THE EFFECT OF MUSCLE GLYCOGEN STATUS ON CONTROL OF SUBSTRATE METABOLISM DURING EXERCISE

Thesis submitted for the degree of Doctor of Philosophy (Medical Physiology)

by

Sandra Mary Weltan

Department of Physiology

University of Cape Town Medical School

Observatory 7925

South Africa

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Dedicated to Andrew and to my parents, Aubrey and Jean Weltan

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DECLARATION

I, Sandra Mary Weltan, do hereby declare the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, or is being, or is to be submitted for another degree to the University of Cape Town, or to any other University.

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ABSTRACT

Glycogen depletion has frequently been shown to result in a decrease in respiratory exchange ratio (RER). However, the metabolic response to glycogen depletion has generally been studied in overnight fasted subjects or in subjects who were already fatigued, or hypoglycaemic, or both, raising the question of whether the differences seen were due to general "carbohydrate deficiency" or due specifically to muscle or liver glycogen depletion. If euglycaemia and especially hyperglycaemia is maintained, the "carbohydrate deficiency" is overcome. In addition, because insulin stimulates muscle glucose uptake and not liver glucose uptake during euglycaemia (except at very high concentrations), insulin infusion would differentiate between liver and muscle glycogen depletion, since if the decrease in RER previously observed is abolished with insulin infusion while euglycaemia is maintained, this would indicate that the decrease is specifically due to muscle glycogen depletion. Thus the aim of this study was to investigate the metabolic effect of glycogen content while an adequate amount or an excess of carbohydrate was provided in the form of an intravenous glucose infusion and when plasma insulin concentrations are raised.

Thirty-two moderately trained cyclists followed an exercise protocol to deplete muscle glycogen, whereafter 21 followed a low- CHO diet (low glycogen; LG) for 48 hours and 11 maintained their normal diet (normal glycogen; NG). All subjects ingested a small meal (±30 g CHO) 3 hours before exercising at 70% of $VO_{2\,max}$ for 145 min. During exercise, 8 LG and 6 NG received an infusion of glucose to maintain euglycaemia (LGE and NGE, respectively) and 5 LG received a euglycaemic, hyperinsulinaemic clamp (LGEI). In addition, 8 LG and 5 NG received a glucose infusion to maintain plasma glucose concentrations at ±9 mmol/l (LGH and NGH, respectively). VO_2 was measured every 20 min during exercise and RER, CHO and fat oxidation were calculated. At the same time-intervals, blood was drawn via an indwelling cannula for determination of plasma concentrations of glucose (G), insulin (INS), glucagon (GG), growth hormone (GH), catecholamines, and serum free fatty acids (FFA). A U-^{14}C-glucose tracer was used for measurement of glucose oxidation. A biopsy was taken from the vastus lateralis before and after completion of the ride for measurement of muscle glycogen content.

Pre-exercise resting FFA and norepinephrine (NE) concentrations were higher and RER lower ($p<0.05$) after glycogen depletion. During exercise, total fat oxidation (F_{tot}) in LGE (126±6 g (mean±SEM)) was significantly ($p<0.05$) higher than in NGE (76±12 g) or LGEI (88±18g) and in LGH (72±7 g) was significantly ($p<0.05$) higher than in NGH (33±11 g). LGEI and LGH were similar with respect to F_{tot}. Rates of glucose oxidation (R_{ox}) increased progressively in all groups ($p<0.05$). Total glucose oxidation (G_{ox}) was significantly ($p<0.05$) lower (389±44 mmol) in LGE than in LGH

or LGEI (840±74 and 785±83 mmol, respectively), which were not significantly different from each other. However, total glucose oxidation did not differ significantly between LGE and NGE (389±44 vs 513±61 mmol; 70±8 vs 92±11 g) but NGE was also lower than LGEI ($p<0.05$). G_{ox} also did not differ significantly between LGH and NGH (840±74 vs 957±95 mmol). Peak rates of glucose oxidation were 9.2±1.7, 8.3 ±1 and 8.4±1 mmol/min (1.66±0.31, 1.51±0.19 and 1.51±0.13 g/min) in NGH, LGH and LGEI, respectively, compared with 5.6±0.9 and 4.5±0.8 mmol/min (1.03±0.16 and 0.82±0.15 g/min) in NGE and LGE, respectively ($p<0.05$). The total amount of glucose infused (G_i) was higher in LGH than in LGEI (1484±125 vs 956 ±121 mmol ($P<0.05$)) and both were significantly higher than LGE (342±69 mmol), but LGE did not differ from NGE (342±69 vs 403±107), neither did LGH differ from NGH (1484±125 and 1461±97 mmol, respectively). G_{ox} matched the total amount of G_i in all the euglycaemic subjects (LGE, LGEI, NGE) irrespective of muscle glycogen content, but the total amount of glucose infused to maintain hyperglycaemia in LGH and NGH was significantly greater than G_{ox}. Muscle glycogen disappearance was greater ($p<0.05$) in NGE and NGH than LGE, LGH or LGEI (90±11 and 78±22 vs 49±9, 41±4 and 28±14 mmol/kg ww, respectively) but there were no significant differences between the latter 3 groups with low muscle glycogen content at the start of exercise. Plasma lactate concentrations did not differ significantly between NGE, LGE and LGEI, but the area under the time vs concentration curve was lower ($p<0.05$) in LGH than NGH during exercise. INS were significantly lower and FFA higher in LGE than NGE and in LGH than NGH but INS were higher and FFA lower in LGEI than in any other group. There were no significant differences in INS or FFA between LGH and NGE. Neither were there any differences between groups in GG, but NE were significantly lower in LGE than in NGE and intermediate between and not significantly different from NGE and LGE in LGEI, but did not differ between LGH and NGH. Plasma epinephrine concentrations (E) were significantly higher ($p<0.05$) in LGEI than in NGE, LGH or LGE and GH were higher in LGEI than LGH but otherwise did not differ significantly between groups.

Muscle glycogen depletion resulted in an increased utilisation of fat at rest and during exercise in both the euglycaemic and hyperglycaemic state, but had no effect on the rate of oxidation of glucose when euglycaemia was maintained by infusion of glucose alone. In LGEI, insulin infusion decreased fat oxidation primarily by increasing glucose oxidation, as did maintenance of hyperglycaemia in LGH and NGH, but even in the latter two groups, the effect of muscle glycogen content on fat oxidation, but not on glucose oxidation, was evident. The association between muscle glycogen depletion and increased fat oxidation may be due to a stimulatory effect of low muscle glycogen content on norepinephrine secretion or an inhibitory effect on insulin secretion, or both.

Muscle metaboreceptors and stimulation of group III and IV muscle afferents in cats have been shown to alter concentrations of hormones and metabolites. It is therefore possible that these receptors and neurons provide the signalling pathway, since: i) the metabolic response to exercise with glycogen depletion is very similar to the metabolic response to exercise in patients with muscle phosphorylase

deficiency; ii) the response to hyperglycaemia in glycogen depleted subjects is very similar to the metabolic response to hyperglycaemia in patients with muscle phosphorylase deficiency and iii) studies of McArdle's disease strongly link the metabolic and cardiovascular defects of this disease with neural feedback from chemoreceptors in contracting muscle. Muscle glycogen depletion was associated with a reduced rate of muscle glycogen disappearance in subjects who started exercise with low muscle glycogen content, but despite the increased rate of glucose oxidation in LGH and LGEI, there was no sparing of muscle glycogen measurable by the muscle biopsy technique. Rates of oxidation of glucose reached a maximum of 1.5 g/min in LGH, NGH and LGEI subjects. Similar rates of glucose oxidation in LGEI and LGH suggest that high insulin concentrations were at least partly responsible for the increase in oxidation rate with hyperglycaemia. With LGE, NGE and LGEI, rates of glucose infusion and oxidation were matched, whereas with LGH and NGH, 43% of the glucose infused was not oxidised.

It can be concluded from these studies that: i) muscle glycogen depletion without fatigue before exercise results in an increase in fat oxidation compared with subjects with normal muscle glycogen content both at rest and during exercise, but has no effect on the rate of oxidation of glucose when euglycaemia is maintained by infusion of glucose alone; ii) when hyperglycaemia is maintained in glycogen-depleted subjects, glucose oxidation is higher than when euglycaemia is maintained in subjects with normal glycogen content during exercise at 70% of $VO_{2\,max}$, but RER is not different; iii) Insulin infusion in glycogen depleted subjects increases glucose oxidation and decreases FFA concentrations and fat oxidation, suggesting that the metabolic effects of a low CHO diet are specifically due to a decrease in intramuscular CHO availability; iv) although rates of glucose infusion and oxidation are matched under euglycaemic conditions, with hyperglycaemia 43% of the glucose infused is not oxidised, thus there is an upper limit of ~ 1.5 g/min to the rate of glucose oxidation during exercise at 70% of $VO_{2\,max}$ with hyperglycaemia irrespective of muscle glycogen status; v) net muscle glycogen utilisation is determined by the muscle glycogen content at the start of exercise even when hyperglycaemia is maintained during exercise, and despite the increased rate of glucose oxidation in LGH and LGEI, there is no sparing of muscle glycogen compared with LGE; vi) the metabolic responses to exercise and hyperglycaemia with glycogen depletion are very similar to those of patients with muscle phosphorylase deficiency (McArdle's disease) and vii) the shift towards lipid metabolism both at rest and during exercise with muscle glycogen depletion may be mediated by a muscle afferent pathway via norepinephrine or insulin.

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CHAPTER 1

INTRODUCTION AND AIMS

Much has been written about the effect of fasting or ingestion of a low carbohydrate diet on substrate metabolism and hormonal changes both at rest and during exercise. The topic is of interest for a number of reasons. Firstly it has been known for a long time that an adequate supply of carbohydrate is needed for exercise performance at moderate to high exercise intensities. Secondly, the growing problem of obesity has proven to be far more complex than simple gluttony and efforts have been made to reach a better understanding of metabolic control over fuel substrate utilisation. A complication of obesity is insulin resistance and many studies have investigated this aspect of both insulin dependent and non-insulin dependent diabetes mellitus. There are also other metabolic diseases such as McArdle's disease which result an inability to utilise available substrates and much effort has been made to trace the causes of the metabolic aberrations seen in these patients.

Metabolic effects that have been described as a result of ingesting a low carbohydrate diet or fasting are a decrease in respiratory exchange ratio (215, 310, 312, 527) and in insulin (215, 268) and lactate (310) concentrations compared with normally fed controls, and an increase in norepinephrine (215, 268), epinephrine (215, 268, 310), glucagon (215, 268), growth hormone (215), cortisol (215), glycerol (312) and free fatty acid (FFA) (215, 268, 310, 312) concentrations.

However, data from these studies (215, 268, 310, 312, 527) do not clearly elucidate whether the effects seen on substrate metabolism and hormonal responses were due to muscle or liver glycogen depletion or, except in cases where glucose was infused during exercise (215, 268), due to a general "carbohydrate deficiency", i.e. a shortage of total carbohydrate available for oxidation. These studies were thus designed to investigate the effect of muscle glycogen depletion when plasma glucose concentrations were prevented from falling or raised above normal thus eliminating the possibility of a whole-body carbohydrate deficiency. Since insulin specifically promotes muscle (145) and not liver glucose uptake unless both plasma glucose and insulin concentrations are very high (107), it was hypothesised that if the metabolic changes previously described were observed during euglycaemia without insulin infusion and abolished with insulin infusion, this would indicate that these changes were specifically due to muscle glycogen depletion.

Maintenance of hyperglycaemia by glucose infusion results in an increase in glucose oxidation during exercise in subjects with normal glycogen content compared with maintenance of euglycaemia, but only about 60% of the glucose infused in a hyperglycaemic clamp is oxidised (265). It was also hypothesised that if the metabolic responses shown in previous studies (215, 257, 268, 310, 312) were

simply due to an inadequate supply of carbohydrate, the "excess" glucose that was not oxidised in subjects with normal muscle glycogen content during hyperglycaemia (265) could be used for oxidation if muscle glycogen is low.

CHAPTER 2

REVIEW OF LITERATURE

THE BALANCE OF FUELS

THE INFLUENCE OF FUEL SUPPLY ON FUEL SUBSTRATE UTILISATION

During sustained submaximal exercise, muscle cells are fuelled by both endogenous (triglycerides and glycogen) and blood-borne (lactate, glucose and fatty acids) substrates (282). Man has a limited capacity to store CHO, the major sites being liver and muscle glycogen (51). The concentration of glycogen in the leg muscles of untrained people eating a normal diet varies from about 80 to 120 mmol/kg of wet muscle (ww) (21, 53, 77, 286, 533), whereas average muscle glycogen content of athletes who ingest a diet high in CHO and are in training, are somewhat higher (229), around 130 mmol/kg ww (579). Values of around 144 - 200 mmol/kg ww are usually found in trained athletes who have not exercised for 24 - 48 hr and who have consumed a high CHO diet (43, 133, 231, 594, 596, 621, 622, 717). According to studies in which muscle biopsy samples were taken (286, 289, 413), either eating a low CHO, high protein-fat diet, or fasting for 6 - 24 hr, results in little change in muscle glycogen concentrations (286, 413) and five days of this dietary regimen causes muscle glycogen levels predominantly in the active muscles to fall by only about 30 - 50% if only normal daily activities are performed (286, 289). However, in a study utilising ^{13}C nuclear magnetic resonance (NMR) where extremely small changes in muscle glycogen concentrations can be detected, whole body muscle glycogen concentrations reached a peak of 100.2±6.7 mmol/l 4 hours after a mixed meal, but decreased by ±12% in the subsequent 3 hours after this peak (644). Muscle glycogen concentrations of 17-45 mmol/kg ww have been reported after an exercise protocol of 30 to 90 minute's duration at intensities of 60-80% of maximal oxygen consumption ($VO_{2\ max}$) followed by a diet consisting of 0-11% CHO for 24-96 hours (215, 230, 231, 267).

Hepatic glycogen content is normally higher than that of muscle and is about 300 mmol/kg ww when a normal diet is consumed (292, 484) and 500 mmol/kg ww when the diet is high in CHO (70%). A liver weighing about 1,8 kg has a total glycogen store of about 160 g when a high CHO diet is eaten (487). However, hepatic glycogen content can fall rapidly as a result of its utilisation for maintaining blood glucose concentration. Glucose is essentially the exclusive fuel used by the brain and because its uptake depends entirely on the concentration gradient of glucose across the blood-brain barrier, it is vitally dependent on maintenance of stable plasma glucose concentrations (222, 338, 608, 649, 694). The brain alone has a daily glucose requirement of about 125 g, which is sufficient to almost deplete the hepatic glycogen stores within 24 hr. Since hepatic glycogen stores fall at a rate of about 9 g/hr, hepatic glycogen depletion could theoretically occur after a fast of only 18 hr (487). This, however,

does not occur in practice because tissue glucose utilisation and total hepatic glucose output decrease during starvation, thus hepatic glycogen depletion occurs only much later, with plasma glucose concentrations being maintained predominantly by gluconeogenesis only after about 48 hr of fasting (123, 484).

A decrease in oxidation of CHO, as indicated by a decreased respiratory exchange ratio (RER) after fasting (49, 102, 213, 365, 369, 379, 394, 421) or after a low CHO diet (215, 231, 257, 267, 268, 310, 312, 424, 501, 527) has been described at rest (215, 231, 312, 397, 622) and during exercise at various intensities (49, 215, 230, 231, 257, 267, 311, 312, 397, 421, 424, 501, 527, 622). Other effects on substrate metabolism and hormonal changes during exercise that have been demonstrated as a result of a low-CHO diet or fasting before exercise are a decrease in plasma insulin (49, 213, 215, 257, 268, 397, 421, 622, 717) and lactate (215, 231, 257, 310, 312, 424, 622, 717) concentrations, and an increase in plasma norepinephrine (49, 213, 268, 312, 394, 397, 413, 585), epinephrine (49, 213, 215, 268, 310, 312, 394, 397), glucagon (213, 215, 268), growth hormone (215) cortisol (213, 215, 394), glycerol (49, 213, 215, 312, 397, 424), ketone (213, 215, 268) and free fatty acid (FFA) (49, 213, 215, 257, 268, 310, 312, 394, 397, 413, 421, 424, 527, 585, 622, 717) concentrations.

However, in these studies it is not clear whether these effects observed on substrate metabolism and hormonal responses were due to muscle or hepatic glycogen depletion or, except in cases where glucose was infused during exercise (215, 268), due to a general "carbohydrate deficiency", that is, a shortage of total CHO available for oxidation. For example, in some studies (215, 424, 622), subjects were overnight fasted and their plasma glucose concentrations were lower than those in controls before (215, 424, 622) or during (215, 424) exercise. Even in the studies by Jansson et al. (310, 312), although subjects ingested an 800 kJ meal 3 hours before the trial, their arterial glucose concentrations decreased to below 4.5 mmol/l during exercise. In an attempt to exclude the effects of an overall CHO deficiency, glucose was infused during exercise in some studies (215, 268). However, these studies are difficult to interpret. In one (268), subjects on a low CHO diet were overnight fasted, while control subjects on a normal diet ate a 60% CHO meal 3 hours before exercise and in another (215), subjects were overnight fasted and glucose infusion was begun only after subjects had reached exhaustion, when norepinephrine concentrations were already increased due to fatigue (592). In addition, in only a few studies (230, 267, 312, 413) were muscle glycogen concentrations measured.

In a study (365) on resting subjects during a 72 hour fast, it was found that plasma insulin concentrations decreased and FFA increased within the first 24 hours of fasting, whereas plasma glucose concentrations only started to decrease after 24 hours. In another study (413) muscle glycogen concentrations were not significantly different and in fact pre-exercise muscle glycogen content was relatively high in two groups in both the fed (168±17 and 186±24 mmol/kg ww) and 24 hour fasted (144±22 and 183±30 mmol/kg ww) states, yet FFA were higher in the fasted state than in the fed

state. In contrast, Ravussin et al. (527) found that subjects on a low-CHO pre-exercise diet had higher FFA during exercise than those who had been on a normal diet, despite the ingestion by subjects in both groups of 100 g of glucose an hour before exercise. In an attempt to specifically deplete hepatic glycogen, Lavoie et al. (394) used a protocol where subjects first carbohydrate loaded, then ingested a low-CHO diet for 24 hours with a period of arm cranking. However, muscle biopsies were not taken before the actual trial to ensure that muscle glycogen concentrations had not decreased in order to provide substrate for gluconeogenesis (80). Possibly the best indication of a specific effect of muscle glycogen depletion on metabolism was obtained in a study (230) in which cyclists exercised with glycogen content 28 mmol/kg ww lower in one leg than the other and it was found that lipid oxidation was higher in the leg with greater glycogen depletion.

INFLUENCE OF EXERCISE INTENSITY ON FUEL SUBSTRATE UTILISATION

Numerous studies have shown that the contribution of CHO to total energy consumption increases from rest to exercise (425) and as the intensity of exercise increases (413, 425, 507); this has also been the subject of a number of reviews (81, 228). The most informative studies on this topic were done by Jones et al. (326) in 1980 and Romijn et al. (563) in 1993. In the first of these two studies (326), plasma ^{14}C-palmitate turnover was measured during 40 min of exercise at 36% of $VO_{2\,max}$ and 70% of $VO_{2\,max}$ on separate days, after an overnight fast. Plasma ^{14}C-palmitate turnover rate did not change from resting values during light work but was decreased by 40% in heavy work. Heavy work was associated with a decrease in the plasma concentrations of free fatty acids, but a greater increase in plasma glycerol in heavy exercise compared with light exercise, from which it was suggested that intramuscular lipolysis was occurring, which led to an efflux of glycerol but not of free fatty acids into plasma. A later study (626) showed that during electrical stimulation of muscle in rats, the greatest utilisation of intramuscular triglycerides was in fast-twitch oxidative fibres. The study by Romijn et al. in 1993 (563) re-examined the observations of Jones et al. (326) with the aim of elucidating the effect of exercise intensity on adipose tissue lipolysis using stable isotope tracers and indirect calorimetry. Trained subjects were studied during exercise intensities of 25, 65, and 85% $VO_{2\,max}$. The results showed conclusively that plasma glucose tissue uptake and muscle glycogen oxidation increased in relation to exercise intensity, but that, in agreement with the previous study (326), while peripheral lipolysis was maximally stimulated at the lowest exercise intensity and fatty acid release into plasma decreased with increasing exercise intensity, muscle triglyceride lipolysis was stimulated only at higher intensities.

