.2%, 68.9%, and 68.3% for 15, 30, 60, 90, and 120 min, respectively.

Muscle glycogen concentration became significantly depleted in both trials as a result of the exercise (Fig 7.26). Related 't'-tests highlighted this fact (t=11.8; P<0.01 for glucose; t=10.4; P<0.01 for saline). A significant difference for the post-exercise muscle glycogen concentration was evident between the trials, with the glucose infusion resulting in a higher concentration (t=3.59; P<0.01). The differences from resting levels to post-exercise were 170.3 μg mol^{-1} dwt after the hyperglycaemic clamp, and 206 μg mol^{-1} dwt after saline infusion. This represents an 8% difference in muscle glycogen use.

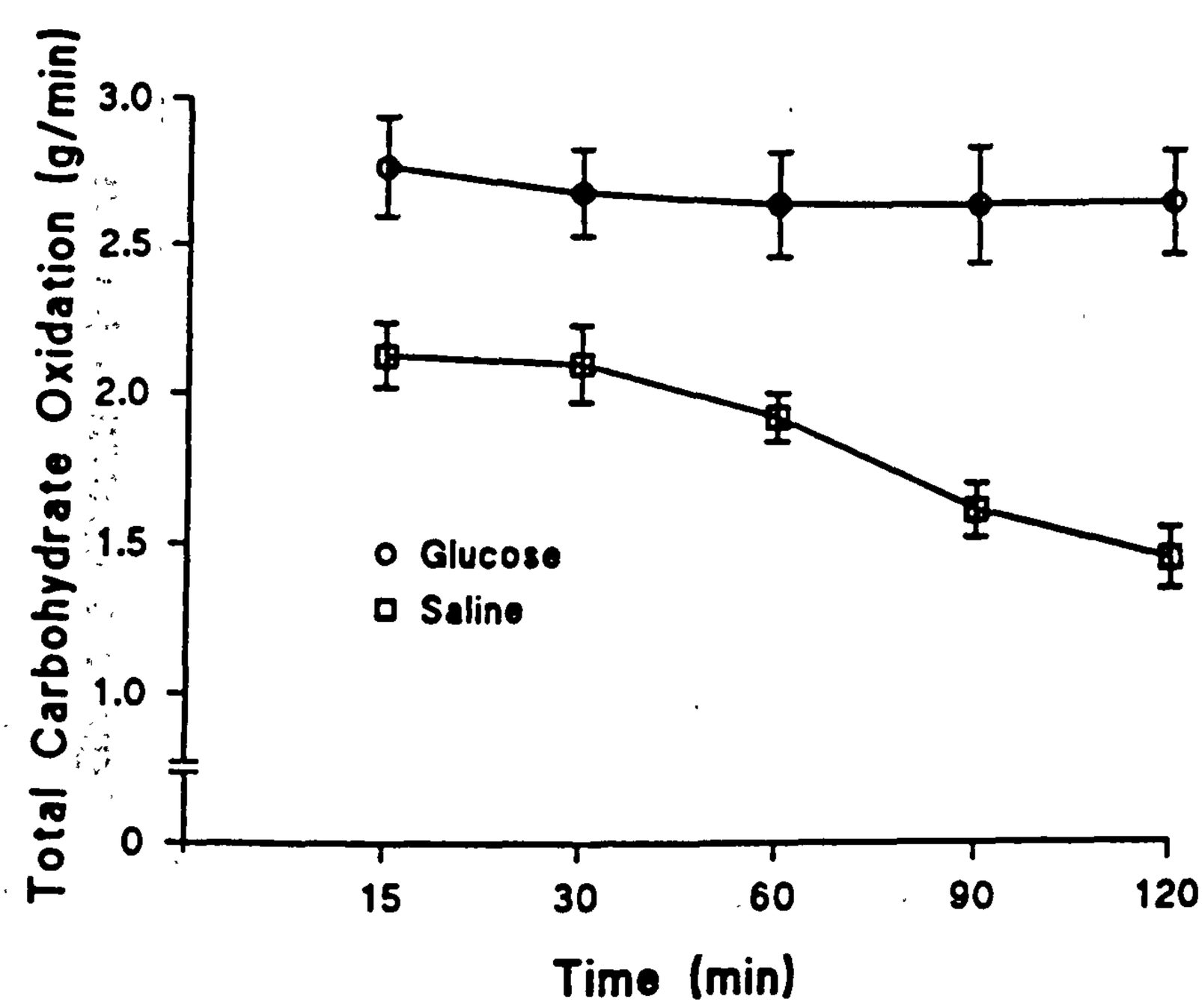


Fig 7.22 Mean (±SEM) total carbohydrate oxidation rates during 120 min of exercise under saline and glucose infusion.

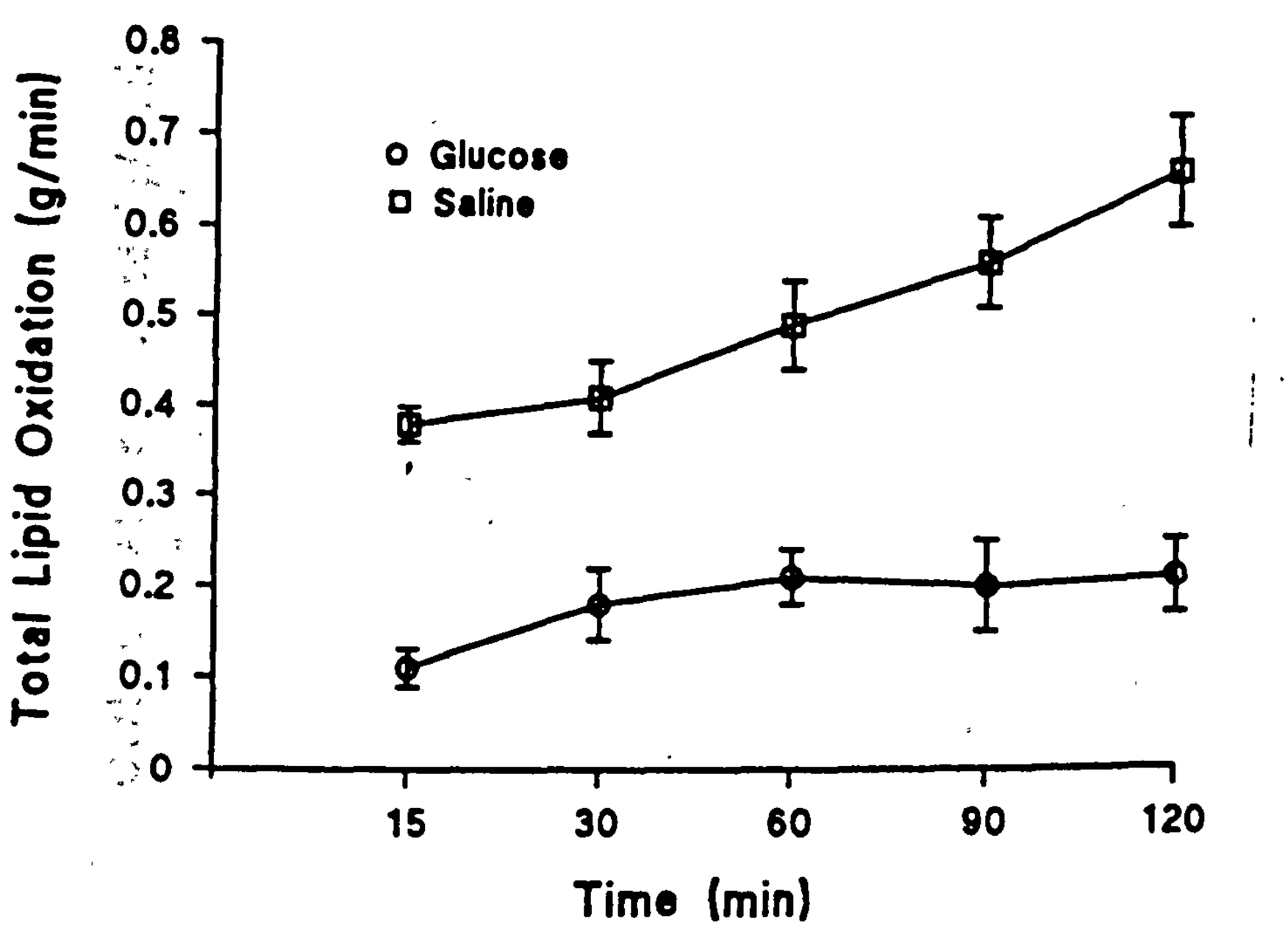


Fig 7.23 Mean (±SEM) total lipid oxidation rates during 120 min of exercise under saline and glucose infusion.

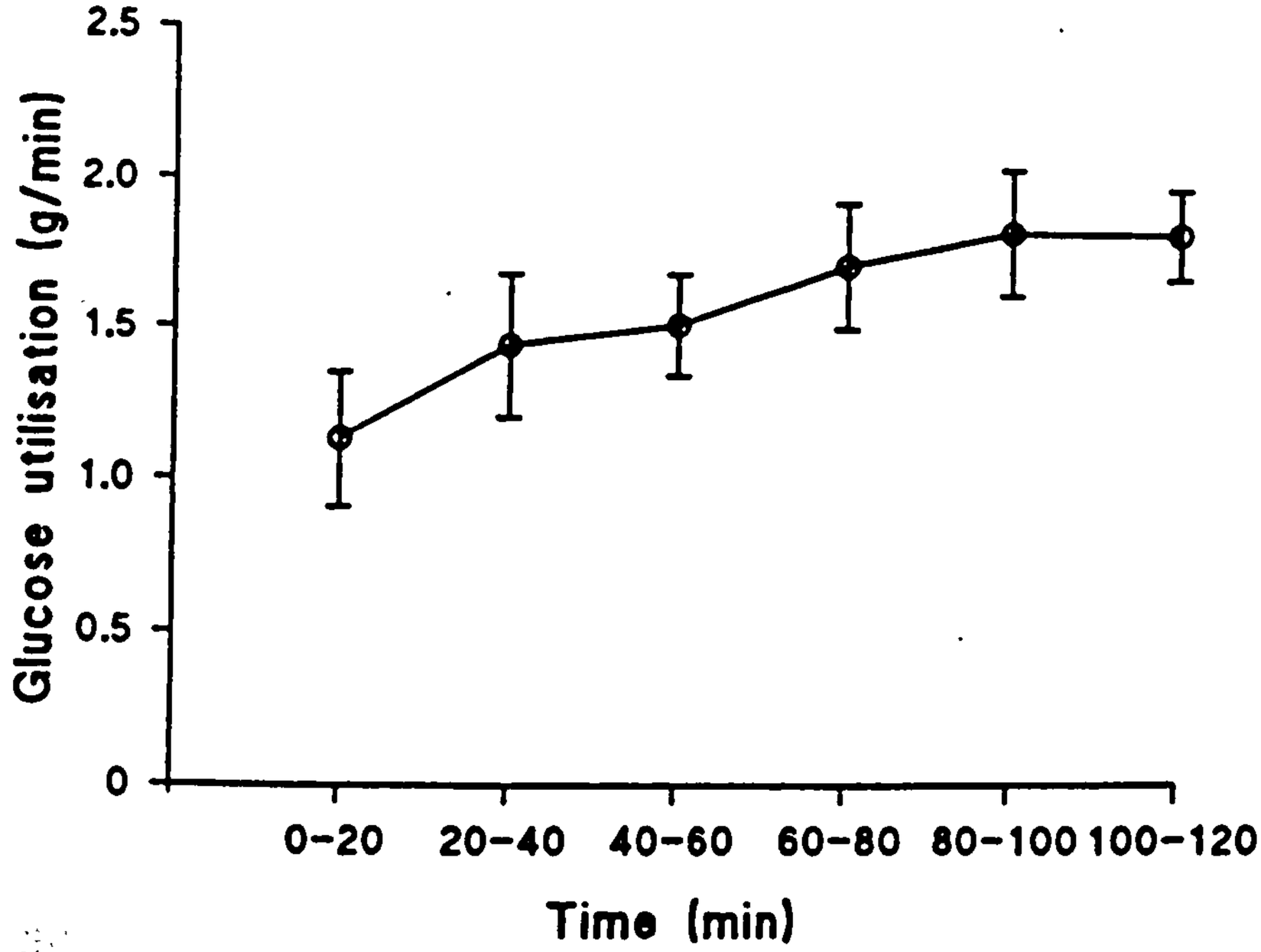


Fig 7.24 Mean (±SEM) glucose utilisation rate (g min^{-1}) during 120 min of exercise under glucose infusion.

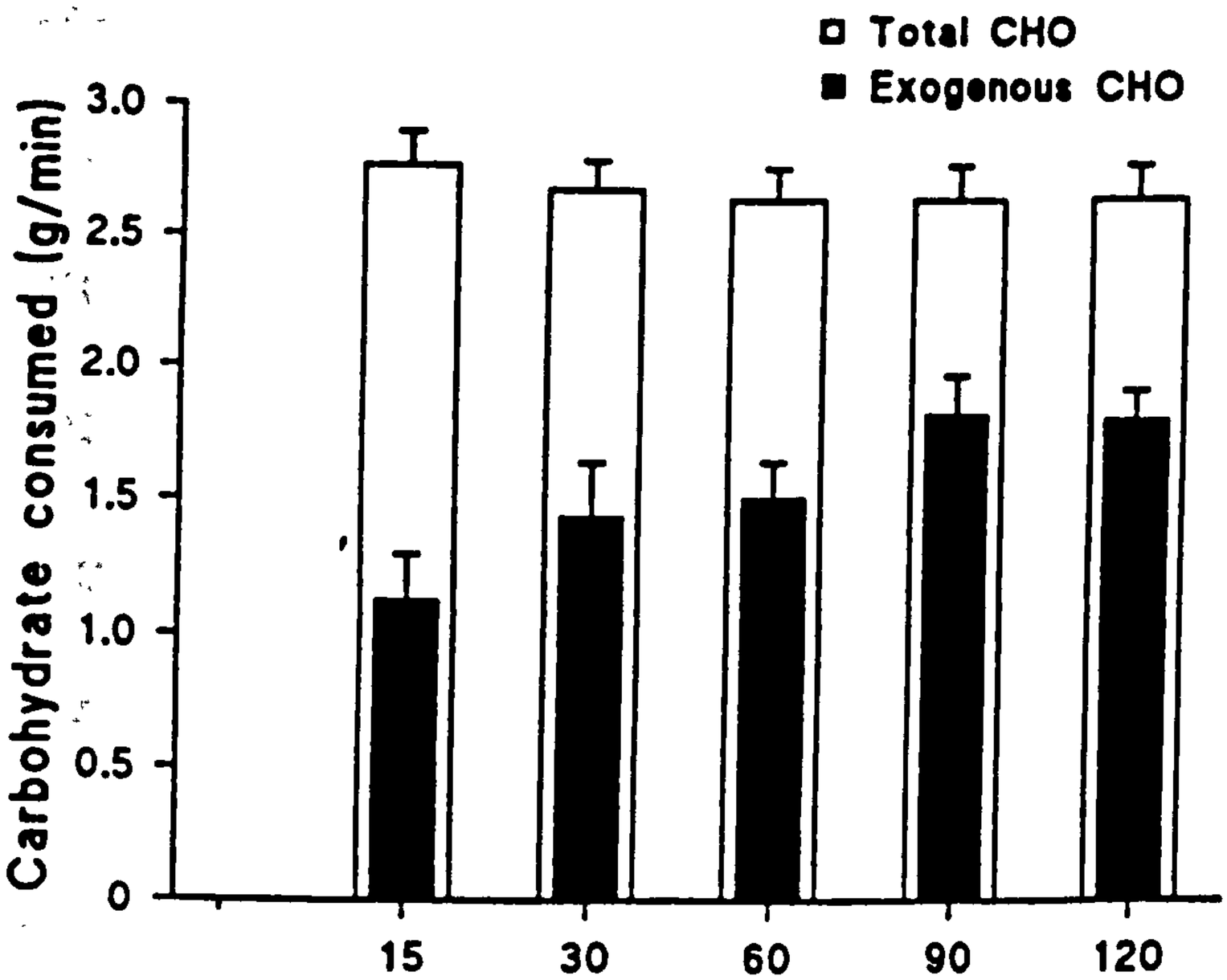


Fig 7.25 Mean (±SEM) rates of glucose utilised (exogenous CHO) and total carbohydrate oxidised (total CHO).

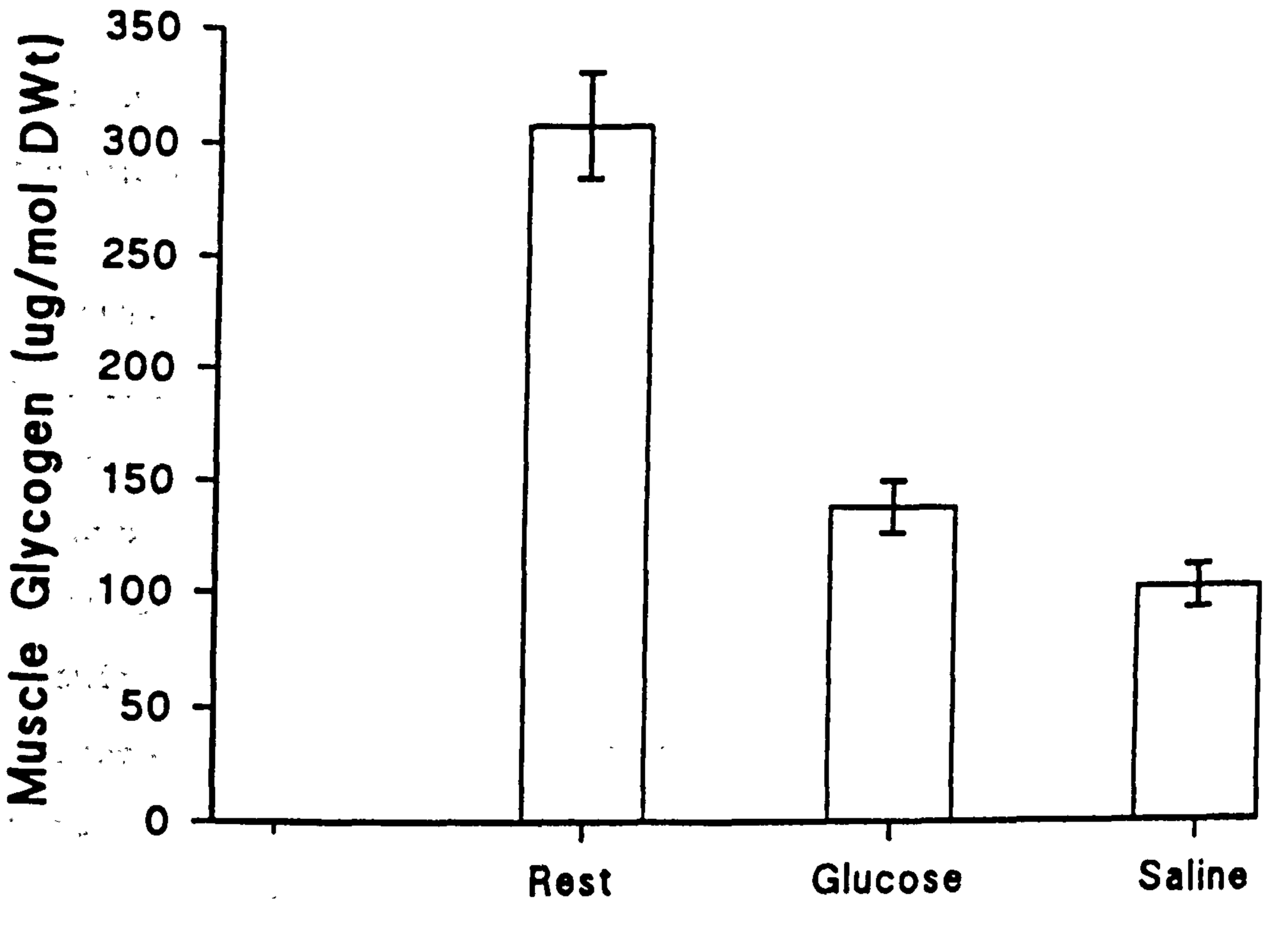


Fig 7.26 Mean (±SEM) muscle glycogen concentrations ($\mu g\ mol^{-1}$ dw) at rest, and after 120 min of exercise under saline and glucose infusions.

7.4 DISCUSSION

Hyperglycaemia was maintained throughout the exercise period with little variation, thus clearly emphasising the suitability of the clamp procedure to situations other than rest. This finding is in keeping with those of Coyle et al. (1991) and Hawley et al. (1994) who were able to clamp their subjects at 10 mM for 120 min of cycling at approximately 70% VO_2max. Since little variation was found, the measure of whole body glucose utilisation from this method appears to be reliable.

The resultant hyperinsulinaemia as a consequence of glucose infusion was expected. The fact that a trend towards a decrease after 60 min was evident is similar to that observed by Coyle et al. (1991). It is quite possible that the elevations in adrenaline which occur during exercise are responsible for this phenomenon. After 60 min, the plasma adrenaline concentration was 0.55 nmol l^{-1}, a rise of 50% above basal concentrations. It could be speculated that a threshold for adrenaline concentration affecting insulin secretion exists, since the insulin response to glucose infusion at rest resulted in continuous elevation of plasma insulin levels. The response under saline infusion was similar to that normally observed during exercise i.e. a gradual decline in insulin concentration. This decrease is due to the sympathoadrenal inhibition of the beta-cells of the pancreas via an increase in adrenaline levels (Christensen & Galbo, 1983).

Maintained hyperglycaemia had a profound attenuating effect on the plasma glucagon concentrations. This is an expected response to hyperglycaemia and illustrates the antagonistic roles of insulin and glucagon. Galbo et al. (1979) have shown that a high-fat diet favours the production of glucagon during exercise, whereas subsequent glucose infusion results in impaired glucagon release. This is probably due to a direct effect of glucose on the pancreatic alpha-cells rather than adrenaline mediated. The effect of a diminished glucagon concentration can be realised in a lowered liver glucose output from glycogenolysis and gluconeogenesis, and hence the infusion rate of glucose may represent the total exogenous uptake by the body during exercise.

The adrenaline response during saline infusion is typical of the response to prolonged, intense exercise, in which impulses from the motor centres in the brain and from working muscle elicit a work-rate dependent increase in sympathoadrenal activity. This response depresses insulin secretion by alpha-receptor mediated mechanisms (Galbo et al., 1977). The diminished response under glucose infusion is due to the available glucose altering the activity of the sympathetic centres in the hypothalamus (Galbo, 1983). Increased availability of glucose in the ventromedial and ventrolateral cells of the hypothalamus would reduce sympathetic activity.

The effect of a high carbohydrate diet, and of glucose infusion has produced an uncertain response with respect to noradrenaline concentrations during exercise (Galbo et al, 1979), although the

levels increase in proportion to exercise intensity and duration. The results in this study support previous findings regarding an increase during exercise, and further corroborate those studies which have shown that elevated plasma glucose concentrations attenuate the normal response to exercise. The greater proportional effect of glucose infusion on adrenaline than noradrenaline is due to the fact that secretion of the former is dependent on both the general level of sympathetic nervous activity and on plasma glucose concentration whilst the latter is secreted in the main in proportion to an increase in sympathetic activity.

At the onset of exercise, impulses from motor centres in the brain and from working muscle elicit an increase in sympathoadrenal activity and in the release of some pituitary hormones. Increases in circulating levels of GH and ACTH, the latter leading to an increase in cortisol, number amongst these. Clearly the results from the saline infusion in this study support the view that prolonged exercise promotes elevation in levels of plasma GH and cortisol. Intensity and duration of exercise are the important factors governing secretion of these hormones (Sutton et al., 1969). The fact that hyperglycaemia results in a suppression of GH has been demonstrated previously (Hansen, 1971), and is probably a result of reduced activation of alpha-receptors by the cells of the hypothalamic ventromedial nucleus which are gluco-receptors (Martin, 1980). The fact that the concentration of GH did not become elevated until 40 min of exercise reflects the fact that it is a steroid hormone, and

therefore has a longer latent period before significant increases in plasma concentration become evident.

The data for cortisol under saline infusion show an extended latent period i.e. 80 min, compared with GH. Many other studies have shown that cortisol is elevated after exercise, particularly if the exercise is not prolonged (Davies & Few, 1973; Gawal et al., 1979). This is because cortisol is stimulated by ACTH from the pituitary and is a steroid hormone; both factors lead to the extended latent period. The 16% decrease in plasma cortisol concentration as a result of the prime infusion of glucose, and the subsequent reduction during exercise gives credence to the view that glucose sensitive receptors are able to modulate the cortisol response. Galbo et al. (1979) have shown that a high-fat diet enhances the secretion of cortisol whilst a high carbohydrate diet attenuates this response. It has also been shown that the plasma cortisol concentration may increase despite an increase in blood glucose levels (Sutton, 1978). The key to interpretation from studies examining cortisol levels are the length of time of the exercise period and/or any infusion of glucose. This study highlighted that no significant change in plasma cortisol was apparent during 120 min of exercise preceeded by a 30 min prime infusion of glucose.

Elevated concentrations of insulin together with diminished levels of catecholamines, cortisol and GH favoured a depression in plasma NEFA and B-OH throughout the exercise period. Insulin is an inhibitor of lipolysis and promoter of lipogenesis. The

increase in glycerol seen during exercise demonstrated that lipolysis was occurring but the fact that NEFA concentrations remained depressed suggest that re-esterification was also evident. The prime glucose infusion significantly reduced concentrations of both glycerol and NEFA, denoting impaired lipolysis. Once exercise began however, the NEFA levels fell over a period of 60 min before rising slowly, whereas the glycerol concentrations rose slowly throughout. This would intimate that lipolysis was occurring in the adipose tissue once exercise was underway, and that the re-esterification of the NEFA was also occurring but at a greater rate than its production. At no stage during exercise was the NEFA concentration elevated above pre-exercise, resting levels. A combination of increased plasma glucose, providing the glycerol for re-esterification, and an elevated insulin concentration, stimulating lipogenesis, was clearly responsible.

Saline infusion resulted in no significant change in NEFA, glycerol or B-OH levels in plasma as a consequence of the prime infusion. Exercise, however, produced a significant increase in concentration of these metabolites due to enhanced hormonally stimulated lipolysis. The suppression of insulin by increasing concentrations of adrenaline, the rise in adrenaline concentration itself, and increases in GH and cortisol lead to a stimulation of cAMP levels in the adipocytes with a resultant enhanced lipolysis.

A notable effect of hyperglycaemia was that carbohydrate oxidation was maintained at over 2.5 g min^{-1} for the duration of the exercise. This is in contrast to a progressive reduction in carbohydrate oxidation to 1.5 g min^{-1} during saline infusion. The results compare favourably with those of Coyle et al.(1991), but the carbohydrate oxidation under glucose infusion values are somewhat lower than the 3.6 g min^{-1} observed by Hawley et al. (1994). Because carbohydrate oxidation was maintained with hyperglycaemia yet it declined during saline infusion, the difference between trials in carbohydrate oxidation became progressively greater. After 120 min of exercise, the rate of carbohydrate oxidation was approximately 40% higher under glucose infusion..

In parallel with the widening difference in the rate of carbohydrate oxidation between trials, the rate of glucose infused (glucose utilisation) increased steadily from 1.1 g min^{-1} at the start of exercise to 1.8 g min^{-1} after 80-100 min, and remained at that level; the increase in rate of glucose utilisation being 40%. Since maintained hyperglycaemia impaired plasma glucagon concentrations and elevated insulin, it is possible to speculate that hepatic glucose output will be totally (or almost totally) suppressed. The rate of glucose infusion thereby reflects whole-body glucose utilisation.

The differences between total carbohydrate oxidation and glucose utilisation seen in Fig 7.25 suggests that in spite of hyperglycaemia, exercising muscles use their own endogenous

glycogen stores. This is supported by the results of the muscle glycogen concentration being significantly reduced after both trials. The difference from pre-exercise to post-exercise muscle glycogen concentration of 170.3 μg mol^{-1} dwt under hyperglycaemia accounted for the approximate total of 190 g of glucose oxidation disparity observed in Fig 7.25.

A major finding of this experiment was that elevation of plasma glucose to 12 mM during 120 min of exercise resulted in significant depletion of muscle glycogen, and that there was a small, but significant 8%, difference in the post-exercise glycogen content between the treatments. Previous studies on carbohydrate ingestion or glucose infusion during exercise have demonstrated muscle glycogen sparing (Bergstrom and Hultman, 1967; Bjorkman et al., 1984; Hargreaves et al., 1984) or no sparing (Coyle et al., 1986, 1991; Hargreaves et al., 1987). Bergstrom and Hultman (1967) intravenously infused glucose into men at a rate of approximately 3 g min^{-1} during 1 h of one-legged exercise, and as a result of an average blood glucose concentration of 21 mM, found that muscle glycogen concentration was reduced by 15 mM kg^{-1} ww compared with the controls. More recently, Coyle et al. (1991) elevated blood glucose to 10 mM in humans and maintained that level during 2 h of exercise. No muscle glycogen sparing was observed. It is quite possible that either the higher levels of glucose being maintained, or the fact that hyperglycaemia was promoted before exercise in my study, could have accounted for the differences observed from Coyle's group. A threshold level above 10 mM blood glucose and/or a

greater stimulation of plasma insulin may be required in order to conserve muscle glycogen stores.

The plasma and red-cells showed marked differences in the fasting concentrations of amino acids taken before the exercise commenced. These observations were remarkably consistent, as values obtained on the two separate occasions were not significantly different. The large standard errors apparent for some of the amino acids reflect the inter-subject variability rather than any imprecision of the assay. The relatively higher concentrations observed in the red-cell has been shown previously for dogs (Elwyn et al., 1972), for sheep (Heitman & Bergman, 1980), and for humans (Hagenfeldt & Arvidsson, 1980).

During exercise, and particularly with maintained hyperglycaemia, significant increases in the red-cell content were noted for eleven of the amino acids studied. This was notably evident for alanine during glucose infusion, where the actual amount increased by fourfold and the rate of uptake was markedly enhanced. An insulin-mediated effect has previously been shown for glutamic acid uptake into the red-cell (Aoki et al., 1972), but hitherto has not been demonstrated for other amino acids. The observed erythrocyte increases are not understood, but may reflect losses from muscle, lower gut, and movement through the plasma compartment (Felig & Wahren, 1973).

Plasma concentrations of alanine increased during exercise irrespective of the infusate. This supports previous findings

where plasma alanine concentrations were observed to change during exercise (Ahlborg et al., 1974). Since the plasma concentrations are the balance between release and uptake, and a-v differences were not assessed in this study, it is not possible to speculate whether the changes in amino acid concentrations were as a result of a greater release or reduced uptake. The circulating plasma levels of alanine, however, have been found to increase during exercise (Viru, 1987) and are associated with the production and release of glucose by hepatic cells (Ahlborg & Felig, 1974). This has been established as being particularly important during prolonged exercise. An interesting observation is that the levels of plasma alanine increased to a greater extent under hyperglycaemia. Could maintained hyperglycaemia have reduced the uptake of alanine by the liver due to an inhibition of gluconeogenesis? This seems quite possible due to the significant reduction in plasma glucagon.

Red-cell amino acid changes during exercise showed net uptakes, particularly for alanine and leucine. This was irrespective of infusate. The large significant uptake of alanine by the red-cell under hyperglycaemia could be due to insulin mediation, since the process appeared to result after the prime infusion period. The net release of alanine by active muscles for the purpose of transport to the liver and resultant glucose production is the basis of the glucose-alanine cycle. Since maintained hyperglycaemia diminishes the need for gluconeogenesis, but elevated red-cell concentrations of alanine are observed, it is possible to speculate that the red-cell may act as a store for

the alanine released by muscle until such time that gluconeogenesis is stimulated. During saline infusion, the uptake of alanine by red-cells was not significant, possibly implying that the glucose-alanine is operating normally. Why the muscle should be releasing alanine under conditions of hyperglycaemia may relate to the action of contractility and nervous stimulation rather than hormonal control, since glucagon and cortisol concentrations were depressed.