INFLUENCE OF TRAINING ON FUEL SUBSTRATE UTILISATION

The effect of training on the balance of fuel utilisation is also superimposed on the effect of the pre-exercise diet and the exercise intensity as discussed in reviews by Gollnick (228) and Brooks et al.

(81). Endurance training results in a shift from CHO towards lipid oxidation (14, 112, 157, 296, 314, 354, 374, 385, 524, 556, 654) with a decrease in muscle glycogen utilisation (127, 190, 242, 243, 296, 314) and possibly an increase in utilisation of intramuscular triglyceride (314). The decrease in muscle glycogen utilisation can occur after only 5-7 days of training for 2 hours at 67% of $VO_{2\ max}$ (241) and is accompanied by an increase in intramuscular creatine phosphate (127, 241) and a decrease in intramuscular creatine (241) and lactate concentrations (127, 190, 241, 242). An increase in muscle oxidative capacity (241, 243, 314) is a later adaptation (241, 243) and this is accompanied by an increase in muscle mitochondrial enzyme concentrations (235, 246, 272, 354). Maximal increases in muscle oxidative capacity takes place after ~ 4 months of training (157).

CONTROL OF LIPOLYSIS

Control of lipolysis appears to be predominantly the domain of the hormones involved in metabolic regulation. Epinephrine and norepinephrine both stimulate lipolysis (138, 476, 636, 678) by increasing intracellular cyclic AMP (cAMP) concentrations, resulting in phosphorylation and activation of hormone sensitive lipase by protein kinase A (476). In untrained subjects, lipolysis increases during exercise (709) in proportion to plasma epinephrine concentrations (587). Catecholamine stimulation also appears to be essential for the increase in lipolysis during exercise in trained subjects (5, 657, 692), since a study using isolated, perfused rat hindquarters (159) indicated that muscle contraction alone did not stimulate lipolysis. The plasma catecholamine concentrations that occur during submaximal exercise result in stimulation of lipolysis but not glycogenolysis (657).

The stimulatory effect of catecholamines on intramuscular triglyceride utilisation during exercise appears to be mediated via β_2-adrenergic stimulation, whereas the increase in adipose tissue lipolysis is due to both β_1 and β_2 stimulation (109). Exercise training increases adipose tissue sensitivity to catecholamines (156, 157, 217) by increasing hormone-sensitive lipase activity per cell, per milligram of protein and per gram of adipose tissue (15). As well as stimulating lipolysis, norepinephrine reduces re-esterification of FFA (375), especially during exercise (709). Glucagon also increases cAMP concentrations and stimulates lipolysis (476), though it does not appear to be involved in the exercise-induced stimulation of lipolysis (192, 212), neither is lipolysis affected by cholinergic activity (150) or short-term thyroxin or glucocorticoid administration (159). However, glucocorticoids are needed for the stimulatory effect of growth hormone on lipolysis. This effect of growth hormone occurs about 1 hour after stimulation of an increase in plasma growth hormone concentrations and involves protein synthesis, possibly including adenyl cyclase, the enzyme which hydrolyses ATP to cAMP (476).

Hyperinsulinaemia results in a decrease in lipolysis (45, 320, 476) by decreasing cAMP concentrations only if they are already raised (476) and the sensitivity of adipose tissue lipolysis to insulin is impaired in insulin-dependent diabetes mellitus. An increase in plasma FFA concentrations

results in a decrease in intramuscular triglyceride utilisation and this feedback mechanism also appears to be mediated by insulin (720). Although plasma insulin concentrations decrease during prolonged exercise, no change in plasma insulin concentration is necessary, at least in rats, for the stimulation of lipolysis during exercise (217). This would imply that the primary stimulus for lipolysis during exercise is the progressive increase in catecholamine concentrations, which would then also cause the progressive decrease in insulin concentrations via α-adrenergic stimulation in the pancreas.

The direct effect of plasma glucose concentrations on lipolysis appears to be controversial. Hyperglycaemia results in suppression of lipolysis (711) and in one study (92), this was shown to be the case despite clamping pituitary and pancreatic hormones at basal concentrations. However, this contradicted the results of an earlier study (93) where it was found that when insulin, growth hormone and somatostatin concentrations were kept constant, there was no effect of hyperglycaemia on lipolysis.

Fasting also increases the sensitivity of lipolysis to epinephrine and decreases its suppression by insulin (321). A decline in plasma glucose concentrations during fasting contributes to, but is not required for, the increase in lipolysis and the increase in epinephrine-stimulated lipolysis that occurs during fasting (363). Although β-adrenergic stimulation increases lipolysis during fasting, some lipolysis continues to occur during β-blockade (364), thus the increase in lipolysis during fasting is not solely due to catecholamine stimulation. Some stimulation of lipolysis may arise from the increase in plasma growth hormone concentrations during fasting (476).

Electrical stimulation of the ventromedial hypothalamus results in adrenal catecholamine release and a β-adrenergic stimulated increase in lipolyis (389), thus central command and/or neural metabolic feedback, both of which will be discussed later in this review, may be involved. Further evidence for this is that intraportal glucose infusion at a rate of 0.125 g/kg/hr in dogs, which does not result in an increase in plasma insulin concentrations, inhibits lipolysis, whereas peripheral glucose infusion at the same rate does not.

CONTROL OF SKELETAL MUSCLE METABOLISM

Control of glucose uptake

Glucose uptake by muscle is a topic which has been widely researched because of its involvement in both insulin-dependent and non-insulin dependent Diabetes Mellitus, and a large number of factors have been found to affect this process. These will be discussed in the sections that follow.

GLUCOSE TRANSPORTERS

Glucose uptake is rate limiting for glucose metabolism in muscle (367). It was recognised in 1984 (22) that glucose transport is facilitated by glycoproteins found in the plasma membrane of cells and in an intracellular membrane pool (169, 330, 366) and that insulin stimulated translocation of these transporters from the intracellular pool to the plasma membrane (330). These glucose transporters were later characterised as a family of glycoproteins (467) of which 6 have thus far been identified (469):

GLUT-1 found in most cells and responsible for basal glucose uptake.

GLUT-2 found in hepatocytes, pancreatic β cells, intestinal and renal epithelium; high K_m for glucose, non-rate limiting.

GLUT-3 found in neurons (low K_m).

GLUT-4 found in insulin-regulatable tissues (304): muscle and adipose tissue.

GLUT-5 fructose transporter.

GLUT-7 present in endoplasmic reticulum (ER) - allows free glucose out of the ER after dephosphorylation of glucose-6-phosphate (G-6-P).

GLUT-1 and GLUT-4 are the transporters found in skeletal muscle (467) and are quantitatively important in whole body glucose uptake, since the level of skeletal muscle glucose transporter protein is reported to be correlated with whole body glucose uptake in man (378). GLUT-1 is found only in the sarcolemma (24, 169), while GLUT-4 is found in the sarcolemma, T-tubules (164) and in an intracellular membrane pool (366). GLUT-1 is responsible for basal glucose uptake (469) and appears to be somewhat regulatable (366), whereas GLUT-4 is regulated by insulin (169, 236, 330), exercise (169, 234) and glucose concetrations (161).

EFFECT OF INSULIN ON GLUCOSE UPTAKE

Insulin is secreted by the β cells of the pancreatic islets (13, 247, 461, 474, 490, 609, 635, 727) and acts via a receptor on cell membranes which has tyrosine kinase activity (97, 158, 298, 331). An increase in insulin binding to its receptors on the sarcolemma results in a calmodulin-dependent (250)

translocation of GLUT-4 receptors from the intracellular pool to the sarcolemma (24, 168, 169, 234, 279, 368) and T-tubules (24).

Insulin stimulates glucose uptake (38, 88, 89, 108, 145, 191, 198, 307, 399, 531, 586, 614, 685, 688), is required for basal glucose metabolism (191) and if euglycaemia is maintained, follows a sigmoidal dose-response curve for glucose uptake (614). The reason for the smaller response at low insulin concentrations is probably related to the time taken for recruitment of transporters (366), while the plateau at the top of the curve is probably due to saturation of insulin receptor sites (87). There appears to be little difference in insulin action whether exogenous insulin is administered continuously or at the same total dose in a pulsatile fashion (495). It has been suggested that the rate-limiting factor in insulin action is its transport from the capillary to the receptor on the target cell (39, 40, 72), although some workers dispute this (87). The promotion of glucose uptake by insulin takes place by a direct effect of recruiting GLUT-4 receptors (168, 169, 236, 238, 279, 366, 368) and increasing their activity (238) and an indirect effect of suppressing FFA release into the circulation, which inhibits glucose uptake in the basal state (88). This dual effect of insulin in the whole body probably explains why a correlation was found between muscle GLUT-4 content and insulin-stimulated glucose uptake in perfused rat muscle (83) but not in vivo in humans (8, 255).

EFFECT OF BLOOD GLUCOSE CONCENTRATION ON GLUCOSE UPTAKE

Short-term hyperglycaemia results in an increase in glucose uptake (3, 180, 203, 239, 418, 548, 685) and the dose-response curve is sigmoidal if hyperinsulinaemia is maintained (180). Glucose uptake is also increased during exercise or muscle contraction if blood glucose concentrations are increased or prevented from falling by CHO ingestion (3, 225) or infusion (685). This is mainly due to a mass action effect across the sarcolemma, i.e. glucose diffuses down a steeper concentration gradient at higher concentrations, until the action of hexokinase becomes rate-limiting (339, 341, 539), since phosphorylation of incoming glucose is essential if the concentration gradient is to be maintained. Hyperglycaemia in the absence of insulin (due to somatostatin infusion) results in intracellular glucose accumulation (339). Hyperinsulinaemia increases glucose uptake during hyperglycaemia, but insulin has a greater effect on glucose uptake than does hyperglycaemia (180, 647). Long term hyperglycaemia results in a decrease in glucose uptake (345, 548) which, if accompanied by hyperinsulinaemia, can occur after only 5 hours (548). This decrease is due to a decrease in GLUT-4 mRNA and in the number of GLUT-4 transporters (161, 162, 367, 586) in both the plasma membrane and the intracellular pool (161). Conversely, GLUT-1 expression increases with hyperglycaemia (161, 162, 367). The decrease in GLUT-4 occurs particularly if hyperglycaemia is accompanied by hyperinsulinaemia as occurs with chronic overfeeding and results in decreased sensitivity to insulin or insulin resistance, which is a feature of non-insulin dependent diabetes mellitus (NIDDM) (468).

EFFECT OF MUSCLE CONTRACTION ON GLUCOSE UPTAKE

Exercise results in a decline in plasma insulin concentrations (278, 299, 532, 710), yet the rate of glucose uptake is increased during muscle contraction (37, 38, 83, 145, 147, 159, 250, 300, 341, 510, 539, 552, 681, 688, 729). This increase can occur in the absence of or at very low insulin concentrations (83, 159, 250, 300, 510, 552, 681, 688), although one study (37) showed in a perfused rat hindquarter that this was not the case since they did not demonstrate an increase in glucose uptake during stimulation of the muscle to contract after washing the preparation with an insulin-free perfusate. However, when Plough et al. (510) repeated the experiment under the even more stringent conditions of using a perfusate containing insulin antiserum, they showed that even in muscle from severely ketoacidotic rats, glucose uptake was increased by muscle contraction. An increase in plasma insulin concentrations during exercise stimulates glucose uptake more than exercise alone (37, 69, 145, 159, 300, 688, 710) and the effects have in most cases been shown to be synergistic (50, 145, 551, 730). In one study (37) using a perfused rat hindquarter preparation, there was no stimulatory effect of muscle contraction on glucose uptake and no additive effect of insulin and contractions, whereas in another study (166) using Zucker rats, the effects of insulin and contractions were synergistic in obese animals but additive in lean animals.

Glucose uptake is also increased after exercise (10, 37, 69, 302, 551, 724), but only in the muscles that were active during exercise (10, 551) and the increase is not associated with an increase in insulin binding (62). There is dispute as to whether insulin is required for post-exercise glucose uptake (37, 552, 724). Whereas it may not be an absolute requirement, it certainly does play a role, since the study of Bourey et al. (69) showed that although glucose uptake was 70% higher during exercise than at rest at maximal insulin concentrations, there was no difference in glucose uptake during recovery at the same insulin concentrations between the previously exercised and non-exercised muscle, indicating maximal stimulation of glucose uptake by insulin, independent of prior contractile activity.

Glucose uptake is greater in slow-twitch oxidative muscle than in fast twitch oxidative-glycolytic muscle (8, 63, 236, 307, 309), possibly because slow twitch muscle has more insulin receptors (309) and demonstrates more insulin binding (63, 309) and tyrosine kinase activity (309) than fast-twitch muscle. Additionally, one study (236) demonstrated higher glucose uptake and higher GLUT-4 content in slow than fast twitch muscle. Citrate synthase, an enzyme which is involved in oxidative metabolism and which is more abundant in the more oxidative slow twitch muscle than in the more glycolytic fast twitch muscle, is quantitatively proportional to GLUT-4 in human muscle (429) and there appear to be correlations between the number of slow-twitch fibres found in a muscle, its GLUT-4 content and its glucose uptake (445).

A number of theories have been proposed for the exercise-induced increase in glucose uptake. Glucose uptake is inversely proportional to the ATP/ADP ratio and phosphocreatine concentration and directly

proportional to lactate concentrations (685). However, glucose uptake is even increased in the non-exercising leg during one-legged exercise in humans (4), which may be due to a change in muscle tone (306), since hyperpolarisation of a muscle segment results in an increase in glucose uptake (728) in that segment. One theory is that neural feedforward or metaboreceptor stimulation occurs, since the ventromedial hypothalamus (VMH) is involved with hormonal and metabolic regulation during exercise (658, 673) and electrical stimulation results in an increase in glucose uptake (460, 600). This mechanism of increased glucose uptake is independent of GLUT-4 transporter number or activity (600) and appears to involve the sympathetic nervous system (460). Another indication of neural involvement is that when arm exercise is added to leg exercise, leg glucose uptake decreases, presumably to limit glucose utilisation and avoid precipitating hypoglycaemia (550).

After exercise, there is an increase in the number of GLUT-4 transporters on the plasma membrane of rats (168, 169, 236, 238) and one study also demonstrated an increase in their turnover number, which is a measure of their intrinsic activity (236). As with insulin, this increase in glucose transporters is specific for GLUT-4 and does not involve GLUT-1. The major difference from the insulin-stimulated increase is that it does not involve translocation of GLUT-4 transporters from an intracellular pool (168, 169). However, McConnell et al. (429) found an inverse relationship between muscle GLUT-4 protein content at the end of exercise and glucose uptake during exercise.

The increase in blood flow which occurs during exercise has been proposed as one of the mechanisms by which glucose uptake is increased during exercise (10, 306, 590), although there is some disagreement (37) and it certainly does not explain how glucose uptake can still be increased 4 hours after exercise (551).

Because glucose uptake increases over the duration of exercise (258, 359), it has been suggested that this and increased glucose uptake after exercise is due to muscle glycogen depletion. Studies in isolated, perfused rat hindquarters have generally shown a negative association between muscle glycogen content and insulin-stimulated (195, 730) and non-insulin-stimulated (274, 510, 544, 652) glucose uptake except in one study (458), where insulin-stimulated glucose uptake was decreased in severely glycogen-depleted muscle and increased in muscle from rats that were carbohydrate-loaded when both were compared with controls. However, in a study in humans, there was no increase in glucose uptake as a result of glycogen depletion (257) probably because plasma insulin concentrations were lower with glycogen depletion than with normal glycogen content. Lower insulin concentrations may have counteracted the increased rates of glucose uptake seen with glycogen depletion in isolated, perfused rat hindquarters (195, 274, 510, 544, 652, 730).

Exercise training also results in an increase in glucose uptake compared with the untrained state (135, 147, 148, 559), though one study showed no effect of training (235) and in another (302) the observed

increase in glucose uptake was ascribed to an effect from the last exercise session (302). Conversely, in the study of Turcotte et al. (654), glucose uptake was higher in untrained than in trained subjects in the last hour of 3 hr of exercise at 60% of maximal dynamic knee extension capacity. Nevertheless, GLUT-4 transporter number increases in the sarcolemma (237, 559) and this is a true effect of training as it was demonstrated 2 and 5 days after the last training session (237). The reverse is also true. Immobilisation of a limb results in a decrease in glucose uptake at rest (549, 670) but not during exercise, while denervation results in a decrease in glucose uptake (94, 270, 281, 445) and GLUT-4 transporter number (94, 270, 445) and an increase in the number of insulin receptors, although these receptors are not functional (281).

EFFECT OF SERUM FREE FATTY ACID CONCENTRATIONS ON GLUCOSE UPTAKE

Most studies that have investigated the effect of FFA on glucose uptake have shown an inhibition of glucose uptake by FFA (55, 56, 256, 318, 353, 491, 683) both at rest (55, 56, 318, 353, 491, 683) and during exercise (256). In some studies, the inhibitory effect of circulating FFA was seen only under certain conditions, for example with hyperinsulinaemia and hyperglycaemia but not with hyperglycaemia and normal plasma insulin concentrations (196). In the study of Yki-Jarvinen et al. (719), forearm glucose uptake was increased by FFA in the basal state but suppressed in the hyperinsulinaemic state, which together with the data from the previous study (196) suggests that reversal of the insulin-induced suppression of FFA release into the circulation was the main reason for the suppression of glucose uptake. In another study, the inhibitory effect of FFA occurred during euglycaemia but not hyperglycaemia (684) and in the study of Wolfe et al. (707) there was no effect of FFA after glucose had been infused at a rate of 8 mg/kg/min overnight and during 4 hours of infusion of fat emulsion and heparin. These latter studies (684, 707) suggest that the effect of FFA on glucose uptake may depend on the availability of glucose.

EFFECT OF OTHER REGULATORY HORMONES ON GLUCOSE UPTAKE

Since counter-regulatory hormones are released during hypoglycaemia, it would be appropriate if they inhibited glucose uptake in order to preserve blood glucose concentrations. This is the case for epinephrine (26, 89, 305, 529, 677) and norepinephrine (401) and occurs by a direct inhibition of insulin-mediated glucose uptake (26, 89, 305) and an inhibition of insulin release from pancreatic β cells via α_2-adrenergic receptor stimulation (322, 323, 468, 567). An increase in serum growth hormone (203, 465, 466) or cortisol (25) concentrations decreases glucose uptake, though this may not be the case if growth hormone concentrations are chronically raised, as in acromegaly (333). In accordance with its role of increasing metabolic rate, thyroid hormone increases fuel supply to muscle cells by increasing insulin binding (163) and stimulating glucose uptake (163, 203).

Control of glycogen synthesis

GLYCOGEN SYNTHASE ACTIVITY RATIO

The classical theory on glycogen synthesis was that the rate-limiting enzyme is glycogen synthase (GSase) which exists in two forms, I and D, interconvertible by phosphorylation (208). The phosphorylated D form is inactive, except at what was considered unphysiologically high concentrations of glucose-6-phosphate (G-6-P) (139). Phosphorylation by a cAMP dependent protein kinase or other protein kinases like phosphorylase kinase (557), results in conversion of GSase I to D. Since phosphorylase kinase is a key enzyme in glycogenolysis which is activated via cAMP (98, 114, 422, 486) and Ca^{2+} (115, 117, 422, 502, 572), it was believed that glycogen synthesis is "switched off" during exercise. On the other hand, insulin (57, 301, 419, 493, 531, 597) or the cessation of exercise (102, 301, 511) results in an increase in the I form of GSase due to increased activity of the enzyme phosphoprotein phosphatase through a mechanism independent of cAMP (116, 301, 391), later proposed to be phosphoprotein phosphatase inhibitor 1 (149). Thus glycogen synthesis was regarded as being dependent on the activity of GSase expressed as the activity ratio [I/(I+D)]. Epinephrine increases the phosphorylation of GSase via β receptor stimulation (99, 305) and thus decreases the activity ratio of GSase (96, 103, 160, 305, 529, 597, 646).