7.5 CONCLUSIONS

The results from this study confirm *hypotheses 14-18*, in that maintained hyperglycaemia promotes factors concerning enhanced carbohydrate oxidation and utilisation, spares muscle glycogen to a limited extent, and that exercise stimulates the release of plasma and red-cell alanine. *Hypothesis 19* was rejected on the basis that hyperglycaemia resulted in a significant increase in red-cell alanine. The conclusion from the latter point being that the red-cell, far from being relatively inert, appears to sequester alanine at an appreciable rate.

8. HORMONAL AND METABOLIC RESPONSES TO MAINTAINED HYPERGLYCAEMIA DURING EXERCISE WHEN CARBOHYDRATE LOADED AND DEPLETED

8.HORMONAL AND METABOLIC RESPONSES TO MAINTAINED HYPERGLYCAEMIA DURING EXERCISE WHEN CARBOHYDRATE LOADED AND DEPLETED

8.1 INTRODUCTION

The carbohydrate loading diet was popularised in the late 1960's after it had been found that muscle glycogen content could be increased by a high-carbohydrate diet (70% carbohydrate) for approximately 3 days before exercise, and that muscle glycogen stores elevated by this technique could enhance subsequent endurance exercise (Ahlborg et al., 1967a). The precise mechanisms underlying this ergogenic effect have not been established. It may be due to the higher muscle glycogen stores causing a delay in the depletion of muscle glycogen (Coyle et al., 1986), although some studies have shown that muscle glycogen utilisation is increased after carbohydrate loading (Richter & Galbo, 1986).

Alternatively, it has been proposed that carbohydrate loading may slow the rate of liver glycogen depletion due to the increased availability of muscle glycogen (Bosch et al., 1993), although a study on rats has shown that rates of hepatic glycogenolysis were accelerated by a high liver glycogen content after carbohydrate loading (Vissing et al., 1989). This experiment uses the hyperglycaemic clamp technique during exercise when subjects are carbohydrate loaded and depleted to establish if the percentage of glucose utilised to total carbohydrate oxidation is affected:-

Hypothesis 20 *Maintained hyperglycaemia during exercise when carbohydrate loaded results in a lower glucose utilisation rate and percentage of glucose utilised to total carbohydrate oxidised than when carbohydrate depleted.*

It has been shown that carbohydrate loading elevates plasma insulin concentrations during exercise whereas carbohydrate depletion results in a diminished response (Galbo, 1983). The resultant of this is likely to be a higher rate of carbohydrate oxidation, reduced fat oxidation, attenuated responses of catecholamines, NEFA, B-OH, and glycerol when carbohydrate loaded. Consequently :-

Hypothesis 21 *Maintained hyperglycaemia results in elevated levels of insulin but diminished levels of catecholamines, NEFA, B-OH, and glycerol when carbohydrate loaded than depleted.*

8.2 METHODS

Subjects. Five healthy, well-trained, male subjects gave their informed written consent in accordance with the procedures approved by the Ethics Committees of the Royal Liverpool University Hospital, and of Liverpool John Moores University. Mean age, body mass, and VO_2 max were 26.8 ± 9.6 years, 69.6 ± 7.7 kg, 4407 ± 335 ml min^{-1}.

Experimental design. Procedures for the determination of VO_2 max, for the hyperglycaemic glucose clamp technique, and for hormonal and metabolite assays have been described in Chapter 7.

Subjects arrived at the laboratory on a Monday morning at 08:00 hours after an overnight fast and under conditions of being either carbohydrate loaded or carbohydrate depleted. The carbohydrate loaded status was achieved by the subjects exercising to exhaustion four days prior to the test and then resting over the subsequent three days whilst eating a large amount of their dietary intake in the form of carbohydrates (i.e. >500 g day^{-1}). All food was weighed by the subjects for analysis of the mass of carbohydrate consumed. A computer program using the McCance and Widdowson Food Composition Tables was employed ('Microdiet', Salford University). The carbohydrate depleted status was achieved by the subjects exercising to exhaustion on days 3 and 2 prior to the test and following a low carbohydrate diet for those days (i.e. <200 g day^{-1}), and also on the day before the test (when no exercise was done). Weighed food intakes were again performed and analysed as stated above. The order of testing was counterbalanced for the subjects, and whereas the exercise time in the previous experiment was 120 min, it was decided to use 90 min of exercise at 70% VO_2 max in this experiment because the subjects were carbohydrate depleted in one of the trials and might desist before 120 min.

In order to check whether appropriate levels of muscle glycogen had been achieved, two of the subjects, on occasions after the

experimental trials, repeated the exercise and dietary interventions and then had a muscle biopsy performed on the *vastus lateralis* as previously described (Chapter 7). The frozen muscle samples were analysed for the determination of glycogen concentration.

Statistics. All data were subjected to two-way analyses of variance with repeated measures. Post-hoc tests were performed when F-values reached statistical significance i.e. when $P<0.05$.

8.3 RESULTS

Hyperglycaemia was maintained in both trials as can be seen in Fig 8.1. Although the rate of glucose utilisation was similar between the carbohydrate loaded and depleted conditions ($F_{1,4}=0.13$; $P>0.05$)), the rate of total carbohydrate oxidised was significantly higher when carbohydrate loaded ($F_{1,4}=10.35$; $P<0.05$). Figures 8.2 and 8.3 illustrate these findings.

When the rate of glucose utilised is examined in relation to the rate of total carbohydrate oxidised (Figs 8.4 and 8.5), it is observed that as the rate of utilisation is increased over the 90 min, the rate of total carbohydrates oxidised is reduced, resulting in an increased reliance on exogenous carbohydrate. The contributions from the glucose infused whilst loaded was 47%, 58%, 66%, and 80% at 20-40, 40-60, 60-80, and 80-90 min respectively. The corresponding values whilst depleted were 58%, 77%, 88%, and 100%.

Elevations in plasma insulin concentrations resulting from the glucose infusion were apparent (Fig 8.6). Mean values at rest were 7.2± 2.5 uU ml^{-1} for the loaded condition and 4.1 ± 2.0 uU

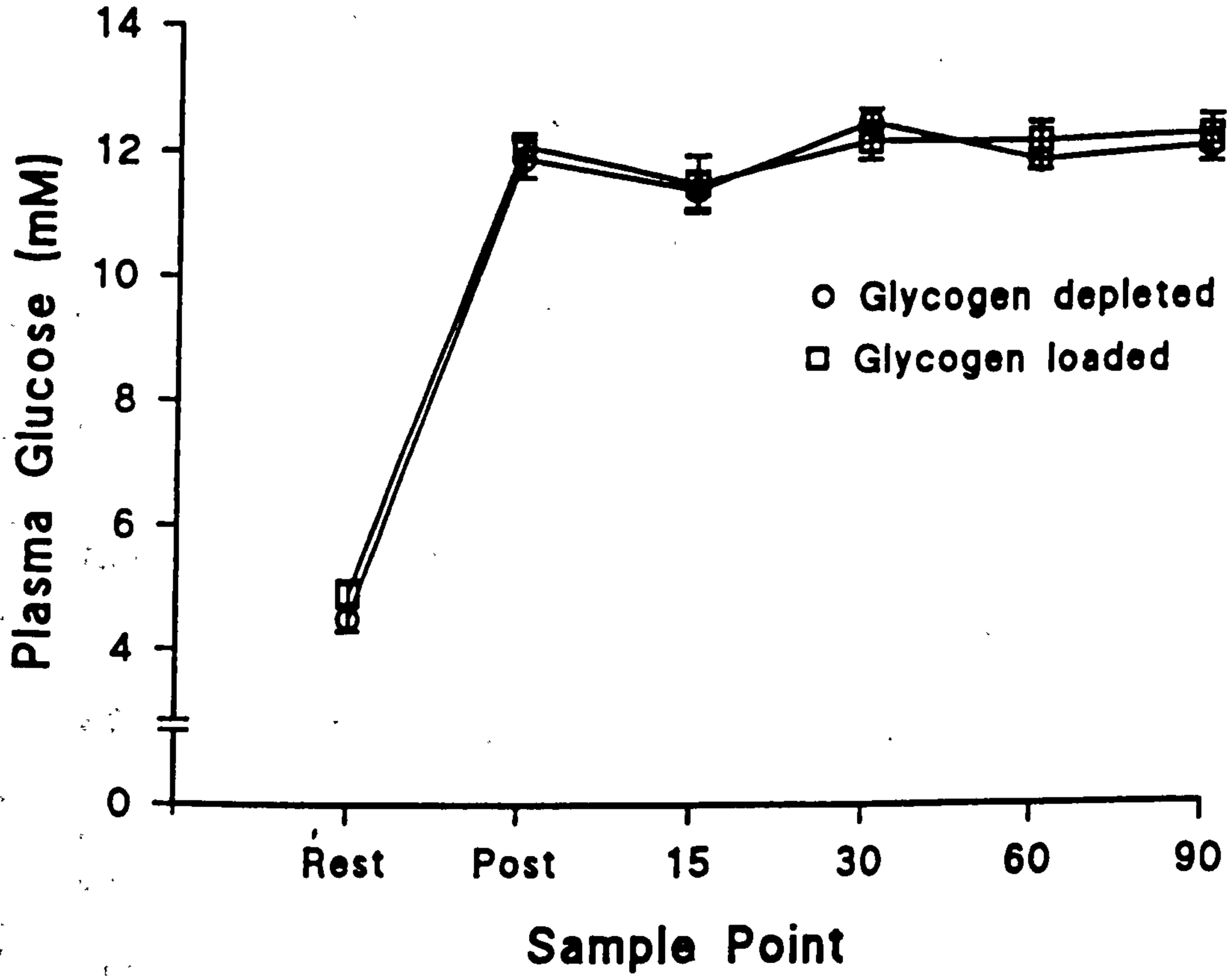


Fig 8.1 Plasma glucose concentrations (mM) at rest, after prime infusion of glucose, and during 90 min of exercise when glycogen loaded and depleted.

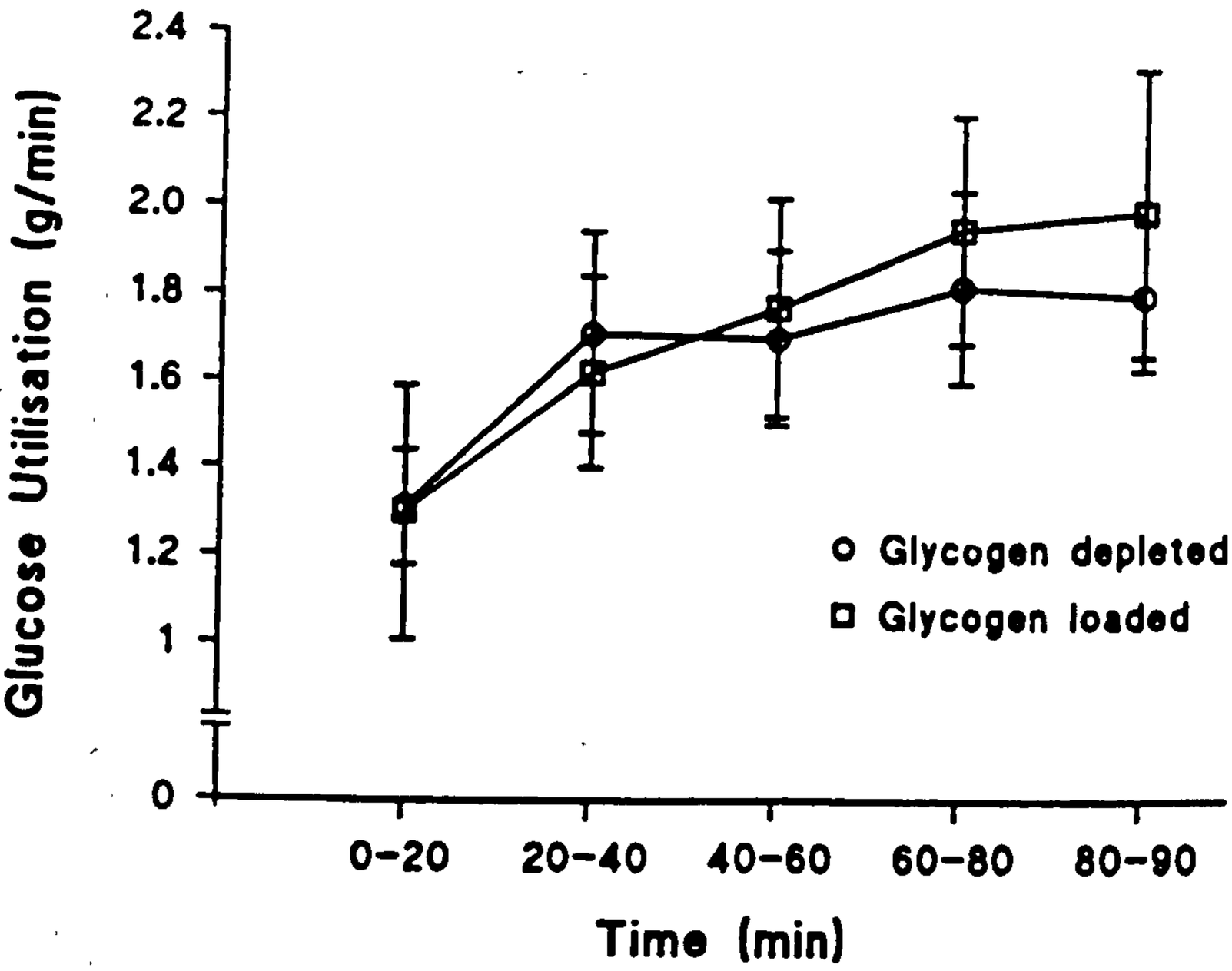


Fig 8.2 : Glucose utilisation rates (g min^{-1}) during 90 min of exercise when glycogen loaded and depleted.

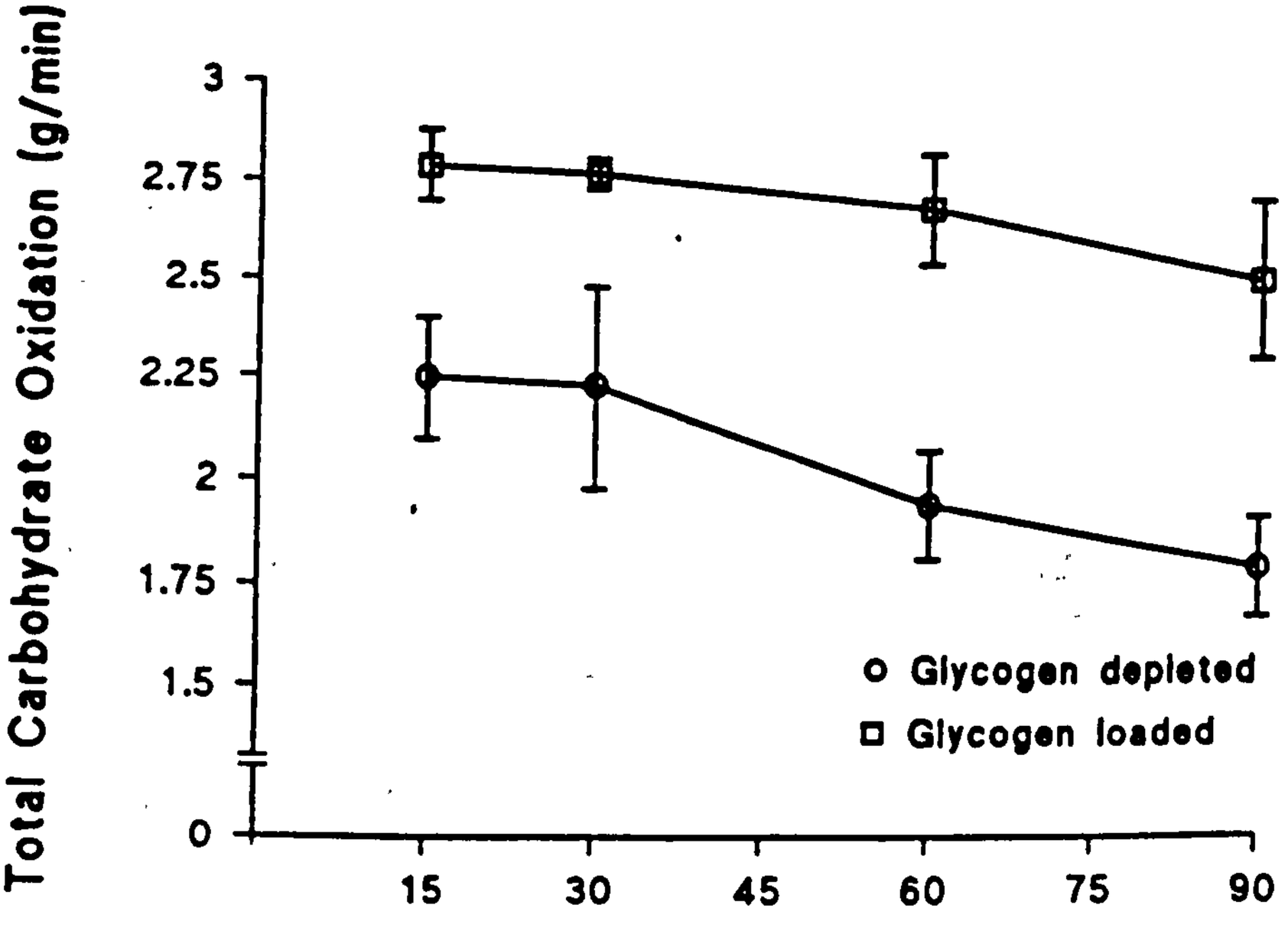


Fig 8.3 : Total carbohydrate oxidation rate (g min^{-1}) during 90 min of exercise when glycogen loaded and depleted.

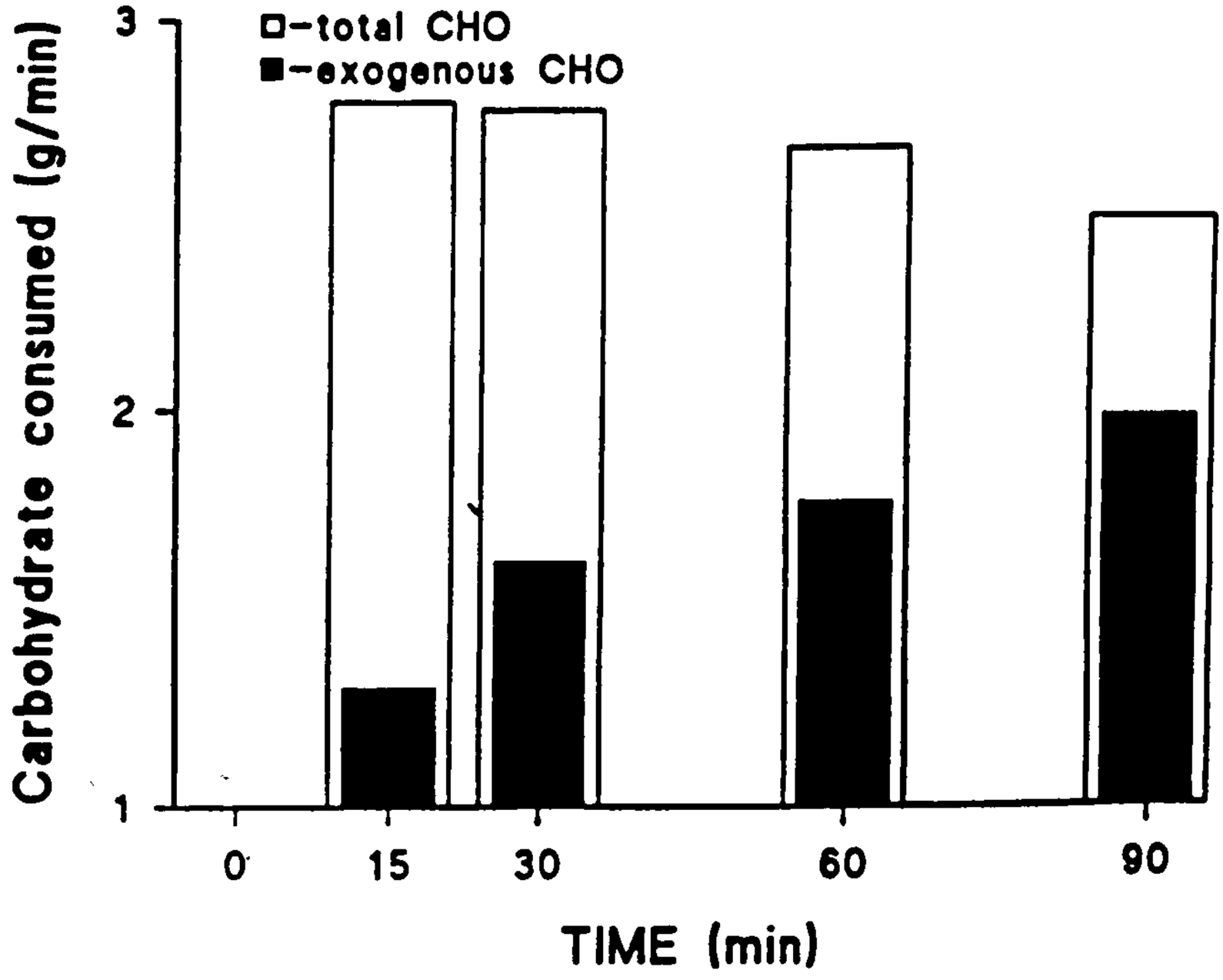


Fig 8.4 Contribution of exogenous glucose to total carbohydrate oxidation during 90 min of exercise when glycogen loaded.

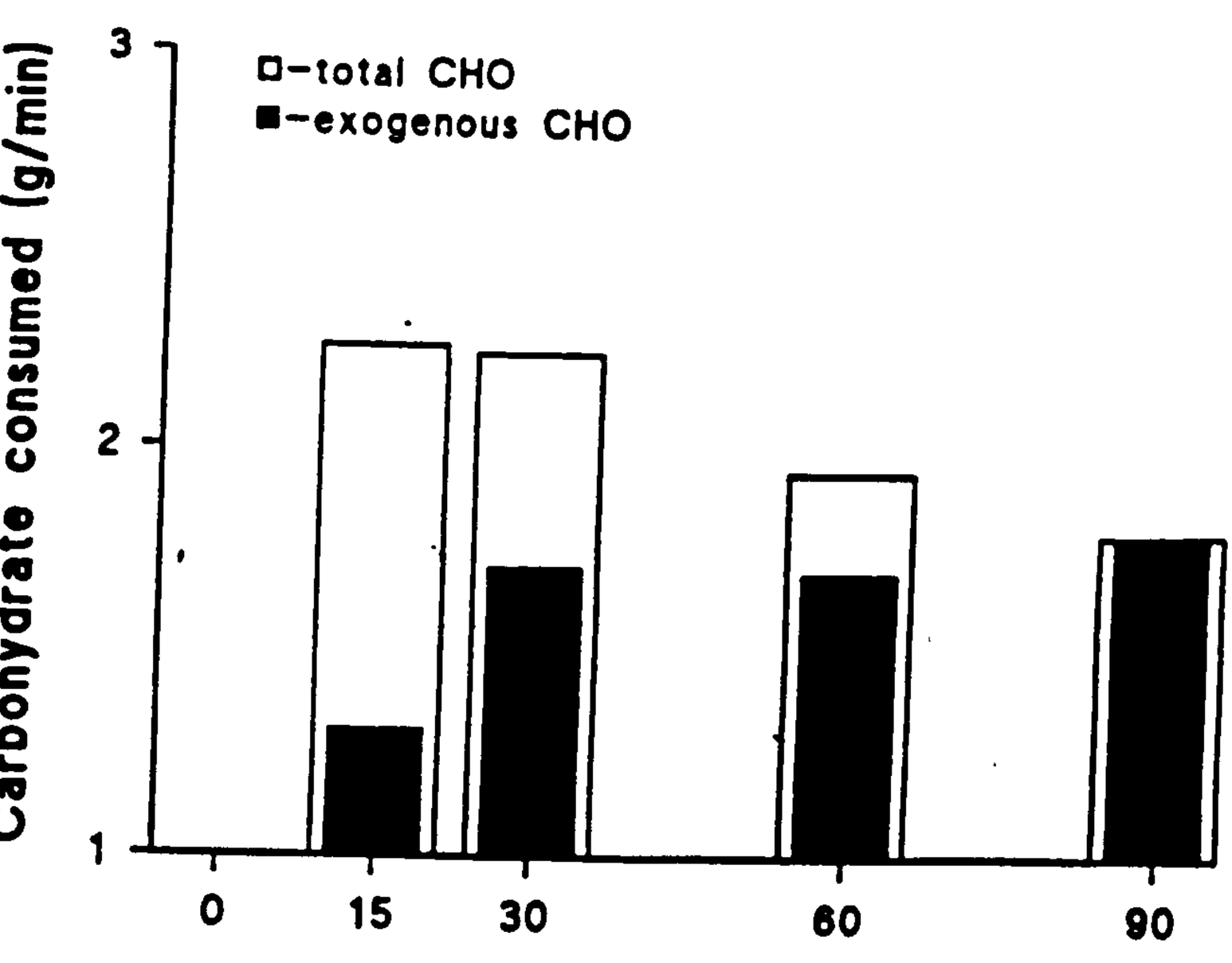


Fig 8.5 Contribution of exogenous glucose to total carbohydrate oxidation during 90 min of exercise when glycogen depleted.

ml^{-1} for the depleted condition. The concentrations became significantly elevated as a result of the prime infusion and exercise under both conditions ($F_{5,20}$=8.96; P<0.01). The loaded condition produced significantly higher insulin concentrations than the depleted condition ($F_{1,4}$=7.78; P<0.05).

Plasma adrenaline concentrations were significantly different between the two conditions ($F_{1,4}$=17.03; P<0.05), with the loaded trial producing significantly lower values than the depleted trial (Fig 8.7). The carbohydrate loaded trial also invoked significantly depressed concentrations of plasma noradrenaline compared with the depleted state ($F_{1,4}$=36.71; P<0.01). The changes with respect to the trial can be seen in Fig 8.8.

Plasma lactate concentrations were significantly elevated under carbohydrate loading compared with carbohydrate depletion ($F_{1,4}$=7.25; P<0.05). This difference was apparent at rest (Fig 8.9).

The lipid responses to the diets, maintained hyperglycaemia, and exercise can be seen in Figs 8.10 - 8.12. Plasma NEFA, B-OH, and glycerol concentrations were all significantly elevated after carbohydrate depletion ($F_{1,4}$=19.12; P<0.05; $F_{1,4}$=23.31; P<0.01; $F_{1,4}$=12.0; P<0.05 respectively). These responses resulted in a significantly elevated total lipid oxidation rate ($F_{1,4}$=8.56; P<0.05) for the depleted state (Fig 8.13).

Examination of the weighed food intakes of the subjects showed that the carbohydrate loaded condition resulted in 561 ± 48 g day^{-1} of carbohydrate being consumed whereas the depleted state resulted in 177 ± 56 g day^{-1} being consumed. These diets led to muscle glycogen concentrations of 377 ug mol^{-1} dw after loading and 159 ug mol^{-1} dw after depletion.

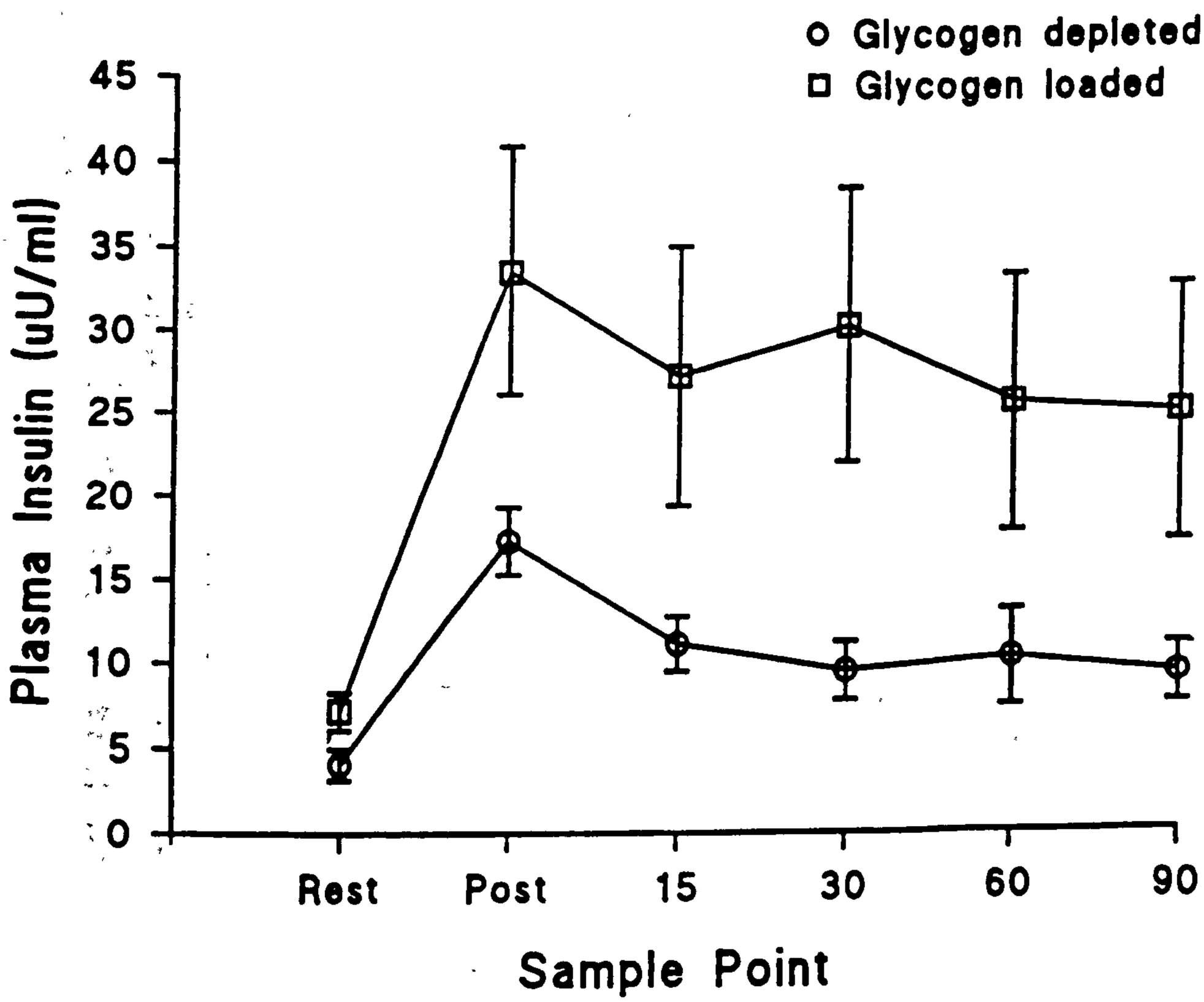


Fig 8.6 Plasma insulin concentrations ($\mu U\ ml^{-1}$) at rest, after prime infusion of glucose, and during 90 min of exercise when glycogen loaded and depleted.

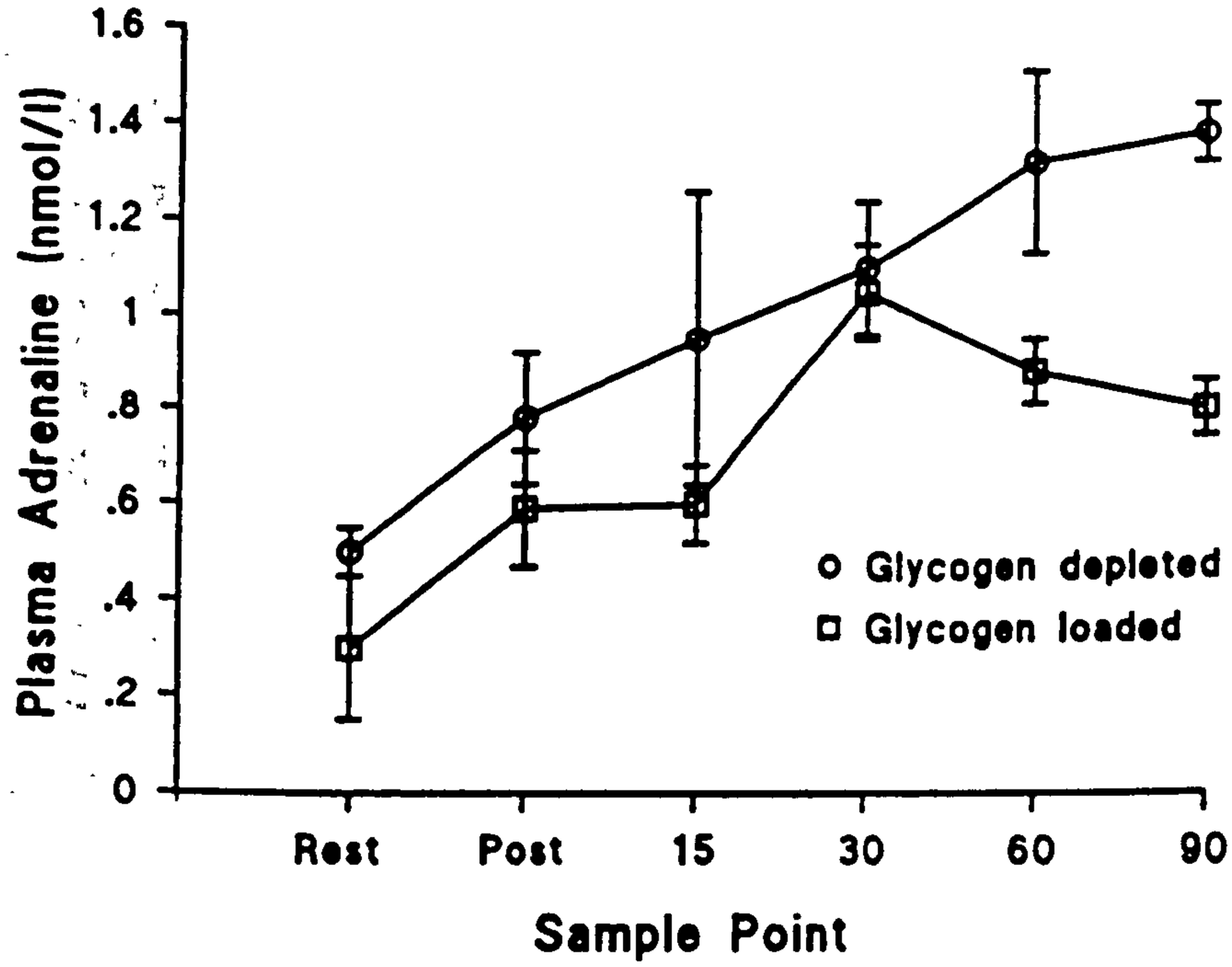


Fig 8.7 Plasma adrenaline concentrations (nM) at rest, after prime infusion of glucose, and during 90 min of exercise when glycogen loaded and depleted.

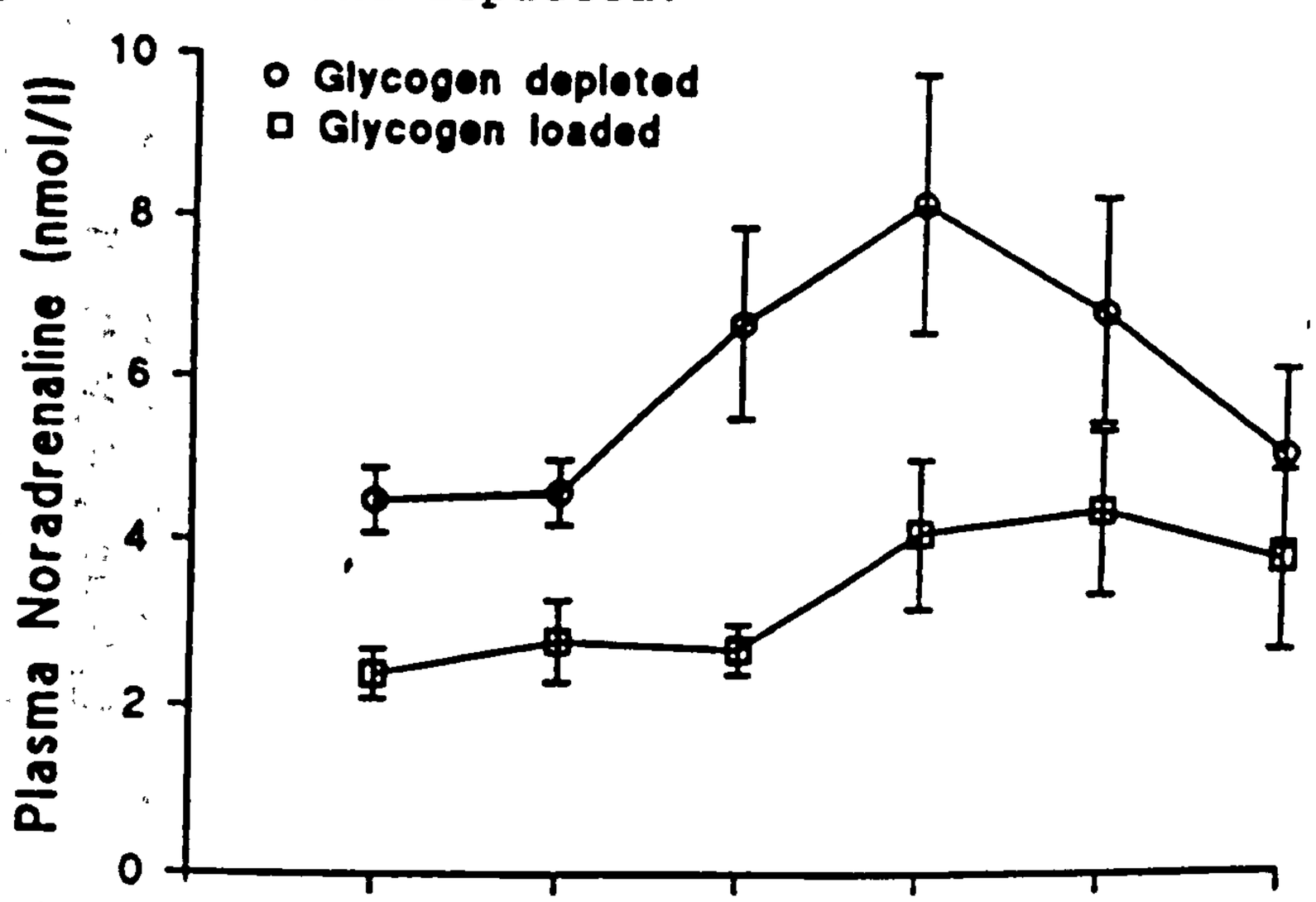


Fig 8.8 Plasma noradrenaline concentrations (nM) at rest, after prime infusion of glucose, and during 90 min of exercise when glycogen loaded and depleted.

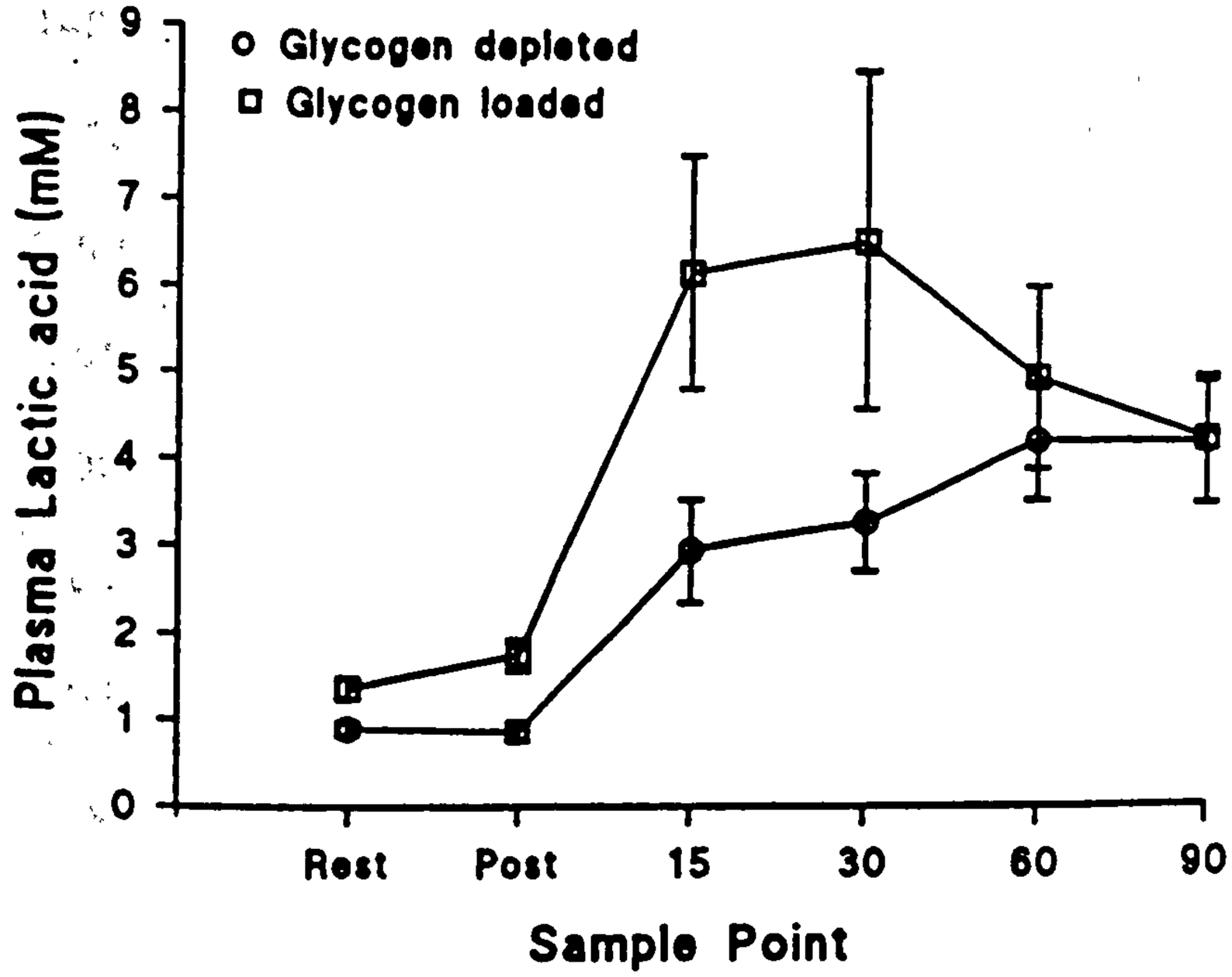


Fig 8.9 Plasma lactate concentrations (mM) at rest, after prime infusion of glucose, and during 90 min of exercise when glycogen loaded and depleted.

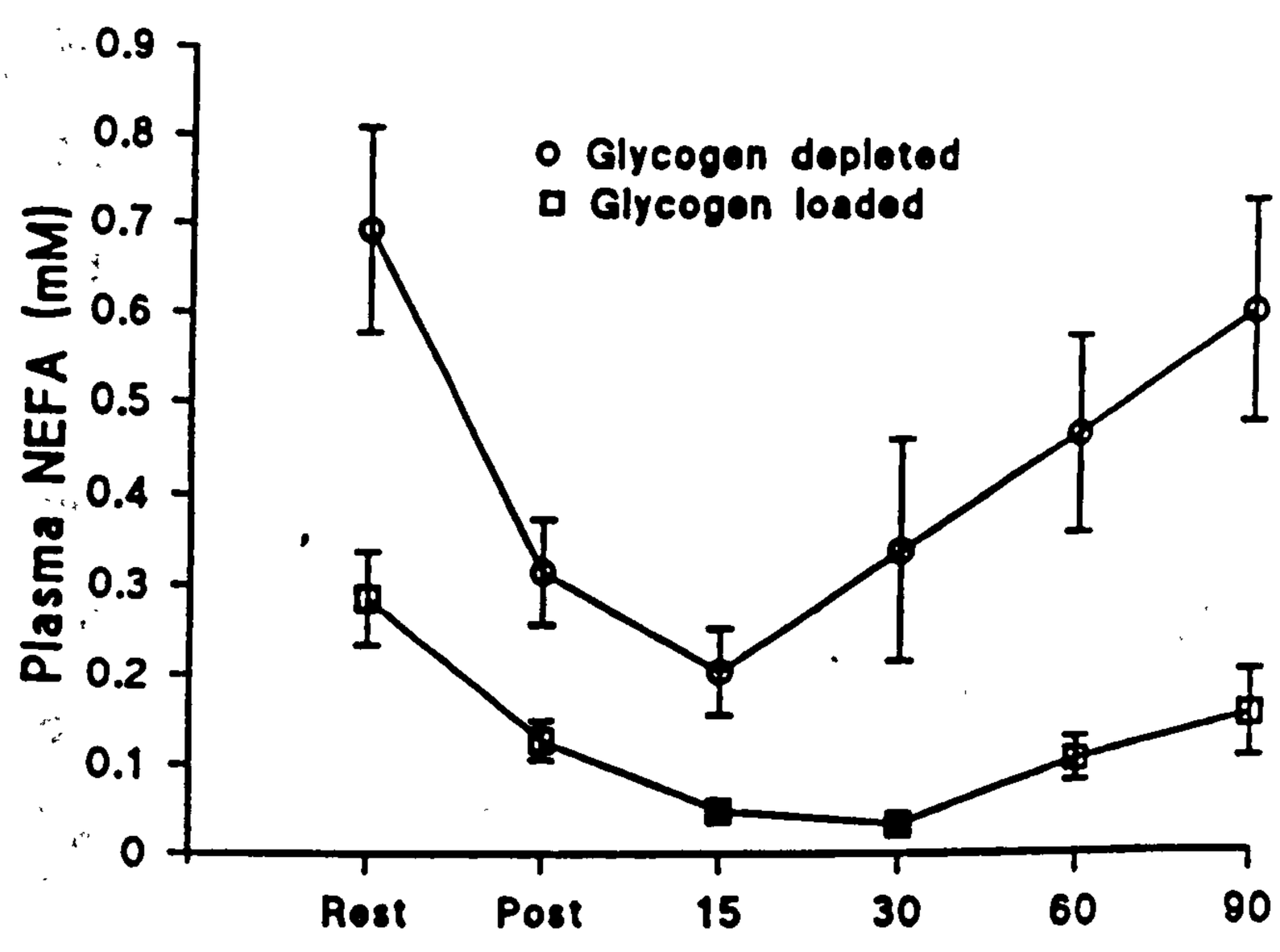


Fig 8.10 Plasma NEFA concentrations (mM) at rest, after prime infusion of glucose, and during 90 min of exercise when glycogen loaded and depleted.

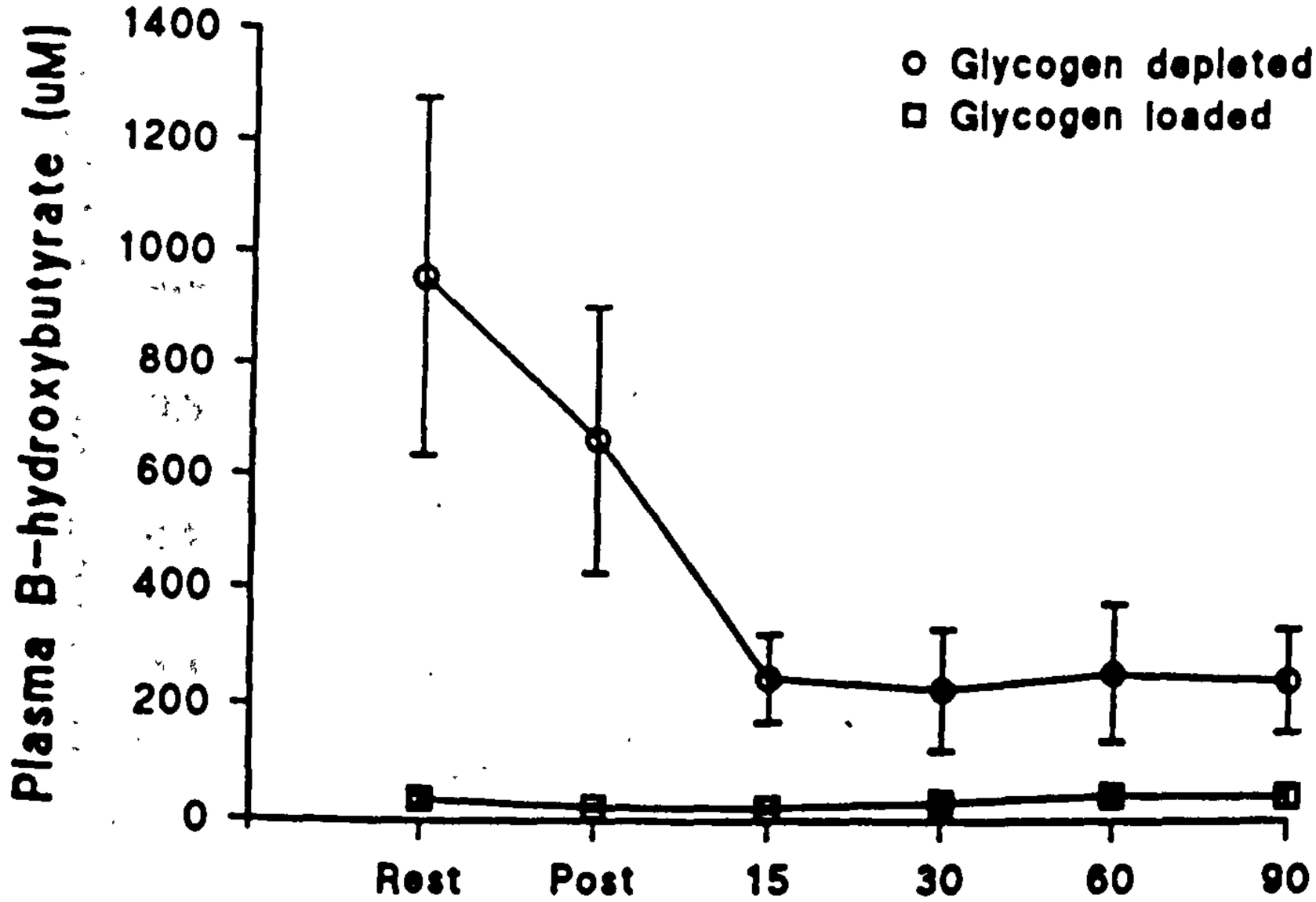


Fig 8.11 Plasma B-OH concentrations (μM) at rest, after prime infusion of glucose, and during 90 min of exercise when glycogen loaded and depleted.

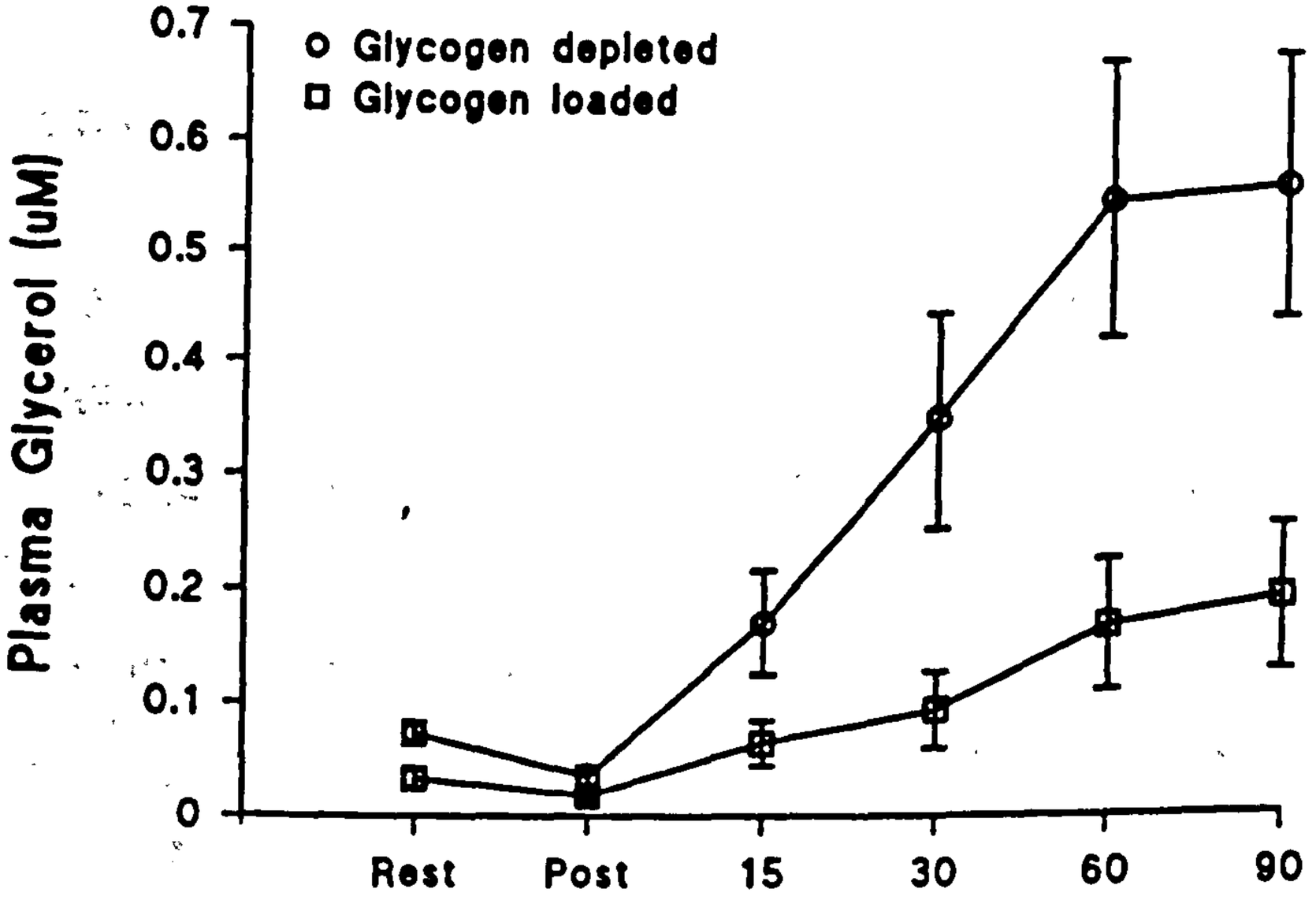


Fig 8.12 Plasma glycerol concentrations (μM) at rest, after prime infusion of glucose, and during 90 min of exercise when glycogen loaded and depleted.

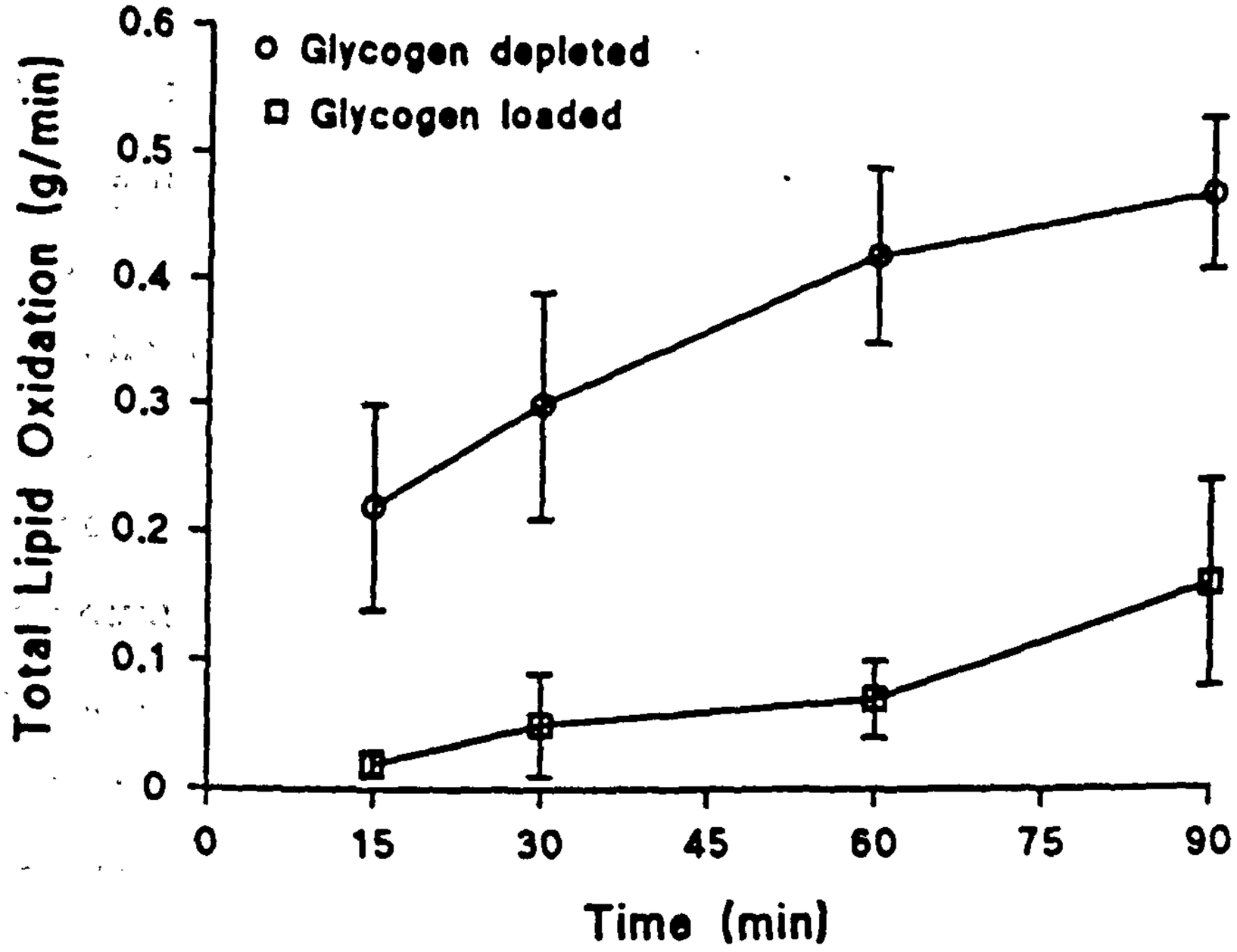


Fig 8.13 Rate of total lipid oxidation (g min^{-1}) during 90 min of exercise when glycogen loaded and depleted.

8.4 DISCUSSION

As in the previous experiment, the hyperglycaemic glucose clamp technique is capable of maintaining 12 mM blood glucose levels quite rigidly, as can be realised on examination of Fig 8.1. Furthermore, similar findings were observed with the rate of glucose utilisation; a progressive increase being determined throughout the 90 min exercise period, and this was irrespective of the carbohydrate status of the subject. The maximal rates of glucose utilisation of 1.82 g min^{-1} and 1.99 g min^{-1} for the loaded and depleted conditions respectively were similar to the value of 1.82 g min^{-1} in the previous experiment. It would appear that muscle cells are incapable of either taking up exogenous

glucose at a rate greater than these levels, in spite of significantly elevated concentrations of insulin. It is possible that this rate represents maximal transport kinetics for glucose across the plasma membrane of skeletal muscle.

Comparison of the glucose utilisation rate with the rate of total carbohydrate oxidation (Figs 8.4 and 8.5) reveals that as exercise progresses, there is an increase in the contribution from exogenous sources. Indeed, there is 100% contribution from the glucose infused in the later stages of exercise in the depleted state. Whether this rate of glucose infusion would have been capable of sustaining this intensity of exercise for a longer period under a depleted state cannot be answered here, although the rate of perceived exertion using the Borg scale (Borg,1973) at the end of the depleted trial was 17 compared with a score of 12 at the end of the loaded trial. It would seem unlikely for subjects to be able to sustain exercise much longer if their perception of effort was so high.

The observed increases in plasma insulin prior to exercise under conditions of glycogen loading were maintained throughout exercise. It is probable that this attenuation of the insulin response after carbohydrate depletion is the result of inhibition of secretion due to elevated adrenaline concentrations (Christensen & Galbo.1983). Increased availability of glucose in the ventromedial and ventrolateral cells of the hypothalamus reduces sympathetic activity and hence reduces adrenaline release. This clearly occurs under conditions of glycogen loading

and results in the attenuated adrenaline response. In turn, the suppression or promotion of adrenaline leads to an inhibition or stimulation of insulin secretion.

The elevation in plasma lactate concentrations both before and during exercise under the loaded condition is an established phenomenon (Jacobs, 1984). An increase in muscle glycogen will favour glycolysis with a concomitant enhanced production of pyruvate, and thereby lactate. Since the exercise is relatively strenuous, an increase in plasma concentration of lactate is inevitable, at least for 30-60 min. The fact that the lactate levels failed to decrease to pre-exercise levels can be attributed to the availability of significant amounts of glucose, even in the glycogen depleted trial.

The elevated levels of lactate, together with the increases in insulin concentration would favour carbohydrate metabolism. This has been established above. However, what was not quite expected was the significant increases in plasma NEFA, B-OH, and glycerol observed during the depleted trials. The resultant of these raised levels was a marked increase in the rate of lipid oxidation. The resting concentration of B-OH for subjects under glycogen depletion reflects a ketotic state, and this is mirrored on examination of pre-exercise levels of NEFA and glycerol. The concentrations of these indicators of lipid metabolism were equivalent to those found under saline infusion in the previous experiment. This occurred in spite of maintained hyperglycaemia. In contrast, there was a marked reduction in NEFA, B-OH, and

glycerol when glycogen loaded; a response similar to that observed in the previous experiment.

8.5 CONCLUSIONS

The results from this study reflect similar trends to those obtained in the previous investigation. Although the rate of total carbohydrate oxidation and the contribution of infused glucose to total carbohydrate oxidation was greater for the depleted state than the loaded state, the actual rates of glucose utilisation were similar (thus refuting *hypothesis 20*). This would imply that glucose uptake may have reached saturation at 1.8-1.9 g min^{-1} (thus supporting *hypothesis 21*).

9. GENERAL DISCUSSION

9 GENERAL DISCUSSION

It is well established that carbohydrates are an important source of energy for prolonged exercise, the result of which may be realised on the variety of research performed and reported in this field. The significance of muscle and liver glycogen stores in prolonging activity has been established since the 1920's and 1930's (Christensen & Hansen, 1939; Dill et al., 1932; Gordon et al., 1925; Levine et al., 1924), although the significance of these early findings were not appreciated until the introduction of the muscle biopsy technique (Ahlborg et al., 1967; Bergstrom et al., 1967). The role of ingested carbohydrate in prolonging endurance activities has been clearly established in the 1980's by the series of experiments from Coggan and Coyle (Coggan & Coyle, 1987, 1988, 1989). The original consideration that the mechanism responsible for promoting endurance exercise by ingesting carbohydrates was related to sparing muscle glycogen has since been discredited (Coyle et al., 1986; Noakes et al., 1988).

The picture emerging from these studies is that muscle glycogen is important for endurance exercise, and that when glycogen levels become depleted fatigue develops. This is particularly relevant on examination of the glycogen stores in muscle fibre types (Vollestad & Blom, 1985). The contribution of exogenous sources of carbohydrate enhancing prolonged activities may be due to maintenance of blood glucose levels and the use of this for oxidative purposes. Figure 9.1 illustrates the concept put

forward by Coyle (1991) to explain the involvement of carbohydrates in energy provision. The tenet of this proposal is that both muscle glycogen and plasma glucose are oxidised by skeletal muscle during prolonged exercise, and although the underlying mechanisms are not clear, there is a gradual shift from intramuscular glycogen towards blood-borne glucose as the predominant carbohydrate energy source. This is particularly so when muscle glycogen stores diminish. The contribution of plasma glucose for oxidation may be limited, however, by a decline in blood glucose concentrations. Since carbohydrate feeding during exercise can prolong the activity by up to 60 min without sparing muscle glycogen but does maintain blood glucose concentrations, the blood glucose appears to be the most important source of energy after 3 h of strenouos cycling.