INTERMEDIATE FORMS OF GLYCOGEN SYNTHASE AND THE FRACTIONAL VELOCITY

It was later demonstrated that glycogen synthase could exist in several intermediate phosphorylated forms with decreased activity ratios but enhanced sensitivity to G-6-P (371, 558). This led to the suggestion that the activation state of GSase be expressed as the fractional activity or velocity (FV_x), defined as the ratio of GSase activity at physiological G-6-P concentration x to the total activity at high G-6-P concentrations of >6 mM (252, 372).

ALLOSTERIC REGULATION OF GLYCOGEN SYNTHASE

The degree of phosphorylation of GSase influences its response to G-6-P (160, 558), thus the stimulatory effect of insulin on glycogen synthesis is greater than that of G-6-P. Conversely, G-6-P binds to GSase and exposes the phosphorylated sites to phosphatase action (90, 665), thus enhancing the effects of insulin and post-exercise stimulation of GSase. Bloch et al. (52) found that after exercise in rats, phosphorylation, which determines the ratio of glycogen synthase I to (I+D), and allosteric control by G-6-P contribute approximately equally to the rate of post-exercise glycogen synthesis. ATP, ADP, AMP, phosphocreatine and P_i are all inhibitors of GSase, regardless of its phosphorylation state, but this inhibition is overcome by G-6-P, making GSase I dependent on G-6-P (505, 506).

POST EXERCISE GLYCOGEN SYNTHESIS

In 1977, Ivy (301) measured resynthesis of muscle glycogen during the first 2 h after exhaustive swimming in rats and found that this phase was partly, but not totally, insulin dependent. An increase in insulin sensitivity and rate of muscle glycogen synthesis was also demonstrated in rats 30 min after a treadmill run (546). Studies on perfused rat skeletal muscle in 1984 (219, 547) showed that glucose uptake after exercise was biphasic (219). During the 2.5 h after a high intensity treadmill run, glucose uptake was increased even in the absence of insulin. However, after 2.5 h, when muscle glycogen content had returned to normal, glucose uptake was entirely insulin dependent. A similar study (547) with nerve simulation led to the conclusion that the first phase of glycogen resynthesis was modulated by local factors in the muscle. This biphasic nature of glucose uptake and glycogen resynthesis after exercise has also been demonstrated in human subjects using ^{13}C NMR, where it was found that when muscle glycogen concentration was <35 mmol/l, glycogen synthesis was independent of insulin, whereas above 35 mmol/l, glycogen synthesis was dependent on insulin (514). In this study (514), it was also noted that the rate of glycogen synthesis decreased 10-fold in the period from the first half-hour to after the first hour of exercise, whereas minor changes occur in GSase I/I+D and FV_x during this period (58, 725). Glycogen synthesis is enhanced if CHO is ingested during the post-exercise recovery period (617).

GLYCOGEN SYNTHESIS DURING EXERCISE

The discovery that phosphorylase kinase (557), converts GSase I to D led to the belief that glycogen synthesis and glycogenolysis were mutually exclusive processes. However, data now exist that show that resynthesis of glycogen also occurs during exercise. In a study by Constable et al. (128), glucose feeding in rats that started exercise in a glycogen depleted state resulted in an increase in muscle glycogen content during 90 min of exercise. In another rat study (297) in which labelled glucose was infused 10 min before the end of 90 min of exercise, it was found that the infused glucose was incorporated into muscle (with the exception of the soleus) and hepatic glycogen. Muscle glycogen resynthesis during exercise has also been shown to occur in trained humans (387), but not in untrained subjects (388).

Using ^{13}C-NMR, Price et al. (513) demonstrated that during low-intensity plantar flexions, muscle glycogen concentrations remained constant after an initial decline. However, when exercise was preceded by glycogen depletion, repletion took place during exercise at a greater rate than at rest until the same steady-state glycogen concentration was reached as in the non-depleted state. Conversely, at rest, the glycogen concentration continued to increase above the concentration in the non-depleted state. Bonen et al. (60) found that mild single-leg exercise (20% of $VO_{2\ max}$) with CHO ingestion resulted in diminished glycogen repletion in exercising and non-exercising muscle after exhaustive exercise, or in further glycogen loss after non-exhaustive exercise compared with rest after exercise.

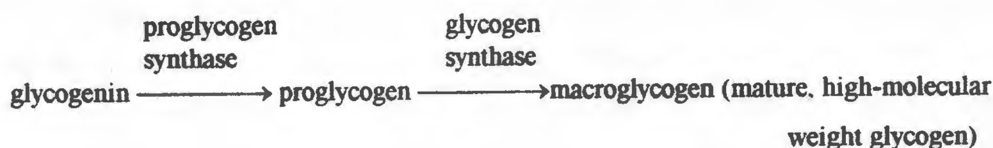
EFFECT OF INSULINAEMIA, GLYCAEMIA, AND GLUCOSE UPTAKE ON GLYCOGEN SYNTHESIS

Although relationships have been found between FV_x and the rate of glycogen synthesis with insulin stimulation (718) during the post-exercise period (19, 717, 725), there have also been several reports of a large change in muscle glycogen content without a substantial change in FV_x (382, 718) or of an increase in GSase FV_x without an increase in net muscle glycogen synthesis (564). In a study by Yki-Järvinen et al. (718) a glucose and insulin infusion which resulted in hyperglycaemia and an insulin concentration more than 10x that at which the maximal GSase FV_{140} was attained, resulted in a twofold increase in forearm glucose uptake compared with an insulin concentration at the maximal GSase FV_{140}. Similarly, in another study (418), an increase in plasma glucose concentrations from 6-12 mmol/l resulted in a doubling of glucose uptake without an increase in GSase FV_{100}. According to results from ^{13}C NMR studies in humans, under conditions of hyperglycaemia and hyperinsulinaemia at rest, almost 90% of glucose uptake is accounted for by muscle glycogen synthesis (605), confirming the results of several earlier studies in rats which had shown a relationship between glucose uptake and glycogen synthesis (307, 602). In transgenic mice in which the human GLUT 1 glucose transporter was overexpressed, a 6.6-7.4- fold increase in glucose transport compared with normal mice was associated with a 10-fold increase in glycogen concentrations in extensor digitorum longus and quadriceps muscle that was not associated with an increase in GSase or a decrease in glycogen phosphorylase activity (539). These discrepancies between GSase activity and the rate of muscle glycogen synthesis and the relationship between glucose uptake and the rate of glycogen synthesis (418, 539, 718) led to a proposal by Schulman et al. (607) of flux control. They proposed that with glucose transport and/or hexokinase (GT/HK) control the flux and covalent phosphorylation of GSase changes the sensitivity of GSase to G-6-P so that G-6-P does not have to rise to unphysiologically high concentrations to match the flux from GT/HK. A reduction in glucose uptake by epinephrine (305), or FFA may partly explain their inhibition of glycogen synthesis, although an increase in serum FFA concentration decrease GSase FV_x (56, 353).

GLYCOGENIN, PROGLYCOGEN AND MACROGLYCOGEN AND GLYCOGEN SYNTHESIS

In 1988, a different explanation was found for the dilemma of the apparent lack of association between glycogen synthase activity and the actual rate of glycogen synthesis. It was discovered that glycogen did not consist entirely of CHO, but contained a PROTEIN called glycogenin (409, 508) which was covalently attached to the glycogen molecule. It was found to be a self-glycosylating molecule with an oligosaccharide chain of variable length which could act as a primer for glycogen synthesis (508). Moreover, its priming glucosyltransferase activity was found to be unaffected by G-6-P concentration or phosphorylation of GSase (508). Lomako et al. (410) later found that glycogenin does not exist in free form in fresh muscle, but in a form with higher molecular mass which they called proglycogen (400 kDa), which breaks down spontaneously to glycogenin (38-39 kDa). Smythe

et al. (615) proposed that glycogenin was not bound to GSase in the glycogen molecule, but did form a 1:1 complex with GSase when free in the cytosol. Since only GSase that was complexed to glycogenin was capable of elongating the primer, there must be some regulatory factor in sarcoplasm controlling glycogen synthesis by modulating the association of GSase and glycogenin. The next discovery was an enzyme called proglycogen synthase (411) and the mechanism that was proposed was:



with proglycogen synthase as the rate-limiting enzyme. Glucose feeding of astrocytes has been found to stimulate G-6-P - mediated glycosylation of proglycogen (411) at a lower concentration of UDP-glucose than that required for stimulation of GSase. This stimulation of glycosylation by glucose feeding explains an increase in glycogen synthesis after increased glucose uptake, but without a corresponding increase in GSase activity, which has been observed in various studies (418, 539, 718).

Control of Glycogenolysis in muscle

REGULATION OF PHOSPHORYLASE BY cAMP AND Ca^{2+}

At rest, the metabolic rate of skeletal muscle is very low, but it is able to respond rapidly to the increased energy demand of exercise (98). Breakdown of muscle glycogen is catalysed by two enzymes, debranching enzyme and glycogen phosphorylase (98, 132). Phosphorylase is the rate-limiting step of muscle glycogenolysis and exists in two forms, *a* and *b*. Phosphorylase *b* is largely inactive in resting muscle and is inhibited by glucose-6-phosphate and ATP, but is active in the presence of AMP (98). Phosphorylase *a* is active in the absence of AMP (131, 165) and is not affected significantly by ATP and glucose-6-phosphate. Phosphorylase *b* is converted to the '*a*' form by reversible phosphorylation by the enzyme phosphorylase kinase which itself is activated by phosphorylation by protein kinase A. Protein kinase A is activated by cAMP and thus is regulated by hormones which utilise cAMP as a second messenger, namely catecholamines and glucagon (98, 114, 422, 486). Transformation of phosphorylase *b* to *a* is also increased by Ca^{2+} binding to the calmodulin subunit of phosphorylase kinase (115, 117, 422, 502, 572). At the onset of muscle contraction, calcium released from the sarcoplasmic reticulum activates phosphorylase kinase (536). This activation, however, reverses after a short time (118, 259, 533, 535, 537), probably due to a decrease in calcium release (295) and inhibition of phosphorylase kinase as the pH of the muscle decreases (101).

CONTROL OF MUSCLE GLYCOGENOLYSIS BY EPINEPHRINE STIMULATION AND ALLOSTERIC EFFECTS

Epinephrine stimulates glycogenolysis (2, 11, 182, 244, 305, 313, 325, 529, 545, 628, 680, 704) via α and β-adrenergic receptors (553). However, Richter et al. reported in 1982 (554) that muscle contractions could stimulate glycogenolysis for a short time in isolated, perfused rat muscle, but epinephrine was needed for sustained glycogenolysis. Numerous studies have subsequently demonstrated a dual control of muscle glycogenolysis by muscle contraction and epinephrine (59, 98, 259, 420). Chasiotis et al. (98, 102) demonstrated a positive allosteric affect of inorganic phosphate (P_i) on phosphorylase *a*. It had been previously thought that the rate of muscle glycogenolysis was dependent on the ratio of phosphorylase *a* to *b* (140, 141, 269, 334), but it was noted in this study (102) and another (103) that although as much as 20% of phosphorylase in resting muscle was in the '*a*' form, the rate of glycogenolysis was low. Moreover, during epinephrine infusion in resting subjects, 80-90% of phosphorylase was in the *a* form, yet the rate of glycogenolysis was still low (98, 103). A dissociation between the percentage of phosphorylase in the *a* form and the rate of glycogenolysis has also been noted in other studies (226, 537). This may be due to low concentrations of P_i in resting muscle, which is rate-limiting for phosphorylase *a* even with epinephrine infusion. During exercise, P_i rises to 20-30 mmol/l, which is in the region of the Michaelis constant for P_i on phosphorylase *a* in the absence of AMP (27 mmol/l) (98, 102).

The allosteric activation of phosphorylase by P_i (98, 100, 102, 259, 534, 535, 623), AMP (245, 259, 535) and IMP (245, 259, 623) explains the sustained glycogenolysis which occurs during muscle contraction (98, 100, 102, 259, 534, 535, 623) and the observation that the rate of muscle glycogen utilisation increases with increasing exercise intensity (18, 54, 210, 226, 444, 538, 676). The latter is also due to the different recruitment of muscle fibre types with increasing exercise intensity (646, 675, 676). Fast twitch fibres have a higher content of phosphorylase *a* and *b*, a greater transformation of phosphorylase into the *a* form during contraction and a higher content of creatine phosphate than slow twitch fibres in rats (100) and these differences are associated with a higher rate of glycogenolysis in fast twitch muscle (244, 676).

EFFECT OF MUSCLE GLYCOGEN CONTENT ON MUSCLE GLYCOGENOLYSIS

Any reaction catalysed by an enzyme is dependent on the substrate concentration and glycogen phosphorylase is no exception. The rate of glycogenolysis is higher when muscle glycogen content at the start of exercise is high (43, 65, 230, 257, 259, 274, 275, 379, 393, 429, 544, 595, 622, 672) and in rats (275), the activity of phosphorylase *a* is greater when muscle glycogen concentrations are higher. Some studies (533, 621) have shown no effect of starting muscle glycogen content on the rate of glycogenolysis, but glycogen content at the start of exercise in these studies was low and the rate of glycogenolysis does not appear to be directly related to muscle content when muscle glycogen concentrations are below 70-80 mmol/kg ww (65, 533). Since rate of net muscle glycogen utilisation

is dependent on the balance of activity between glycogen phosphorylase and glycogen synthase (222, 669), the reduced rate of glycogenolysis described may be due to increased activity of glycogen synthase when muscle glycogen content is low (513, 717). The effect of muscle glycogen content also appears to be diminished at very high exercise intensities, since Bangsbo et al. (21) found in a study in which one leg was carbohydrate-loaded and the other not, that there was no difference in glycogen utilisation between the glycogen loaded leg and non loaded leg during supramaximal exercise.

CONTROVERSY AROUND THE EFFECT OF FREE FATTY ACIDS ON MUSCLE GLYCOGENOLYSIS

The effect of an increase in plasma FFA concentrations on muscle glycogenolysis during exercise is controversial. In some studies in rats (165, 384, 542) a glycogen sparing effect was observed with increased plasma FFA concentrations, but in two of these studies, the rats were fasted (165, 384) and the rate of glycogenolysis may have been decreased for the same reason as discussed above, namely a reduced availability of glycogen, rather than an increased availability of FFA. This possibility is supported by other studies (12, 256) where FFA were acutely raised during exercise in human subjects by infusion of triglyceride emulsion (Intralipid) and heparin and by caffeine injections in overnight fasted rats (12). Increased FFA did not inhibit muscle glycogenolysis in either of these studies. However another rat study (542) did show a glycogen sparing effect when FFA were raised by feeding corn oil and injecting heparin before exercise. Dyck et al. (179) also showed glycogen sparing in human subjects during 15 minutes of exercise at 85% of $VO2_{max}$. The protocol used by Dyck et al. (179) was somewhat different from that used by Hargreaves et al. (256) in that the subjects ate a high CHO meal 2-4 hours before the trial and Intralipid infusion commenced 30 minutes before exercise. Heparin was given 30 (1000 IU) and 15 (500 IU) minutes before and at the start of exercise (500 IU) and the rate of Intralipid infusion was increased from 1.7 ml/min at rest to 3 ml/min during exercise. In the study by Hargreaves et al. (256), subjects were overnight fasted and exercised one leg at 80% of knee-extensor maximum work capacity for an hour, then rested for 50-70 minutes before the first heparin bolus and commencement of the Intralipid infusion 30 minutes before a second exercise bout at the same intensity using the other leg. During the hour of exercise, 1000 IU of heparin was administered and the Intralipid infusion rate remained constant at 1.7 ml/min during rest and exercise. The major differences between these trials were thus the pre-trial feeding and exercise and the Intralipid infusion rate during exercise and either the feeding or the higher FFA concentrations may have resulted in glycogen sparing during this short exercise duration.

Inhibition of lipolysis with nicotinic acid does lower FFA concentrations and increase the rate of glycogenolysis (44, 126, 230, 283, 500, 630) but this is not surprising, since this procedure forces a total reliance on CHO during exercise, whereas under more physiological conditions, oxidation of plasma FFA and muscle triglycerides still supplies roughly 28% of total energy at 85% of $VO_{2\ max}$ (563).

Intracellular control of muscle metabolism

Most of the energy required for muscle contraction is generated by the hydrolysis of ATP (290, 416) and its resynthesis during high intensity exercise is from PCr (104, 121, 273, 282, 283, 290, 294, 575, 580, 612) and muscle glycogenolysis (104, 186, 227, 273, 283, 290, 444, 507, 563). Breakdown of glucose or G-6-P by glycolysis to pyruvate and lactate is a rapid means of generating ATP for high intensity exercise of short duration (79). Glycolysis results in a rapid production of lactate (104, 273, 283), which may be released from the muscle (38, 79) or oxidised (38, 79, 436), or may accumulate in the muscle (121). Muscle ATP content decreases during sprinting (61) and isometric exercise (342), though some studies have shown that changes in ATP during high intensity exercise are small (273). Muscle pH declines (86, 273, 283, 627) during high intensity exercise, which inhibits glycolysis (273, 638). If glycolysis is blocked and contraction persists, muscle ATP, total adenine nucleotides (576) and muscle lactate concentrations (638) decrease.

Glycolysis is inefficient in terms of mmol ATP/mol of glucose produced and the first step in the release of the remaining energy is the conversion of pyruvate to acetyl- CoA, which is also formed from the oxidation of fatty acids particularly during sustained exercise (228, 416). Acetyl-CoA is oxidatively decarboxylated in the tricarboxylic acid cycle producing CO_2 and the reduced forms of pyridine and flavin nucleotides. The hydrogen atoms of the reduced nucleotides are transported in the form of electrons through the electron transport (respiratory) chain to molecular O_2 to form H_2O. The resulting free energy change is used to phosphorylate ADP to ATP. Enzymes of fatty acid oxidation, tricarboxylic acid cycle and respiration are located inside mitochondria (416).

The two main fuels for oxidative metabolism are carbohydrate and fat (186, 189, 498). At exercise intensities between 70 and 100% $VO_{2\ max}$, carbohydrate is the main fuel after the intake of normal (mixed) or high-carbohydrate diets (287). There are a number of limitations to the usefulness of fat as a fuel, particularly at higher exercise intensities: i) the maximum rates of ATP production are ~100% higher from CHO than fat (287); ii) the time taken to reach maximal ATP production rate is longer for fat than CHO (573) and iii) the ATP yield per mol of O_2 is lower when FFA are oxidised than when glucose is oxidised (287).

The fuel selection of muscle fibres at rest is dependent on substrate availability (287). However, during exercise, fuel selection is dependent on the availability of fuels, training status and the intensity and duration of exercise, all which have been discussed previously in this review.

CREATINE PHOSPHATE

The most immediate source of energy for contracting muscle besides ATP itself is creatine phosphate (phosphocreatine, PCr) (288). The degradation of PCr begins at the onset of exercise at the same time

as (42, 210, 287) or slightly before (293) glycolysis is accelerated, though the degree of stimulation of PCr degradation depends on the muscle fibre type (291), since fast twitch muscle fibres have a higher content of PCr than slow twitch fibres (181). Although PCr degradation occurs at exercise intensities as low as 40% of $VO_{2\,max}$ (580), a higher rate of degradation takes place at high exercise intensities (104, 121, 273, 282, 294, 575, 580, 612). However, the amount of PCr in muscle is fairly small and in human subjects at a stimulation frequency and voltage adjusted to produce an initial tension of 50-75% of the maximum voluntary contraction force, the PCr store decreased exponentially during contraction and was practically depleted after 50 seconds (293).

CONTROL OF GLYCOLYSIS

The starting point of glycolysis is glucose-6-phosphate (G-6-P). The product of glycogenolysis is glucose-1-phosphate, which is converted to (G-6-P) by phosphoglucomutase (666). Glucose 1,6 bisphosphate (G-1,6-P_2) is formed as in intermediate step in this reaction, through a side reaction of phosphoglucomutase with fructose 1,6 bisphosphate or with 1,3-diposphoglycerate or through a side reaction of phosphofructokinase with glucose-1-phosphate and ATP and is an important regulatory molecule (34, 574). Because phosphoglucomutase is the link between glycogenolysis and glycolysis, its concentration in muscle is regarded as being representative of the capacity of a tissue for glycogen metabolism (34). The other major source of G-6-P is glucose from plasma or interstitial fluid which enters the cell and is phosphorylated by hexokinase, which as previously discussed, is rate-limiting for glucose uptake (264, 339, 341, 539). Two other major points of regulation of carbohydrate metabolism are the reactions catalysed by phosphofructokinase (PFK) and the pyruvate dehydrogenase complex (PDH).

Glucose 1,6 bisphosphate

G-1,6-P_2 is a potent stimulator of phosphoglucomutase, phosphofructokinase (PFK) and pyruvate kinase and an inhibitor of hexokinase, hepatic fructose 1,6 bisphosphatase, and 6-phosphogluconate dehydrogenase, an enzyme involved in the pentose phosphate pathway (34). Thus an increase in G-1,6-P_2 concentration in muscle would promote glycolysis (336) and inhibit glucose uptake and a decrease would inhibit glycolysis and increase glucose uptake. A strong positive relationship has also been observed between muscle G-1,6-P_2 content and the mean rate of CHO oxidation during hyperinsulinaemia with euglycaemia (336).

Formation of G-1,6-P_2 is increased by cAMP and decreased by cyclic GMP (cGMP) and Ca^{2+}. It is increased by β-adrenergic stimulation (via cAMP) (34, 701) but decreased by α-adrenergic stimulation, serotonin, bradykinin and phospholipase A_2 (34). The concentration of the enzyme which degrades G-1,6-P_2, glucose-1,6-bisphosphatase is increased by Ca^{2+}, calmodulin and cGMP and decreased by cAMP (34).

During fasting (34) or insulin-induced hypoglycaemia (700), G-1,6-P_2 concentrations decrease in the liver, promoting gluconeogenesis, and increase in skeletal muscle, promoting glycolysis and lactate release (700) and decreasing glucose uptake (336). In exercising fasted rats (702), the increase in G-1,6-P_2 in skeletal muscle was observed only in fast-twitch muscle. However if muscle glycogen content is low, G-1,6-P_2 concentrations are low in skeletal muscle both at rest and following exercise (621). G-1,6-P_2 content is decreased in skeletal muscle of rats after exercise training (174) and probably plays a role in both the decreased utilisation of muscle glycogen which occurs after training, since the decrease in glycolytic flux with training occurs before PFK in the glycolytic pathway (113), and in the decrease in RER that has been observed after a low CHO diet. The latter concept is supported by the finding that muscle content of G-1,6-P_2 is low in patients with muscle phosphorylase deficiency (McArdle's disease (McAD)) (715) a disorder in which RER is low in relation to exercise intensity (402).

An increase in insulin results in an increase in G-1,6-P_2 in rat diaphragm muscle in a process involving calmodulin (105), though as discussed earlier, calmodulin is also associated with a decrease in G-1,6-P_2. Skeletal muscle G-1,6-P_2 content is also increased in human subjects during euglycaemic hyperinsulinaemia at rest (125, 336, 338) especially when circulating epinephrine concentrations are also raised (529), but not during hyperglycaemia at rest when hyperinsulinaemia is prevented by simultaneous infusion of somatostatin (339).

The influence of G-1,6-P_2 on glycolysis and glucose uptake during exercise is less clear cut. During the first 5 minutes of submaximal exercise (75% of $VO_{2\,max}$) in the study of Katz et al. (341) in human subjects, G-1,6-P_2 and G-6-P increased, but thereafter both decreased continuously. However, in an earlier study (343) no relationship was seen during exercise at 40 and 75% of $VO_{2\,max}$ between changes in muscle G-1,6-P_2 content and flux through PFK or hexokinase. However, this observation may be due to the multiple factors controlling the activity of these enzymes as will be discussed subsequently in this review, since another study using electrically stimulated rat skeletal muscle did show a relationship between intramuscular G-1,6-P_2 and lactate concentrations (29). Paradoxically, during chronic low frequency stimulation of rabbit muscle (240) G-1,6-P_2 increased from 3 to 12-24 hours of stimulation, which coincided with glycogen resynthesis, despite the inhibitory effect of increased G-1,6-P_2 on glucose uptake.

Hexokinase

Hexokinase (HK) consists of a family of isoenzymes I to IV of which type IV has the highest K_m for glucose and is called glucokinase. It is found in the hepatic parenchymal cells and the pancreatic β cells (694). Type II HK is found in skeletal muscle and other insulin-dependent tissues, although type I may be of significance in human muscle (561). HK is mostly bound to the outer mitochondrial membrane (464, 613, 694, 713) and appears to preferentially utilise mitochondrially generated ATP in its phosphorylation of glucose (36). Mg^{2+}ATP is required as an intermediate in its action and it is

inhibited by ADP (339), G-6-P (264, 305, 337, 341, 509, 694) and G-1,6-P_2 (34) The latter two molecules also cause dissociation of HK from the mitochondrial membrane, which increases its susceptibility to their inhibition. The inhibitory effect of G-6-P and G-1,6-P_2, is antagonised by P_i in type I, but to a much lesser extent in type II HK (694). During the first 5 minutes of submaximal exercise (75% of $VO_{2\ max}$), glucose accumulates intracellularly, but then, as concentrations of G-6-P and G-1,6-P_2 (694) and PCr (541) decrease, HK activity increases and intracellular free glucose concentrations decrease (341). During chronic, low intensity stimulation of rat muscle, HK binding to mitochondria increases at the onset of exercise, but decreases after 3 weeks of stimulation, probably because of a shift to lipid oxidation (689). High-intensity exercise is associated with inhibition of HK and intracellular glucose accumulation.

Muscle HK content increases with training (85, 271, 400, 540) and this is an early adaptation, as it occurs after only 24 (85) to 48 hours (540). Insulin stimulation increases mitochondrially bound HK through a calcium-calmodulin-dependent process (105), and also increases transcription of the HK gene (664).

Phosphofructokinase and fructose 2,6 bisphosphate

The irreversible phosphorylation of fructose-6-phosphate by PFK is one of the rate-limiting steps in glycolysis (187, 645) and a deficiency of this enzyme causes a decrease in exercise tolerance (403, 407) and low RER (407) during exercise. Not surprisingly, the activity of PFK is greater in glycolytic than in oxidative muscle fibres (623). The control of PFK activity is complex, involving stimulation and inhibition by a variety of molecules, largely related to energy state and concentrations of substrate and product. Its activity is also dependent on whether it is bound to cytoskeletal actin (35). Thus PFK activity is increased by its substrate, fructose-6-phosphate, ADP (284, 621, 623, 624), AMP (284, 443, 621, 624) and P_i (119, 284, 624) and decreased by ATP (284, 629, 663), 3-phosphoglycerate (284) and citrate (30, 284). Paradoxically, PFK is also stimulated by its product, fructose-1,6-bisphosphate (9, 30, 284, 504, 624, 648), though not in the presence of physiological concentrations of AMP and P_i (503). Stimulation of PFK by G-1,6-P_2 has been discussed in a previous section.

The most potent stimulator of PFK is fructose-2,6-bisphosphate (F-2,6-P_2) (284, 443, 504, 648, 661, 663). This molecule, which also inhibits fructose-1,6-bisphosphatase and thus gluconeogenesis in the liver (662), has a greater affinity for PFK than fructose 1,6 bisphosphate and both these have greater affinity than G-1,6-P_2. All 3 of these molecules compete for the same binding site on PFK and are thus antagonistic to one another (201, 663). F-2,6-P_2 is formed from and degraded to fructose-6-phosphate by an enzyme that has dual activity, depending on its phosphorylation state (284, 660). In its dephosphorylated state (phosphofructokinase II), the enzyme catalyses formation of F-2,6-P_2 from frucose-6-phosphate and ATP, while the phosphorylated form of the enzyme (fructose-2,6-bisphosphatase) catalyses dephosphorylation of F-2,6-P_2. Phosphorylation of the enzyme is controlled

by a cAMP-dependent protein kinase (659). Formation of F-2,6-P_2 in the liver is stimulated by insulin and α-adrenergic stimulation and inhibited by citrate, phosphoenolpyruvate, 3-phosphoglycerate, β-adrenergic stimulation and glucagon. Dephosphorylation of F-2,6-P_2 is stimulated by ATP, 3-phosphoglycerate, β-adrenergic stimulation and glucagon and inhibited by fructose-6-phosphate (284).

In the liver, F-2,6-P_2 plays a vital role in the co-ordination of glycolysis and gluconeogenesis (284). In muscle, the control of and effects of F-2,6-P_2 are less clear. One study has shown no relationship between the intramuscular concentrations of F-2,6-P_2 and the rate of glycolysis (459). However, Hue et al. (285) demonstrated a stimulation of lactate production, but a decrease in F-2,6-P_2 during electrical stimulation of a perfused rat hindlimb. More recently, Winder et al. (702) showed a decrease in F-2,6-P_2 in the liver, but an increase in fast-twitch muscles and no change in slow-twitch muscles in rats during exercise after fasting, indicating a role in glycolytic muscle fibres of providing gluconeogenic substrate (80).

Pyruvate kinase/ oxaloacetate decarboxylase and lactate dehydrogenase

Pyruvate kinase (PK) catalyses the irreversible (under normal physiological conditions) conversion of phosphoenolpyruvate (PEP) to pyruvate (224).

Pyruvate kinase is associated with the sarcoplasmic reticulum (714) and the activity of oxaloacetate decarboxylase is also inherent in the same protein (136, 137, 171, 329). PK requires Mg^{2+}ATP as a cofactor (170, 470), is a cAMP dependent molecule and is activated by F-1,6-P_2 (91, 106, 726), PEP (91, 449), Mg^{2+}(91, 470) and P_i (23). Phenylalanine is a competitive inhibitor of PEP for activation of PK. The activity of PK is decreased after fasting.

The interconversion of pyruvate and lactate is a fully reversible reaction, catalysed by lactate dehydrogenase (LDH). An increase in the concentration of pyruvate and particularly in NADH drives the reaction in the direction of lactate formation. Lactate production in muscle occurs under fully aerobic conditions (120, 340) and the concentration of lactate in muscle is proportional to the glycolytic carbon flow (78). Factors that favour lactate production include: i) the V_{max} of LDH, which is several times greater than the combined activities of enzymes involved in alternative pathways of pyruvate metabolism; ii) the K_m of LDH for pyruvate, which is sufficiently low to ensure maximal stimulation of LDH in the conversion of pyruvate to lactate; and iii) the K_{eq} of pyruvate to lactate conversion, which exceeds 1000.

The pyruvate dehydrogenase complex

The pyruvate dehydrogenase complex (PDH) is a multi-enzyme complex that catalyses the irreversible oxidative decarboxylation of pyruvate to acetyl-CoA and as such regulates the rate of carbohydrate oxidation (152, 287, 497). It is activated (PDHa) by dephosphorylation (262, 263, 569), which is

catalysed by the enzyme PDH phosphatase (398, 525) and inactivated by phosphorylation which is catalysed by PDH kinase (251, 261, 263, 512). PDH phosphatase in turn is activated in vitro by an increase in NAD^+ (287), Ca^{2+} (151, 153, 287, 398, 430, 432, 435, 477, 569) and Mg^{2+} (152, 287, 398) and inhibited by NADH (287), while PDH kinase is activated by acetyl-CoA, NADH (152, 287) and an increase in the acetyl CoA/CoASH concentration ratio (287) and inhibited by pyruvate (152), ADP (152, 287), NAD^+ and CoASH (287). Epinephrine (155, 431) (via α_1 receptors (16)), glucagon (16, 431) and insulin (17) all increase PDH activity. Epinephrine and glucagon may both increase intramitochondrial calcium concentrations (16) and possibly use inositol trisphosphate as a second messenger (373).

During muscle contraction, cytoplasmic and mitochondrial Ca^{2+} concentrations increase (151, 153, 154, 432, 433, 536), phosphocreatine is degraded and P_i released (210, 580), ADP and AMP are formed (327) and glycogenolysis is activated as discussed earlier, resulting in an increased formation of pyruvate. All these are factors that activate PDH and thus not surprisingly, its activity rises during exercise (129, 179, 516), with a five- to sevenfold increase having been observed during exercise at 85% of $VO_{2\ max}$ (179). After only 3 minutes of exercise at 75% of $VO_{2\ max}$, there is almost total transformation of PDH into the active form and at this exercise intensity, it was not inhibited by an increase in acetyl CoA/CoASH concentration ratio (130).

Activation of PDH varies with exercise type and intensity (687). In this study (687), 40% of the enzyme was in the active form at rest, 88% after progressive aerobic exercise to exhaustion, 60% after intermittent supramaximal short-term exercise, and 39% after isometric maximal exercise of 65 s duration. Muscle glycogen depletion was greatest with intermittent exercise and least with isometric maximal exercise, but the increase in muscle lactate was least with progressive exercise, intermediate in intermittent maximal exercise and greatest after isometric exercise. During incremental dynamic exercise where subjects exercised for 3-4 minutes on a bicycle ergometer at work loads corresponding to 30, 60 and 90% of their $VO_{2\ max}$, the active form of pyruvate dehydrogenase complex increased progressively from rest to 90% of $VO_{2\ max}$, there was a continuous increase in muscle lactate and both acetyl-CoA and acetylcarnitine increased at the two highest work loads, with a corresponding fall in CoASH and free carnitine contents (129). In another study on intermittent maximal exercise (516), glycolytic flux was 13-fold greater than PDHa, resulting in substantial lactate accumulation, despite an increase in mitochondrial oxidation state as reflected by a fall in mitochondrial NADH/NAD. PDHa remained high during recovery and was probably facilitated by lower ATP/ADP and NADH/NAD and increased concentrations of pyruvate and H^+, resulting in continued oxidation of the lactate load between exercise bouts. Exercise training results in a greater ratio of active to total enzyme compared with the untrained state (687).

The influence of substrate availability on PDH was first suggested by Randle et al. (526) in 1963, when they presented experimental evidence that an increased availability of FFA resulted in

accumulation of acetyl CoA and NADH, which caused inhibition of PDH in rat hearts. They therefore proposed that if FFA were available to skeletal muscle in increased concentrations, CHO oxidation may be inhibited. However, with the exception of one study in which PDHa was inhibited by exogenous FFA during hyperinsulinaemia at rest (353), PDH in skeletal muscle does not appear to be influenced by increasing (179, 582) or decreasing (319, 656) the availability of FFA by infusion of Intralipid or inhibition of lipolysis respectively, either at rest (319, 582, 656) or exercise (179), despite an increase in glucose (582, 656) or a decrease in lipid oxidation (319) with decreasing FFA availability.

In contrast, PDH decreases during fasting or starvation (262, 263, 383, 434, 637). When PDHa and acetyl group accumulation were examined in human skeletal muscle at rest and during exercise after different diets (518), resting PDHa was lower after a low CHO diet and this coincided with a greater intramuscular acetyl-CoA/CoASH ratio, acetyl-CoA content, and acetylcarnitine content. PDHa increased during exercise at 75% of $VO_{2\ max}$ in both conditions but at a lower rate after the low CHO diet than after the high CHO diet. During exercise, muscle acetyl-CoA and acetylcarnitine content and the acetyl-CoA/CoASH ratio decreased in the low CHO diet condition but increased in the high CHO diet condition. Pyruvate dehydrogenase activity, acetyl group, and citrate accumulation were also examined (517) in human skeletal muscle while 400 mmol of sodium acetate was infused at a constant rate during 20 min of rest, 5 min of cycling at 40% of $VO_{2\ max}$ and 15 min of cycling at 80% of $VO_{2\ max}$. At rest PDHa was regulated by variations in acetyl-CoA/CoASH secondary to enhanced acetate metabolism. Conversely, during exercise PDHa regulation appeared independent of variations in acetyl-CoA/CoASH. In a study by Mandarino et al. (418), PDHa was increased during hyperglycaemia in resting subjects. When the evidence from these studies (179, 262, 263, 319, 383, 418, 434, 517, 518, 582, 637, 656) is considered together, it becomes apparent that at least at rest, PDHa is influenced not by an increased or decreased availability of fat, but by the availability of CHO.

BETA OXIDATION OF FATTY ACIDS

Fatty acids undergo beta oxidation in the mitochondria to acetyl-CoA (287). Earlier studies indicated that fatty acid uptake into muscle cells is not rate-limiting for FFA oxidation and that the rate of FFA oxidation is proportional to the plasma FFA concentration (249), but more recently, it has been shown that fatty acid uptake into muscle cells is saturable (653), though it is increased by exercise training (583). Medium-chain triglycerides can cross the mitochondrial membrane (204, 209, 438), but longer chain fatty acids must first be activated in sarcoplasm by coenzyme A and transferred into the mitochondria by way of a carnitine-dependent shuttle mechanism (73, 204, 287). The enzyme which catalyses the conversion of a fatty acyl CoA to acylcarnitine is carnitine acyltransferase (204, 209) and since palmitate is the most abundant FFA in mammalian tissue, the reaction catalysed by carnitine palmitoyltransferase (CPT-1) is the rate-limiting step for oxidation of FFA (73, 204, 408, 437, 439, 698). If CPT-1 is inhibited in rats and a greater dependence on FFA oxidation is induced by fasting or

exercise, glycolysis is stimulated and lipid oxidation reduced compared with controls (408). However, CPT-1 is not directly stimulated by high FFA during a hyperinsulinaemic, euglycaemic clamp even though lipid oxidation increases (582). There is also no difference in rate of glucose oxidation between rats with CPT-1 blockade and controls when euglycaemia is maintained and CHO supply is thus not limited (319). β oxidation in the heart and probably also in skeletal muscle is also controlled by the energy demand, since 2 enzymes in the pathway, 3-hydroxyacyl CoA dehydrogenase and 3-ketoacyl CoA thiolase are inhibited by NADH and acetyl CoA, respectively (686).

Malonyl CoA is the committed step in fat synthesis (204, 440), but it is also an important controller of FFA oxidation (440, 442), since it is a potent inhibitor of CPT-1 (440, 442). Malonyl CoA is synthesised from acetyl CoA and HCO_3^- by a cyclic AMP-dependent enzyme, acetyl CoA carboxylase. In the liver, glucagon stimulation results firstly in phosphorylation and inactivation of acetyl CoA carboxylase (204, 439, 442) and secondly in inhibition of glycolysis, resulting in a reduction in availability of acetyl CoA (70, 442). The combined effect is a decrease in malonyl CoA concentrations, relieving inhibition of CPT-1 and stimulating fatty acid oxidation (204, 442). Thus the effect of starvation, which results in an increase in plasma glucagon concentrations, is to stimulate β oxidation and inhibit synthesis of FFA in the liver (437, 438). Conversely, insulin stimulation results in dephosphorylation and activation of acetyl CoA carboxylase (64, 82, 414, 705, 706), thus inhibiting oxidation and promoting synthesis of FFA. Oxidation of plasma FFA appears to be more sensitive to suppression by insulin than is total lipid oxidation (248).

Malonyl CoA and acetyl CoA carboxylase are also found in muscle (650, 651). In rats, malonyl CoA concentrations in muscle decrease during muscle contraction (172, 625, 697, 698), occurring 20 minutes and 30 minutes after the onset of muscle contraction in fast and slow twitch muscle fibres respectively (698). This has been shown to be independent of any humoral influence (699, 703) and independent of cAMP (415). The decrease in malonyl CoA in rats during muscle contraction is attenuated by glucose infusion (184), but insulin is also needed to prevent the decrease (173). It has recently been shown (492) that malonyl CoA concentrations in human muscle are lower than in rats and do not decrease significantly during 70 minutes of exercise at 65% of $VO_{2\ max}$, though as in previous studies (172, 625, 697, 698), there was a significant decline in rats. However, this does not completely rule out a similar control of FFA oxidation in muscle of humans since the K_m for malonyl CoA for inhibition of CPT-1 varies between species and between tissues within the same species (441) and some chemically related compounds have a similar effect on FFA oxidation as malonyl CoA (441). The results of the study in humans (492) may also be have been influenced by the fact that the subjects in the study ingested a high- carbohydrate meal 2-4 hours before exercise, which may have increased plasma glucose and insulin concentrations.