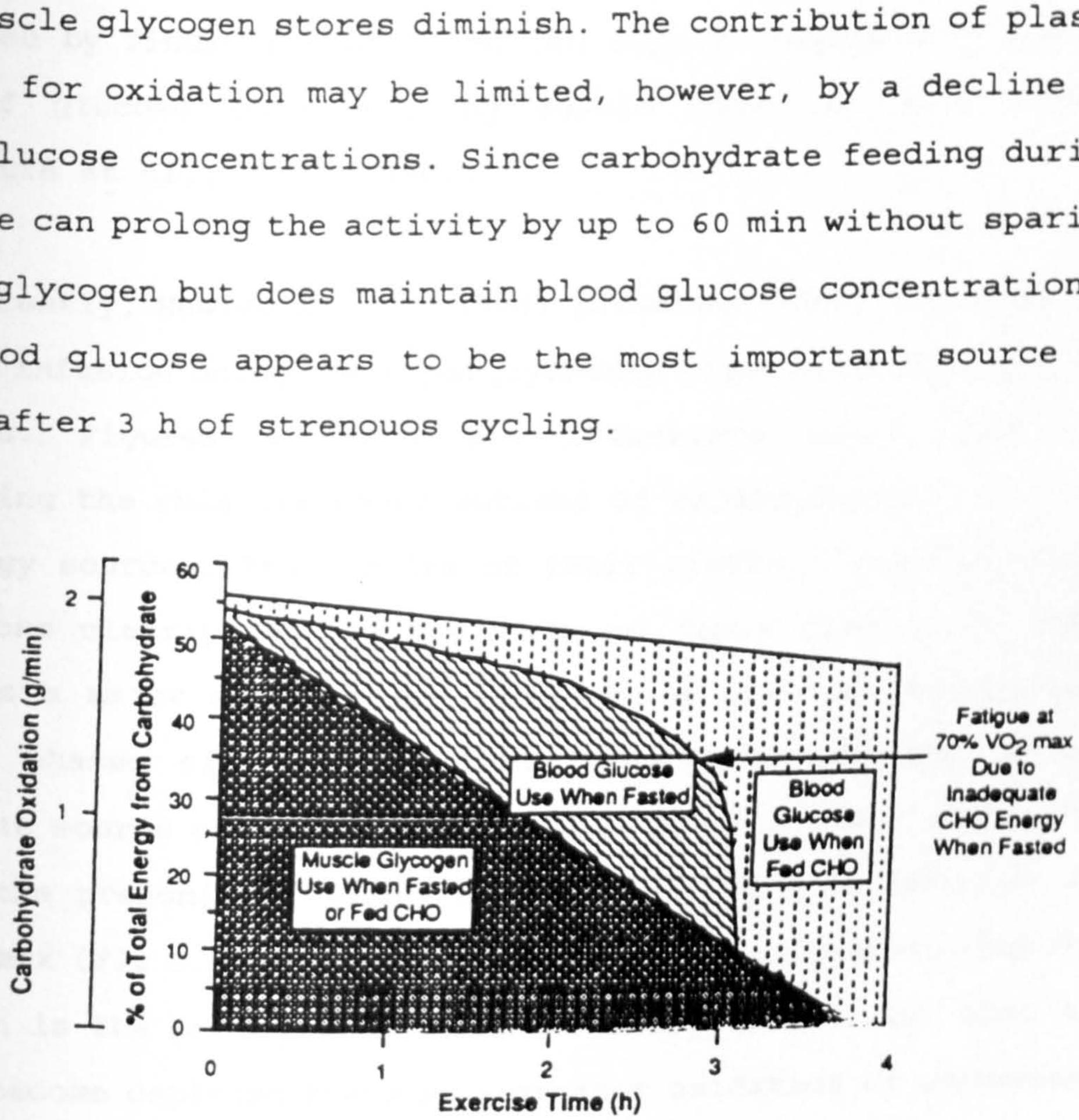


Fig 9.1 The percentage of energy and the absolute rate of carbohydrate oxidation which is derived from muscle glycogen and blood glucose during prolonged cycling (from Coyle, 1991).

hat is worthy of note from the proposal by Coyle (1991) is that carbohydrates provide approximately 50% of the total energy even in the last hour of exercise when fed carbohydrates, the contribution being approximately 40% or less immediately before fatigue when no carbohydrates are given. Furthermore, the rate of carbohydrate oxidation is approximately 2 g min^{-1} when fed carbohydrates (even just prior to fatigue). This view is supported by findings that blood can supply between 0.7- 1.0 g min^{-1} of glucose to exercising muscle (Katz et al., 1986; Massicotte et al., 1989, 1990).

More recently, Hawley et al. (1994) presented their findings on glucose infusion using the hyperglycaemic clamp technique and on controls. Figures 9.2 and 9.3 illustrate their findings concerning the relative contributions of carbohydrates and fats as energy sources. The results of their findings under control conditions clearly confirm the views of Coyle (1991), in that there is a major contribution from muscle glycogen during the earlier phases of exercise; that blood-borne glucose is an important source of carbohydrate energy later on; and that fats become the predominant energy source at 120 min of exercise at 70% VO_2 max (Fig 9.2). Under conditions of hyperglycaemia, muscle glycogen is the predominant source of energy (80%), but that as levels become depleted there is a greater oxidation of exogenous glucose (Fig 9.3). Under these conditions, fats do not contribute more than 18% of the total energy.

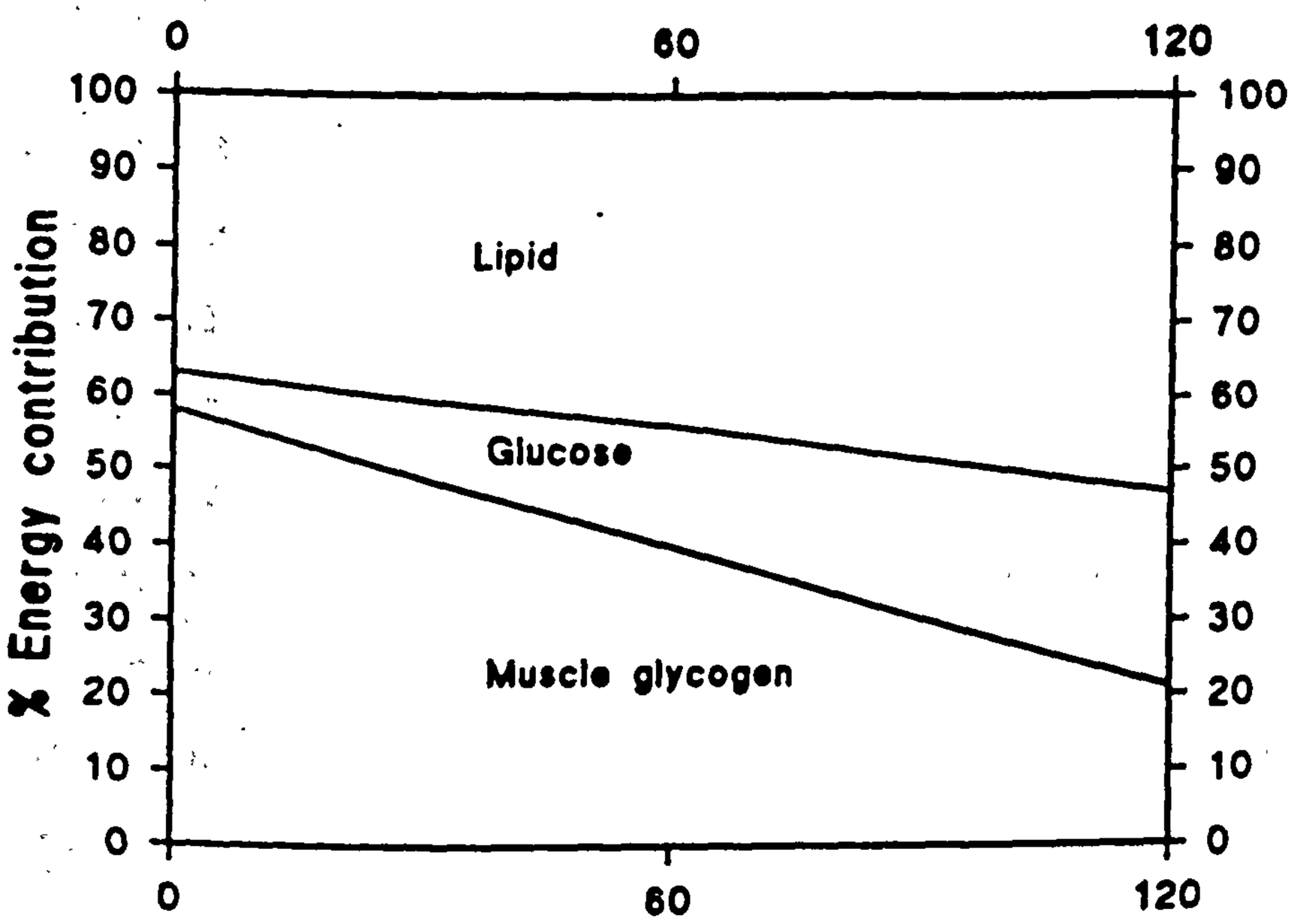


Fig 9.2 The relative contribution of fuel substrates to total energy during 120 min cycling at 70% VO_2. max under control conditions.

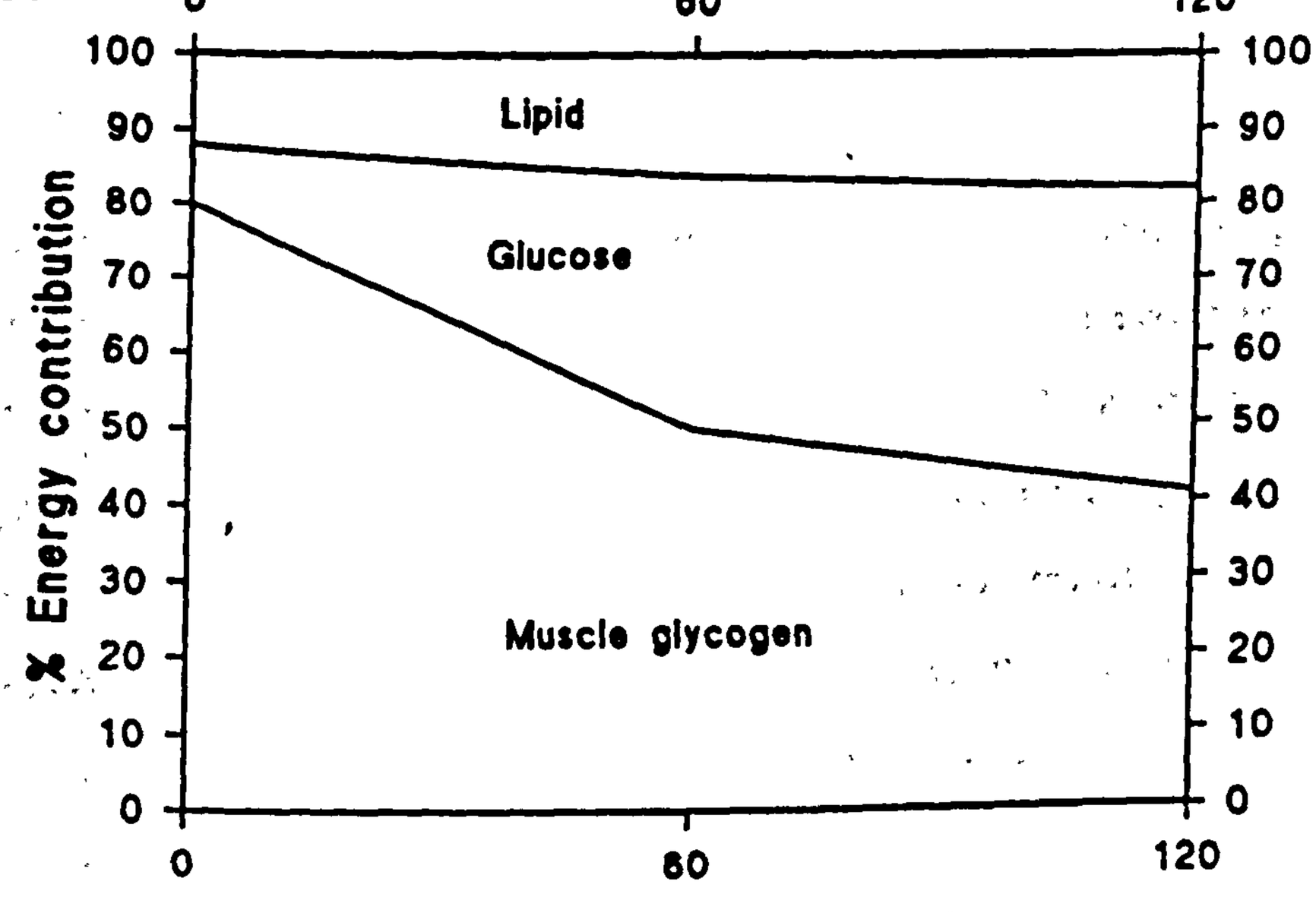


Fig 9.3 The relative contribution of fuel substrates to total energy during 120 min of cycling at 70% VO_2 max when hyperglycaemic.

With regard to the contribution of carbohydrates to the total energy provision during cycling for 90 min at 65% VO_2 max, this thesis reveals that there is a steady decrease in the provision of energy from carbohydrate stores (Fig 9.4). However, when carbohydrates are ingested there is a significantly greater proportion of energy derived from carbohydrates than fats (Fig 9.5). Glucose infusion using the hyperglycaemic glucose clamp technique, on the other hand, resulted in approximately 85% of the energy being contributed from carbohydrates under a normal dietary status (Fig 9.6), 90% from carbohydrates when on a high carbohydrate diet (Fig 9.7), and 60% from carbohydrates when on a low carbohydrate diet (Fig 9.8). It is clear that carbohydrate 'feeding' results in a stimulation of carbohydrate metabolism, with the result that carbohydrates are a preferred source of energy.

The results from the two studies on carbohydrate ingestion provide support for the conclusions of previous investigations that carbohydrate oxidation rates in excess of 2 g min^{-1} are unlikely when carbohydrates are ingested. It was not possible in this thesis to decide the proportion of carbohydrate oxidation from exogenous sources since a study using radiolabelled carbohydrate for ingestion would be necessary for such a determination.

The use of the hyperglycaemic glucose clamp technique did allow for estimations to be made concerning exogenous glucose use, based on the qualification that maintained hyperglycaemia at a

concentration of 12 mM would totally inhibit hepatic glucose production. Although the maximal rate of total carbohydrate oxidation of approximately 3 g min^{-1} was observed, the maximal rate of glucose utilisation (rate of glucose infused) was no more than 2 g min^{-1}. These maximal rates were independent of the carbohydrate status of the subjects, and may represent the maximal possible rate of glucose transport across skeletal muscles. Even so, it is unlikely that subjects would be capable of exercising at 70% VO_2 max with maintained hyperglycaemia when muscle glycogen depleted.

The hormonal control of metabolism whilst exercising has been quite well established (Galbo, 1983), although there is a comparative lack of literature with regard to exercise and carbohydrate ingestion, and a relative dearth with regard to exercise and glucose infusion. This thesis confirmed the earlier findings that exercise results in an inhibition of plasma insulin concentrations, but stimulates the catecholamines, glucagon, growth hormone, and cortisol. The resultant is a rise in circulating NEFA, B-OH, and glycerol, thereby indicating stimulation of fat metabolism. An attenuated response of these hormones was observed under conditions of glucose ingestion, with the exception of insulin concentrations, which were elevated; probably due to a combination of increased blood glucose and reduced adrenaline.

The prime infusion of glucose in the hyperglycaemic clamp studies caused a drastic increase in plasma insulin concentrations prior

to the exercise starting. This resulted in a significant fall in concentrations of the catecholamines, glucagon, GH, and cortisol, and favoured the metabolism of carbohydrates. Despite the exercise intensity being sufficiently high (70% VO_2 max) to cause a rise in adrenaline concentrations and thereby inhibit insulin secretion, the expected suppression of insulin did not occur until after 60 min of exercise.

The effect of the carbohydrate status of the subjects on the hormonal and metabolic responses to exercise under hyperglycaemia supports the contention that glycogen loading further stimulates carbohydrate metabolism, and that glycogen depletion favours lipid metabolism. The fact that insulin levels were depressed and catecholamine levels stimulated under glycogen depletion are probably the controlling factors in determining substrate use. The factors which result in these hormonal changes, which were observed at rest, could be related to subtle changes in blood glucose level being monitored by the receptors in the hypothalamus.

This study supports the findings that have reported muscle glycogen sparing due to carbohydrate 'feedings'. The significantly higher post-exercise muscle glycogen stores found in this study could solely be attributed to glucose infusion and maintained hyperglycaemia at 12 mM. These findings are in contrast with those obtained by Coyle et al.(1991), who clamped their subjects at 10 mM, but support the study by Bergstrom & Hultman (1967) who infused glucose at a rate of 3 g min^{-1} and

thereby elevated plasma glucose to 21 mM. It is quite reasonable to speculate that a concentration of 11-12 mM of blood glucose is a threshold above which some muscle glycogen sparing occurs.

Finally, an objective of this thesis was to re-appraise the glucose-fatty acid, the glucose-lactate, and the glucose-alanine cycles in the light of maintained hyperglycaemia and exercise. The results are equivocal in support of the existence of the glucose-fatty acid cycle since hyperglycaemia clearly diminished fat metabolism under normal carbohydrate and high carbohydrate dietary conditions, yet under carbohydrate depleted conditions resulted in an upsurge of fat metabolism in spite of hyperglycaemia. If the glucose-fatty acid cycle were operating, there would be an expected decrease in fat metabolism in the light of hyperglycaemia irrespective of low carbohydrate stores in the body. The role of insulin is of crucial importance in regulating this cycle, and it appears that if the levels of insulin are reduced, even when hyperglycaemic, then fat metabolism is promoted.

The glucose-alanine cycle appeared to be compromised under conditions of maintained hyperglycaemia. Increased levels of alanine were found to accumulate in the red-cell during exercise when hyperglycaemic, and this in spite of plasma glucagon concentrations being below resting levels. The original proponents of this cycle examined plasma concentrations of alanine only, and failed to realise the importance of the erythrocytes as transporters and storers of alanine. The release

of alanine, presumably from skeletal muscle, occurs during exercise irrespective of hyperglycaemia. This would imply that the release of alanine maybe due to muscle contractility and not solely hormonally mediated.

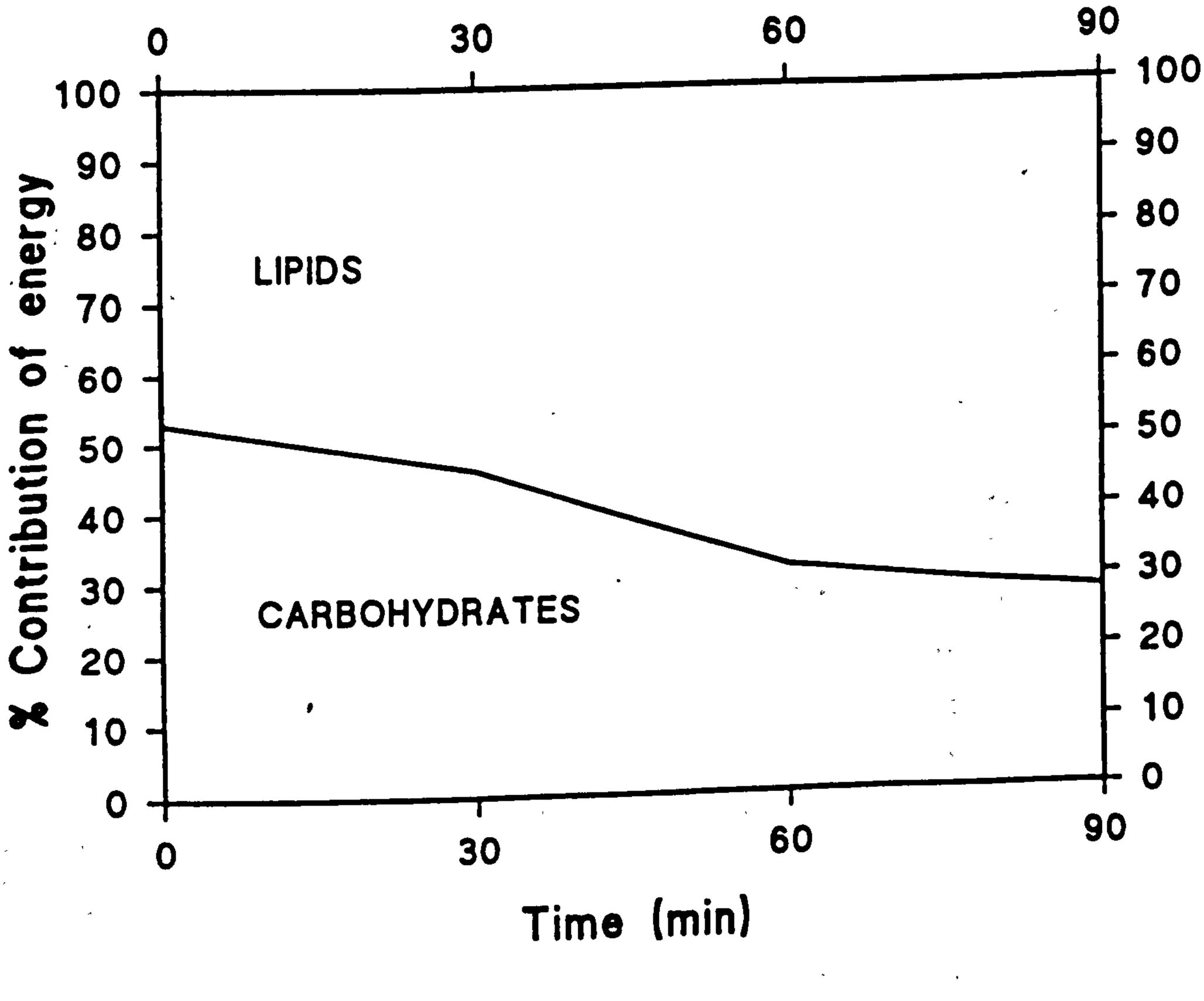


Fig 9.4 The relative contribution of carbohydrates and lipids to total energy during 90 min of cycling at 65% VO_2 max (from chapter 4).

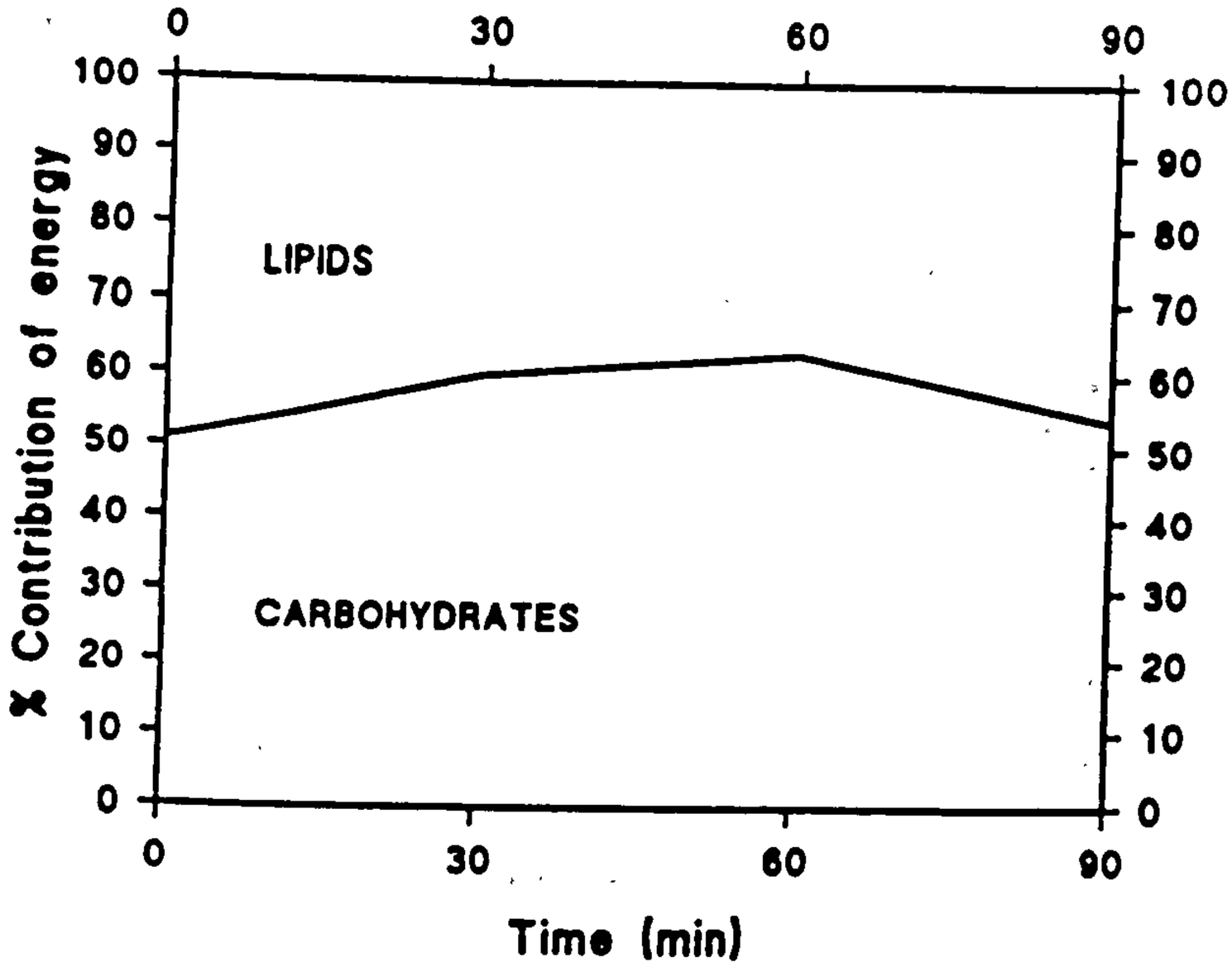


Fig 9.5 The relative contribution of carbohydrates and lipids to total energy during 90 min of cycling at 65% VO_2 max when carbohydrate was ingested immediately prior to exercise (from chapter 4).

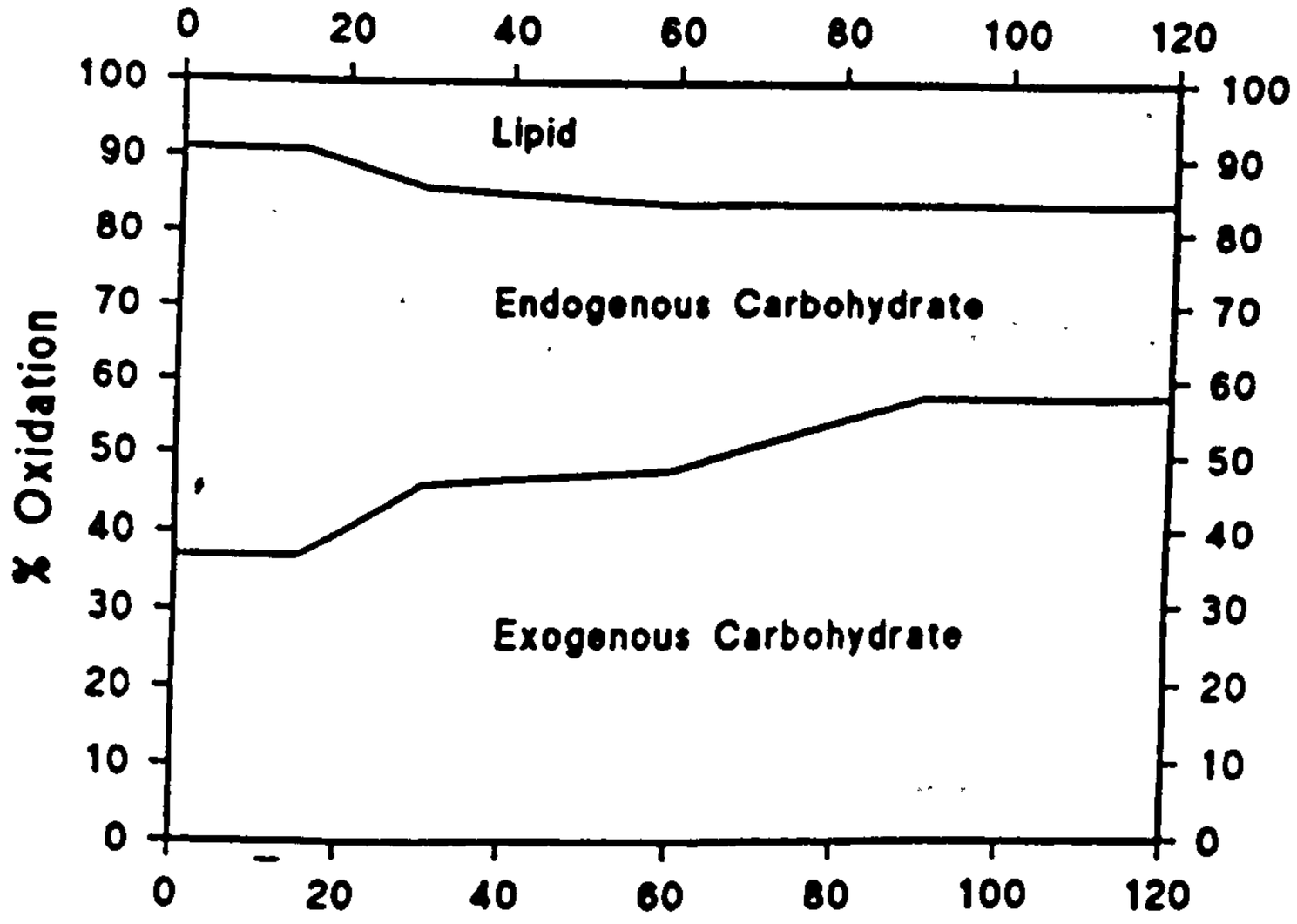


Fig 9.6 The relative contribution of fuel substrates to total energy during 120 min cycling at 70% VO_2 max when hyperglycaemic clamped and under normal dietary conditions (from chapter 7).

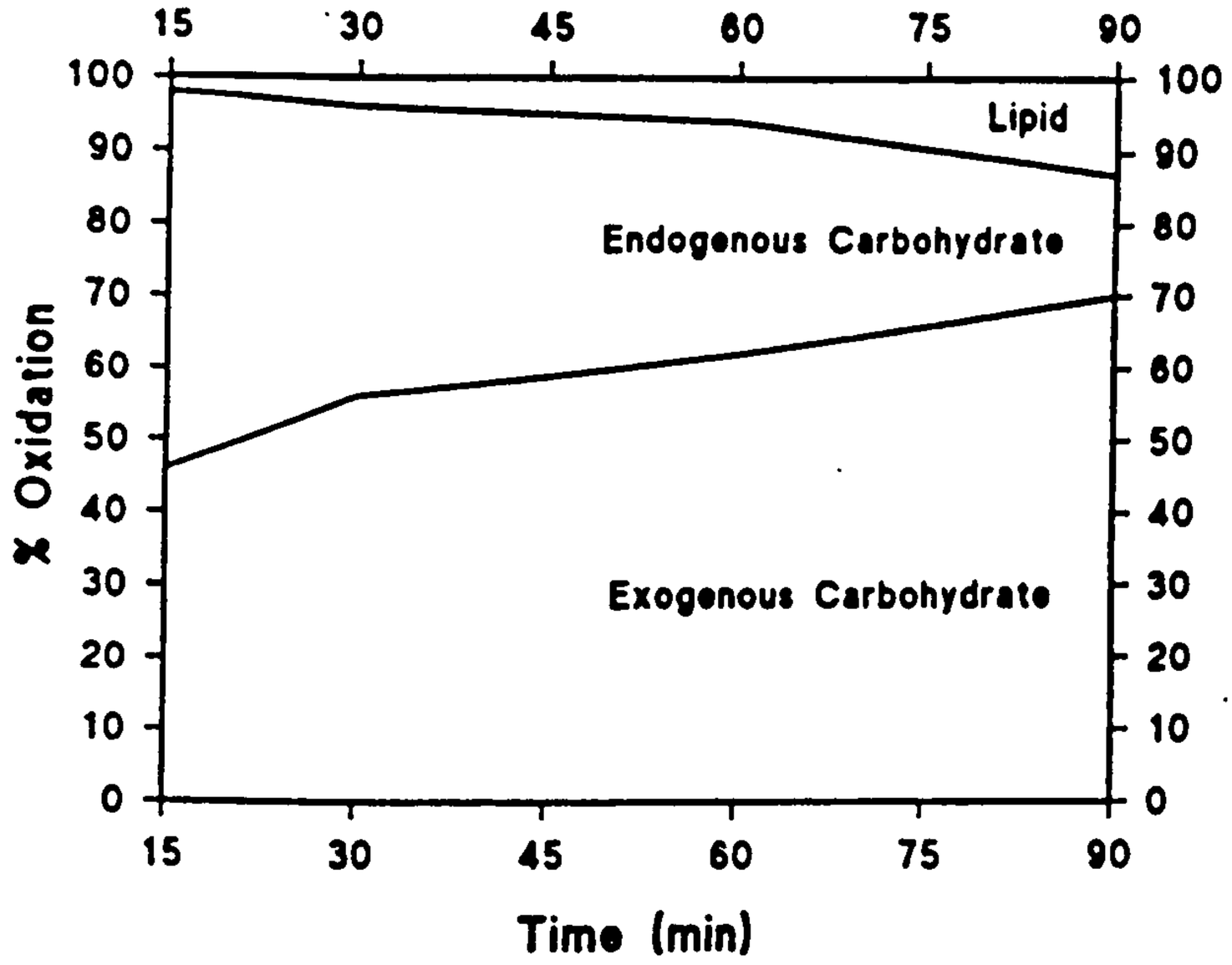


Fig 9.7 The relative contribution of fuel substrates to total energy during 90 min cycling at 70% VO_2 max using the hyperglycaemic clamp when carbohydrate loaded (from chapter 8).