The muscle concentrations of acetyl-CoA and acetylcarnitine increase during exercise in humans (129, 130, 179) and rats (625), whereas infusion of Intralipid in humans does not affect acetyl-CoA concentrations at rest or during exercise (179). In rats (625), perfusion of hindquarter muscles with

high FFA concentrations increased acetyl-CoA and acetylcarnitine compared with fat-free perfusion which had no effect. During stimulation, however, there was no effect of increased FFA on acetyl-CoA and acetylcarnitine. At submaximal exercise intensities, the increase in acetyl-CoA is less than the increase in acetylcarnitine (130, 179), but during incremental exercise in one study (129), there was a close relationship between acetyl-CoA and acetylcarnitine accumulation in muscle during exercise, with a binding of approximately 500 mol acetyl groups to carnitine for each mole of acetyl-CoA accumulated. It was suggested that the carnitine store in muscle functions as a buffer for excess formation of acetyl groups from pyruvate catalysed by the pyruvate dehydrogenase complex. Chronic muscle stimulation in rabbits results in increases in total cellular activities of enzymes of β oxidation (3-hydroxyacyl- CoA dehydrogenase, carnitine palmitoyl-CoA transferase, 3-keto-acyl-CoA thiolase) as well as citrate synthase, NADH-cytochrome c oxidoreductase, succinate-cytochrome c oxidoreductase, and cytochrome c oxidase (530). This increased capacity for oxidation of FFA may partly explain the increase in lipid oxidation that occurs with exercise training (14, 112, 296, 314, 354, 374, 385, 524, 556, 654).

REGULATION OF THE TRICARBOXYLIC ACID CYCLE AND OXIDATIVE PHOSPHORYLATION

The tricarboxylic acid (TCA) cycle, coupled with oxidative phosphorylation, is the dominant source of ATP (403). The main regulatory sites of the TCA cycle are isocitrate dehydrogenase and α-ketoglutarate dehydrogenase (152). Both are inhibited by increased ratios of ATP/ADP and $NADH/NAD^+$ and stimulated by NAD^+. As with PDH, calcium is a significant activator of both these enzymes (151, 153, 154, 430, 432, 433, 477). The rate of oxidative phosphorylation is regulated mainly by intramitochondrial ADP concentrations (20, 202, 221, 390) or the ATP/ADP+P_i ratio, as well as by substrate supply ($NADH/NAD^+$) and availability of O_2 (693), which has been shown in dogs to be limiting during maximal exercise (176).

The effect of training on oxidative metabolism is further evidenced by increases in citrate synthase (CS) (48, 113, 220, 235, 260, 271, 272, 280, 355), succinate dehydrogenase (SDH) (167, 260, 272, 560), malate dehydrogenase (MDH) (260, 271) and cytochrome c oxidase (220, 308, 475) activities. An increase in SDH and CS was observed after 10 days of chronic, low grade muscle stimulation in rats (642), but there was no effect of exercise training on concentrations of muscle mitochondrial oxidative enzymes in humans after 10-12 days (243), although changes had taken place by 4 weeks (242). Chronic β-adrenergic stimulation has also been shown to increase concentrations of CS, MDH, and SDH in muscle (260), but neither epinephrine nor sympathetic nervous stimulation are associated with the training effect on the oxidative enzymes (272).

MUSCLE GLYCOGEN DEPLETION AND METABOLIC DISEASES-CONSEQUENCES OF INADEQUATE CARBOHYDRATE OXIDATION

The changes in metabolic hormones, plasma lactate, FFA, glycerol and ketones that occur as a result of fasting or a pre-exercise diet which is low in CHO have been described earlier in this review, and may be as a result of the changes that occur within the muscle when oxidative metabolism is blocked or when carbohydrate substrate supply is limited. Blockade of glycolysis results in a decrease in total adenine nucleotide (TAN) content of muscle, ATP (576) and lactate (638). Blockade at PFK as in PFK deficiency results in accumulation of glucose-1-phosphate, an increase in F-2,6-P_2 and a decrease in G-1,6-P_2 (715). There are many similarities in the metabolite concentrations in glycogen depleted and phosphorylase deficient muscle and these are summarised in Table 2.1 with respect to normal, matched controls in the case of McArdle's disease (McAD) and subjects with normal or high muscle glycogen content in the case of glycogen depletion.

A problem with measuring these metabolites after a prolonged exercise bout to fatigue (76, 77, 579, 622) or a short, high-intensity bout of exercise (21, 621, 691) is that it is difficult to separate the effects of fatigue from those of glycogen depletion, especially if one exercise bout is followed by another after a period of rest (76, 77). However, in one study (488), biopsies were taken after 1 hour of exercise at 70% of $VO_{2\,max}$ and fibres were separated into fibre types and further subdivided according to glycogen content. Although there were no differences between glycogen-depleted and glycogen-containing fibres in content of ATP, ADP and AMP, but the IMP content of the glycogen-depleted fibres was greater than in the glycogen-containing fibres of both type I and II. AMP deaminase, which catalyses the reaction AMP $\rightarrow$ IMP +NH_3 is mainly activated by a high ATP turnover (342, 577) and accumulation of AMP (175, 352, 577, 621) and ADP (406, 412, 621) and can be activated under these conditions even when muscle lactate is low (342, 621) as opposed to fatigued muscle in which lactate may be high. Since IMP formation is increased compared with controls during exercise when glycogen phosphorylase is low (as in McAD), a shortage of its substrate, glycogen, could have the same effect. Thus, the elevated IMP concentrations in glycogen-depleted fibres in the study (488) discussed earlier may have been a direct effect of glycogen depletion. By the time the muscle fibres were separated and sorted, it is possible that the adenine nucleotides had been regenerated from other substrates, but this does not rule out the possibility that ADP and AMP were elevated during exercise.

Table 2.1 Comparison of metabolite changes in glycogen-depleted muscle and glycogen phosphorylase deficient muscle.

	GLYCOGEN DEPLETION	McARDLE'S DISEASE
G-1-P	D (77, 621, 622)	D (377)
G-1,6-P_2	D (621)	D (715)
G-6-P	D (621, 622)	D (377, 523, 574)
F-6-P	D (621, 622)	D (377)
F-2,6-P_2	-	I (715)
Triose phosphates	D (621)	D (377)
Lactate	D (77, 622)	D (484, 574, 578)
TCA cycle intermediates	D (579, 621)	D (578)
ATP	D (77, 533, 622)	D (574)
		N (406)
ADP	I (622)	I (574)
	N (621)	
AMP	N (621, 622)	I (574)
[ATP]/([ADP] + [AMP])	D (691)	D (402)
IMP	I (21, 77, 488, 579, 621, 622, 691)	I (574)
Hypoxanthine	I (691)	-
Xanthine	I (622)	-
NH_3	I (76, 77)	I (377, 402)
P_i	N (46)	I (406)

D, decreased with respect to controls; I, increased with respect to controls; N, not different from controls; -, no information available; G-1-P, glucose-1-phosphate; G-1,6-P_2, glucose 1,6 bisphosphate; G-6-P, glucose-6-phosphate; F-6-P, fructose-6-phosphate; F-2,6-P_2, fructose-2,6-bisphosphate, TCA, tricarboxylic acid.

An increase in NH_3 content of muscle is generally associated with an increase in IMP concentrations (77), but it may also be increased as a result of increased deamination of amino acids as concluded in the study by Wagenmakers et al. (679). In that study, there was an increase in plasma NH_3, but no differences in IMP, TAN, or ATP content in glycogen depleted subjects compared with those with normal glycogen content. Branched-chain 2-oxoacid dehydrogenase complex activity was significantly higher in muscle of glycogen-depleted subjects. However, the conclusion that the NH_3 did not arise from AMP deamination is questionable since plasma NH_3 was only raised in the subjects in the glycogen depletion trial during the first 25 minutes of exercise, but the workload was then decreased,

which would have reduced the dependence on carbohydrate oxidation and allowed IMP and adenine nucleotides to return to normal by the end of 2 hours of exercise. In the trial after carbohydrate-loading, however, the subjects exercised at 70-75% of maximal workload for the full 2 hours and muscle glycogen content would by then have been quite low, even though still higher than in the depleted subjects. Thus the adenine nucleotide content of the muscles at the end of exercise may have converged.

In the study of Spencer and Katz (621) muscle was analysed at rest, before and after exercise. The only difference found in muscle at rest between glycogen-depleted subjects and subjects with normal muscle glycogen content was a lower G-1,6-P_2 content in the glycogen-depleted muscle. There were no other significant differences in glycolytic or TCA cycle intermediates or in high energy phosphates or purine nucleotide catabolites between non-exercised, glycogen depleted muscle and muscle with normal glycogen content. With McAD, the deficiency in glycogen phosphorylase does not appear to cause major changes in muscle metabolites at rest, although one study (574) showed decreased concentrations of ATP, ADP and AMP.

CENTRAL COMMAND AND NEURAL FEEDBACK IN METABOLIC CONTROL

The traditional view of metabolic regulation has been that of feedback mechanisms involving blood-borne molecules (193, 317). However, the rapid cardiovascular and metabolic response to the start of exercise suggested neural involvement (618). In the early 1970's, it was realised that the cardiac and respiratory response to exercise was mediated by descending signals from higher motor centres (central command) (232, 233). Moreover, it was also found at around the same time that cardiovascular responses are controlled by reflex neural feedback from contracting muscles via group III and IV muscle afferents (426, 428) and this has been confirmed and further investigated in many studies (216, 303, 347, 423, 682). In 1979, Galbo (215) suggested that the sympathetic nervous system is activated in response to workload (214) and that this resulted in the decrease in plasma insulin concentrations observed during exercise (211). This hypothesis was supported by a number of studies that followed (356, 589, 668, 671, 674). It has since been shown that both central command (207, 360, 589, 620, 668, 671, 672) and neural feedback mechanisms (200, 347, 349, 370, 404, 405, 407, 528, 570, 571, 667, 674) exist in metabolic control. Central command has been studied in humans during exercise by comparing cardiovascular and metabolic responses to exercise with and without neuromuscular blockade. Exercise with neuromuscular blockade resulted in increased plasma catecholamine concentrations at the same oxygen uptake (216).

The posterior hypothalamic motor region is involved in the initiation of locomotion. In decorticate cats, stimulation of this region, with simultaneous neuromuscular blockade to prevent feedback from

muscle afferents, results in increases in rate of hepatic glucose appearance (Ra), plasma glucose, epinephrine, norepinephrine and glucagon and decreases in plasma insulin concentrations (668). Based on the results of a study in rats, Vissing et al. (671) suggested that hepatic glucose output during exercise was regulated by central command from CNS motor centres and that this primary setting may be modulated by metabolic feedback mechanisms. A similar conclusion has been reached by others who have investigated both types of control mechanism (75, 361, 362, 655).

Group III afferents are mainly stimulated by the mechanical effects of muscle contraction (347, 348, 446) and in cats are stimulated predominantly during static muscle contraction (350), while group IV fibres are more likely to be stimulated by the metabolic products of muscle contraction (347, 348) and appear to be equally stimulated by static and rhythmic muscle contraction (350). Stimulation of Group III and IV muscle afferents in cats at 20 x motor threshold results in an increase in Ra and in plasma glucose and ACTH concentrations, while stimulation at 140 x motor threshold results in increases in Ra, plasma glucose, ACTH, β-endorphin, met-enkephalin and a decrease in insulin concentrations (667). Kjaer et al. (362) showed an inhibition of the normal exercise-induced increases in ACTH and β-endorphin in subjects who performed dynamic exercise during epidural anaesthesia which causes afferent nerve blockade, and smaller responses to static exercise at 15% maximum voluntary contraction (MVC) in blood pressure, heart rate, β-endorphin, ACTH, epinephrine and norepinephrine compared with controls (361), suggesting that a similar afferent feedback mechanism from contracting muscle may occur in humans. Due to its lack of specificity, epidural anaesthesia has several disadvantages in investigations of this type. Firstly, it reduces muscle strength (361, 362). Thus at the same absolute workload, the influence of central command cannot be excluded (216). This problem can largely be overcome by exercising subjects at the same relative workload, as was done in these studies (361, 362). Secondly, at least in the experiment on static exercise (361), the post-exercise pressor reflex was not abolished, indicating that blockade of unmyelinated fibres may have been incomplete (206) and thirdly, since thresholds for lidocaine blockade are the same for pain and temperature sensing fibres, which were blocked, and efferent sympathetic fibres, it is possible that the latter were also blocked, though the sympathetic projections from the spinal cord terminate at L2 (223), which is just proximal to the injection site (361).

In added support of feedback via muscle afferents is the observation that patients with muscle phosphorylase deficiency (McArdle's disease) or PFK deficiency have a greater than normal change in heart rate for a given change in oxygen uptake ($\Delta Q/\Delta VO_2$) during exercise. This has been linked to a deficiency of oxidisable fuel (404, 405, 407), since the $\Delta Q/\Delta VO_2$ decreases when the enzymatic defect is bypassed by infusing glucose in McArdle's disease (402, 405) and lactate or FFA in PFK deficiency (407) and increases when nicotinic acid is used to block mobilisation of FFA in McArdle's disease.

The actual chemical stimulants or inhibitors for group III and IV afferents and their relationship to the metabolic state of the muscle are not yet known. Hypoxia *per se* has little effect (277), but group III and IV muscle afferents are known to be responsive in cats to substances such as lactic acid (566, 610), potassium (200, 348, 349, 370, 570, 571), histamine and serotonin (200), bradykinin (200, 370, 447, 448) and cyclo-oxygenase metabolites of arachadonic acid (348, 565, 566). Monobasic phosphate and 2-chloroadenosine have very little effect and sodium lactate even less (566). In a study in humans, glycogen depletion resulted in attenuated lower limb metaboreceptor mediated pressor and forearm vasoconstrictor responses which normally occur during exercise (611). However, it is unlikely that glycogen itself has an effect on muscle afferents, since patients with McArdle's disease have been shown to have reduced muscle sympathetic nerve activity during static exercise (515) and yet have adequate muscle glycogen content, although they cannot metabolise it (402).

Group III and IV muscle afferents travel via the dorsal roots to the ipsilateral spinal cord where they synapse in the substantia gelatinosa (328) and release substance P during muscle contraction (276, 328, 695). The second order (or higher) neurons cross to the contralateral side of the spinal cord (328) and travel rostrally in the spinocervical tract (254). Muscle afferents and the hypothalamic locomotor region both project to the nucleus reticularis gigantocellularis (NGC) and bilateral electrolytic lesion of the NGC at the pontomedullary border in cats causes greater responses in tidal volume, respiratory frequency and heart rate to stimulation of the hypothalamic locomotor region than the responses recorded prior to lesioning (543). As mentioned earlier, stimulation of the posterior hypothalamic motor region results in a metabolic pattern similar to that normally seen during exercise in the same species (668). In rats, anaesthesia of the ventromedial hypothalamus (VMH) resulted in increased resting plasma concentrations of glucose, lactate, glycerol, epinephrine and norepinephrine. During exercise, initial hepatic glucose production, plasma catecholamines, subsequent plasma glucose concentrations and overall hepatic glycogenolysis were lower in VMH-anaesthetised rats compared with control rats (673) and anaesthesia of the paraventricular nucleus (PVN) resulted in an attenuation of the exercise-induced increase in plasma epinephrine and cortisol (658). There are interconnections between hypothalamic nuclei (335, 634, 639, 640, 722), and the VMH (581, 616, 673, 723) and PVN (74, 696, 721) have extensive connections with the sympathetic nervous system and with the hypophysis (641, 658).

Hypothalamic areas, particularly the VMH, lateral area (LHA) and PVN that are involved in the regulation of blood glucose and plasma free fatty acid (FFA) levels also contribute to the regulation of food intake (634). A description is presented in a review by Steffens et al. (634) of the intrahypothalamic connections and the pathways between the hypothalamus and the motor areas of both the sympathetic system in the spinal cord (the intermediolateral column IML) and the parasympathetic system in the brainstem (the dorsal motor nucleus of the vagus and the nucleus ambiguus). Noradrenergic stimulation of the LHA, VMH and PVN can alter blood glucose, plasma

FFA and insulin concentrations independently of each other (634). Noradrenergic stimulation of the VMH leads to an increase in plasma insulin, glucose and FFA concentrations (589, 599, 632, 634), but bilateral lesion of the VMH leads to an exaggerated increase in plasma insulin concentrations, due to vagal pancreatic stimulation (315). Exercise induced increases in plasma glucose concentrations are suppressed by α-adrenergic blockade of the LHA (589, 634) VMH (589, 634) and PVN (634). Alpha-adrenergic blockade of the VMH during exercise causes an exaggerated increase in plasma FFA concentrations (589, 634) probably due to blockade of presynaptic inhibitory alpha-adrenergic receptors whereas α-blockade of both the LHA and PVN does not change the normal exercise induced increase in plasma FFA (634) However, norepinephrine infusion into the LHA results in a decrease in plasma FFA (632) due to β-adrenergic stimulation (589) and an increase in insulin concentrations (633, 634). Thus combination of the results of the various studies discussed in this section may explain the integrated metabolic control via central command, neuromuscular reflexes and humoral metabolic feedback.

THE EFFECT OF HEPATIC GLYCOGEN CONTENT ON METABOLIC CONTROL

FACTORS WHICH AFFECT HEPATIC GLYCOGEN CONTENT

Hepatic glycogen content in humans after a 12 hour overnight fast is between 160 and 274 mmol/l (measured by NMR) (33) and has been shown to decrease to 22-55 mmol/kg ww (measured by liver biopsy) after 3 days on a low-CHO diet (484). Little hepatic glycogen repletion takes place with glucose infusion. After infusing glucose at a mean rate of 5.8 mmol/min for 4 hours in resting subjects, Nilsson and Hultman (485) showed an increase of only 76 mmol/kg wet weight even though plasma glucose concentrations remained above 10 mmol/l after 15 minutes of infusion and rose to a peak of ± 20 mmol after 90 minutes of exercise. In addition, in the study of DeFronzo et al. (143) even when plasma glucose concentrations were raised in humans to ~13 mmol/l with plasma insulin at ~40μU/ml, little splanchnic uptake of glucose took place. However, exogenous glucose does result in suppression of hepatic glucose output (1, 143).

The reason for the low rate of repletion with glucose infusion is probably that glucose is a poor substrate for hepatic glycogen synthesis (70, 344), since the liver has limited capacity for glucose phosphorylation (381). Isolated hepatocytes only begin to take up glucose and synthesise glycogen when the glucose concentration of the incubation medium is raised to 10-12 mmol/l and the rate of glycogen synthesis only starts to match that from a mixture of glucose, lactate and glutamine when the glucose concentration reaches 50-60 mmol/l (344). The rate of hepatic glycogen synthesis directly from glucose is also low in vivo (473, 520) and most hepatic glycogen is formed from gluconeogenic precursors, as demonstrated by the fact that inhibition of gluconeogenesis in fasted rats at the

phosphoenolpyruvate carboxykinase step results in an 85% reduction in hepatic glycogen synthesis, but infusion of glycerol, which enters the gluconeogenic pathway distal to the phosphoenolpyruvate carboxykinase step, restores glycogen synthesis (475).

Hepatic glycogen synthesis in rats is greater from ingested glucose than from infused glucose, even when hyperglycaemia and hyperinsulinaemia are maintained, but the percentage of glycogen synthesised via the direct and indirect pathways is not influenced by the route of administration (603). In one study in humans (520), however, only 10 g of hepatic glycogen was formed from 100g of ingested glucose. Estimates of hepatic glycogen synthesis by the direct pathway from glucose vary from 31-36% in rats (332, 606) whereas in humans ingesting a 75 g glucose load, 40-50% of glycogen is synthesised via the indirect pathway (124, 472).