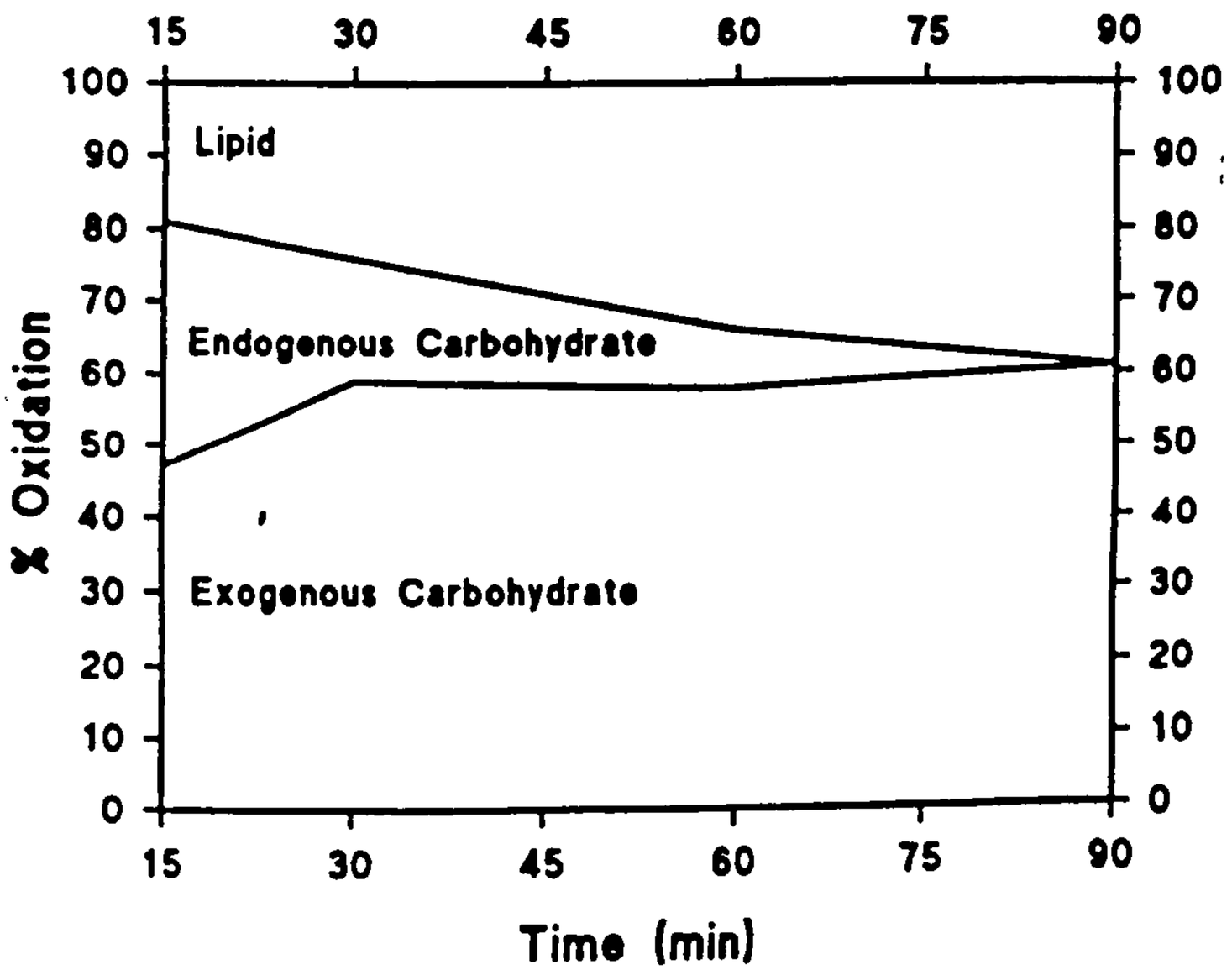


Fig 9.8 The relative contribution of fuel substrates to total energy during 90 min of cycling at 70% VO_2 max using the hyperglycaemic clamp when carbohydrated depleted (from chapter

10. CONCLUSIONS AND RECOMMENDATIONS.

10. CONCLUSIONS AND RECOMMENDATIONS

The aims of this thesis were to:-

i) assess the metabolic and biochemical responses to prolonged exercise under conditions of carbohydrate ingestion and hyperglycaemia using trained subjects, and

ii) develop the use of the hyperglycaemic glucose clamp technique in calculating the rate of glucose utilization during exercise after varying dietary regimens.

The specific objectives concerned with the effects of carbohydrate ingestion on the hormonal and metabolite responses during exercise, and the form of carbohydrate ingested were addressed in chapters 4 and 5. The objectives relating to the hormonal and metabolite responses to maintained hyperglycaemia at rest and during exercise were the focus of chapters 6, 7, and 8.

10.1 CARBOHYDRATE INGESTION AND EXERCISE

The first two experiments in this work were concerned with establishing the hormonal and metabolite responses to carbohydrate ingestion both before (chapter 4) and during (chapter 5) exercise. Although there had been many studies reported on the effects of carbohydrates on prolonged exercise,

and on the rates of oxidation of endogenous and exogenous carbohydrates, there is a lack of literature concerning the relationships with hormones. These two studies presented an integrated approach in that performance, rates of oxidation of total carbohydrate, and the hormonal and metabolite responses were examined.

With respect to the effect of carbohydrate ingestion on performance, both studies highlighted the benefits of carbohydrate ingestion compared with placebo (*supports hypothesis 1*), although the form of carbohydrate did not appear to make a difference (*refutes hypothesis 10*). Despite the lack of significant differences for the times to exhaustion between the carbohydrate treatments, maltodextrin ingestion did present a 20.9% (chapter 4) and 8.5% (chapter 5) improvement compared with glucose ingestion. The addition of guar gum made no significant difference to times to exhaustion, although a 13.7% improvement was noted for glucose and a 7.3% for maltodextrin.

As a consequence of carbohydrate ingestion the rates of total carbohydrate oxidation were enhanced when compared with ingestion of placebo irrespective of whether the carbohydrate was ingested before or during exercise (*supports hypotheses 2 and 7*). A reciprocal finding was evident on examination of the rates of total lipid oxidation. The differences between the rates of total carbohydrate oxidation from the placebo and carbohydrate ingestion trials were similar in both studies i.e. the timing of ingestion made no difference to the rate of oxidation (*refutes*

hypothesis 8). The maximal rates of total carbohydrate oxidation when carbohydrates were ingested were also similar between the studies (*refutes hypotheses 5 and 9*), and were equivalent to those reported in other studies (i.e. 1.8-2.0 g min^{-1}).

The ingestion of a carbohydrate solution either before or during exercise elevated the plasma glucose response and attenuated the plasma lipid responses (*supports hypothesis 3*). The type of carbohydrate ingested (i.e glucose or maltodextrin) did not influence the plasma glucose or lipid concentrations (*refutes hypothesis 9*).

The expected increases in plasma insulin concentration together with a concomitant decrease in catecholamines and glucagon were found during exercise when carbohydrates were ingested (*supports hypothesis 4*). Adding guar gum to the ingested carbohydrate did not have the expected attenuating effect on plasma glucose and insulin concentrations (*refutes hypothesis 6*).

10.2 CARBOHYDRATE INFUSION AND REST

The study on the hormonal and metabolite responses to maintained hyperglycaemia at rest fulfilled two purposes, and these were to become familiar with the procedures of the hyperglycaemic glucose clamp technique, and to provide the base-line for the next two investigations when the 'clamp' would be used during exercise. As expected, the maintenance of hyperglycaemia at rest elevated

hypothesis 8). The maximal rates of total carbohydrate oxidation when carbohydrates were ingested were also similar between the studies (*refutes hypotheses 5 and 9*), and were equivalent to those reported in other studies (i.e. 1.8-2.0 g min^{-1}).

The ingestion of a carbohydrate solution either before or during exercise elevated the plasma glucose response and attenuated the plasma lipid responses (*supports hypothesis 3*). The type of carbohydrate ingested (i.e glucose or maltodextrin) did not influence the plasma glucose or lipid concentrations (*refutes hypothesis 9*).

The expected increases in plasma insulin concentration together with a concomitant decrease in catecholamines and glucagon were found during exercise when carbohydrates were ingested (*supports hypothesis 4*). Adding guar gum to the ingested carbohydrate did not have the expected attenuating effect on plasma glucose and insulin concentrations (*refutes hypothesis 6*).

10.2 CARBOHYDRATE INFUSION AND REST

The study on the hormonal and metabolite responses to maintained hyperglycaemia at rest fulfilled two purposes, and these were to become familiar with the procedures of the hyperglycaemic glucose clamp technique, and to provide the base-line for the next two investigations when the 'clamp' would be used during exercise. As expected, the maintenance of hyperglycaemia at rest elevated

carbohydrate oxidation, diminished fat oxidation, and decreased plasma NEFA and glycerol concentrations throughout the trial (*supports hypothesis 11*). Raising plasma glucose and maintaining it at a concentration of 12 mM stimulated the release of insulin and inhibited the secretions of glucagon, the catecholamines, and cortisol (*supports hypothesis 12*). Since the subjects in this study were normal, healthy volunteers, the rate of glucose utilisation was steadily increased throughout the trial (*supports hypothesis 13*).

10.3 CARBOHYDRATE INFUSION AND EXERCISE

The maintenance of hyperglycaemia during exercise preceded by a 30 min prime infusion period resulted in a rise in plasma insulin up to the 60th minute whereafter the concentration began to decrease. The changes were not significant with respect to time during exercise. There was a clear suppression of counterregulatory hormones and of plasma lipids (*supports hypothesis 14*).

Due to the enhanced availability of glucose, the rate of glucose utilisation determined by 'clamp' technique increased to a maximal rate of 1.8 g min^{-1} at 80-100 min of exercise before a plateau was reached. This was different from the resting study where a continual rise in glucose utilisation was noted, and was not predicted (*refutes hypothesis 15*). Furthermore, the rise in glucose utilisation rate expressed as a percentage of the total rate of carbohydrate oxidation demonstrated that the two

processes were not matched (*refutes hypothesis 15*).

The glucose utilisation rate was higher throughout exercise than at rest (*supports hypothesis 16*). The rise was accommodated by the magnitude of the energy demand during exercise compared with rest.

Previous investigations had generally reported that no muscle glycogen sparing was evident with glucose ingestion. As hyperglycaemia was being maintained at 12 mM throughout this study, there was evidence of muscle glycogen sparing (*supports hypothesis 17*).

The amino acid response to exercise resulted in an elevated plasma and red-cell concentration of alanine (*supports hypothesis 18*), whereas the concentrations of the branched chain and other amino acids produced variable findings (*refutes hypothesis 18*). The maintenance of hyperglycaemia was expected to highlight an attenuating response of alanine and the other amino acids, but this was not borne out by the data (*refutes hypothesis 19*).

The hyperglycaemic clamp technique was also employed during exercise when subjects were glycogen depleted and glycogen loaded. The rate of glucose utilisation was similar between the two conditions (*refutes hypothesis 20*) although the percentage of glucose utilised to the total carbohydrate oxidised was lower when glycogen loaded (*supports hypothesis 20*).

Elevated concentrations of insulin and diminished levels of catecholamines, NEFA, B-OH, and glycerol were observed (*supports hypothesis 21*).

10.4 FULFILMENT OF OBJECTIVES

The objectives set out for the thesis were achieved by the 5 experiments undertaken. The two experiments on the effects of carbohydrate ingestion before and during exercise on the hormonal, metabolite, and metabolic responses highlighted the stimulation of carbohydrate metabolism and the attenuation of lipid metabolism. The effects were mediated by hormonal influences and resulted in enhanced exercise performance. Maltodextrin (an 8-11 chain glucose polymer) ingestion did not confer any significant advantages over glucose ingestion with regard to performance, carbohydrate or lipid metabolism, or changes in concentrations of hormones and metabolites.

The objectives relating to the hyperglycaemic clamp technique being employed at rest and during exercise under varying dietary regimens were pursued in 3 experiments. The results emphasised the stimulatory effect that maintained hyperglycaemia had on carbohydrate metabolism and the inhibitory effect on lipid metabolism; the hormonal and metabolite changes contributing to the metabolic responses observed. Maximal rates of glucose utilisation were determined using the clamp technique, and led to the speculation that the maximal rate of glucose transport across skeletal muscle was the determining factor. The glucose-

fatty acid cycle appeared to be compromised by hyperglycaemia, and this became evident on examination of the red-cell alanine concentrations.

An attempt was made, in chapter 9, to synthesise the metabolic data from this thesis, and to present the relative contributions of carbohydrates and lipids for total oxidation. An overview of the hormonal responses integrating metabolism was also proferred.

10.5 RECOMMENDATIONS FOR FURTHER STUDIES

Numerous studies have been performed on the effects of the ingestion of carbohydrates on exercise metabolism, and it is difficult to envisage how useful and innovative any further investigations in this domain are likely to be. However, few investigations have reported on the influence of carbohydrate ingestion when subjects are carbohydrate depleted or loaded, and in particular the rates of oxidation and hormonal response in these conditions. The use of radiolabelled tracers incorporated into various types of carbohydrate to be ingested and the subsequent rate of oxidation when loaded or depleted may provide information as to the most useful form of carbohydrate to ingest.

The use of the hyperglycaemic clamp technique during exercise has only been reported in a few investigations to date. The taking of serial muscle biopsies during exercise may be helpful in

determining the rate of muscle glycogen use during exercise when hyperglycaemic. The time-point wherein there is some sparing may become apparent. The effect of hyperglycaemia on muscle glycogen utilisation has not been studied in subjects under carbohydrate loaded or depleted conditions, and clearly this is an area for further investigation.

The maximal rates of glucose utilisation have been suggested as being approximately 2.0 g min^{-1} in this study, yet higher rates have been observed in two other studies where hyperglycaemia was maintained at 10 mM glucose. Infusing glucose to maintain hyperglycaemia at different concentrations should help to establish the rate at which glucose is utilised in terms of a dose response.

The infusion of stable-isotope glucose in order to maintain hyperglycaemia and the assessment of muscle glycogen concentrations could further elucidate the contribution of exogenous and endogenous sources of carbohydrate to total carbohydrate oxidation. Studies employing cannulation for the determination of a-v differences across an exercising muscle would contribute to the understanding of the rates of uptake of exogenously supplied glucose. As previously mentioned, it is possible that glucose transport limits glucose uptake by skeletal muscle during exercise when hyperglycaemic. The effects of maintained hyperglycaemia on GLUT-4 transporters may elucidate mechanisms relating to the control of glucose transport.

Investigations into the effect of maintained hyperglycaemia on the glucose-alanine cycle clearly need the assessment of a-v differences across exercising muscle and the cannulation of hepatic blood vessels (if possible) in order to assess a-v differences associated with the liver. These methods could prove invaluable in clarifying whether the glucose-alanine is indeed compromised by hyperglycaemia.

REFERENCES

Abumrad,N.N., Rabin, D., Diamond, M.P., and Lacy, W.W. (1981). Use of a heated superficial hand vein as an alternative site for the measurement of amino acid concentrations and for the study of glucose and alanine kinetics in man. *Metabolism,* 30, 936-940.

Ahlborg, G., and Bjorkman, O. (1987). Carbohydrate utilization by exercising muscle following pre-exercise glucose ingestion. *Clinical Physiology,* 7, 181-195.

Ahlborg, G., and Felig, P. (1976). Influence of glucose ingestion on fuel-hormone response. *Journal of Applied Physiology,* 41, 683-688.

Ahlborg, G., and Felig,P. (1982). Lactate and glucose exchange across the forearm, legs, and splanchnic bed during and after prolonged leg exercise. *Journal of Clinical Investigation,* 69, 45-54.

Ahlborg, G., Bergstrom, J., Brohult, L.-G., Hultman, E., and Maschio, G. (1967a). Human muscle glycogen content and capacity for prolonged exercise after different diets. *Foersvarsmedicin,* 3, 85-99.

Ahlborg, G., Bergstrom., J., Eklund, L.-G., and Hultman, E. (1967b). Muscle glycogen and muscle electrolytes during prolonged physical exercise. *Acta Physiologica Scandanavica,* 70, 129-142.

Ahlborg, G., Felig, P., Hagenfeldt, L., Hendler, R., and Wahren, J. (1974). Substrate turnover during prolonged exercise in man: Splanchnic and leg metabolism of glucose, free fatty acids and amino acids. *Journal of Clinical Investigation,* 53, 1080-1090.

Ahlborg, G., Hagenfeldt, L., and Wahren, J. (1975). Substrate utilisation by the inactive leg during one-leg or arm exercise. *Journal of Applied Physiology,* 39, 718-723.

Andersen, P.H., Lund, S., Schmitz, O., Junker, S., Kahn, B.B., and Pedersen, O. (1993). Increased insulin-stimulated glucose uptake in athletes: the importance of GLUT4 mRNA, GLUT4 protein and fibre type composition of skeletal muscle. *Acta Physiologica Scandanavica*, **149**, 393-404.

Aoki, T.T., Brennan, M.F., Muller,W.A., Moore, F.D., and Cahill, G.F. (1972). Effect of insulin on muscle glutamate uptake. *Journal of Clinical Investigation*, **51**, 2889-2894.

Arner,P., Kreigholm, E., Engfeldt, P., and Bolinder, J. (1990). Adrenergic regulation of lipolysis in situ at rest and during exercise. *Journal of Clinical Investigation*, **85**, 893-898.

Ashbrook, J.D., Spector, A.A., Santos, E.C., and Fletcher, J.E. (1975). Long chain fatty acid binding to human plasma albumin. *Journal of Biological Chemistry*, **6**, 2333-2338.

Bagby, G.J., Green, H.J., Katsuta, S., and Gollnick, P.D. (1978). Glycogen depletion in exercising rats infused with glucose, lactate, or pyruvate. *Journal of Applied Physiology*, **45**, 425-429.

Barstow, T.J., Cooper, D.M., Epstein, S., and Wasserman, K. (1989). Changes in breath $^{13}CO_2/^{12}CO_2$ consequent to exercise and hypoxia. *Journal of Applied Physiology*, **66** , 936-942.

Berger, M., Hagg, S.A., and Ruderman, N.B. (1975). Glucose metabolism in perfused skeletal muscle: interaction of insulin and exercise on glucose uptake. *Biochemical Journal*, **146**, 231-238.

Berger, M., Hagg, S.A., Goodman, M.N., and Ruderman, N.B. (1976). Glucose metabolism in perfused .skeletal muscle: effects of starvation, diabetes, fatty acids, acetoacetate, insulin, and exercise. *Biochemical Journal*, **158**, 191-202.

Bergmeyer, H.U., and Bernt, E. (1974). Determination of D-glucose with glucose oxidase and peroxidase: method with ABTS. In *Methods of Enzymatic Analysis* (edited by H.U.Bergmeyer) pp 1212-1215.

Bergstrom, J., and Hultman, E. (1966). The effect of exercise on muscle glycogen and electrolytes in normals. *Scandinavian Journal of Clinical and Laboratory Investigation*, **18**, 16-20.

Bergstrom, J., and Hultman, E. (1967). A study of the glycogen metabolism during exercise in man. *Scandinavian Journal of Clinical and Laboratory Investigation*, **19**, 218-228.

Bergstrom, J., Hermansen, L., Hultman, E., and Saltin, B. (1967). Diet, muscle glycogen, and physical performance. *Acta Physiologica Scandanavica*, **71**, 140-150.

Bergstrom, J., Hultman, E., and Roch-Norland, A.E. (1972). Muscle glycogen synthase in normal subjects: Basal values, effect of glycogen depletion by exercise and of a carbohydrate-rich diet following exercise. *Scandinavian Journal of Clinical Laboratory Investigation*, **29**, 231-236.

Bergstrom, J., Hultman, E., Jorfeldt, L., Pernow, B., and Wahren, J. (1969). Effect of nicotinic acid on physical work capacity and on metabolism of muscle glycogen in man. *Journal of Applied Physiology*, **26**, 170-176.

Bjorkman, O., Sahlin, K., Hagenfeldt, L., and Wahren, J. (1984). Influence of glucose and fructose ingestion on the capacity for long-term exercise. *Clinical Physiology*, **4**, 483-494.

Blackburn, N.A., Holgate, A.M., and Read, N.W. (1984). Does guar gum improve post-prandial hyperglycaemia in humans by reducing small intestinal contact area? *British Journal of Nutrition*, 52, 197-204.

Bonen, A., Clune, P.A., and Tan, M.H. (1986). Chronic exercise increases insulin binding in muscles but not liver. *American Journal of Physiology*, **251**, E196-E203.

Bonen, A., Tan, M.H., and Watson-Wright, W.M. (1981). Insulin binding and glucose uptake differences in rodent skeletal muscles. *Diabetes*, **30**, 702-704.

Bonen, A., Tan, M.H., and Watson-Wright, W.M. (1984). Effects of exercise on insulin binding and glucose metabolism in muscle. *Canadian Journal of Physiology and Pharmacology*, **62**, 1500-1504.

Borg, G.A.V. (1973). Perceived exertion: A note on "history" and methods. *Medicine and Science in Sports*, **5**, 90-93.

Bosch, A.N., Dennis, S.C., and Noakes, T.D. (1993). Influence of carbohydrate-loading on fuel substrate turnover and oxidation during prolonged exercise. *Journal of Applied Physiology*, **74**, 1921-1927.

Brooke, J.D., Davies, G.J., and Green, L.F. (1975). The effects of normal and glucose syrup work diets on the performance of racing cyclists. *Journal of Sports Medicine*, **15**, 257-265.

Brooks, G.A. (1986). The lactate shuttle during exercise and recovery. *Medicine and Science in Sports and Exercise*, **18**, 360-368.

Brouns, F., Saris, W.H.M., and Beckers, E. (1989). Metabolic changes induced by sustained exhaustive cycling and diet manipulation. *International Journal of Sports Medicine*, **10** (*Supplement 1*), S49-S62.

Bulow, J. (1981). Human adipose tissue blood flow during prolonged exercise: effect of β-adrenergic blockade, nicotinic acid and glucose infusion. *Scandinavian Journal of Clinical Laboratory Investigation*, **41**, 415-424.

Bulow, J. (1982). Subcutaneous adipose tissue blood flow and triacylglycerol mobilization during prolonged exercise in dogs. *Pflugers Archive*, **392**, 230-234.

Bulow, J. (1983). Adipose tissue blood flow during exercise, *Danish Medical Bulletin*, **30**, 85-100.

Bulow, J. (1988). Lipid mobilization and utilisation. In: *Principals of Exercise Biochemistry*, (edited by J.R. Poortmans) pp140-163. Basel:Karger.

Bulow, J., and Madsen, J. (1986). Regulation of lipid mobilization during exercise. *Scandinavian Journal of Sports Science*, **8**, 19-26.

Bulow, J., and Tondevold,E. (1982). Blood flow in different adipose tissue depots during prolonged exercise in dogs. *Pflugers Archive*, **392**, 235-238.

Bulow, J., Madsen, J., Astrup, A., and Christensen, N.J. (1985). The effect of high free fatty acid/albumin molar ratios on the perfusion of adipose tissue in vivo. *Acta Phsiologica Scandanavica*, **125**, 661-667.

Chasiotis, D. (1983). The regulation of glycogen phosphorylase and glycogen breakdown in human skeletal muscle. *Acta Physiologica Scandanavica* (Suppl.) **518**, 1-68.

Chasiotis, D., Sahlin, K., and Hultman, E. (1982). Regulation of human skeletal muscle at rest and during exercise. *Journal of Applied Physiology*, **53**, 707-715.

Christensen, E.H., and Hansen, O. (1939). Hypoglycamie, arbeitsfahigkeit und Ermuding. *Scandinavisches Archiv fur Physiologie*, 81 , 172-179.

Christensen, N.J., and Galbo, H. (1983). Sympathetic nervous activity during exercise. *Annual Review of Physiology*, 45, 139-153.

Coggan, A.R., and Coyle, E.F. (1987). Reversal of fatigue during prolonged exercise by carbohydrate infusion or ingestion. *Journal of Applied Physiology*, 63, 2388-2395.

Coggan, A.R., and Coyle, E.F. (1988). Effect of carbohydrate feedings during high-intensity exercise. *Journal of Applied Physiology*, 65, 1703-1709.

Coggan, A.R., and Coyle, E.F. (1989). Metabolism and performance following carbohydrate ingestion late in exercise. *Medicine and Science in Sport and Exercise*, 21, 59-65.

Coggan, A.R., and Coyle, E.F. (1991). Carbohydrate ingestion during prolonged exercise: effects on metabolism and performance. *Exercise and Sports Science Reviews*, 19, 1-40.

Coggan, A.R., Kohrt, W.M., Spina, R.J., Kirwan, J.P., Bier, D.M., and Holloszy, J.O. (1992). Plasma glucose kinetics during exercise in subjects with high and low lactate thresholds. *Journal of Applied Physiology*, 73, 1873-1880.

Consolazio, C.R., Johnson, R.E., and Pecora, L.T. (1963). *Physiological Measurements of Metabolic Functions in Man*. pp 72-87. McGraw; New York.

Copack, S.W., Frayn, K.N., Humphreys, S.M., Dhar, H., and Hockaday, T.D.R. (1989). Effects of insulin on human adipose tissue metabolism in vivo. *Clinical Science*, 77, 663-670.

Copeland, G.P., Al-Sumidaie, A.M., Leinster, S.J., Davis, J.C., and Hipkin,L.H. (1987). Glucose metabolism in patients with gastrointestinal malignancy but without excessive weight loss. *European Journal of Surgical Oncology*, **13**, 11-16.

Copeland, G.P., Leinster, S.J., Davis, J.C., and Hipkin, L.H. (1988). Postoperative glucose metabolism in patients with gastrointestinal malignancy. *European Journal of Surgical Oncology*, **14**, 677-683.

Cori, C.F. (1931). Mammalian carbohydrate metabolism. *Physiological Reviews*, **11**, 143-275.

Costill, D.L., Bennett, A., Branam, G., and Eddy, D. (1973). Glucose ingestion at rest and during prolonged exercise. *Journal of Applied Physiology*, **34**, 764-769.

Costill, D.L., Coyle, E.F., Dalsky, G., Evans, W., Fink, W., and Hoopes, D. (1977). Effects of elevated plasma FFA and insulin on muscle glycogen usage during exercise. *Journal of Applied Physiology*, **43**, 695-699.

Coyle, E.F. (1991). Timing and method of increased carbohydrate intake to cope with heavy training, competition and recovery. *Journal of Sports Sciences*, **9**, 29-52.

Coyle, E.F., and Coggan, A.R. (1984). Effectiveness of carbohydrate feeding in delaying fatigue during prolonged exercise. *Sports Medicine*, **1**, 446-458.

Coyle, E.F., Coggan, A.R., Hemmert, M.K., and Ivy, J.L. (1986). Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *Journal of Applied Physiology*, **61**, 165-172.

Coyle, E.F., Hagberg, J.M., Hurley, B.F., Martin, W.H., Ehsani, A.A., and Holloszy, J.O. (1983). Carbohydrate feeding during prolonged strenuous exercise can delay fatigue. *Journal of Applied Physiology*, 55, 230-235.

Coyle, E.F., Hamilton, M.T., Alonso, J.G., Montain, S.J., and Ivy, J.L. (1991). Carbohydrate metabolism during intense exercise when hyperglycaemic. *Journal of Applied Physiology*, 70, 834-840.

Craig, B.W., Hammons, G.T., Garthwaite, S.M., Jarret, L., and Holloszy, J.O. (1981). Adaptations of fat cells to exercise: response of glucose uptake and oxidation to insulin. *Journal of Applied Physiology*, 51, 1500-1506.

Cushman, S.W., and Wardzala, L.J. (1980). Potential mechanism of insulin action on glucose transport in the isolated rat adipose cell. *Journal of Biological Chemistry*, 255, 4758-4762.

Czech, M. (1985). The nature and the regulation of the insulin receptor: structure and function. *Annual Review of Physiology*, 47, 357-381.

Davies, C.T.M., and Few, J.D. (1973). Effects of exercise on adrenocortical function. *Journal of Applied Physiology*, 35, 887-891.

Decombaz, J., Sartori, D., Arnaud, M.J., Thelin, A.L., Schurch, P., and Howald,H. (1985). Oxidation and metabolic effects of fructose or glucose ingested before exercise. *International Journal of Sports Medicine*, 6, 282-286.

DeFronzo, R.A., Tobin, J.D., and Andrea, R. (1979). Glucose clamp technique: a method for quantifying insulin secretion and resistance. *American Journal of Physiology*, 237, E214-E223.

Dela, F., Handberg, A., Mikines, K.J., Vinten, J., and Galbo, H. (1993). GLUT4 and insulin receptor binding and kinase activity in trained human muscle. *Journal of Physiology*, **469**, 615-624.

Despres, J.P., Bouchard, C., Savard, R., Tremblay, A., Marcotte, M., and Threiault, G. (1984). The effect of a 20-week endurance training program on adipose tissue morphology and lipolysis in men and women. *Metabolism*, **33**, 235-239.

Devlin, J.T., Calles-Escandon, J., and Horton, E.S. (1986). Effect of pre-exercise snack feeding on endurance cycle exercise. *Journal of Applied Physiology*, **60**, 980-985.

Dill, D.B., Edwards, H.T., and Talbot, J.H. (1932). Studies in muscular activity: factors limiting the capacity for work. *Journal of Physiology*, **77**, 49-62.

Dill, D.B., and Costill, D.L. (1974). Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *Journal of Applied Physiology*, **37**, 247-248.

Dohm, G.L., Kasperek, G.J., and Barakat, H.A. (1985). Time course of changes in gluconeogenic enzyme activities during exercise and recovery. *American Journal of Physiology*, **249**, E6-E11.

Dole, V.P. (1956). A relation between non-esterified fatty acids in plasma and the metabolism of glucose. *Journal of Clinical Investigation*, **35**, 150-154.

Douen, A.G., Ramlal, T., Rastogi, S., Bilan, P.J., Cartee, G.D., Vranic, M., Holloszy, J.O., and Klip, A. (1990). Exercise induces recruitment of the "insulin-responsive glucose transporter": evidence for distinct intracellular insulin- and exercise-recruitable transporter pools in skeletal muscle. *Journal of Biological Chemistry*, **265**, 13427-13430.

Ebeling, P., Bourey, R., Koranyi, L., Touminen, J.A., Groop, L.C., Henriksson, J., Mueckler, M., Sovijarvi, A., and Koivisto, V.A. (1993). Mechanism of enhanced insulin sensitivity in athletes: increased blood flow, muscle glucose transport protein (GLUT-4) concentration, and glycogen synthase activity. *Journal of Clinical Investigation*, **92**, 1623-1631.

Edwards, R.H.T., Young, A., and Wiles, C.M. (1980). Needle biopsy of skeletal muscle in the diagnosis of myopathy and the clinical study of muscle function and repair. *New England Journal of Medicine*, **302**, 261-271.

Ellis, R.E., Burley, V.J., Leeds, A.R., and Peterson, D.B. (1988). A guar-enriched wholemeal bread reduces postprandial glucose and insulin response. *Journal of Human Nutrition and Dietetics*, **1**, 77-84.

Elwyn, D.H., Launder, W.J., Paraki, H.C., and Wise, E.M. (1972). Roles of plasma and erythrocytes in interorgan transport of amino acids in dogs. *American Journal of Physiology*, **222**, 1333-1342.