In a study using NMR in humans (33), glucose uptake by the liver took place 8 minutes after the commencement of a hyperglycaemic, hyperinsulinaemic clamp, yet glycogen synthesis was only evident after an infusion of ~ 60 minutes. The time delay was probably due to the conversion of the glucose to 3-C products and this may be the reason for the observation that muscle glycogen is repleted at the expense of hepatic glycogen after exercise in rats (511) and in humans (197, 386, 417). Two recent studies by Taylor et al. (643, 644) using NMR have possibly provided a further explanation for these observations. The first study (644) followed the time course of muscle glycogen synthesis after subjects ingested a solid mixed meal (914 kcal; 60% CHO, 21% fat; 19% protein) following an overnight fast and found that from an overnight-fasted muscle glycogen content of ~ 83 mmol/l (NMR measurement), gastrocnemius muscle glycogen concentrations started to rise after 60-120 mins, reached a peak of ~100 mmol/l 294 mins after the meal and then fell to ~ 90 mmol/l 420 mins postprandially. In the other study (643) a liquid mixed meal (824 kcal: 67% CHO, 19% fat, 14% protein) was ingested after 3 days on a controlled diet and an overnight fast. Hepatic glycogen content rose from ~207 mmol/l to ~316 mmol/l after 318 min, then began to decline. The meals in the two studies were of similar composition and if the results are compared, it would appear that the decline in muscle glycogen may be due to release of lactate and other gluconeogenic precursors for use in hepatic glycogen synthesis. While the NMR results of the two studies concur, a puzzling aspect of the second study (643) is that the percentage hepatic glycogen supposedly derived from the direct pathway, estimated by a technique measuring urinary excretion of ^{14}C-acetaminophen, was 46% from 120 to 240 min after meal ingestion and 68% from 240 to 360 min. It would be expected that if the muscle were releasing gluconeogenic precursors from ~290 mins, this would be the period during which the greatest proportion of hepatic glycogen synthesis would be from the **indirect** pathway.

As in muscle, hepatic glycogen synthesis and glycogenolysis can take place simultaneously, especially in the fed state (604). Insulin does not influence the rate of hepatic glycogen synthesis if substrate concentrations are optimal (70) and insulin infusion alone does not increase hepatic glycogen

synthesis (107), but it does potentiate hepatic glucose uptake during hyperglycaemia (1, 27, 143, 144). The main stimulus for an increase in hepatic glycogenolysis during fasting and exercise is an increase in plasma glucagon concentrations (194, 222). Epinephrine also stimulates hepatic glycogenolysis via β receptor stimulation, but glycogenolysis is inhibited by α-adrenergic stimulation (305).

The liver is richly innervated by sympathetic and parasympathetic nerves (716), more so in the human and guinea pig than in rats (32). The effect of hepatic nerve stimulation depends on the firing rate, duration and the hormonal environment (584). Hepatic nerve stimulation results in an increase in glucose appearance (Ra) (32, 482, 584) and a decrease in lactate uptake by the liver (32, 584). The functional implication in glucoregulation of sympathetic nerves has been well-documented, while that of parasympathetic nerves remains less understood (716). Sympathetic stimulation leads to rapid activation of hepatic glycogenolytic enzymes (185, 598). Prostaglandins may mediate this effect since the stimulatory effect of hepatic nerves on hepatic glycogenolysis is reduced when prostaglandin inhibitors are infused into the liver (32). However, blockade of the celiac ganglion which blocks adrenal medullary secretion and hepatic sympathetic nerve supply, does not affect Ra in humans during exercise, but does result in lower plasma FFA and glycerol concentrations (358). Parasympathetic stimulation in the liver potentiates the effect of insulin in stimulation of glycogen synthesis only in the presence of sympathetic blockade (218) in rats and this was inhibited in humans with muscarinic blockade only when the islet clamp technique (somatostatin infusion with insulin, glucagon and growth hormone replacement at fixed rates) was used (71). Thus parasympathetic stimulation is dominated by sympathetic effects if the hepatic nerves are non-specifically stimulated in the absence of sympathetic blockade (218).

EFFECT OF HEPATIC GLYCOGEN CONTENT ON METABOLIC CONTROL

Hepatic glucoreceptors have been postulated to be coupled with afferent nerves, conveying sensory signals of blood glucose concentration or hepatic glycogen content to the central nervous system (396, 471, 478, 483, 568, 588), although a recent study (392) cast some doubt on the importance of hepatic afferent feedback on plasma epinephrine and norepinephrine responses during exercise with hypoglycaemia. However, there seems to be little doubt about the effect of portal venous concentrations in hepatic afferent activity (319, 380). Infusion of 0.25 mmol/min glucose into the portal vein of dogs during treadmill exercise resulted in attenuation of the normal exercise-induced increase in plasma norepinephrine and glycerol concentrations, whereas peripheral infusion of glucose at the same rate had no effect (380) on either. In a similar study, Bernal et al. (45) found that intraportal infusion of glucose in small amounts into dogs, although not increasing plasma glucose or insulin levels, resulted in decreased FFA mobilisation from adipose tissue and in a reduction of the plasma norepinephrine response to exercise.

In a study by Vissing et al. (672) which investigated the effect of hepatic glycogen levels on Ra during treadmill running for 35 min, Ra and plasma glucose concentrations increased more and hepatic glycogenolysis was higher in fasted-refed compared with control rats which had been fed rat chow *ad lib*, even though the stimuli for Ra were higher in control rats, as the plasma concentrations of insulin and glucose were lower and glucagon and catecholamines higher. These data indicate that hepatic glycogenolysis during exercise is directly related to hepatic glycogen content. Studies by Lavoie et al. (395, 396) with rats also support the concept that hepatic glucoreceptors are sensitive to changes in hepatic glycogen concentration. During exercise, muscle glycogen utilisation was greater in rats with hepatic vagotomy and hepatic glycogen depletion than in sham-operated rats (395, 396) while there was a decreased response in plasma FFA concentrations to glycogen depletion at rest but not during exercise (395). Hepatic vagotomy with fasting also resulted in lower plasma insulin concentrations both at rest and during exercise (395) but not after food restriction when hepatic glycogen content was somewhat higher (396).

CHAPTER 3

CHOICE AND DEVELOPMENT OF METHODS

GLUCOSE CLAMP TECHNIQUES

To isolate the effect of muscle glycogen content on metabolic regulation during exercise in these studies, it was essential that plasma glucose concentrations were prevented from falling and kept constant. The techniques used were based on the euglycaemic and hyperglycaemic clamps described by DeFronzo et al. (146).

The formula of DeFronzo et al. (146) is:

$$S_i = \frac{(G_d - G_i) \times 10 \times (0.19 \times \text{body weight})}{G_{inf} \times 15} \times PF + \frac{(SM_{i-2}) \times (G_d - G_b)}{(G_i - G_b)} \times (FM_{i-1})$$

where, S_i is the infusion rate required, G_d is the desired blood glucose concentration, G_i is the actual blood glucose concentration at time point i, G_{inf} is the glucose concentration of the infusate, G_b is the basal blood glucose concentration, PF is the factor which converts a rate in ml/min to the units used in the infusion pump, SM_{i-2} is the calculated metabolic component of the infusion rate 2 iterations (10 min) previously and FM_{i-1} is a dimensionless correction factor.

As these formulae were developed for subjects at rest, initial pilot studies were performed to determine their suitability for the experiments in this thesis. The first pilot study was a hyperglycaemic clamp and it was found that the rate of adjustment of the infusion rate was not rapid enough to meet the progressively increasing rate of glucose oxidation which occurs during moderate intensity exercise, and thus the plasma glucose concentrations fell.

Examination of data from previous studies (65, 67) in this unit gave an estimated rate of increase of plasma glucose oxidation at 70% of VO2 max of ~8 mg/min. Since a blood sample was taken every 5 minutes for adjustment of the clamp, this amounted to an increase of ~ 40 mg every 5 min. Accordingly, the original formula was modified to:

$$Si = \frac{(Gd - Gi) \times 10 \times (0.19 \times \text{body weight}) \times PF}{Ginf \times 15} + \frac{(SMi\text{-}2) \times (Gd - Gb) \times (FMi\text{-}1)}{(Gi - Gb)} + \frac{\Delta G}{Ginf}$$

where ΔG was the estimated increase in glucose disappearance (mg) during the last 5 minute period. The pilot study was then repeated starting with ΔG set at 40 mg. After analysis of the blood glucose

concentrations, ΔG was further adjusted to take into account changes in demand during the course of exercise, which meant that at some time points ΔG was 20 mg, while at others, particularly near the beginning of the exercise period ΔG was as much as 70 mg. These adjustments to the formulae resulted in maintenance of the desired, constant glucose concentration during exercise.

INSULIN INFUSION RATE

Since insulin specifically promotes muscle (145) and not liver glucose uptake unless both plasma glucose and insulin concentrations are very high (107), it was hypothesised that if the metabolic changes previously described after ingestion of a low carbohydrate diet (215, 268, 310, 312, 527) were observed during euglycaemia without insulin infusion and abolished with insulin infusion, this would indicate that these changes were mainly due to muscle glycogen depletion (since the insulin infusion would increase uptake of glucose in the muscle, thus compensating for a low muscle glycogen content.

In order to distinguish between the effects of muscle and liver glycogen depletion, plasma insulin concentrations during euglycaemia had to remain below 40U/l (143) in order to stimulate muscle but not hepatic glucose uptake. From a series of studies examining dose-response relationships for insulin infusions, (451, 457, 551), Mikenes (450) reported average plasma insulin concentrations of 20 and 50 μU/ml for infusion rates of 0.2 and 0.7 mU/min/kg, respectively. Therefore, an initial pilot study was carried out on a cyclist exercising at 70% of $VO_{2\ max}$ with an insulin infusion rate of 0.5 mU/min/kg and a euglycaemic clamp. Plasma insulin concentrations increased from 5.6 U/l at rest to 67 U/l after 60 min of exercise, which was much higher than the desired concentrations for these studies. In a study by Wolfe et al. (710), at a lower exercise intensity and with a somatostatin infusion and glucagon replacement, a plasma insulin concentration of ~20 μU/ml was obtained with an infusion rate of 0.2 mU/kg/min. Thus a second pilot study was performed at this infusion rate and a maximum plasma insulin concentration of 18 μU/ml was obtained after 60 minutes of exercise. Since it was important for the dose of insulin to be low enough so as to avoid stimulating hepatic glucose uptake during the glucose infusion, this infusion rate of 0.2 mU/kg/min was chosen for these studies.

USES AND LIMITATIONS OF TRACER METHODOLOGY

Carbohydrate molecules labelled with radioactive or stable isotopes have been used in studies of carbohydrate metabolism to measure glucose and lactate turnover, ingested carbohydrate oxidation and blood glucose or lactate oxidation, or both.

i) Stable vs radiolabelled tracers.

The stable isotope of carbon is ^{13}C, whereas the radioactive isotope is ^{14}C. The main advantage of a stable isotope is that, it is non radioactive and can be used many times in the same subject, making it "easier" to reach statistical significance when differences are found. However, they are difficult to measure in vivo due to the low percentage of ^{13}C that occurs naturally in the food chain, dictating that any tracer has to be artificially enriched with ^{13}C glucose and that makes the costs unaffordable in this country.

A methodological problem with naturally enriched ^{13}C-glucose is that since *most* foods in South Africa are naturally enriched with ^{13}C because of the C-4 photosynthetic pathway of South African sugar cane and grass species, the normal diet of the population contains appreciable amounts of ^{13}C. Thus muscle and liver glycogen and fat is enriched with ^{13}C. A failure to correct for the release of this ^{13}C during exercise would result in an overestimation of plasma glucose oxidation or an underestimation of hepatic glucose appearance (Ra) as a consequence of glycogenolysis during exercise. Glycogenolysis would result in increased $^{13}CO_2$ production unrelated to an increase in blood ^{13}C-glucose oxidation or, in turnover studies, appearance from the liver of ^{13}C-glucose.

Although not relevant to the studies in this thesis, the use of stable isotopes for measurement of splanchnic glucose appearance does not provide the same degree of sensitivity of measurement as radioactive isotopes. This necessitates the use of more than negligible amount of tracer, thereby breaking one of the fundamental rules in the use of tracers and thus rendering it unsuitable as a tracer for turnover studies unless very sensitive measurement equipment is available (712) or the glucose tracer is artificially enriched. Under such circumstances, estimation of glucose flux in dogs using stable isotope techniques has been compared with simultaneous measurements made in the same animal with radioactive isotope methods and similar rates of glucose turnover were found (47). Because of the problems described, it was decided to persist with our use of radiolabelled tracers for determination of rate of oxidation of glucose in the studies which follow.

ii) Measurement of rate of splanchnic glucose appearance using radiolabelled tracers

Although Ra was not measured in these studies, a discussion on this topic may be important to illustrate differences between the technique for measuring glucose turnover and that for measuring glucose oxidation. One of the methods for calculating of the rate of total splanchnic (endogenous plus exogenous) glucose Ra, the rate of glucose appearance from exogenous carbohydrate (Ra_{exog}) and appearance of endogenous glucose from liver (Ra_{end}) is the use of Steele's equations for non-steady

state exercise (631).This has been elegantly validated by Radziuk et al. (522). The equations are shown below:

$Ra_{tot} = (I - (pV \times Glu_{tot} \times dSA/dt))/SA$

$Ra_{exog} = (I - (pV \times Glu_{lab} \times dSA/dt))/SA$

$Ra_{end} = Ra - Ra_{exog}$.

where, Ra, Ra_{exog}, and Ra_{end} are as defined above, in mmol/min; I is the infusion rate of labelled glucose in dpm/min; p is the pool fraction (0.75) in which rapid changes in glucose concentration and specific activity take place (316, 522); V is the glucose distribution volume (19.6% of body mass in l) at rest (316); Glu_{tot} is the mean plasma glucose concentration (^{14}C-labelled and non-labelled) in mmol/l in consecutive samples; Glu_{lab} is the mean plasma glucose concentration of ^{14}C-labelled glucose (^{14}C glucose radioactivity (dpm) of the sample divided by the specific activity (SA) of the ^{14}C ingested glucose) in mmol/l in consecutive samples; dSA/dt is the change in plasma labelled glucose specific activities in dpm/mmol over the sample interval in minutes; SA is the mean dpm/mmol glucose specific activity in successive samples and dGlu/dt is the mmol/l/min change in total glucose concentration.

Since measurements of Ra depend on tracer dilution, the calculation depends on measurements of changes in specific activity over time. For this method to be accurate, the specific activity of glucose should only change due to an alteration in glucose output from the liver or some exogenous source. This necessitates a constant and known rate of introduction of glucose tracer and steady-state conditions. However, because a number of assumptions are made in the calculation, there are a number of other factors that must be taken into account:

i) Choice of radiolabelled tracer

Depending on the nature and position of the label, different values are obtained in calculation of Ra from isotope dilution measurements (708). Since the use of ^{14}C-glucose tracers would introduce the problem of re-cycling of the ^{14}C label (7, 177, 522) resulting in an underestimation of Ra, a non-recycling (^{3}H-glucose) tracer appears to be the tracer of choice.

The ^{3}H-label of glucose labelled in the second carbon position is lost in the glucose 6-phosphate - fructose 6-phosphate equilibrium in the glycolytic and gluconeogenic pathways, and thus the incorporation of ^{3}H-glucose into hepatic glycogen is minimised (317), particularly during the pre-exercise infusion period. Thus, with 2-^{3}H-glucose the potential incorporation and subsequent hydrolysis of ^{3}H-glucose labelled glycosyl residues of glycogen, and consequent underestimation of glucose appearance, is limited. However, when glucose is labelled on the first or third carbon, ^{3}H can

be incorporated into hepatic glycogen, which can be subsequently released during glycogenolysis. leading to an underestimation of hepatic glucose appearance (317). However, 3-^{3}H-glucose is often used in turnover studies. An underestimation of glucose turnover will also result if isotope that has been cleared is recycled into the systemic circulation (for example 6-^{3}H-glucose into hepatic glycogen) (84).

ii) Model for computation of glucose kinetics

The validity of the tracer technique depends on several assumptions, one of which is that the selected model of glucose kinetics is valid. Under non steady state conditions this has recently been challenged (84) for the most commonly used single compartment model of Steele (631).

The model proposed by Steele (631) to compute rates of appearance and disappearance of glucose in non- steady state (a pool fraction model) has been extensively used in experiments in which rate of hepatic glucose appearance has been calculated. The assumption inherent in this equation, and similar other equations, is that the body glucose pool is a single, readily mixable pool. Since the equation was first used in 1959, the fact that this condition is not met has been recognised, and thus part of the equation includes a "pool fraction" (p), and represents the fraction of the total glucose pool that behaves as an ideal, readily mixing pool and compensates for non-ideal pool behaviour. The problems and limitations will be discussed subsequently.

Steele's model has been subjected to theoretical analysis by Cobelli et al. (110). They showed that the model introduces errors dependent on the volume of the compartment and configuration of the system, which depend on the time course of changes in specific activity. This analysis suggests that there is no single pool fraction value satisfactory under all non steady state conditions and that tracer specific activity should be as constant as possible during experiments measuring turnover. In a 1983 paper, Cobelli et al. (111) concluded that the quantitative reliability of predictions provided by the pool fraction model was quite poor. Subsequently, Caumo and Cobelli (95)suggested a method of estimation of hepatic glucose production which is based on deconvolution and use of a two compartment minimal model to describe a time varying impulse response of glucose Ra.

Finegood et al. (199) have suggested a variable one compartment model of glucose kinetics and step increases in rates of tracer infusion in which infusion of the tracer was increased two or three fold when it was anticipated that endogenous glucose appearance would change rapidly, such as the onset of exercise. This technique was found to provide a more accurate estimate of glucose appearance than the standard fixed volume model and constant tracer infusion.

However, the validation study by Radziuk et al. (522) appears to have been forgotten. In that study the effects of different models to calculate Ra were evaluated. Data were analysed using a single

compartment model with a number of different volumes of distribution, a two compartment model, and a generalised dispersion (impulse response) model. The calculated rates of infusion (Ra) were 9.5, 8.4, and 7.8% higher than the actual rate for the three different models, respectively. Thus it appears that all models tend to overestimate glucose appearance, but that there is little difference in accuracy between the more complex models and the pool fraction (single compartment) models. In a comparison of tracer determined Ra with a known glucose infusion rate, Koivisto et al. (376) compared turnover rates when euglycaemia was maintained by infusing unlabelled glucose mixed with 3-^{3}H or 6-^{3}H-glucose. The isotopically determined glucose disposal rate was virtually identical to the exogenous glucose infusion rate with both tracers (376). Therefore, the single compartment model described by Steele (631) remains as good as any to calculate Ra.

iii) Pool fraction

Many investigators have attempted to determine the most suitable value for the glucose pool fraction (p), used in the equations for calculating glucose appearance. Values for the glucose pool fraction range between 0.5 - 0.8 (521), 0.65 - 0.75 (522), and 0.5 - 0.75 (489). Searle (593) states that "Steele now proposes that 0.77 would be a more appropriate value for the calculation of non-steady state events when one employs a continuous infusion technique and assumes a simple mono-compartment system". However, these values were determined at rest and have mostly employed the use of glucose infusions at known rates to determine the accuracy of a simultaneous tracer-determined rate. Most experiments were designed to produce fairly rapid changes in blood glucose concentrations. However, it is known that the slower the change in blood glucose concentration, the higher the value of 'p' becomes, until at an infinitely slow rate of change in glucose concentration the 'p' would be 1.0 (134). The data of Cowan and Hetenyi (134) suggest that 'p' values becomes larger when rates of glucose appearance increase. During exercise, rates of glucose appearance are much higher than the rates reported in those experiments where 'p' was determined to be 0.65. Cowan and Hetenyi (134) state that the value of 0.65 in their (non-exercising) dogs is probably a minimum value and that 'p' would be higher under more physiological conditions (smaller changes in glucose concentrations).