Eriksson, J., Koranyi, L., Bourey, R., Schalin, J.C., Widen, E., Mueckler, M., Permutt, A.M., and Groop. L.C. (1992). Insulin resistance in Type 2 (non-insulin-dependent) diabetic patients and their relatives is not associated with a defect in the expression of the insulin-responsive glucose transporter (GLUT-4) gene in human skeletal muscle. *Diabetologia*, **35**, 143-147.

Erickson, M.A., Schwarzkopf, R.J., and McKenzie, R.D. (1987). Effects of caffeine, fructose, and glucose ingestion on muscle glycogen utilization during exercise. *Medicine and Science in Sports and Exercise*, **19**, 579-583.

Essen, B., Hagenfeldt, L., and Kaijser, L. (1977). Utilization of blood-borne and intramuscular substrates during continuous and intermittent exercise in man. *Journal of Physiology*, **265**, 489-506.

Federspil, G., Lefebvre, P., Luyckx, A., and De Palo, C. (1975). Endocrine mechanisms of exercise-induced fatty acids mobilization in rats. In: *Metabolic adaptations to prolonged exercise* (Edited by H. Howald and J.R. Poortmans) pp301-307. Basel:Birkhauser Verlag.

Felig, P. (1973). The glucose-alanine cycle. *Metabolism*, **22**, 179-207.

Felig, P. (1977). Amino acid metabolism in exercise. *Annals of the New York Academy of Sciences*, **301**, 56-63.

Felig, P., and Wahren, J. (1971). Amino acid metabolism in exercising man. *Journal of Clinical Investigation*, **50**, 2703-2714.

Felig, P., and Wahren, J. (1974). Protein turnover and amino acid metabolism in the regulation of gluconeogenesis. *Federation Proceedings*, **33**, 1029-1037.

Felig, P., and Wahren, J. (1979). Role of insulin and glucagon in the regulation of hepatic glucose production during exercise. *Diabetes*, **28**, *Supplement* **1**, 71-75.

Felig, P., Wahren, J., and Raf, L. (1973). Evidence of interorgan amino acid transport by blood cells in humans. *Proceedings of the National Academy of Sciences*, **70**, 1775-1779.

Fellows, I.W., and MacDonald, I.A. (1985). An automated method fot the measurement of oxygen consumption and carbon dioxide excretion in man. *Clinical Physics Physiological Measurements*, **6**, 349-355.

Ferrannini, E. (1988). The theoretical bases of indirect calorimetry. A review. *Metabolism*, **37**, 287-301.

Ferrannini, E., Barrett, E.J., Bevilacqua, S., and DeFronzo, R.A. (1983). Effect of fatty acids on glucose production and utilization in man. *Journal of Clinical Investigation*, **72**, 1737-1747.

Fielding, R.A., Costill, D.A., Fink, W.J., King, D.S., Hargreaves, M., and Kovaleski, J.E. (1985). Effect of carbohydrate feeding frequency and dosage on muscle glycogen use during exercise. *Medicine and Science in Sport and Exercise*, **17**, 472-476.

Flynn, M.G., Costill, D.L., Hawley, J.A. (1987). Influence of selected carbohydrate drinks on cycling performance and glycogen use. *Medicine and Science in Sport and Exercise*, **19**, 37-40.

Foster, C., Costill, D.L., and Fink, W.J. (1979). Effects of pre-exercise feedings on endurance performance. *Medicine and Science in Sport and Exercise*, **11**, 1-5.

Frayn, K.N. (1983). Calculations of substrate oxidation rates in vivo from gaseous exchange, *Journal of Applied Physiology*, **55**, 628-634.

Frayn, K.N. (1989). Adipose tissue metabolism. *Clinical Dermatology*, **7**, 48-61.

Frayn, K.N., Coppack, S.W., Humphreys, S.M., and Whyte, P.L. (1989). Metabolic characteristics of human adipose tissue in vivo. *Clinical Science*, **76**, 509-516.

Frayn, K.N., and Maycock, P.F. (1983). Sample preparation with ion-exchange resin before liquid-chromatographic determination of plasma catecholamines. *Clinical Chemistry*, **29**, 1426-1428.

Froberg, S.O., and Mossfeldt, F. (1971). Effect of prolonged strenuous exercise on the concentration of triglycerides, phospholipids and glycogen in muscle of man. *Acta Physiologica Scandanavica*, **82**, 167-171.

Galbo, H. (1981). Endocrinology and metabolism in exercise. *International Journal of Sports Medicine*, **2**, 203-211.

Galbo, H. (1983). *Hormonal and metabolic adaptations to exercise.* New York: Georg Thieme Verlag.

Galbo, H. (1992), Exercise physiology: humoral function. *Sports Science Review*, **1**, 65-93.

Galbo, H., Holst, J.J., and Christensen, N.J. (1979). The effect of different diets and insulin on the hormonal response to prolonged exercise. *Acta Physiologica Scandanavica*, **107**, 19-32.

Galbo, H., Holst, J.J., Christensen, N.J., and Hilsted, J. (1976). Glucagon and plasma catecholamines during beta-receptor blockade in exercising man. *Journal of Applied Physiology*, **40**, 855-863.

Gammeltoft, S. (1984). Insulin receptors: binding kinetics and structure-function relationship of insulin. *Physiological Reviews*, **64**, 1321-1378.

Gawel, M.J., Alaghband-Zadeh, J., Park, D.M., and Rose, F.C. (1979). Exercise and hormonal secretion. *Postgraduate Medical Journal*, **55**, 373-376.

Gerich, J.E. (1993). Control of glycaemia, *Baillieres Clinical, Endocrinology, and Metabolism*, **7**, 551-586.

Gibson, H., and Edwards, R.H.T. (1985). Muscular exercise and fatigue. *Sports Medicine*, **2**, 120-132.

Gleeson, M., Maughan, R.J., and Greenhaff, P.L. (1986). Comparison of the effects of pre-exercise feedings of glucose, glycerol and placebo on endurance and fuel homeostasis in man. *European Journal of Applied Physiology*, 55, 645-653.

Goldrick,R.B., Ashley, B.C.F., and Lloyd, M.L. (1974). Effect of prolonged incubation and cell concentration on lipogenesis from glucose in isolated human omental fat cells. *Journal of Lipid Research*, 10, 253-259.

Gollnick, P.D., and Saltin, B. (1982). Significance of skeletal muscle oxidative enzyme enhancement with endurance training. *Clinical Physiology*, 2, 1-12.

Gordon, B., Kohn, L.A., Levine, S.A., Matton, M., Scriver, W. De M., and Whiting, W.B. (1925). Sugar content of the blood in runners following a marathon race. *Journal of the American Medical Association*, 185, 508-509.

Green, C.J. (1990). Energy balance in multiple organ failure. PhD thesis, University of Liverpool.

Green, L.F., and Bagley, R. (1972). Ingestion of a glucose syrup drink during long distance canoeing. *British Journal of Sports Medicine*, 16, 125-128.

Guezennec,C.Y., Satabin,P., duforez, F., Merino, D., Peronnet, F., and Koziet, J. (1989). Oxidation of corn starch, glucose, and fructose ingested before exercise. *Medicine and Science in Sport and Exercise*, 21, 45-50.

Gutmann, I., and Wahlefeld, A.W. (1974). L(+)lactate determination with lactate dehydrogenase and NAD. In *Methods of Enzymatic Analysis* (edited by H.U.Bergmeyer) pp 1464-1468.

Hagenfeldt, L. Metabolism of free fatty acids and ketone bodies during exercise in normal and diabetic man. *Diabetes*, **28** (suppl. 1), 66-70.

Hagenfeldt, L., and Arvidsson, A. (1980). The distribution of amino acids between plasma and erythrocytes. *Clinica Chimica Acta*, **100**, 133-141.

Hansen, A.P. (1971). The effect of adrenergic receptor blockade on the exercise-induced serum growth hormone rise in normals and juvenile diabetics. *Journal of Clinical Endocrinology*, **33**, 807-812.

Hargreaves, M. (1990). Skeletal muscle carbohydrate metabolism during exercise. *Australian Journal of Science and Medicine in Sport (June)*, 35-38.

Hargreaves, M., and Briggs, C.A. (1988). Effect of carbohydrate ingestion on exercise metabolism. *Journal of Applied Physiology*, **65**, 1553-1555.

Hargreaves, M., Costill, D.L., Coggan, A.R., Fink, W.J., and Nishibata, I. (1984). Effect of carbohydrate feedings on muscle glycogen utilization and exercise performance. *Medicine and Science in Sport and Exercise*, **16**, 219-222.

Hargreaves, M., Costill, D.L., Fink,W.J., King, D.S., and Fielding, R.A. (1987). Effect of pre-exercise carbohydrate feedings on endurance cycling performance. *Medicine and Science in Sport and Exercise*, **19**, 33-36.

Hargreaves, M., Costill, D.L., Katz, A., and Fink, W.J. (1985). Effect of fructose ingestion on muscle glycogen usage during exercise. *Medicine and Science in Sport and Exercise*, **17**, 360-363.

Hargreaves, M., and Proietto, J. (1994). Glucose kinetics during exercise in trained men. *Acta Physiologica Scandanavica*, **150**, 221-225.

Hargreaves, M., Kiens, B. and Richter, E.A. (1991). Effect of increased plasma free fatty acid concentrations on muscle metabolism in exercising men. *Journal of Applied Physiology*, **70**, 194-201.

Havel, R.J., Pernow, B., and Jones, N.L. Uptake and release of free fatty acids and other metabolitsm in the leg of exercising man. *Journal of Applied Physiology*, **23**, 90-96.

Hawley, J.A., Bosch, A.N., Weltan, S.M., Dennis, S.C., and Noakes, T.D. (1994a). Effects of glucose ingestion or glucose infusion on fuel substrate kinetics during prolonged exercise. *European Journal of Applied Physiology*, **68**, 381-389.

Hawley, J.A., Bosch, A.N., Weltan, S.M., Dennis, S.C., and Noakes, T.D. (1994b). Glucose kinetics during prolonged exercise in euglycaemic and hyperglycaemic subjects. *Pflugers Archive*, **426**, 378-386.

Hawley, J.A., Dennis, S.C., and Noakes, T.D. (1992). Oxidation of carbohydrate ingested during prolonged endurance exercise. *Sports Medicine*, **14**, 27-42.

Hawley, J.A., Dennis, S.C., Laidler, B.J., Bosch, A.N., Noakes, T.D., and Brouns, F. (1991). High rate of exogenous carbohydrate oxidation from starch ingested during prolonged exercise. *Journal of Applied Physiology*, **71**, 1801-1806.

Haymond, M.W., and Miles, J.M. (1982). Branched-chain amino acids as a major source of alanine nitrogen in man. *Diabetes*, **31**, 86-89.

Heitman, R.N., and Bergman, E.N. (1980). Transport of amino acids in whole blood and plasma of sheep. *American Journal of Physiology*, **239**, E242-E247.

Hellerstein, M.K., Christiansen, M., and Kaempfer, S. (1991). Measurement of de novo hepatic lipogenesis in humans using stable isotopes. *Journal of Clinical Investigation*, **87**, 1841-1852.

Hermansen, L., Hultman, E., and Saltin, B. (1967). Muscle glycogen during prolonged severe exercise. *Acta Physiologica Scandanavica*, **71**, 129-139.

Hetenyi, G.J. (1982). Correction for the metabolic exchange of ^{14}C for ^{12}C atoms in the pathway of gluconeogenesis in vivo. *Federation Proceedings*, **41**, 104-109.

Hirsch, I.B., Marker, J.C., Smith, L.J., Spina, R.J., Parvin, C.A., Holloszy, J.O., and Cryer, P.E. (1991). Insulin and glucagon in prevention of hypoglycemia during exercise in humans. *American Journal of Physiology*, **260**, E695-E704.

Hirshman, M.F., Wallberg-Henriksson, H., Wardzala, L.J., Horton, E.D., and Horton, E.S. (1988). Acute exercise increases the number of plasma membrane glucose transporters in rat skeletal muscle. *FEBS letters*, **238**, 235-239.

Hodgetts, V., Coppack, S.W., Frayn, K.N., and Hockaday, D.R. (1991). Factors controlling fat mobilization from human subcutaneous adipose tissue during exercise. *Journal of Applied Physiology*, **71**, 445-451.

Holt, S., Heading, R.C., Carter, D.C., Prescott, L.F., and Tothill, P. (1979). Effect of gel fibre on gastric emptying and absorption of glucose and paracetamol. *Lancet*,**i**, 636-639.

Hood, D.A., and Terjung, R.L. (1990). Amino acid metabolism during exercise and following endurance training. *Sports Medicine*, 9, 23-35.

Houlzer, D.R., Dalsky, G.P., Clutter, W.E., Shah, S.D., Holloszy, J.O., and Cryer, P.E. (1986). Glucoregulation during exercise: hypoglycemia is prevented by redundant glucoregulatory systems, sympathochromaffin activation and changes in islet hormone secretion. *Journal of Clinical Investigation*, 77, 217-221.

Houmard, J.A., Hortobagyi, T., Johns, R.A., Bruno, N.J., Nute, C.C., Shinebarger, M.H., and Welborn, J.W. (1992). Effects of short-term training cessation on performance measures in distance runners. *International Journal of Sports Medicine*, 13, 572-576.

Hughes, V.A., Fiatarone, M.A., Fielding, R.A., Kahn, B.B., Ferrara, C.M., Shepherd, P., Fisher, E.C., Wolfe, R.R., Elahi, D., and Evans, W.J. (1993). Exercise increases muscle GLUT-4 levels and insulin action in subjects with impaired glucose tolerance. *American Journal of Physiology*, 264, E855-E862.

Hultman, E. (1967). Physiological role of muscle glycogen in man, with special reference to exercise. *Circulation Research*, 20-21 (*Supplement 1*), I99-I114.

Hurley, B.F., Nemeth, P.M., Martin, W.H., Hagberg, J., Dalsky, G., and Holloszy, J.O. (1986). Muscle triglyceride utilisation during exercise: effect of training. *Journal of Applied Physiology*, 60, 562-567.

Issekutz, B. (1978). Role of beta-adrenergic receptors in mobilization of energy sources in exercising dogs. *Journal of Applied Physiology*, 44, 869-876.

Issekutz, B., and Vranic, M. (1980). Role of glucagon in regulation of glucose production in exercising dogs. *American Journal of Physiology*, **238**, E13-E20.

Jacobs, I. (1984). Blood lactate: implications for training and sports performance. *Sports Medicine*, **3**, 10-25.

Jahoor, F., Peters, E.J., and Wolfe, R.R. (1990). The relationship between gluconeogenic substrate supply and glucose production in humans. *American Journal of Physiology*, **258**, E288-E296.

Jakeman, P., and Davies, B. (1979). The characteristics of a low resistance breathing valve designed for the measurement of high aerobic capacity. *British Journal of Sports Medicine*, **13**, 81-83.

James, D.E., Kraegen, E.W., and Chisholm, D.J. (1985). Muscle glucose metabolism in exercising rats: comparison with insulin stimulation. *American Journal of Physiology*, **248**, E575-E580.

James, D.E., Strube, M., and Mueckler, M. (1989). Molecular cloning and characterisation of an insulin-regulatable glucose transporter. *Nature*, **338**, 83-87.

Jandrain, B., Krzentowski, G., Pirnay, F., Morosa, F., Lacroix, M., Luyckx, A.S., and Lefebvre, P.J. (1984). Metabolic availability of glucose ingested 3 h before prolonged exercise in humans. *Journal of Applied Physiology*, **56**, 1314-1319.

Jansson, E., Hjemdahl, P., and Kaijser, L. (1986). Epinephrine-induced changes in muscle carbohydrate metabolism during exercise in male subjects. *Journal of Applied Physiology*, **60**, 1466-1470.

Jansson, E., and Kaijser, L. (1982). Effect of diet on the utilisation of blood-borne and intramuscular substrate during exercise in man. *Acta Physiologica Scandanavica*, **115**, 19-30.

Jansson, E., and Kaijser, L. (1987). Substrate utilisation and enzymes in skeletal muscle of extremely endurance-trained men. *Journal of Applied Physiology*, **62**, 999-1005.

Jenkins, A.B., Chisholm, D.J., James, D.E., Ho, K.Y.,and Kraegen, E.W. (1985). Exercise-induced hepatic glucose output is precisely sensitive to the rate of systemic glucose supply. *Metabolism*, **34**, 431-436.

Jenkins, D.J.A., Wolever, T.M.S., Hockaday, T.D.R., Leeds, A.R., Howarth, R., Bacon, S., Apling, E.C., and Dilawari, J. (1977). Treatment of diabetes with guar gum. Reduction of urinary glucose loss in diabetics. *Lancet*, **ii**, 779-780.

Jequier, E., and Felber, J.P. (1987). Indirect calorimetry, *Baillieres Clinical, Endocrinology, and Metabolism*, **1**, 911-935.

John-Alder, H.B., McAllister, R.M., and Terjung, R.L. (1986). Reduced running endurance in gluconeogenesis-inhibited rats. *American Journal of Physiology*, **251**, R137-R142.

Kather, H., and Simon, A. (1980). Catecholamine-sensitive adenylate cyclase of human fat cell ghosts. A comparative study using different beta-adrenergic agents. *Metabolism*, **24**, 1179-1182.

Katz, A., Broberg, S., Sahlin, K., and Wahren, J. (1986). Leg glucose uptake during maximal dynamic exercise in humans. *American Journal of Physiology*, **251**, E65-E70.

Kelada, A.S., Macaulay, S.L., and Proietto, J. (1992). Cyclic AMP acutely stimulates translocation of the major insulin-regulatable transporter. *Journal of Biological Chemistry*, **267**, 7021-7025.

Kern, M., Wells, J.A., Stephens, J.M., Elton, C.W., Friedman, J.E., Tapscott, E.B., Pekala, P.H., and Dohm, G.L. (1990). Insulin responsiveness in skeletal muscle is determined by glucose transporter (Glut4) protein level. *Biochemical Journal*, **270**, 397-400.

Kjaer, M., Engfred, K., Fernandes, A., Secher, N.H., and Galbo, H. (1990). Influence of sympathoadrenergic activity on hepatic glucose production during exercise in man. *Medicine and Science in Sport and Exercise*, **22**, (Suppl.2), S54.

Kjaer, M., Engfred, K., Sonne, B., Rasmussen, K., Galbo, H., and Keiding, S. (1992). Regulation of hepatic glucose production during exercise in liver-transplanted subjects. *Scandinavian Journal of Gastroenterology*, **17**, 235.

Kjaer, M., Farrell, P.A., Christensen, N.J., and Galbo, H. (1986). Increased epinephrine response and inaccurate glucoregulation in exercising athletes. *Journal of Applied Physiology*, **61**, 1693-1700.

Kjaer, M., Kiens, B., Hargreaves, M, and Richter, E.A. (1991). Influence of active muscle mass on glucose homeostasis during exercise in humans. *Journal of Applied Physiology*, **71**, 552-557.

Kjaer, M., Secher, N.H., Bach, F.W., and Galbo,H. (1987). Role of motor center activity for hormonal changes and substrate mobilization in humans. *American Journal of Physiology*, **253**, R687-R695.

Koivisto, V.A., Harkonen, M., Karonen, S., Groop, P.H., *Elovainio, R., Ferrannini, E., Sacca, L., and DeFronzo, R.A.* (1985). Glycogen depletion during prolonged exercise: influence of glucose, fructose, or placebo. *Journal of Applied Physiology*, **58**, 731-737.

Koivisto, R., Karonen, S.-L., and Nikkila, E.O. (1981). Carbohydrate ingestion before exercise: comparison of glucose, fructose and sweet placebo. *Journal of Applied Physiology*, **51**, 783-787.

Koranyi, L.I., Bourey, R.E., Vuorinen-Markkola, H., Koivisto, V.A., Mueckler, M., Permutt, M.A., and Yki-Jarvinen, H. (1991). Level of skeletal muscle glucose transporter protein correlates with insulin-stimulated whole body glucose disposal in man. *Diabetologia*, **34**, 763-765.

Kraus-Friedman, N. (1984). Hormonal regulation of hepatic gluconeogenesis. *Physiological Reviews*, **64**, 170-259.

Krzentowski, G.B., Jandrain, B., Pirnay, F., Morosa, F., Lacroix, M., Luyckx, A.S., and Lefebvre, P.J. (1984). Availability of glucose given orally during exercise. *Journal of Applied Physiology*, **56**, 315-320.

Kuipers, H., Costill, D.L., Porter, D.A., Fink, W.J., and Morse, W.M. (1986). Glucose feeding and exercise in trained rats: mechanisms for glycogen sparing. *Journal of Applied Physiology*, **61**, 859-863.

Landsberg, L., and Young, J.B. (1978). Fasting, feeding and regulation of the sympathetic nervous system. *New England Journal of Medicine*, **298**, 1295-1300.

Levine, S.A., Gordon, B., and Derick, C.L. (1924). Some changes in the chemical constituents of blood following a marathon race. *Journal of the American Medical Association*, **82**, 1778-1779.

Livesey, G., and Elia, M. (1988). Estimation of energy expenditure, net carbohydrate utilisation, and net fat oxidation and synthesis by indirect calorimetry evaluation of errors with special reference to the detailed composition of foods, *American Journal of Clinical Nutrition*, **47**, 608-628.

Madsen, J., Bulow, J., and Nielsen, N.E. (1986). Feed back regulation of fatty acid mobilization by arterial free fatty acid concentration. *Acta Physiologica Scandanavica*, **127**, 161-166.

Mallette, L.E., Exton, J.H., and Park, C.R. (1969). Control of gluconeogenesis from amino acids in perfused rat liver. *Journal of Biological Chemistry*, **244**, 5713-5723.

Martin, S.E., and Bockman, E.L. (1986). Adenosine regulates blood flow and glucose uptake in adipose tissue in dogs. *American Journal of Physiology*, **250**, H1127-H1135.

Massicotte, D., Peronnet, F., Allah, C., Hillaire-Marcel, C., Ledoux, M., and Brisson, G. (1986). Metabolic response to [^{13}C] glucose and [^{13}C] fructose ingestion during exercise. *Journal of Applied Physiology*, **61**, 1180-1184.

Massicotte, D., Peronnet, F., Brisson, G., Bakkouch, K., and Marcel, C.H. (1989). Oxidation of glucose polymer during exercise: comparison with glucose or fructose. *Journal of Applied Physiology*, **66**, 179-183.

Massicotte, D., Peronnet, F., Brisson, G., Boivin, L., and Marcel, C.H. (1990). Oxidation of exogenous carbohydrate during prolonged exercise in fed and fasted conditions. *International Journal of Sports Medicine*, **11**, 253-258.

Maughan, R.J. (1991). Fluid and electrolyte loss and replacement in exercise. *Journal of Sports Sciences*, **9**, 117-142.

McConell, G., Fabris, S., Proietto, J., and Hargreaves, M. (1994). Effect of carbohydrate ingestion on glucose kinetics during exercise. *Journal of Applied Physiology*, **77**, 1537-1541.

McLeod, A.A., Brown, J.E., Kitchell, B.B., Sedor, F.A., Kuhn, C., Shand, D.G., and Williams, R.S. (1984). Hemodynamic and metabolic responses to exercise after adrenoceptor blockade in humans. *Journal of Applied Physiology*, **56**, 716-722.

McMahon, M.M., Marsh, H.M., and Rizza, R.A. (1991). Comparison of extracellular and net glucose oxidation measured isotopically and by indirect calorimetry during high and low glucose turnover. *American Journal of Clinical Nutrition*, **53**, 1138-1142.

McMurray, R.G., Wilson, J.R., and Kitchell, B.S. (1983). The effects of fructose and glucose on high intensity endurance performance. *Research Quarterly*, **54**, 156-162.

Megeney, L.A., Neufer, P.D., Dohm, G.L., Tan, M.H., Blewett, C.A., Elder, G.C.B., and Bonen, A. (1993). Effects of muscle activity and fibre composition on glucose transport and GLUT-4. *American Journal of Physiology*, **264**, E583-E593.

Mitchell, J.H. (1990). Neural control of the circulation during exercise. *Medicine and Science in Sport and Exercise*, **22**, 141-154.

Mitchell, J.B., Costill, D.L., Houmard, J.A., Fink, W.J., Pascoe, D.D., and Pearson, D.R. (1989). Influence of carbohydrate dosage on exercise performance and glycogen metabolism. *Journal of Applied Physiology*, **67**, 1843-1849.

Moodley, D., Noakes, T.D., Bosch, A.N., Hawley, J.A., Schall, R., and Dennis, S.C. (1992). Oxidation of exogenous carbohydrate during prolonged exercise: the effects of the carbohydrate type and its concentration. *European Journal of Applied Physiology*, **64**, 328-334.

Mole, P.A., Baldwin, K.M., Terjung, R.L., and Holloszy, J.O. (1973). Enzyme pathways of pyruvate metabolism in skeletal muscle: adaptations to exercise. *American Journal of Physiology*, **224**, 50-54.

Moller, N., Jorgensen, J.O.L., Schmitz, O., Moller, J., Christiansen, J.S., Alberti, K.J.M., and Orskov, H. (1990). Effects of a growth hormone pulse on total and forearm substrate fluxes in humans. *American Journal of Physiology*, **258**, E86-E91.

Muckle, D.S. (1973). Glucose syrup ingestion and team performance in soccer. *British Journal of Sports Medicine*, **7**, 340-343.

Murray, R. (1987). The effects of consuming carbohydrate-electrolyte beverages on gastric emptying and fluid absorption during and following exercise. *Sports Medicine*, **4**, 322-351.

Murray, R., Paul, G.L., Siefert, J.G., Eddy, D.E., and Halaby, G.A. (1989). The effects of glucose, fructose, and sucrose ingestion during exercise. *Medicine and Science in Sport and Exercise*, **21**, 275-282.

Nazar, K., Brzezinska, Z., Kozlowski, S. (1975). Sympathetic activity during prolonged exercise in dogs. In: *Metabolic adaptation to prolonged exercise* (edited by J.A. Poortmans, and H. Howald) pp204-210. Basel: Birkhauser Verlag.

Nesher, R., Karl, I.E, and Kipnis, D.M. (1985). Dissociation of effects of insulin and contraction on glucose transport in rat epitrochlearis muscle. *American Journal of Physiology*, **248**, C226-C232.

Neufer, P.D., Costill, D.L., Flynn, M.G., Kirwan, J.P., Mitchell, J.B., and Houmard, J. (1987). Improvements in exercise performance: effects of carbohydrate feedings and diet. *Journal of Applied Physiology*, **62**, 983-988.

Nillson, N.O., Stralfors, P., Fredrikson, G., and Belfrage, P. (1980). Regulation of adipose tissue lipolysis: effects of noradrenaline and insulin on phosphorylation of hormone-sensitive lipase and on lipolysis in intact rat adipocyte. *FEBS Letters*, **111**, 125-130.

Noakes, T.D. (1990). The dehydration myth and carbohydrate replacement during prolonged exercise. *Cycling Science*, 23-29.

Noakes, T.D. (1991). Energy utilization and repletion during endurance exercise: an historical perspective. *Journal of Human Nutrition and Dietetics*, **4**, 45-55.

Noakes, T.F., Lambert, E.V., Lambert, M.I., McArthur, P.S., Myburgh, K.H., and Benade, A.J.S. (1988). Carbohydrate ingestion and muscle glycogen depletion during marathon and ultramarathon racing. *European Journal of Applied Physiology*, **57**, 482-489.

Noakes, T.D., Maughan, R.J., and Rehrer, N.J. (1991). The importance of volume in regulating gastric emptying. *Medicine and Science in Sports and Exercise*, **23**, 307-313.

Odessey, R., Khairallak, E.A., and Goldberg, A. (1974). Origin and possible significance of alanine production by skeletal muscle. *Journal of Biological Chemistry*, **249**, 7623-7629.

Pallikarikas, N., Jandrain, B., Pirnay, F., Morosa, F., and Lacroix, M. (1986). Remarkable metabolical availability of oral glucose during long-duration exercise in humans. *Journal of Applied Physiology*, **60**, 1035-1042.

Peden, C., Sherman, W.M., D'Acquisto, L., and Wright. D.A. (1989). 1 h pre-exercise carbohydrate meals enhance performance. *Medicine and Science in Sport and Exercise*, **21**, S59.

Peronnet, F., Massicotte, D., Brisson, G., and Hillaire-Marcel, C. (1990). Use of ^{13}C substrates for metabolic studies in exercise: methodological considerations. *Journal of Applied Physiology*, **69**, 1047-1052.

Picton, C., Klee, C.B., and Cohen, P. (1981). The regulation of muscle phosphorylase kinase by calcium ions, calmodulin and troponin C. *Cell Calcium*, **2**, 281-294.

Pilkis, S.J., and Claus, T.H. (1991). Hepatic gluconeogenesis/glycolysis: regulation and structure/function relationships of substrate cycle enzymes. *Annual Review of Nutrition*, **11**, 465-515.

Pirnay, F., Lacroix, M., Mosora, F., Luyckx, A., and Lefebvre, P. (1977). Glucose oxidation during prolonged exercise evaluated with naturally labelled [^{13}C] glucose. *Journal of Applied Physiology*, **43**, 258-261.

Ploug, T., Galbo, H., and Richter, E.A. (1984). Increased muscle glucose uptake during contractions: no need for insulin. *American Journal of Physiology*, **247**, E726-E731.

Ploug, T., Galbo, H., Vinten, J., Jorgensen, M., and Richter, E.A. (1987). Kinetics of glucose transport in rat muscle: effects of insulin and contractions. *American Journal of Physiology*, **253**, E12-E20.

Randle, P.J., Garland, R.B., Hales, C.N., and Newsholme, E.A. (1963). The glucose-fatty acid cycle: its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet*, **1**, 785-789.

Randle, P.J., Newsholme, E.A., and Garland, P.B. (1964). Regulation of glucose uptake by muscle. *Biochemical Journal*, **93**, 652-665.

Ravussin, E., Bogardus, C., Scheidegger, K., Lagrange, B., Horton, E.D., and Horton, E.S. (1986). Effect of elevated FFA on carbohydrate and lipid oxidation during prolonged exercise in humans. *Journal of applied Physiology*, **60**, 893-900.

Ravussin, E., Pahud, P., Dorner, A., Arnaud, M.J., and Jequier, E. (1979). Substrate utilization during prolonged exercise preceded by ingestion of ^{13}C-glucose in glycogen depleted and control subjects. *Pflugers Archive*, **382**, 197-202.

Rehrer, N.J., Wagenmakers, A.J.M., Beckers, E.J., Halliday, D., Leiper, J.B., Brouns, F., Maughan, R.J., Westerterp, K., and Saris, W.H.M. (1992). Gastric emptying, absorption, and carbohydrate oxidation during prolonged exercise. *Journal of Applied Physiology*, **72**, 468-475.

Rennie, M.J., Edwards, R.H.T., Davies, C.T.M., Krywawych, S., and Halliday, D. (1980). Protein and amino acid turnover during and after exercise. *Biochemical Society Transactions*, 6, 499-501.

Rennie, M.J., Winder,W.W., and Holloszy, J.O. (1976). A sparing effect of increased plasma fatty acids on muscle and liver glycogen content in exercising rats. *Biochemical Journal*, **156**, 647-655.

Richter, E.A. (1984). Influence of the sympathoadrenal system on some metabolic and hormonal responses to exercise in the rat. *Acta Physiologica Scandanavica*, Suppl. **528**.

Richter, E.A., and Galbo, H. (1986). High glycogen levels enhance glycogen breakdown in isolated contracting muscle. *Journal of Applied Physiology*, **61**, 827-831.

Richter, E.A., Ploug, T., and Galbo, H. (1985). Increase muscle glucose uptake after exercise: no need for insulin during exercise. *Diabetes*,**34**, 1041-1048.

Richter, E.A., Ruderman, N.B., Gavras, H., Belue, E.R., and Galbo, H. (1982). Muscle glycogenolysis during exercise: dual control by epinephrine and contractions. *American Journal of Physiology*, **242**, E25-E32.

Rizza, R.A., Cryer, P.E., and Gerich, J.E. (1979). Role of glucagon, epinephrine and growth hormone in human glucose counterregulation: effects of somatostatin and adrenergic blockade on plasma glucose recovery and glucose flux rates following insulin-induced hypoglycemia. *Journal of Clinical Investigation*, **64**, 62-71.