Most experiments, including those of Radziuk et al. (522) validating the equations for determining rate of glucose appearance and 'p' values, indicate that a change in the value of 'p' from 0.5 to 0.65 has little effect on the "goodness" of fit of a tracer determined glucose Ra and a known rate of glucose infusion. Wolfe et al. (710) reported that 40 - 210 ml/kg (which corresponds to a range in 'p' from 0.2 - 1.0) is an acceptable range for calculation of rate of glucose appearance, since the difference in calculated glucose Ra was not more than 10%. These calculations demonstrate that it would only make a 3% difference glucose Ra if for example, 0.5 was used for 'p' instead of 0.75.

iv) Constant infusion vs single injection of tracer

The question of whether a constant infusion of tracer or a single injection of tracer is the better technique was investigated by Allsop et al. (6). They calculated glucose Ra from changes in plasma glucose specific radioactivity after a single intravenous injection of labelled glucose and compared the values with an actual constant infusion rate of unlabelled glucose into an anaesthetised dog with all sources of endogenous glucose production surgically removed. The mean glucose Ra calculated from the area under the specific radioactivity versus time curve was 7% higher than the actual infusion rate (n = 4), but the difference was not statistically significant. The variability in the glucose Ra calculated in this manner was, however, greater than the variability reported with rates determined from a primed constant infusion of glucose tracer (6).

v) Tracer equilibration in blood

Radziuk et al. (522) used an unprimed infusion of 80 - 100 min in their experiments, as did Jenkins et al. (316). Katz et al. (346) stated that "unless the conditions require a very short experimental period, a priming bolus is unnecessary". In the studies of Bosch et.al (65, 67, 68), pilot experiments showed that tracer equilibration, as evidenced by a constant plasma glucose specific activity, took a minimum of 90 min to be reached, and was somewhat variable. It was found that a small priming dose equivalent to 45 min of constant infusion at 20μCi ensured that equilibration always occurred between 60-75 min after the start of infusion.

vi) Non-glucose contaminants

One potential problem with commercially available isotopes is that there have been reports that some may contain non-glucose radioactive contaminants which have a slower rates of clearance than glucose under conditions of high glucose turnover. This would result in overestimates of specific activity and underestimates of glucose appearance (84, 499, 591). However, others have not found any non-glucose contaminants in the labelled glucose that was used in their experiments (376). The glucose used in this laboratory was provided by the Amersham radiochemical laboratory and has been found to be contaminant free.

Calculation of exogenous carbohydrate oxidation

The rates of exogenous carbohydrate oxidation (R_{exog}) in g/min can be calculated from the following equation:

$$R_{exog} = (^{14}CO_2 \times 6)/SA_{exog}) \times VCO_2 \times 1.35$$

where, $^{14}CO_2 \times 6$ is the dpm/mmol value multiplied by 6, as there are 6 carbon atoms per molecule of ^{14}C-glucose; SA_{exog} is the specific activity of the carbohydrate ingested in dpm/mmol; VCO_2 is the volume of expired CO_2 in l/min; and 1.35 is the number of grams of glucose oxidised to produce 1 l of CO_2.

In measurements of exogenous carbohydrate oxidation, it is important that the tracer chosen behaves in an identical manner to the carbohydrate molecule of interest as regards digestion, absorption, metabolic processing and oxidation. If, for instance, the tracer is absorbed without needing be digested, as is the case with a glucose monomer and the ingested carbohydrate is a long glucose polymer which has to be cleaved into glucose monomers before absorption, the tracer may enter circulation and be oxidised faster than the ingested carbohydrate. In this case, the rate of total plasma glucose oxidation will be correct as will be explained later in this chapter, but the rate of ingested carbohydrate oxidation will be over-estimated.

Calculation of plasma glucose oxidation

The rates of total (endogenous + exogenous) plasma glucose oxidation (R_{ox}) in g/min were calculated from the following equation:

$$R_{ox} = ((^{14}CO_2 \times 6)/SA\ glu) \times VCO_2 \times 1.35$$

where, $^{14}CO_2 \times 6$ is the dpm/mmol value multiplied by 6, as there are 6 carbon atoms per molecule of ^{14}C-glucose; SA glu is the plasma ^{14}C-glucose specific activity in dpm/mmol, VCO_2 is the volume of expired CO_2 in l/min; and 1.35 is the number of grams of glucose oxidised to produce 1 l of CO_2.

In calculating exogenous carbohydrate oxidation, the ratio between expired $^{14}CO_2$ specific activity (SA) and the SA of the ingested carbohydrate is used. Basically, if only ingested carbohydrate was oxidised, then the ratio of the SA of the expired $^{14}CO_2$ to that of the ingested carbohydrate would equal 1/6. Thus in the equation to calculate the ingested carbohydrate oxidised, the first term would equal 1/6, leaving the oxidation rate equal to VCO_2 multiplied by 6 x 1.35g/l. In reality, this situation is unlikely to occur, and in addition to the ingested carbohydrate, other carbohydrate fuels contribute towards CO_2 production. The ratio of SA of CO_2 to SA of the ingested carbohydrate gives the fraction of all CO_2 that results from the oxidation of the ingested carbohydrate.

Similarly, it is the specific activity of the blood glucose at the time that the specific activity of the $^{14}CO_2$ is measured that is used in the calculation of blood glucose oxidation. Since it is the "instantaneous" ratio of $^{14}CO_2$ to ^{14}C blood glucose SA that is important, it does not matter by what route the labelled glucose gets into the blood. Once in the blood, the labelled glucose will follow the

same path in oxidation to be measured as expired $^{14}CO_2$. Nevertheless, when the blood sample is drawn for determination of blood glucose specific activity, the glucose being oxidised at that time intracellularly, and the resultant $^{14}CO_2$ may not be of the same specific activity as the blood glucose due to any delay in transit of a glucose molecule between the blood glucose pool and cellular oxidation. Thus there could be a "mismatch" between blood glucose specific activity and that of the expired $^{14}CO_2$. This would result in either an over- or under-estimation of blood glucose oxidation rate depending on whether the blood glucose specific activity was increasing or decreasing during the time at which the glucose molecule was being oxidised to CO_2. Thus any substantial variation in the rate of glucose entry into the blood might result in either over or under-estimation of the blood glucose oxidation. However, variation is unlikely to be substantial. We have found that $^{14}CO_2$ was present in measurable amounts in the expired air of a cyclist 30s exercising at 70% of $VO_{2\ max}$ after a bolus injection of 15μCi of ^{14}C-glucose (Bosch, 1997; unpublished observations. Moreover, measurement of blood glucose specific activity after ingestion of labelled carbohydrate shows that change in glucose Ra is gradual (Figure 3.1) and thus unlikely to be a major source of error. As shown in Figure 3.1, during the first 40 min of exercise when blood glucose specific activity is increasing, there is potential for under-estimation of blood glucose oxidation rate. However, since the lag phase is only 30s, the error would only be about 2%. This is true whether the tracer is ingested or infused, as even when infused, there is an increase in specific activity during the early phases of the experiment. With ingestion of tracer, blood glucose specific activity declines somewhat after 40 minutes of exercise, which if anything will result in over-estimation of rate of oxidation.

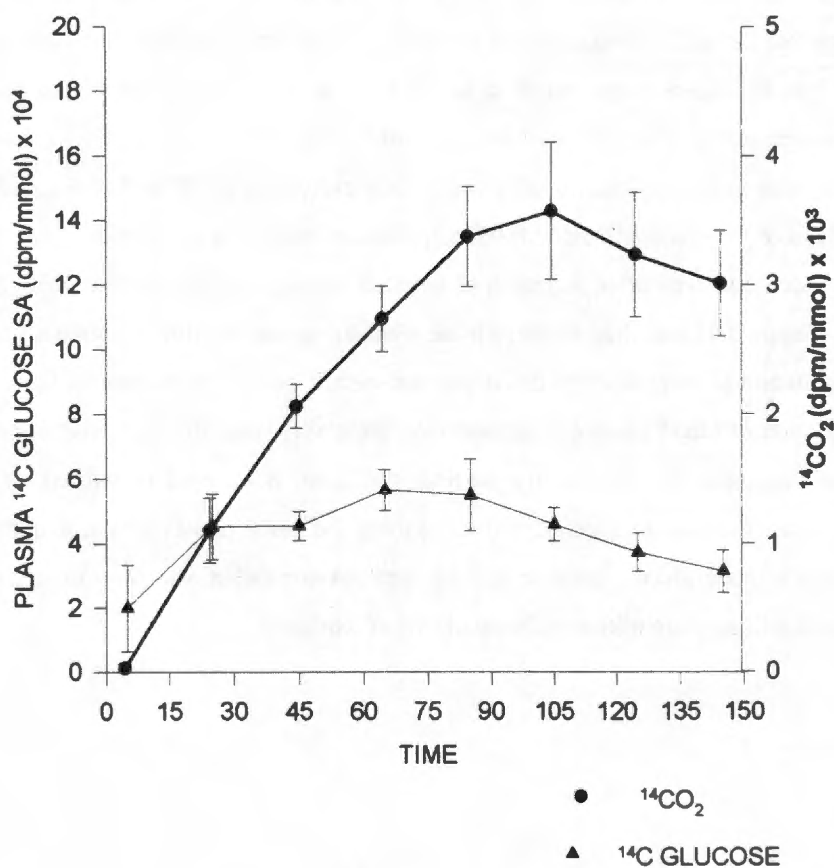


Figure 3.1 Plasma ^{14}C-glucose specific activity and $^{14}CO_2$ specific activity during 145 minutes of exercise.

Poor absorption of the ingested carbohydrate would result in a lowered blood specific activity, reflecting the lower rate of entry of the ingested carbohydrate into blood, but this would not influence the calculation of oxidation rate of the blood glucose since the specific activity of expired CO_2 would be correspondingly lower, reflecting the lower blood glucose specific activity as explained in Figure 3.2.

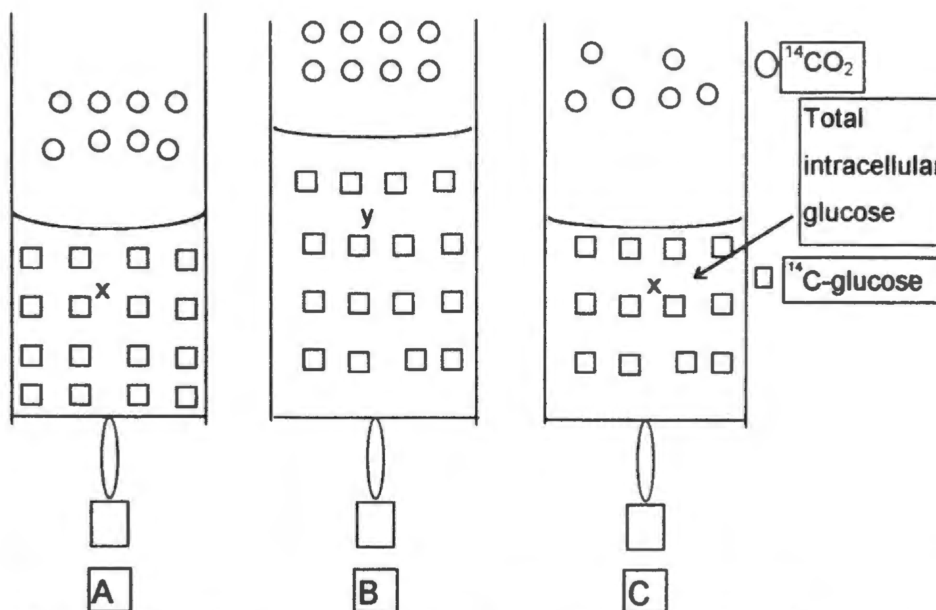


Figure 3.2 Schematic diagram of use of tracer methodology to measure glucose oxidation. In A, the total amount of glucose in the cell (x) is less than in B (y), i.e. the specific activity is higher (16/x vs 16/y). If all the glucose in both cells is oxidised, the same amount of $^{14}CO_2$ (8/t) is produced in both cases, but the total amount of glucose oxidised is greater in B than in A (proportional to 8x/16=x/2 vs 8y/16=y/2) . In C, the amount of glucose is the same as in A, but the specific activity is lower, thus less $^{14}CO_2$ is produced in the oxidation of all the glucose because the ratio of specific activities in A and C are the same (8x/16=x/2 vs 6x/12=x/2), the calculation of glucose oxidation would give the same result in A and C.

Loss of $^{14}CO_2$ into the bicarbonate pool would result in an underestimation of the rate of oxidation until the pool has equilibrated. This is discussed in detail subsequently.

Calculation of endogenous glucose oxidation

Oxidation of endogenous glucose can be calculated by difference between R_{ox} and R_{exog} in experiments where this is applicable.

Bicarbonate pool

The formulae to calculate plasma and exogenous glucose oxidation do not take into account the $^{14}CO_2$ retained in the bicarbonate (HCO_3^-) pool. The body bicarbonate pool can act as a sink for labelled carbon atoms used in the determination of exogenous carbohydrate and plasma glucose oxidation and can account for discrepancies between total and exogenous glucose oxidation in relation to the peak time of occurrence, as well as the absolute quantities (494).

The time required to equilibrate $^{14}CO_2$ with the plasma CO_2/HCO_3^- pool during exercise performed at 60-70% $VO_{2\,max}$ has been stated to vary from 5 min (519), to 15 - 20 min (28, 562), to 30 min (619), to 45 min (142), and to as long as 75 min (555). However, a study by Barstow et al. (28) presents

strong evidence that 90% of equilibration has occurred after 16 min of moderate intensity exercise. In the present study, plasma ^{14}C glucose, (which would not be affected by the HCO_3^- pool) took ~40 min to reach equilibrium, with $^{14}CO_2$ specific activity closely tracking the increase in plasma ^{14}C glucose specific activity (Figure 3.1). It is therefore unlikely that any lag in $^{14}CO_2$ appearance was due solely to retention of $^{14}CO_2$ in the HCO_3^- pool. Data on bicarbonate pool kinetics from this laboratory indicate equilibration of the pool within 45 min (A. N. Bosch and S. M. Weltan, unpublished observations).

A more or less complete equilibration of the bicarbonate/CO_2 pool in 15 to 30 min would be predicted from the calculated flux of CO_2 through the body bicarbonate stores:- In a 70 kg male, body water content is around 40 l. Of this, 25 l are extracellular fluid which contains 25 mmol/l bicarbonate and 15 l are intracellular water which contains approximately 10 mmol/l bicarbonate. Hence total body bicarbonate stores are around 775 mmol, which corresponds to 17.5 l of CO_2. Since 85% of the CO_2 produced is carried to the lungs as bicarbonate, a typical exercise VCO_2 of 2.5 l/min would turn over 2.1 l of the CO_2 from bicarbonate per min. Most of the bicarbonate should therefore (theoretically) turn over in 17.5/ 2.1 min i.e. 8 min (66). Furthermore, any slight underestimation of the rate of plasma glucose oxidation as a result of HCO_3^- turnover would be similar among groups and therefore would not affect the comparisons.

ADMINISTRATION OF RADIOLABELLED TRACER FOR CALCULATION OF PLASMA GLUCOSE OXIDATION

In 1994, Beckers et al. (31) published a paper describing the theoretical dangers of ingestion or infusion of ^{14}C labelled glucose. However, the real risks are much smaller. By analysing the results from previous studies in this department at similar workloads and comparing the total amount of label recovered in expired CO_2 with the total dose administered, it was calculated that, of the 40 μCi of U-^{14}C-glucose ingested in this study, on average 55% of the ^{14}C would be recovered as $^{14}CO_2$ during the trial. This would leave a total of 18 μCi in the body at the end of the trial, the biological half-life of which is 12 days. According to calculations performed by Dr. M. Shackleton of the Department of Medical Physics, University of Cape Town, this translates to a cumulative activity of :

1.44 x 18 μCi x 12 d x 24 hr

= 7465 μCi.hr

Where: 1.44 is a constant that takes into account the decay and excretion characteristics;

18 μCi is the amount of ^{14}C remaining in the body at the end of the trial and

12 d is the biological (as opposed to the physical) half life of ^{14}C glucose.

$$S_{(Total\ Body \leftarrow Total\ Body)} = 1.5 \times 10^{-6}\ rad/\mu Ci.hr$$

Where S is the absorbed dose per unit cumulative activity for ^{14}C.

The total absorbed dose is thus:

$$D_{(Total\ Body \leftarrow Total\ Body)} = 7465\ \mu Ci.hr \times 1.5 \times 10^{-6}\ rad/\mu Ci.hr$$

$$= 11\ mrad$$

$$= 0.11\ mSv$$

The recommended dose limits in South Africa are 1 mSv in a year for the public and 20 mSv per year for occupational exposure, average defined over 5 years.

In comparison, the radiation dose received by subjects in this trial would be 2.5% of what they would receive from bone scintigraphy and 7% of what they would receive from a chest X-ray, which are commonly performed radiological examinations. Assuming linear extrapolation from established data (which may not be entirely appropriate) (183), the risk coefficient (probability of biological change) would be $1,85 \times 10^{-6}$ for the whole body and 4.48×10^{-7} for the gonads and the dose used in this trial would be 0.011% of the estimated dose required to double the "normal" human mutation rate.

As described previously, in experimental protocols in which the rate of oxidation of ingested carbohydrate (CHO) is determined, the glucose tracer is added to the CHO that is ingested. Similarly, if blood glucose oxidation is to be determined, the tracer should be infused rather than ingested. However, in the studies described in this thesis, it was decided to have subjects ingest the tracer rather than infuse it since the original earlier design of the study made provision for including the testing of a group of subjects with low glycogen content who would ingest carbohydrate of which the oxidation rate was to be measured. However, after experimental work had commenced, this question was answered in another study (265) and the research emphasis was changed to accommodate this. This made carbohydrate ingestion unnecessary, but since subjects had already been tested, for the sake of consistency, tracer administration in the drink was continued. The technique of having subjects ingest a drink containing a radiolabelled glucose tracer and using the blood glucose specific activity to measure the rate of blood glucose oxidation has been used previously for 180 min of exercise (65, 67, 68, 265, 266). However, a further pilot study was performed to determine whether ^{14}C-glucose tracer administered by ingestion or infusion gave similar results for the determination of blood glucose oxidation rate.

METHODS

Six moderately trained male endurance cyclists took part in the study which was approved by the Research and Ethics Committee of the Faculty of Medicine of the University of Cape Town. As radio-labelled tracers (total radiation dose 0.11 mSv) were infused or ingested and blood samples taken, the procedures and risks were explained to the subjects and written informed consent obtained.

Measurement of maximum oxygen consumption

Each cyclist first came to the laboratory for determination of his maximum oxygen uptake ($VO_{2\,max}$). This was measured during a progressive exercise test on an electrically braked cycle ergometer (Lode, Groningen, Netherlands), modified to the configuration of a racing bicycle. The starting work rate was calculated as 3.3 W/kg, with a 50 W increment after 150 sec followed by 25 W increments every 150 sec until exhaustion. This information was used to adjust the work rate in the experimental trial so that each subject exercised at an intensity corresponding to ~70% of VO_2 max.

During the test, the subject was connected on-line to an Oxycon Alpha (Jaeger Mijnhardt, Netherlands) for determination of oxygen uptake (VO_2), carbon dioxide production (VCO_2) and respiratory exchange ratio (RER) every 15 sec.

Measurement of endogenous glucose oxidation during exercise

Each cyclist returned to the laboratory on a further 2 occasions after an overnight fast for the experimental trials. In random order, the subjects cycled for 60 min at 70% of VO_2 max while either ingesting a drink labelled with U-^{14}C-glucose tracer or receiving an infusion of ^{14}C-glucose tracer during the 60 min of exercise.

In infusion experiments, an 18G Jelco (Johnson and Johnson, Halfway House, South Africa) cannula was inserted into the antecubital vein of one forearm for blood sampling every 15 min and a 20G cannula was inserted into the antecubital vein of the other forearm and connected to a 3-way stop-cock for continuous infusion of ^{14}C -glucose tracer (Amersham International, Buckinghamshire, UK).

In order to calculate rates of blood glucose oxidation, tracer was either ingested or infused. When ingested, 400 ml of the drink containing 111 Kbq/l was ingested at the start of exercise and a further 100 ml ingested every 10 min thereafter, so that 600 ml was ingested every hour. The rate of infusion was determined to approximate the blood specific activity that was expected to be obtained with ingestion and then infused at a constant rate. Thus a total of 200 kBq of a sterile, pyrogen free 7400 kBq/mmol U-^{14}C-glucose (Amersham International, Buckinghamshire, UK) tracer was infused.