Robert, J.J., Kozet, T., Chauvet, D., Darmaun, D., Desjeux,J.F., and Young, V.R. (1987). Use of ^{13}C-labelled glucose for estimating glucose oxidation: some design considerations. *Journal of Applied Physiology*, 63, 1725-1732.

Rodnick, K.J., Henriksen, E.K., James, D.E., and Holloszy, J.O. (1992). Exercise training, glucose transporters, and glucose transport in rat skeletal muscle. *American journal of Physiology*, **262**, C9-C14.

Saris, W.H.M., Goodpaster, B.H., Jeukendrup, A.E., Brouns, F., Halliday, D., and Wagenmakers, A.J.M. (1993). Exogenous carbohydrate from different carbohydrate sources during exercise. *Journal of Applied Physiology*, 75, 2168-2172.

Savard, R., Despres, J.P., Marcotte, M., Theriault, G., Tremblay, A., and Bouchard, C. (1987). Acute effects of endurance exercise on human adipose tissue metabolism. *Metabolism: clinical and experimental*, 36, 480-485.

Schoeller,D.A., Klein, P.D., Watkins, J.B., Heim, T., and Maclean, W.C.(1980). ^{13}C abundance of nutrients and the effect of variations in ^{13}C isotopic abundance of test meals formulated for $^{13}CO_2$ breath tests. *American Journal of Clinical Nutrition*, 33, 2375-2385.

Shaw, W.A.S., Issekutz, J.B., and Issekutz, B. (1975). Interrelationship of FFA and glycerol turnovers in resting and exercising dogs. *Journal of Applied Physiology*, 39, 30-36.

Sherman, W.M., Costill, D.L., Fink, W.J., and Miller, J.M. (1981). Effect of exercise-diet manipulation on muscle glycogen and its subsequent utilization during performance. *International Journal of Sports Medicine*, 2, 114-118.

Sherman, W.M., and Wimer, G.S. (1991). Insufficient dietary carbohydrate during training: does it impair athletic performance? *International Journal of Sport Nutrition*, 1, 28-44.

Silverman, M. (1991). Structure and function of Hexose transporters. *Annual Review of Biochemistry*, 60, 757-794.

Simard, C., Tremblay, A., and Jobin, M. (1988). Effects of carbohydrate intake before and during an ice hockey match on blood and muscle energy substrates. *Research Quarterly for Exercise and Sport*, 59, 144-147.

Spriet, L.L., Ren, J.M., and Hultman, E. (1988). Epinephrine infusion enhances muscle glycogenolysis during prolonged electrical stimulation. *Journal of Applied Physiology*, 64, 1439-1444.

Steele, R. (1959). Influences of glucose loading and of injected insulin on hepatic glucose output. *Annals of the New York Academy of Sciences*, 82, 420-430.

Sutton, J.R., Young, J.D., Lazarus,L., Hickie, J.B., and Maksvytis, J. (1969). The hormonal response to physical exercise. *Australian Annals of Medicine*, 18, 84-90.

Tan, M.H., and Bonen, A. (1985). The in vitro effects of corticosterone on insulin binding and glucose metabolism in mouse skeletal muscles. *Canadian Journal of Physiology and Pharmacology*, 62, 1460-1465.

Tappy, L., Acheson, K., Normand, S., Pachiaudi, C., Riou, J.P., and Jequier, E. (1994). Overnight glucose metabolism in obese non insulin dependent diabetic patients and in healthy lean individuals. *Clinical Physiology*, 14, 251-265.

Tappy, L., Paquot, N., Tounian, P., Schneiter, P., and Jequier, E. (1995). Assessment of glucose metabolism in humans with the simultaneous use of indirect calorimetry and tracer techniques. *Clinical Physiology*, **15**, 1-12.

Tuttle, K.R., Marker, J.C., Dalsky, G.P., Schwartz, N.S., Shah, S.D., Clutter, W.E., Holloszy, J.O., and Cryer, P.E. (1988). Glucagon not insulin, may play a secondary role in defense against hypoglycemia during exercise. *American Journal of Physiology*, **254**, E713-E719.

Van Handel, P.J., Fink, W.J., Branham, G., and Costill, D.L. (1980). Fate of ^{14}C glucose ingested during prolonged exercise. *International Journal of Sports Medicine*, **1**, 127-131.

Viru, A. (1987). Mobilisation of structural proteins during exercise. *Sports Medicine*, **4**, 95-128.

Vissing, J., Wallace, J.L., and Galbo. H. (1989). Effect of liver glycogen content on glucose production in running rats. *Journal of Applied Physiology*, **66**, 318-322.

Vollestad, N.K., and Blom, P.C.S. (1985). Effect of varying exercise intensity on glycogen depletion in human muscle fibres. *Acta Physiologica Scandanavica*, **125**, 395-405.

Vollestad, N.K., Vaage, O., and Hermansen, L. (1984). Muscle glycogen depletion patterns in type I and subgroups of type II fibres during prolonged severe exercise in man. *Acta Physiologica Scandanavica*, **122**, 433-441.

Vranic, M., Kawamori, R., Pek, S., Kovacevic, N., and Wrenshall, G.A. (1976). The essentiality of insulin and the role of glucagon in regulating glucose utilization and production during strenuous exercise in dogs. *Journal of Clinical Investigation*, **57**, 245-255.

Vukovich, M.D., Costill, D.L., Hickey, M.S., Trappe, S.W., Cole, K.J., and Fink,W.J. (1993). Effect of fat emulsion and fat feeding on muscle glycogen utilization during cycle exercise. *Journal of Applied Physiology*, **75**, 1513-1518.

Wagenmakers, A.J.M., Schepens, J.T.G., and Veerkamp, J.H. (1984). Effect of starvation and exercise on actual and total activity of the branched-chain 2-oxo acid dehydrogenase complex in rat tissues. *Biochemical Journal*, **223**, 815-821.

Wahren, J., Felig, P., Ahlborg, G., and Jorfeldt, L. (1971). Glucose metabolism during leg exercise in man. *Journal of Clinical Investigation*, **50**, 2715-2725.

Wahren, J., Felig, P., Hagenfeldt, L., Handler, R., and Ahlborg, G. (1975). Splanchnic and leg metabolism of glucose, free fatty acids and amino acids during prolonged exercise in man. In: *Metabolic adaptations to prolonged physical exercise*, (edited by H. Howald and J. Poortmans) pp144-153. Basel: Birkhauser Verlag.

Wallberg-Henriksson, H. (1987). Glucose transport in skeletal muscle. Influence of contractile activity, insulin, catecholamines and diabetes mellitus. *Acta Physiologica Scandanavica (Supplement* **564***)*.

Wallberg-Henriksson, H., and Holloszy, J. (1984). Contractile activity increases glucose uptake by muscle in severely diabetic rats. *Journal of Applied Physiology*, **57**, 1045-1049.

Wasserman, D.H., and Cherrington, A.D. (1991). Hepatic fuel metabolism during muscular work: role and regulation. *American Journal of Physiology*, **260**, E811-E824.

Wasserman, D.H., Mohr, T., Kelly, P., Lacy,D., and Bracy, D. (1992). Impact of insulin deficiency on glucose fluxes and muscle glucose metabolism during exercise. *Diabetes*, **41**, 1229-1238.

Wasserman, D.H., Spalding, J.A., Lacy, D.B., Colburn, C.A., Goldstein, R.E., and Cherrington, A.D. (1989a). Glucagon is a primary controller of hepatic glycogenolysis during muscular work. *American Journal of Physiology*, **257**, E108-E117.

Wasserman, D.H., Williams, P.E., Lacy, D.B., Green, D.R., and Cherrington, A.D. (1989b). Exercise-induced fall in insulin and hepatic carbohydrate metabolism during muscular work. *American Journal of Physiology*, **257**, E500-E509.

White, R.H., Frayn, K.N., Little, R.A., Threlfall, C.J., Stoner, H.B., and Irving, M.H. (1987). Hormonal and metabolic responses to glucose infusion in sepsis studied by the hyperglycaemic glucose clamp technique. *Journal of Parenteral and Enteral Nutrition*, **11**, 345-353.

Williams, C., Nute, M.G.L., Broadbank, L., and Vinall, S. (1990). Influence of fluid intake on endurance running performance. *European Journal of Applied Physiology*, **60**, 112-119.

Winder, W.W., Arogyasami, J., and Yang, H.T. (1988). Effects of glucose infusion in exercising rats. *Journal of Applied Physiology*, **64**, 2300-2305.

Winder, W.W., Hagberg, J.M., Hickson, R.C., Ehsani, A.A., and McLane, J.A. (1978). Time course of sympathoadrenergic adaptation to endurance exercise training in man. *Journal of Applied Physiology*, **45**, 370-374.

Wolfe, B.M., Klein, S., Peters, E.J., Schmidt, B.F., and Wolfe, R.R. (1988). Effect of elevated free fatty acids on glucose oxidation in normal humans. *Metabolism*, **37**, 323-329.

Wolfe, R.R. (1992). *Radioactive and Stable Isotope Tracers in Biomedicine*, Wiley-Liss; New York.

Wolfe, R.R., Allsop, J.R., and Burke, J.F. (1979). Glucose metabolism in man: Responses to intravenous glucose infusion. *Metabolism*, **28**, 210-220.

Wolfe, R.R., Durkot, M.J., and Wolfe, M.H. (1981). Investigation of kinetics og integrated metabolic response to adrenergic blockade in conscious dogs. *American Journal of Physiology*, **241**, E385-E395.

Wolfe, R.R., Goodenough, R.D., Wolfe, M.H., Loyle, G.T., and Nadel, E.R. (1982). Isotopic analysis of leucine and urea metabolism in exercising humans. *Journal of applied Physiology*, **52**, 458-466.

Wolfe, R.R., Klein, S., Carraro, F., and Weher, J.M. (1990). Role of triglyceride-fatty acid cycle in controlling fat metabolism in humans during and after exercise. *American Journal of Physiology*, **258**, E382-E389.

Wolfe, R.R., Nadel, E.R., Shaw, J.H.F., Stephenson, L.A., and Wolfe, M.H. (1986). Role of changes in insulin and glucagon in glucose homeostasis in exercise. *Journal of Clinical Investigation*, **77**, 900-907.

Wright, D.A., Sherman, W.M., and Dernbach, A.R. (1991). Carbohydrtae feedings before, during, or in combination improve cycling endurance performance. *Journal of Applied Physiology*, **71**, 1082-1088.

Yki-Jarvinen, H., Young, A.A., Lamkin, C., and Foley, J.E. (1987). Kinetics of glucose disposal in whole body and across the forearm in man. *Journal of Clinical Investigation*, 79, 1713-1719.

Young, J.B., Rowe, J.W., Pallotta, J.A., Sparrow, D., and Landsberg, L. (1980). Enhanced plasma norepinephrine response to upright posture and oral glucose administration in elderly human subjects.*Metabolism*, 29, 532-539.

Ziel, F.H., Venkatesan, N., and Davidson, M.B. (1988). Glucose transport is rate limiting for skeletal muscle glucose metabolism in normal STZ-induced diabetic rats. *Diabetes*, 37, 885-890.

Zinker, B.A., Lacy, D.B., Bracy, D.P., and Wasserman, D.H. (1993). Role of glucose and insulin loads to the exercising limb in increasing glucose uptake and metabolism. *Journal of Applied Physiology*, 74, 2915-2921.

Zuntz, N. (1900). Uber die Bedentung der verschiedener Nahr-Stoffe als Erzeuger der Muskeldraft. *Pflugers Archive*, 83, 557-582.

APPENDICES

APPENDIX A.

Thermal equivalent of oxygen for non-protein respiratory exchange ratio (RER), including grams derived from carbohydrates and fat (after Zuntz, 1900).

Nonprotein RQ	kcal per Litre Oxygen Consumed	Grams per Litre O_2 Consumed	
		Carbohydrate	Fat
0.707	4.686	0.000	.496
.71	4.690	.012	.491
.72	4.702	.051	.476
.73	4.714	.090	.460
.74	4.727	.130	.444
.75	4.739	.170	.428
.76	4.751	.211	.412
.77	4.764	.250	.396
.78	4.776	.290	.380
.79	4.788	.330	.363
.80	4.801	.371	.347
.81	4.813	.413	.330
.82	4.825	.454	.313
.83	4.838	.496	.297
.84	4.850	.537	.280
.85	4.862	.579	.263
.86	4.875	.621	.247
.87	4.887	.663	.230
.88	4.899	.705	.213
.89	4.911	.749	.195
.90	4.924	.791	.178
.91	4.936	.834	.160
.92	4.948	.877	.143
.93	4.961	.921	.125
.94	4.973	.964	.108
.95	4.985	1.008	.090
.96	4.998	1.052	.072
.97	5.010	1.097	.054
.98	5.022	1.142	.036
.99	5.035	1.186	.018
1.00	5.047	1.231	.000

Hormonal and Metabolite Responses to Glucose and Maltodextrin Ingestion With or Without the Addition of Guar Gum

D. P. M. MacLaren [1], T. Reilly [1], I. T. Campbell [2], K. N. Frayn [3]

[1] School of Human Sciences, Liverpool John Moores University, Mountford Building, Byrom Street, Liverpool L3 3AF
[2] Dept. of Anaesthesia, Royal Liverpool University Hospital
[3] MRC Trauma Unit, Hope Hospital, Manchester (now at Sheikh Rashid Diabetes Unit, Radcliffe Infirmary, Oxford)

Abstract

D. P. M. MacLaren, T. Reilly, I. T. Campbell and K. N. Frayn, Hormonal and Metabolite Responses to Glucose and Maltodextrin Ingestion With or Without the Addition of Guar Gum. Int. J. Sports Med., Vol. 15, No. 8, pp. 466–471, 1994.

Accepted after revision: April 20, 1994

The purpose of this study was to examine the effects of ingesting water (P), a glucose solution (GL), a maltodextrin solution (Md), a glucose solution with 8% guar gum (GL + G), and a maltodextrin solution with 8% guar gum (Md + G), on the hormonal and metabolite responses during cycling, and on subsequent time to exhaustion. Five male subjects undertook five 90 min rides on a bicycle ergometer at an exercise intensity corresponding to 65% VO_2max after having ingested 1 g kg^{-1} body weight of the test product in 400 ml of water immediately before the exercise. Blood samples were taken during the trials for analyses of adrenaline, noradrenaline, insulin, glucagon, glucose, lactate and non-esterified fatty acids (NEFA). Respiratory measures were also undertaken during the trials for the determination of oxygen consumption (VO_2) and respiratory exchange ratio (RER), from which the carbohydrate oxidation rates were calculated. Rates of perceived exertion (RPE) were also assessed. Ten minutes after the 90 min ride, subjects exercised to volitional exhaustion at an exercise intensity of 75% VO_2max. ANOVA revealed that there were significant differences between the treatments for adrenaline ($p < 0.01$), insulin ($p < 0.05$), glucose ($p < 0.01$), lactate ($p < 0.01$), NEFA ($p < 0.01$), RER ($p < 0.001$) and carbohydrate oxidation rate ($p < 0.01$). Post hoc analyses showed that significantly higher levels of adrenaline were found for P compared to Md ($p < 0.05$), significantly lower levels of insulin for P compared to Md ($p < 0.05$), significantly lower levels of glucose and lactate for P compared to all carbohydrate treatments ($p < 0.05$), significantly higher levels of NEFA for P compared to the carbohydrate treatments ($p < 0.05$), and significantly lower RER and carbohydrate oxidation rates for P compared to the carbohydrate trials ($p < 0.05$). Furthermore, tests highlighted that significantly longer times to exhaustion were achieved by the carbohydrate treatments compared to placebo ($p < 0.05$). These results support the use of carbohydrate supplementation prior to prolonged exercise, although the type of carbohydrate is not important.

Key words

Carbohydrates, hormones, metabolism, guar gum

Introduction

Carbohydrate ingestion during exercise has been shown to enhance endurance performance (7). The exogenous carbohydrate appears to maintain plasma glucose levels (8,25), although conflicting results have been obtained regarding muscle glycogen sparing (2,8,17,18). Furthermore, exogenous carbohydrate has been shown to be an available substrate for exercising muscle, contributing from 5 to 68% of the glucose oxidized during prolonged exercise (19).

Glucose ingestion in the period 10–60 min before endurance exercise may have adverse effects on metabolism during the early stages (22). It has been proposed that such treatments increase the rate of glycogen breakdown in active muscle and thereby reduce endurance capacity (6,11). This arises as a consequence of an elevated plasma insulin level at the start of exercise which inhibits lipolysis and fat mobilisation from adipocytes, and thereby increases reliance on carbohydrate metabolism. Thus the timing of ingestion of a carbohydrate source seems to be important.

During the last decade much attention has been focused on the optimum form and type of carbohydrate beverages to prolong physical performance. Various studies have reported on the efficacy of glucose, fructose, maltose, and glucose polymer based drinks (23,24,25). Solutions of maltodextrins, comprising polymerised glucose units of varying chain lengths, exert less osmotic pressure than equimolar solutions of glucose or other simple sugars. This characteristic is considered to be advantageous in the light of research indicating the role

Int J Sports Med 15 (1994) 466–471
© Georg Thieme Verlag Stuttgart · New York

played by osmolality in determining gastric emptying rates (25). Although results from studies examining gastric emptying rates of glucose and glucose polymer drinks have proved variable, there have been no adverse reports of gastric emptying rates involving glucose polymers (19).

The addition of guar gum, an endosperm extract of the Indian Cluster bean, to carbohydrate meals and drinks has been shown to attenuate post-prandial hyperglycaemia in normal and diabetic humans (10). This is thought to be due to a reduction in the rate of glucose absorption, either as a result of a delay in the delivery of carbohydrate to the small intestine (20) and/or an inhibition of convection within the intestinal lumen, thus preventing access of glucose to the intestinal epithelium (3). The possible beneficial effects therefore of combining guar gum with a carbohydrate source ingested prior to prolonged exercise could be realised with a slower, 'phased' uptake of glucose combined with a lowered insulin response, leading to a reduced reliance on carbohydrates.

This study was set up to examine the effects of ingesting two carbohydrate sources, glucose and maltodextrin (an 8–12 chain glucose polymer), with and without the addition of guar gum on hormonal and metabolic responses to prolonged exercise, and on subsequent time to exhaustion.

Methods

Subjects

The subjects were five male athletes who regularly participated in and trained for endurance-based activities. They were familiar with prolonged exercise on a cycle ergometer. Means (±SD) for age, body mass, height, and maximal oxygen uptake were 27.6±8.3 years 74.6±8.9 kg, 1.79±0.04 m, and 51.1±13.2 ml·kg^{-1}·min^{-1}, respectively.

Preliminary testing

Maximal oxygen uptake was determined on a Monark 91 cycle ergometer using a continuous, incremental test to volitional exhaustion. Pedal frequency was maintained at 60 revs·min^{-1} throughout the test, with 30 watt increments every 2 min until the subject could no longer maintain the pace. Oxygen consumption was monitored by subjects breathing through a low resistance valve (21) to an automated gas analysis system (P. K. Morgan, Rainham). The latter incorporated a Fleisch No. 3 pneumotachograph for measuring ventilation, a paramagnetic O_2 analyser and an infra-red CO_2 analyser. Values for $\dot{V}O_2$ were obtained at 1 min intervals up to the point of volitional exhaustion. Results from the $\dot{V}O_2$max test were used to establish the exercise intensity that corresponded to 65% and 75% of each subject's $\dot{V}O_2$max for use in the subsequent trials.

Experimental design

Subjects visited the laboratory on five separate occasions, with each visit separated by a maximum period of 3 weeks. Immediately prior to each ergometer ride at 65% $\dot{V}O_2$max for 90 min, the subjects consumed 400 ml of either water, or 1 g·kg^{-1} body weight of glucose (GL) or maltodextrin (Md) or 1 g·kg^{-1} bodyweight of glucose (GL + G) or maltodextrin (Md + G) with 8% guar gum. All drinks were orange flavoured. The choice of 400 ml was based on a compromise between not having too concentrated a solution (drinks varied in concentration between 16% and 22.5% dependent on the subjects' body mass) and not having too large a volume in the stomach at the start of the ride. The carbohydrate drinks were therefore isocaloric for each subject. The order of treatments was randomised.

After an overnight fast of approximately 12 hours, each subject arrived at the laboratory, voided urine and lay down before an indwelling cannula was inserted into an antecubital vein under local anaesthesia. Following 30 min rest, two 10 ml samples of blood were withdrawn in lithium heparin tubes (one with Trasylol added for later glucagon assay). Subjects then ingested the trial drink over a 2–3 min period before cycling at an exercise intensity corresponding to 65% $\dot{V}O_2$max. After 15, 30, 45, 60, 75 and 90 min of exercise, ratings of perceived exertion (RPE) were obtained using the Borg scale (4) as were measures of oxygen uptake ($\dot{V}O_2$) and respiratory exchange ratio (RER). Further duplicate 10 ml samples of blood were taken at 15, 30, 60 and 90 min exercise. After 90 min the subjects were allowed to rest for 10 min before a timed ride to exhaustion was undertaken at an exercise intensity corresponding to 75% $\dot{V}O_2$max. A final duplicate set of blood samples was taken at the point of exhaustion.

Analyses

Venous blood samples were collected in lithium heparin tubes and used for the determination of haemoglobin by the cyanmethhaemoglobin method and for packed cell volume using a micro-haematocrit centrifuge (Hawksley Ltd.). Changes in plasma volume were estimated according to the method of Dill and Costill (1974). Plasma samples were obtained by centrifugation for 20 min, aliquoted, and then stored at −20 °C before being analysed for glucose (1), lactate (16) and non-esterified fatty acids (NEFA kit from Alpha laboratories, London). Plasma insulin was analysed using a Coat-a-Count solid-phase ^{125}I-insulin RIA kit (Diagnostic Products Ltd., Oxford, UK) whilst glucagon was analysed using an RIA kit (Novo, Denmark).

Plasma samples for adrenaline and noradrenaline were frozen at −70 °C before analysis using high pressure liquid chromatography (12).

Results from the plasma and respiratory analyses, and for RPE were subjected to analysis of variance (ANOVA) with repeated measures. Where significant differences were found, a post-hoc HSD test was employed. Results for the times to exhaustion were examined using paired t-tests. Significance was accepted at the 0.05 level.

Results

ANOVA revealed significant differences between the drinks ingested for plasma adrenaline concentrations ($F = 3.64$; $p < 0.01$). The post-hoc test established that the adrenaline concentrations for the placebo were significantly higher than for the maltodextrin treatment ($p < 0.05$). No significant differences were found between the carbohydrate treatments (Fig. 1a).

Mean plasma noradrenaline concentrations (Fig. 1b) showed a significant increase during the course of the exercise irrespective of the treatment ($F = 16.43$; $p < 0.001$).

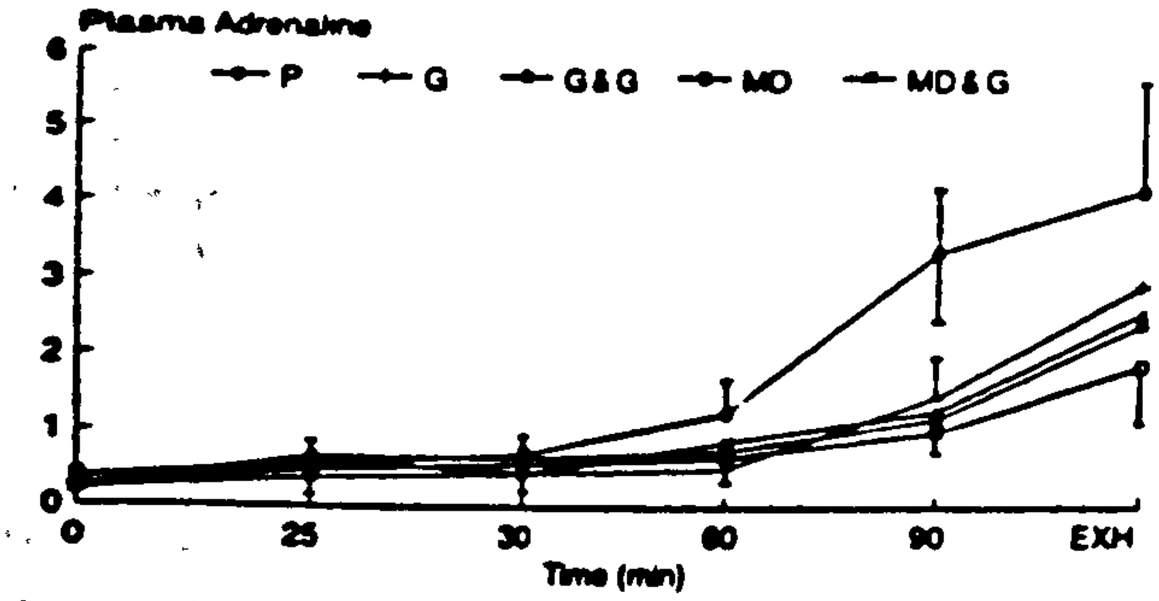


Fig. 1a Mean (± SEM) plasma adrenaline concentrations during the 5 trials.

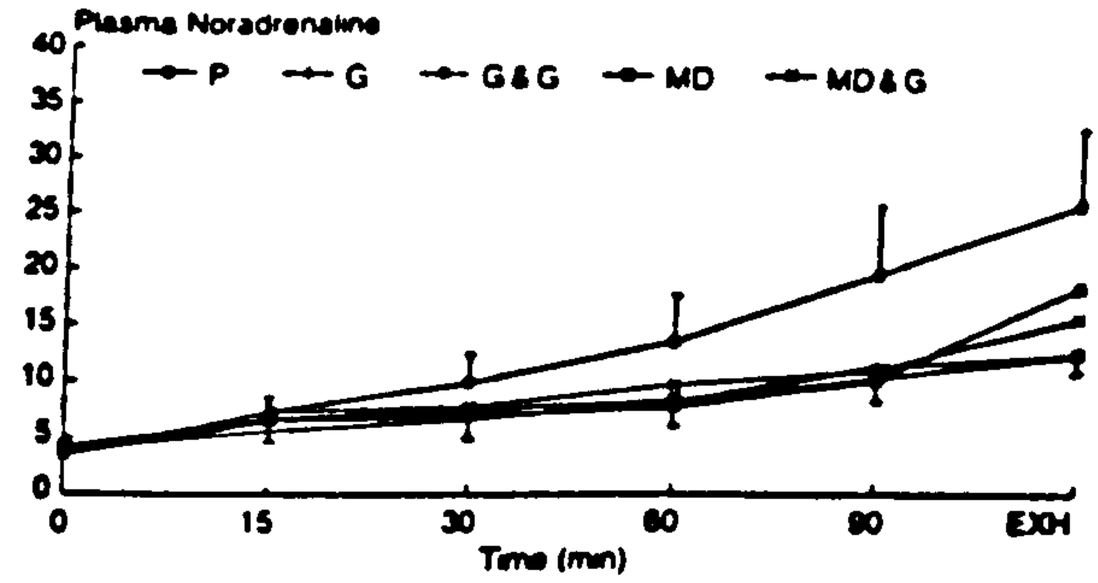


Fig. 1b Mean (± SEM) plasma noradrenaline concentrations during the 5 trials.

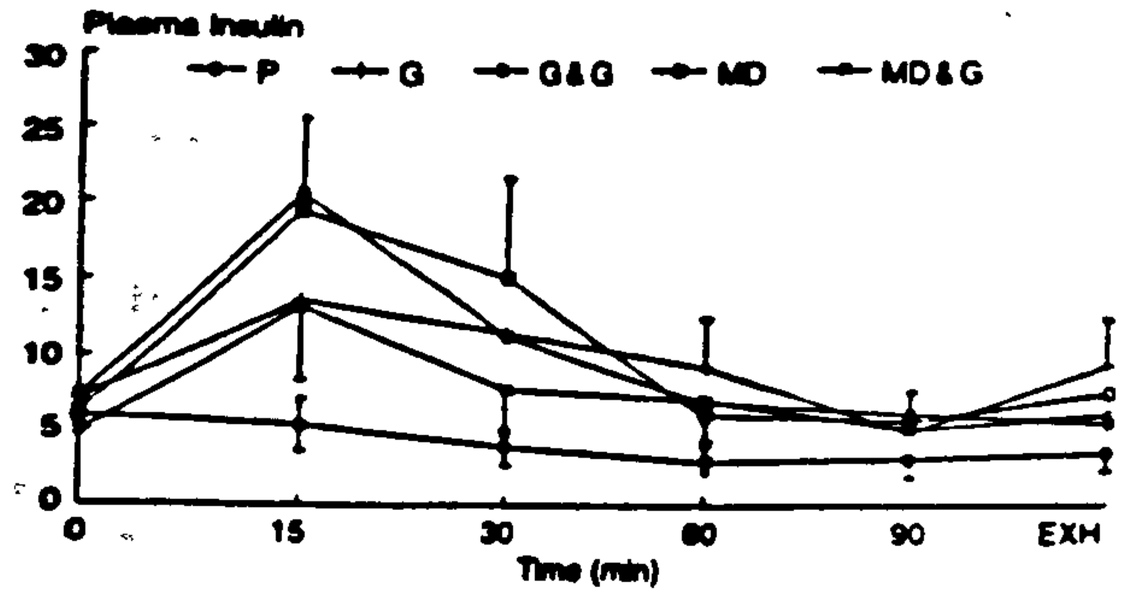


Fig. 1c Mean (± SEM) plasma insulin concentrations during the 5 trials.

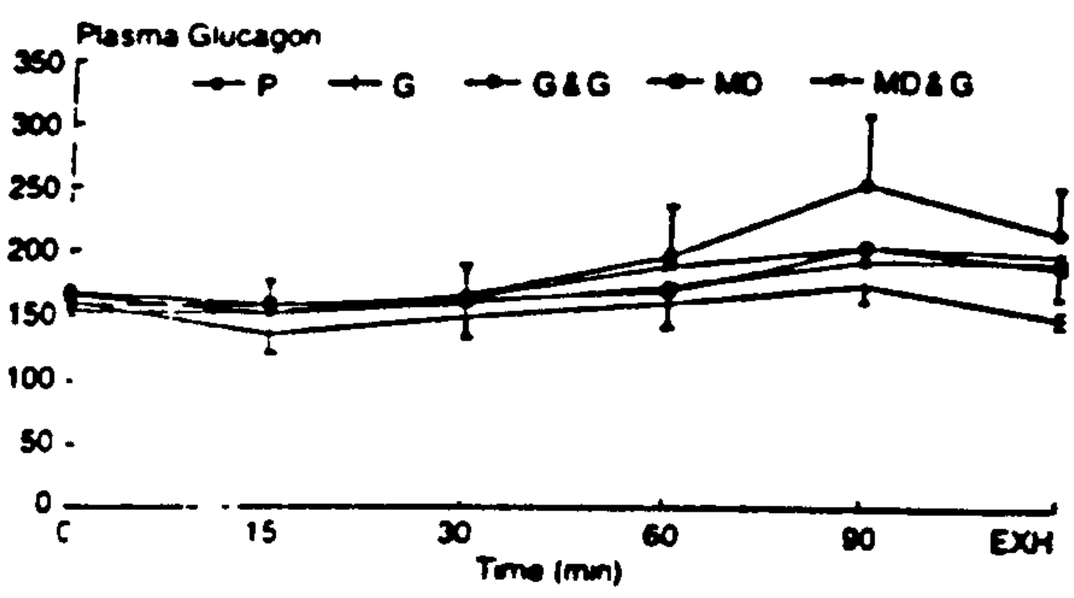


Fig. 1d Mean (± SEM) plasma glucagon concentrations during the 5 trials.

The ANOVA gave no significant main effect of the experimental treatments ($F = 1.19$; $p > 0.05$).

The plasma insulin concentrations decreased during exercise in the placebo condition (Fig. 1c), although all carbohydrate treatments resulted in significant increases in plasma insulin concentration within the first 30 min ($p < 0.01$) before decreasing. The effects of the experimental treatments were significant ($F = 4.30$; $p < 0.05$), with the significantly higher concentrations being for maltodextrin compared to placebo ($p < 0.05$). No differences were apparent between the carbohydrate treatments.

The results for plasma glucagon showed a significant rise during exercise ($F = 10.0$; $p < 0.01$), but no differences were found between the drinks ingested ($F = 0.54$, $p > 0.05$). Fig. 1d highlights these changes.

Mean plasma glucose concentrations were similar for all conditions at rest. Values fell slowly during exercise with the placebo, although ingestion of carbohydrate maintained the plasma glucose levels above resting values throughout the exercise period (Fig. 2). Significant differences were found between treatments ($F = 5.41$; $p < 0.05$), with the post-hoc HSD tests highlighting the difference between placebo and the carbohydrate treatments ($p < 0.05$). No differences were found between the carbohydrate treatments.

The placebo treatment resulted in a gradual and significant increase in NEFA concentration whereas ingestion of carbohydrate attenuated this response (Fig. 2b). There were significant differences between the treatments ($F = 6.24$; $p < 0.01$) with the post-hoc tests emphasising the differences between the placebo and the carbohydrate treatments ($p < 0.05$). The differences between the carbohydrate treatments were not significant.

Lactate concentrations increased significantly from rest (Fig. 2c) and were notably higher after the ride to exhaustion at 75% $\dot{V}O_2max$ ($F = 39.77$; $p < 0.001$). There were significant differences between the placebo treatment and the carbohydrate treatments ($F = 9.08$; $p < 0.01$).

The respiratory exchange ratio and oxygen consumption differed between the carbohydrate trials and the placebo ($F = 20.6$; $p < 0.001$ and $F = 5.22$; $p < 0.05$) with the former providing significantly higher values than the placebo. These findings resulted in significantly elevated rates of oxidation of carbohydrate (Table 1) for the carbohydrate trials compared to the placebo ($F = 26.22$; $p < 0.001$).

The times to exhaustion at 75% $\dot{V}O_2max$ showed significant differences between the carbohydrate trials and the placebo trial. Paired t-tests highlighted the improvements in time to exhaustion for GL (372 ± 155s; $p < 0.05$) GL - G (433 ± 156S, $p < 0.05$), Md (450 ± 186s; $p < 0.05$), and Md - G (483 ± 165S, $p < 0.01$) compared with P (163 ± 53s). No

significant differences were apparent between the carbohydrate trials.

The rate of perceived exertion scale (RPE) was not significantly different between the trials according to ANOVA ($F = 1.65$).

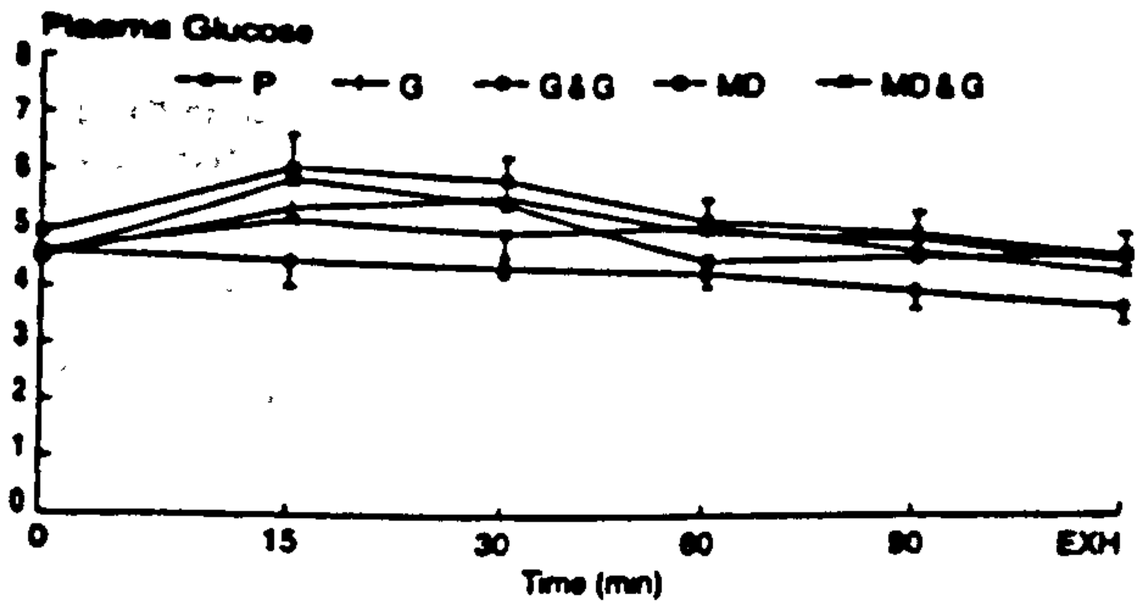


Fig. 2a Mean (± SEM) plasma glucose concentrations during the 5 trials.

Discussion

Concentrations of adrenaline and noradrenaline were found to increase significantly with time, the noradrenaline concentration being consistently higher. The values in our study reflect the stress on the subjects. Carbohydrate ingestion was found to attenuate the rise in adrenaline (particularly maltodextrin) although no significant effect was noted with respect to noradrenaline concentration. It is well documented that changes in the availability of glucose alters the activity of sympathetic centres in the hypothalamus (13). Glucose infusion during exercise has been shown to cause a marked decrease in plasma adrenaline levels (14) whereas a reduction in plasma glucose has resulted in elevated adrenaline concentrations during exercise (15). Our results highlight the inhibitory effect of carbohydrate ingestion on plasma catecholamines.

Due to the reduced availability of NEFA following carbohydrate ingestion, it would be expected that a greater proportion of energy be derived from carbohydrate stores. The significantly elevated plasma glucose levels and reduced plasma NEFA levels after carbohydrate ingestion point to the possibility of more energy being derived from carbohydrates under such conditions. Estimation of carbohydrate oxidation from oxygen consumption and RER values confirmed the

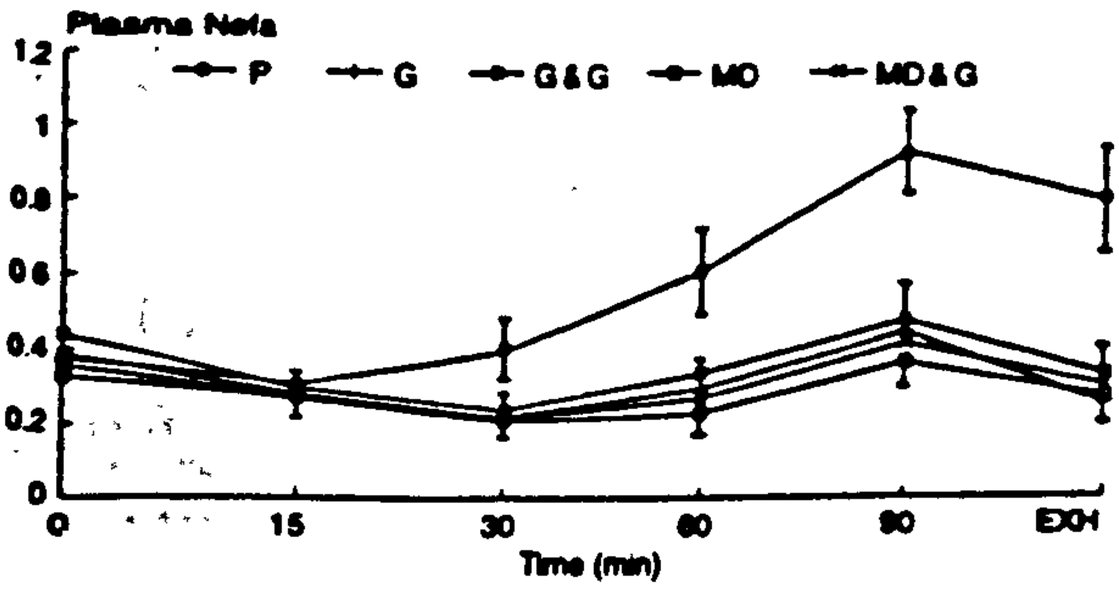


Fig. 2b Mean (± SEM) plasma NEFA concentrations during the 5 trials.

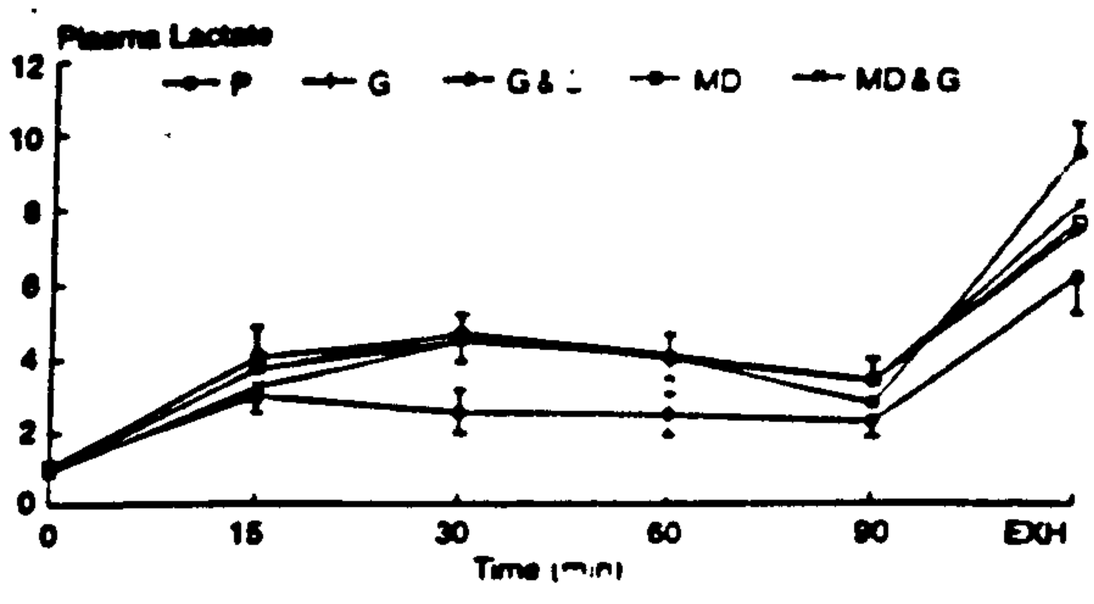


Fig. 2c Mean (± SEM) plasma lactate concentrtions during the 5 trials.

Table 1 Respiratory exchange ratio and carbohydrate oxidation rates during the 5 trials.

Time (min)	Respiratory Exchange Ratio P	GL	GL + G	Md	Md + G	Carbohydrate Oxidation (g/min) P	GL	GL + G	Md	Md + G
15	0.86 ± 0.015	0.86 ± 0.016	0.86 ± 0.018	0.87 ± 0.047	0.86 ± 0.017	1.36 ± 0.36	1.50 ± 0.52	1.43 ± 0.44	1.49 ± 0 44	1.50 ± 0.33
30	0.84 ± 0.005	0.88 ± 0.026	0.88 ± 0.020	0.89 ± 0.012	0.88 ± 0.020	1.24 ± 0.34	1.85 ± 0.59	1.75 ± 0.57	1.93 ± 0.63	1.82 ± 0.51
45	0.81 ± 0.013	0.90 ± 0.019	0.88 ± 0.016	0.90 ± 0.015	0.88 ± 0.015	0.96 ± 0.26	2.04 ± 0.68	1.80 ± 0.63	2.03 ± 0.69	1.91 ± 0.55
60	0.80 ± 0.008	0.89 ± 0.013	0.89 ± 0.019	0.89 ± 0.020	0.87 ± 0.021	0.91 ± 0.30	1.92 ± 0.64	1.95 ± 0.66	1 93 ± 0 67	1.89 ± 0 60
75	0.79 ± 0.011	0.89 ± 0.037	0.88 ± 0.024	0.89 ± 0.022	0.85 ± 0.021	0.89 ± 0.30	1.81 ± 0.44	1.89 ± 0.61	1 99 ± 0 59	1.80 ± 0 49
90	0.79 ± 0.008	0.87 ± 0.028	0.86 ± 0.023	0.87 ± 0.013	0.83 ± 0.027	0.85 ± 0.27	1.71 ± 0.48	1.74 ± 0 55	' 79 ± 0 46	' 69 ± 0 42

significantly higher energy being derived by exercising muscles from carbohydrates when carbohydrates were ingested compared to placebo. This is similar to other studies which have investigated the oxidation rates of carbohydrates ingested during prolonged exercise (24). Indeed it would appear from our calculations that all the carbohydrate ingested immediately prior to exercise may have been oxidised i.e. approximately 70 g in 90 min. This is not dissimilar to rates of 50–70 g in 120 min as found by Massicotte et al. (1989).

Carbohydrate ingestion has been associated with enhanced exercise performance during prolonged exercise, and that exogenous carbohydrate may serve as a supplementary fuel at a time when muscle glycogen stores are compromised (8). Reductions in blood glucose have been shown to result in lowered muscle glucose uptake, a decrease in the rate of carbohydrate oxidation, and to impaired performance, whereas elevated RER values due to carbohydrate ingestion have resulted in enhanced work output (5, 8, 26).

The results from this study are clearly in accord with the above findings in that carbohydrate ingestion promoted plasma glucose concentrations, enhanced carbohydrate oxidation and elevated RER. As a consequence, there was a significant improvement in time to exhaustion. Although muscle glycogen concentrations were not determined, the enhanced performance was likely to result either from a muscle glycogen sparing and or an enhanced availability of exogenous glucose to the active muscles. The enhanced performance at 75% VO_2max after 90 min of steady state exercise as observed in the carbohydrate trials may have resulted from an enhanced availability, uptake and utilization of plasma glucose.

The form of carbohydrate ingested and the addition of guar gum did not significantly affect carbohydrate availability nor performance. This is not entirely surprising in so far as the same total amount of carbohydrate and fluid was ingested in all carbohydrate trials. Although the osmolalities of the drinks would have varied, it has been reported that caloric content and volume are the most important considerations for gastric emptying of beverages whilst exercising (25). The delay in the elevation of plasma glucose and the expected impaired insulin response with guar gum did not materialise. It is quite possible that this finding was due to a combination of exercise and the concentration of guar gum used. Clearly this is an area for further investigation.

In summary, this study indicates that ingestion of glucose, glucose + 8% guar gum, maltodextrin, and maltodextrin + 8% guar gum resulted in similar hormonal, metabolic, sensory and performance responses. When compared to a placebo trial however, there were significantly elevated concentrations of plasma glucose and lactate, but significantly reduced levels of NEFA. The consequences of such metabolic changaes were an enhanced oxidation of carbohydrates and an improved time to exhaustion in the carbohydrate trials. Clearly no significant advantage was found when guar gum was added to carbohydrates, yet neither were there any disadvantages.

References

1 Bergmeyer H. U., Bernt E.: Determination of D-glucose with glucose oxidase and peroxidase method with ABTS. in Bergmeyer H. U. (ed): Methods of Enzymatic Analysis, Vol. 3, 1974, pp 1212–1215.

2 Bjorkman O., Sahlin K., Hagenfeldt L., Wahren J.: Influence of glucose and fructose ingestion on the capacity for long term exercise in well-trained man. *Clin Physiol* 4: 493–494, 1984.

3 Blackburn N. A., Redfern J. S., Jarjis H., Holgate A. M., Hanning I., Scarpello J. H. B., Johnson I. T., Read N. U.: The mechanism of action of guar gum in improving glucose tolerance in man. *Clin Sci* 66: 329–336, 1984.

4 Borg G.: Sample rating methods for estimation of perceived exertion, in Borg G. (ed): Physical Work and Effort. Pergamon, New York, 1975, pp 39–46.

5 Coggan A. R., Coyle E. F.: Reversal of fatigue during prolonged exercise by carbohydrate infusion or ingestion. *J Appl Physiol* 63: 2388–2395, 1987.

6 Costill D. L., Coyle E. F., Dalsky G., Evans W., Fink W. J., Hoops D.: Effects of elevated plasma FFA and insulin on muscle glycogen usage during exercise. *J Appl Physiol* 43: 695–699, 1977

7 Coyle E. F., Coggan A. R.: Effectiveness of carbohydrate feeding in delaying fatigue during prolonged exercise. *Sports Med* 1: 446–458, 1984.

8 Coyle E. F., Coggan A. R., Hemmert M. K., Ivy J. L.: Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *J Appl Physiol* 61: 165–172, 1986

9 Dill D. B., Costill D. L.: Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration *J Appl Physiol* 37: 247–248, 1974.

10 Ellis P. R., Burley V. J., Leeds A. R., Peterson D. B.: A guar-enriched wholemeal bread reduces postprandial glucose and insulin responses. *J Hum Nut Dietetics* 1: 77–84, 1988

11 Foster C., Costill D. L., Finks W. J.: Effect of pre-exercise feeding on endurance performance. *Med Sci Sports Exerc* 11: 1–5, 1979.

12 Frayn K. N., Maycock P.: Sample preparation with ion-exchange resin before liquid-chromatographic determination of plasma catecholamines. *Clin Chem* 29: 1426–1428, 1983.

13 Galbo H.: Hormonal and metabolic adaptations to exercise. Thieme Verlag, New York, 1983.

14 Galbo H., Christensen J. N., Holst J. J.: Glucose-induced decrease in glucagon and epinephrine responses to exercise in man. *J Appl Physiol* 42: 525–530, 1977.

15 Galbo H., Holst J. J., Christensen N. J.: The effect of different diets and of insulin on the hormonal response to prolonged exercise. *Acta Physiol Scand* 107: 19–32, 1979

16 Gutmann I., Wahlefeld A. W.: L(+)lactate determination with lactate dehydrogenase and NAD. in Bergmeyer H. U. (ed): Methods of Enzymatic Analysis, Vol. 3, 1974, pp 1464–1468.

17 Hargreaves M., Briggs M. C.: Effect of carbohydrate ingestion on exercise metabolism. *J Appl Physiol* 65: 1553–1555, 1988

18 Hargreaves M., Costill D. L., Coggan A., Fink W. J., Nishibata I.: Effect of carbohydrate feedings on muscle glycogen utilization and exercise performance. *Med Sci Sports Exerc* 16. 219–222, 1984

19 Hawley J. A., Dennis S. C., Noakes T. D.: Oxidation of carbohydrate ingested during prolonged endurance exercise *Sports Med* 14 27–42, 1992.

20 Holt S., Heading R. C., Carter D. C., Prescott L. F., Tothill P.: Effect of gel fibre on gastric emptying and absorption of glucose and paracetamol *Lancet* 1: 636–639, 1979.

21 Jakeman P., Davies B.: The characteristics of low resistance breathing valve designed for the measurement of high aerobic capacity. *Br J Sports Med* 13: 81–83, 1979.

22 Koivisto V. A., Karonen S. L., Nikkila E. A.: Carbohydrate ingestion before exercise: comparison of glucose, fructose and sweet placebo *J Appl Physiol* 51: 783–787, 1981.

23 Massicotte D., Peronnet F., Brisson G., Bakkouch K., Hillaire-Marcel C.: Oxidation of a glucose polymer during exercise: Comparison with glucose and fructose. *J Appl Physiol* 66 [illegible] 1989

APPENDIX C.

Calculation of Glucose Utilisation Rate.

1. Glucose delivery.

Glucose delivery was calculated for each 20 min period as follows:-

a) the length of time of each rate of infusion within each 20 min period was calculated from records made at the time of the clamp.

b) delivery of glucose for each rate was calculated using the formula :-

$$\textbf{Delivery (mol min}^{-1}\textbf{)} = \frac{k \times f \times c}{60 \times 180}$$

where k = rate of infusion (ml h^{-1})
f = time of infusion (min)
c = concentration of glucose infused (mg ml^{-1}) *
60 = time of correction
180 = molecular weight of glucose

* The concentration of glucose infused was obtained from analysis of a 1 in 200 dilution of the infusate using the Analox glucose analyser for the clamp studies. Thus:-

$$\text{Actual concentration of glucose (mg ml}^{-1}\text{)} = mc \times 200 \times \frac{180}{1000}$$

where mc = measured concentration (mM)
200 = correction for dilution
180 = molecular weight of glucose

c) Total delivery for each 20 min period was calculated by summation of the individual deliveries at different rates.

2. Urinary glucose loss.

Urinary glucose concentration was measured at the end of the clamp and was assumed to be constant over the period of the clamp, since a square wave of hyperglycaemia was created (DeFronzo et al., 1979). Urinary glucose loss was expressed as mM kg^{-1} min^{-1}.

3. Glucose utilisation rate.

Mean glucose utilisation rate (uM kg^{-1} min^{-1})for each successive 20 min period was calculated as follows :-

$$\text{Glucose utilisation rate} = \frac{D}{W \times 20} - U - [(g_2 - g_1) \times 0.0095] \times 1000$$

where D = total glucose delivery (mol min^{-1})
W = body mass
U = urinary glucose loss (mM kg^{-1} min^{-1})
g_1 = blood glucose at start of 20 min (mM)
g_2 = blood glucose at end of 20 min (mM)
$(g_2 - g_1) \times 0.0095$ = space correction factor
20 = time correction

APPENDIX D.

AMINO ACID	INFUSION	PLASMA		ERYTHROCYTE	
		REST	POST PRIME	REST	POST PRIME
Alanine	Glucose	418±48	379±48	1422±308	1665±304
	Saline	361±46	327±46	1187±220	1136±220
Arginine	Glucose	100±15	83±14	38±12	68±36
	Saline	97±6	99±3	34±9	48±16
Asparagine	Glucose	39±11	38±9	258±49	356±100
	Saline	46±14	42±13	280±44	331±61
Aspartic Acid	Glucose	27±14	13±2	415±132	454±130
	Saline	21±9	22±11	494±149	401±177
Cysteine	Glucose	95±20	69±20	13±7	8±7
	Saline	112±5	99±13	14±8	10±5
Glutamine	Glucose	483±57	495±74	127±21	215±84
	Saline	449±82	424±76	141±26	142±29
Glutamate	Glucose	632±48	616±55	427±66	619±106
	Saline	530±74	494±77	552±78	551±43
Glutamic Acid	Glucose	149±38	121±32	330±58	405±44
	Saline	81±11	69±8	410±70	410±67
Glycine	Glucose	257±16	216±10	1202±191	1660±450
	Saline	246±14	230±20	1261±197	1293±239
Histidine	Glucose	82±7	75±5	290±70	438±129
	Saline	87±7	95±9	228±39	230±53
Isoleucine	Glucose	57±7	48±6	169±49	257±113
	Saline	57±10	50±9	143±21	138±38
Leucine	Glucose	114±14	97±15	402±42	476±36
	Saline	131±15	118±15	402±21	454±62
Lysine	Glucose	90±11	75±7	632±134	693±329
	Saline	134±31	152±51	709±241	647±380
Methionine	Glucose	24±5	22±6	118±31	125±32
	Saline	27±1	24±2	113±14	118±30
Phenylalanine	Glucose	55±6	47±6	275±67	297±54
	Saline	53±7	48±6	232±32	259±67
Serine	Glucose	120±22	101±20	581±123	887±231
	Saline	123±12	123±11	513±102	632±149

Threonine	Glucose	83±7	79±3	418±130	621±200
	Saline	97±12	95±11	354±69	432±128
Tryptophan	Glucose	50±3	40±3	59±13	70±15
	Saline	65±17	54±15	70±11	82±19
Tyrosine	Glucose	58±7	50±8	227±60	335±118
	Saline	46±10	43±9	257±47	257±75
Valine	Glucose	197±13	186±17	614±175	935±424
	Saline	243±54	224±47	468±88	411±83

AMINO ACID	PLASMA		ERYTHROCYTE	
	Glucose	Saline	Glucose	Saline
Alanine	-9%	-9%	+17%	-4%
Arginine	-17%	+2%	+79%	+41%
Asparagine	-2.5%	-9%	+38%	+18%
Aspartic Acid	-52%	+4%	+9%	-19%
Cysteine	-27%	-12%	-38%	-29%
Glutamine	+2%	-6%	+122%	---
Glutamate	-2%	-7%	+45%	---
Glutamic Acid	-19%	-15%	+23%	---
Glycine	-16%	-7%	+38%	+3%
Histidine	-9%	+9%	+51%	---
Isoleucine	-16%	-12%	+52%	-3%
Leucine	-15%	-10%	+18%	+13%
Lysine	-17%	+13%	+10%	-9%
Methionine	-8%	-11%	+6%	+4%
Phenylalanine	-13%	-9%	+8%	+12%
Serine	-16%	---	+53%	+23%
Threonine	-5%	-2%	+49%	+4%
Tryptophan	-20%	-11%	+19%	+17%
Tyrosine	-13%	-6%	+48%	---
Valine	-6%	-8%	+52%	-12%

Percentage changes in amino acid concentration after the 30 minute prime infusion.

Mean (±SEM) plasma and erythrocyte amino acid concentrations (μM/L) under glucose and saline infusion during 120' exercise.

AMINO ACID	INFUSION	PLASMA CONC $\mu M.L^{-1}$				ERYTHROCYTE CONC $\mu M.l^{-1}$			
		30'	60'	90'	120'	30'	60'	90'	120'
Alanine	Glucose	471±72	539±63	600±61	629±82	3181±673§	3181±501§	3868±732§	4388±1061*
	Saline	506±64	527±52	529±75	514±164	1211±264	1581±352	1507±557	1741±873
Arginine	Glucose	89±15	119±27	150±17	120±24	69±16	99±25	107±25	77±11
	Saline	108±11	153±40	134±24	131±29	53±17	92±26	120±48	57±15
Asparagine	Glucose	40±9	44±9	50±10	45±10	412±102	426±93	609±150	515±160
	Saline	46±13	61±14	55±14	58±14	316±68	424±68	377±79	410±77
Aspartic Acid	Glucose	16±4	15±4	25±6	25±3	403±99	338±75	317±72	362±65
	Saline	19±9	30±12	27±11	32±13	363±116	293±63	299±42	377±88
Cysteine	Glucose	66±18	62±15	71±5	69±8*	6±5	5±4	5±2	4±3
	Saline	103±9	86±5	84±11	97±5	8±4	7±4	4±3	7±4
Glutamine	Glucose	497±58	574±72	529±122	512±171	206±52	237±76	278±72	234±58
	Saline	483±109	533±106	604±108	629±147	161±20	158±22	200±19	181±26
Glutamate	Glucose	626±58	711±52	720±78	746±102	743±127	833±138	966±180	719±163
	Saline	549±107	613±102	687±112	719±163	684±93	722±165	729±221	632±187
Glutamic Acid	Glucose	129±40	137±32*	191±48*	235±71*	537±109	596±91	688±142	650±180
	Saline	67±10	80±17	84±19	90±24	523±112	564±156	529±203	451±174
Glycine	Glucose	215±16	255±12	285±14	293±23	1801±315	1754±51	2246±262	2111±420
	Saline	257±31	290±44	280±26	266±27	1350±274	2032±482	1708±498	1928±785
Histidine	Glucose	81±7*	92±8	93±12	99±15	477±115	518±81▲	592±121▲	567±201
	Saline	101±10	111±20	118±27	134±45	286±68	301±78	299±106	272±127
Isoleucine	Glucose	49±7	48±7	52±5	49±4	270±60*	284±25	319±45	325±65
	Saline	50±5	59±3	55±6	51±6	136±29	212±66	239±91	233±98
Leucine	Glucose	101±19	100±15*	114±12*	102±9	613±34	775±58	863±27	941±186
	Saline	135±15	149±18	137±12	126±16	501±73	681±151	670±117	738±198
Lysine	Glucose	82±11	94±13	121±9	126±24	782±255	1007±306	1353±514	1064±369
	Saline	161±38	181±36	150±39	181±73	620±263	1127±649	1286±743	1456±1100
Methionine	Glucose	23±8	28±8	36±5	34±7	198±42	211±20	287±78	231±44
	Saline	27±2	30±4	35±5	36±5	119±21	[illegible]	221±87	155±83
Phenylalan-ine	Glucose	45±8	60±8	71±10	67±2	436±121	[illegible]	722±192	686±247
	Saline	55±4	72±11	70±7	71±9	228±41	[illegible]	443±207	411±254

Serine	Glucose	107±30	132±30	163±32	166±33	958±185	994±114	1138±187§	1131±289*
	Saline	132±16	163±48	147±32	158±41	580±170	760±204	649±165	643±191
Threonine	Glucose	67±4	91±7*	108±9	119±12	719±168	818±130	1043±225§	867±259
	Saline	97±12	124±10	118±7	111±11	451±119	535±150	515±171	487±187
Tryptophan	Glucose	34±2	42±3	52±2	51±3	111±35	135±34	150±40	135±38
	Saline	56±15	63±15	62±17	60±16	85±17	120±38	101±28	72±20
Tyrosine	Glucose	48±10	59±9	70±7	70±7	407±128	423±120	546±173	497±210
	Saline	49±12	52±7	58±8	56±10	272±68	345±86	359±136	256±91
Valine	Glucose	163±10	177±16	192±9	198±16	1018±244*	1057±237*	1412±403*	1087±317*
	Saline	247±54	263±46	242±45	247±52	453±84	547±147	571±137	426±143

Rate of uptake/release (μM/min) of amino acids during exercise under glucose or saline infusion.

AMINO ACID	PLASMA			ERYTHROCYTE		
	Glucose	Saline		Glucose	Saline	
Alanine	2.10▲	1.33+	↑	20.45▲	5.03	↑▲
Arginine	0.45	0.30	↑	0.18	0.29	↓
Asparagine	0.09*	0.14*	↓	1.72*	0.72	↑
Aspartic Acid	0.11+	0.09	↑	-0.91	-0.37	↓
Cysteine	0.01	-0.08	↑	-0.03	-0.04	↑
Glutamine	0.22	1.77+	↓*	0.37	0.39	↓
Glutamate	1.18+	1.96+	↓	2.51*	0.68	↑
Glutamic Acid	0.96+	0.19*	↑▲	2.14+	0.29	↑*
Glycine	0.75▲	0.32	↑	4.49	5.43	↓
Histidine	0.20*	0.32*	↓	1.23	0.32	↑
Isoleucine	0.02	0.02		0.61	0.97*	↓
Leucine	0.08	0.06	↑	3.93▲	2.45*	↑
Lysine	0.47▲	0.16	↑	4.38*	7.61*	↓
Methionine	0.12▲	0.10+	↑	1.01+	0.59	↑
Phenylalanine	0.23+	0.21+	↑	3.54+	1.73	↑*
Serine	0.62▲	0.28	↑	2.22	0.31	↑
Threonine	0.41▲	0.17	↑	2.73*	0.58	↑
Tryptophan	0.13+	0.06	↑	0.63+	-0.01	↑
Tyrosine	0.21▲	0.12+	↑	1.55	0.28	↑
Valine	0.16	0.12	↑	3.10	0.49	↑

APPENDIX E

Subject data (Experiment 1).

Subject	Age	Body Mass (kg)	Height (cm)	VO_2max (ml min^{-1})
1	23	92	186	4329
2	44	70	174	3945
3	22	66	180	3360
4	26	75	178	3551
5	23	70	179	3436
Mean	27.6	74.6	179	3724
(±SD)	±8.3	±9.2	±4	±363

Subject data (Experiment 2).

Subject	Age	Body Mass (kg)	VO_2max (ml min^{-1})
1	33	69	3863
2	28	66	3872
3	32	75	4534
4	22	85	5150
5	27	66	3527
6	22	76	4715
Mean	27.3	72.8	4243
±SD	±4.3	±6.7	±613

Subject data (Experiment 3).

Subject	Age	Body Mass (kg)	Height (cm)
1	40	83	188
2	22	82	184
3	34	95	188
4	23	60	172
5	42	74	169
Mean	32.2	78.8	180
±SD	±8.4	±11.5	±8

Subject data (Experiment 4).

Subject	Age	Body Mass (kg)	Height (cm)	VO_2max (ml min^{-1})
1	24	63	169	3120
2	45	70	174	3945
3	49	65	169	3612
4	24	66	173	4012
5	22	85	185	5150
6	32	72	177	4256
7	52	65	168	3988
8	45	65	168	3866
Mean	36.6	68.9	173	3994
±SD	±11.6	±6.7	±6	±543

Subject data (Experiment 5).

Subject	Age	Body Mass (kg)	VO_2max (ml min^{-1})
1	46	72	4545
2	21	57	3912
3	22	66	4127
4	24	80	4813
5	21	73	4636
Mean	26.8	69.6	4407
±SD	±9.6	±7.7	±335