VO_2, VCO_2, and $^{14}CO_2$ measurements

Every 15 min during exercise, VO_2 and VCO_2 were determined as previously described and expired air was trapped for the later determination of $^{14}CO_2$ specific activity. The CO_2 trapping mixture has been described previously (65, 67) and consisted of 1 ml of hyamine hydroxide in methanol (Packard, Illinois, USA), 1 ml of ethanol and 2 drops of 1% phenolphthalein indicator. Expired air was bubbled through the trapping mixture until the solution became clear, at which point 1 mmol of CO_2 had been absorbed. Liquid scintillation cocktail (Beckman Ready Gel, Fullerton, USA) was then added (10 ml) and $^{14}CO_2$ radioactivity was counted in a liquid scintillation counter (Packard Tri-Carb 4640, Illinois, USA). All ^{14}C counts were automatically corrected for quenching and background activity.

Plasma glucose concentrations and specific activity

Plasma glucose concentrations (G) were determined with a glucose analyser 2 (Beckman Instruments Inc., Fullerton, USA) after collection of blood (5ml) into chilled tubes containing potassium oxalate and sodium fluoride and centrifugation at 500 x g for 15 min.

Plasma glucose specific activity

A 1 ml aliquot of each of the plasma samples used for G was deproteinised by addition of 70 µl of $HClO_4$ (3.5 M) which also served to drive off ^{14}C-bicarbonate as $^{14}CO_2$. The samples were then centrifuged at 4°C and the protein-free supernatant removed and kept cold. The precipitate was then washed by resuspension in 0.76 ml of 0.13M $HClO_4$, re-centrifuged and the supernatant added to that previously saved. The last step was then repeated. The pH of the combined supernatants was returned to between 7.0-8.0 by the addition of 132 µl of 3M K_2CO_3 in 0.01M Tris-HCl buffer. The sample was kept on ice for 10 min then re-centrifuged as before to remove the precipitate. The supernatant was passed through an anion exchange column (Extra-Sep RC SAX, Chromatography Research Supplies, Addison, Il, USA). The void volume, which contained some glucose, was collected and the remaining glucose was eluted with distilled water (3 x 1 ml). Lactate was then eluted with (2 x 1 ml) 1 M $CaCl_2$ (pH 2). Five control samples were processed and run concurrently with the test samples each time the analysis was performed. One was a normal plasma sample containing no radioactivity, so that any contamination by radioactivity during the processing would be subtracted as background in the scintillation counting (blank). The radioactivity in these samples was always very low. Two control samples were spiked with a known amount of U-^{14}C-glucose so that the dpm values of experimental samples could be corrected for small losses which occurred during processing. The last two were spiked with a known amount of U-^{14}C-lactate. A complete separation of glucose and lactate could thus be confirmed if no radioactivity was found in the lactate elution of the U-^{14}C-glucose controls and no radioactivity was found in the glucose elution of U-^{14}C-lactate controls.

In order to reduce the water/ liquid scintillation cocktail ratio during radioactivity counting, the eluates (~7 ml) were evaporated to near dryness (~0.3 ml) at 60°C over approximately 20 h. One ml of distilled water was then added to the residue and mixed with 15 ml of Ready Gel (Beckman, Fullerton, USA) liquid scintillation cocktail for ^{14}C radioactivity determinations as described earlier. Since the 1 ml aliquot of plasma used for radiation counting was from the same plasma sample as previously used for the determination of glucose concentration, the specific activity in dpm/ mmol glucose could be calculated after the small (~4%) loss of radioactivity during the preparation of the sample was determined from the control plasma samples.

Plasma glucose oxidation

The rates of plasma glucose oxidation (R_{ox}) in g/min were calculated from the following equation:

$$R_{ox} = ((^{14}CO_2 \times 6)/SA\ glu) \times VCO_2 \times 1.35$$

where, $^{14}CO_2 \times 6$ is the dpm/mmol value multiplied by 6, as there are 6 carbon atoms per molecule of ^{14}C-glucose; SA glu is the plasma ^{14}C-glucose specific activity in dpm/mmol; VCO_2 is the volume of expired CO_2 in l/min; and 1.35 is the number of grams of glucose oxidised to produce 1 l of CO_2.

Statistical analyses

All results are presented as means ± SEMs for 6 subjects. Statistical significance ($p < 0.05$) of differences were assessed by a two-way repeated measures ANOVA.

RESULTS

Subject characteristics are presented in Table 3.1. All were highly trained, reaching high peak workloads and VO_2 max values during the VO_2 max tests.

Table 3.1. Characteristics of subjects.

Age (yr)	23±3
VO_2 max (l/min)	5.11±0.18
Peak work rate (W)	414±16
Mass (kg)	76.7±3.4

Values are Means ± (SEM).

Mean VO_2 during the U-^{14}C-glucose ingestion and infusion trials were not significantly different (3.40 ± 0.15 vs 3.40 ± 0.14 l/min, respectively); neither was VCO_2 (3.02 ± 0.28 and 2.99 ± 0.18 l/min, respectively) or RER (0.87 ± 0.06 vs 0.87 ± 0.03) significantly different. Mean plasma glucose concentrations were also not significantly different between ingestion and infusion trials (4.69 ± 0.24 vs 4.90 ± 0.30 mmol/l, respectively). Plasma ^{14}C-glucose specific activities are presented in Figure 3.3. As expected, these were not significantly different between trials. There were no differences between the $^{14}CO_2$ specific activity between ingestion and infusion trials (Figure 3.4). Neither were there differences in the ration in the ratio between $^{14}CO_2$ and glucose specific activities. Glucose oxidation rates were not significantly different between trials and are shown in Figure 3.5. Values at 60 minutes of exercise were 0.42±0.06 and 0.35±0.05 g/min in ingestion and infusion trials, respectively.

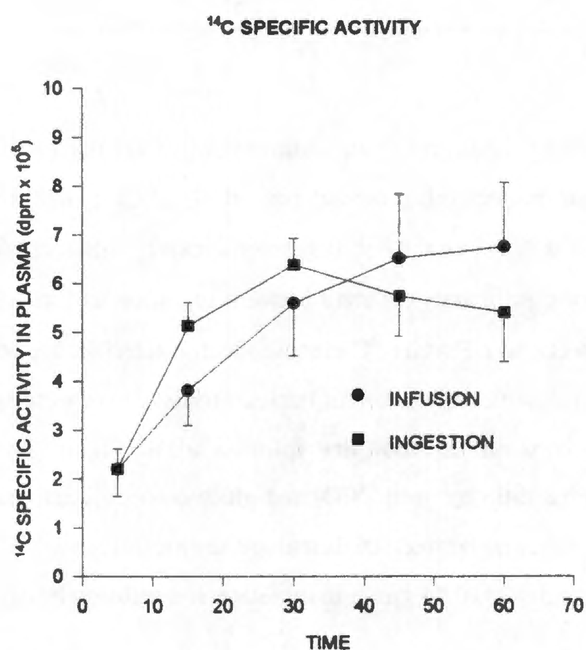


Figure 3.3. Plasma ^{14}C specific activity during 60 minutes of exercise with either ingestion or infusion of ^{14}C-glucose tracer. As was designed, no significant difference existed between routes of administration of tracer.

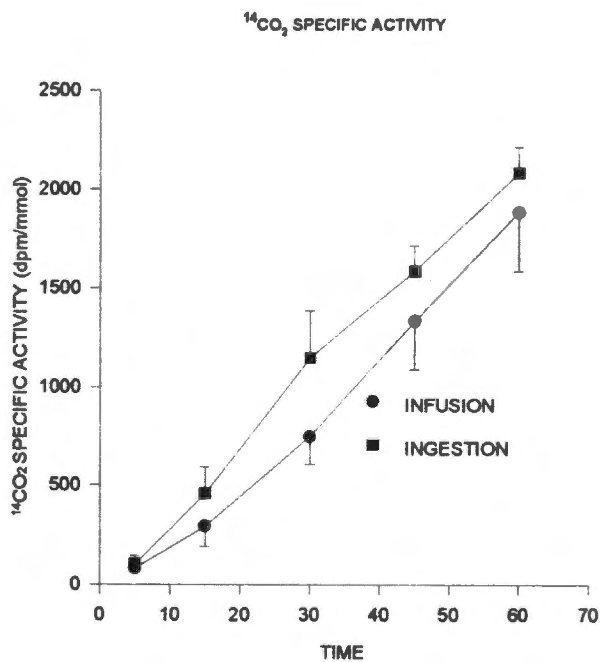


Figure 3.4. $^{14}CO_2$ specific activity during 60 minutes of exercise with either ingestion or infusion of ^{14}C-glucose tracer. No significant differences between routes of administration of tracer.

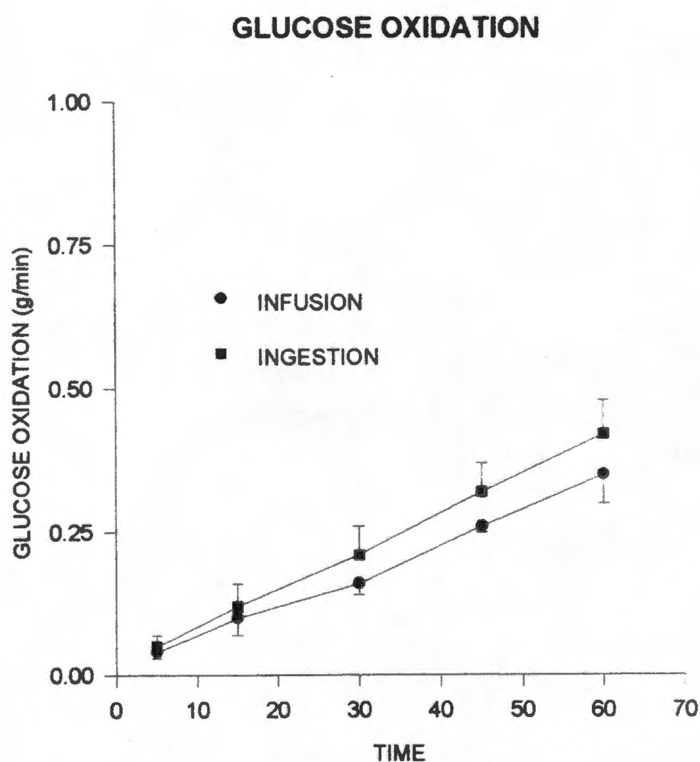


Figure 3.5. Plasma glucose oxidation (g/min) during 60 minutes of exercise with either ingestion or infusion of ^{14}C-glucose tracer. No significant difference between routes of administration of tracer.

DISCUSSION

The comparison of rates of plasma glucose oxidation calculated from tracer glucose that was either ingested or infused (Figure 3.5) showed that there was no significant difference ($p < 0.05$) between the two routes of administration. The reason for the initial apparent (but not significant) faster rise in plasma specific activity with ingestion of the tracer (Figure 3.3) is that a bolus of 400 ml of drink containing 111 kBq of ^{14}C- glucose was ingested. Figure 3.1 shows the relationship between $^{14}CO_2$ and

plasma ^{14}C glucose specific activities during the trial described in Chapter 6 of this thesis. It should be noted that here too, there is a rapid rise in plasma ^{14}C-glucose specific activity during the first 45 minutes due to the initial bolus dose of 111 kBq of ^{14}C-glucose. There is then no significant change in plasma ^{14}C-glucose specific activity from 45 to 85 minutes. After 85 minutes, plasma ^{14}C-glucose specific activity declines. This could indicate an increase in rate of glucose oxidation and this contention is supported by the increase in the rate of glucose infusion required to maintain euglycaemia (oxidation is proportional to $^{14}CO_2$ specific activity ÷ ^{14}C glucose specific activity; see formula on page 44).

Glucose oxidation rates are not significantly different between trials and are shown in Figure 3.5. Values at 60 minutes of exercise were 0.42±0.06 and 0.35±0.05 g/min in ingestion and infusion trials, respectively. It should be noted, however, that this validation of the technique was for a period of 60 minutes, whereas the actual experimental trials were 150 minutes in duration. Thus it is only assumed that the techniques remains valid for the entire duration of the experiment.

CHAPTER 4

GENERAL METHODS

SUBJECTS

Thirty-two endurance-trained male cyclists participated in the study, which was approved by the Research and Ethics Committee of the Faculty of Medicine of the University of Cape Town. Cyclists were selected who regularly trained ≥250 km a week and who had recently completed a 105 km cycle race in less than 3h30. Subject characteristics are given in Table 4.1. As radio-labelled tracers were ingested and muscle biopsies and blood samples were taken, the procedures and risks were explained to the subjects and their written informed consents were obtained.

The total radiation dose received by each subject was approximately 0.11 mSv. As discussed in Chapter 3, a radiation dose of 1 mSv/yr is accepted as safe for the public and 20 mSv/yr is regarded as safe for occupational exposure to radioactivity (Information courtesy of Dr M. Shackelton of the Department of Medical Physics, Groote Schuur Hospital, Cape Town).

MEASUREMENTS TAKEN PRIOR TO MANIPULATION OF MUSCLE GLYCOGEN CONCENTRATIONS.

On the first day of the trial, subjects ingested a small breakfast (~1200 kJ, 30 g carbohydrate (CHO)) 3 hours before arrival in the laboratory at 09h15. The subject was first weighed and body fat percentage was estimated using standard formulae (178) from the sum of biceps, triceps, subscapular and supra-iliac skin-folds.

MEASUREMENT OF MAXIMAL OXYGEN UPTAKE

Maximum oxygen uptake ($VO_{2\ max}$) of each subject was determined on an electrically braked cycle ergometer (Lode, Groningen, Netherlands) as discussed in Chapter 3. This information was used to adjust the work rate in the subsequent phases of the trial so that each subject exercised at an intensity corresponding to 70% of $VO_{2\ max}$. Since metabolic and hormonal responses appear to be related to relative rather than absolute workload (357), work rate was adjusted during the trial if necessary to maintain exercise at this intensity.

MANIPULATION OF MUSCLE GLYCOGEN CONTENT AND COMPOSITION OF EXPERIMENTAL GROUPS

After the $VO_{2\ max}$ test, all subjects rested for 20 min and then rode for a further 90 min at 70% of $VO_{2\ max}$ with 5 minute intervals at 90% of $VO_{2\ max}$ every 20 min in order to deplete muscle glycogen. Following this procedure, subjects were randomly assigned to one of 5 groups:

LGE: Subjects started exercise with low muscle glycogen content (LG) and received a variable rate glucose infusion to maintain euglycaemia (E).

NGE: Subjects started exercise with normal (NG) muscle glycogen content and received a variable rate glucose infusion to maintain euglycaemia (E).

LGEI: Subjects started exercise with low mucle glycogen content (LG) and received an insulin infusion (I) and a variable rate glucose infusion to maintain euglycaemia (E).

LGH: Subjects started exercise with low muscle glycogen content (LG) and received a variable rate glucose infusion to maintain hyperglycaemia (H).

NGH: Subjects started exercise with normal (NG) muscle glycogen content and received a variable rate glucose infusion to maintain hyperglycaemia (H).

Subjects in the low glycogen groups (LGE, LGEI and LGH) ingested a low CHO diet for the 36 hours following the glycogen depletion regimen. They were given a list of food choices that would provide a daily energy intake of ~ 6800 kJ (16% CHO). NGE and NGH subjects followed an unrestricted diet. All subjects were instructed to do only light training on the second day (approximately 1 hour of low-intensity cycling). The low carbohydrate diet in the former groups, combined with light training during this period, was designed to limit muscle glycogen resynthesis while allowing recovery from the fatiguing effects of the depletion ride. In contrast, the normal diet in the latter groups combined with the light training allowed repletion of muscle glycogen content to normal (confirmed by measurement of muscle glycogen content before the start of exercise).

EXPERIMENTAL PROTOCOL AFTER MANIPULATION OF MUSCLE GLYCOGEN CONTENT

Resting measurements

Subjects were again instructed to ingest a small breakfast (~1200 kJ, 30 g CHO) 3 hours before arrival in the laboratory at 09h15 on the third day of the trial. Shortly after the subject's arrival in the

laboratory, an 18 g teflon cannula (Jelco, Johnson and Johnson, Halfway House, South Africa) was placed into his forearm vein and connected to a three-way stopcock (Uniflex, Mallinckrodt Medical, Hennef-Sieg, Germany). A blood sample was only taken 20 minutes later, in case venepuncture caused a rise in circulating catecholamine concentrations. Resting oxygen uptake (VO_2) and carbon dioxide production (VCO_2) were measured on-line with the subject seated and relaxed, using a computerised system (Oxycon Alpha), whereafter a urine sample was collected for determination of urinary ketones. Twenty minutes after cannulation, a 20 ml blood sample was obtained for measurement of concentrations of plasma glucose (G), lactate (L), insulin (INS), glucagon (GG), epinephrine (E) and norepinephrine (NE) and serum free fatty acids (FFA) and growth hormone (GH). Aliquots of the blood sample were placed into tubes containing potassium oxalate and sodium fluoride for glucose and lactate determination; lithium heparin for insulin, epinephrine and norepinephrine determination; EDTA and 100 µl Aprotinin (Midran, Novo Nordisk, Johannesburg, South Africa) for glucagon and serum separating tubes (SST, Beckton Dickinson) for free fatty acid and growth hormone concentrations.

The tubes were immediately placed on ice and after 20 min, centrifuged at 500 x g and 4°C for 20 min. The supernatant (plasma or serum) was then transferred to plastic test tubes, sealed and stored at -20°C for later measurements of G, L, INS and GG or at-80°C for later determinations of E, NE, GH and FFA.

Leg muscle glycogen disappearance

The muscle biopsy technique of Bergstrom (41), as modified by Evans (188), was performed to sample muscle from the vastus lateralis muscle before the start of and immediately on completion of exercise. The sample was immediately placed in liquid nitrogen for later determination of muscle glycogen concentration using conventional methods (496).

Glucose and insulin infusion

A 20g teflon cannula was placed in a left forearm vein for infusion of glucose (all groups) and insulin (LGEI) during the entire 145 minutes of cycling at 70% of $VO_{2\,max}$. Plasma glucose concentration was maintained at ~9 mmol/l (LGH and NGH) or ~4.5 mmol/l (LGE, LGEI and NGE) by infusing a sterile 25% (m/v) glucose solution using the hyperglycaemic (LGH and NGH), euglycaemic (LGE and NGE) and euglycaemic, hyperinsulinaemic (LGEI), glucose clamp techniques (146), modified as described in the previous chapter. In LGEI, insulin (Actrapid, Novo Nordisk, South Africa) was infused in normal saline at a rate determined as described in Chapter 3 (0.2 mU/kg/min). All infusions were controlled using calibrated auto syringe pumps (Travenol Laboratories, Hooksett, NJ). Blood samples were obtained at 5 min intervals to measure blood glucose concentrations using a pocket glucometer (Accutrend, Boehringer Mannheim, Mannheim, Germany) for adjustment of the

glucose infusion rate in order to maintain the selected blood glucose concentration. At the end of exercise, a second urine specimen was obtained to determine whether the glucose infusion had resulted in glycosuria.

Measurement of substrate oxidation and hormone concentrations during exercise

Rates of endogenous glucose oxidation were measured using a U-^{14}C glucose tracer. Two hundred kBq of a 7400 kBq/mmol U-^{14}C-glucose (Amersham International, Buckinghamshire, UK) tracer was added to 1.8 l of artificially flavoured water. As discussed in the previous chapter, U-^{14}C plasma glucose specific activity, $^{14}CO_2$ specific activity and calculated rates of plasma glucose oxidation were the same irrespective of whether the tracer was ingested or infused. While warming up (~ 5 mins) prior to the 145 min exercise test, subjects drank a 400 ml bolus of the drink containing 111 kBq/l of U-^{14}C-glucose tracer. Every 10 min after commencement of the exercise test, they ingested a further 100 ml, giving a total of 600 ml/hr.

After 5 minutes of exercise and at 20 min intervals during exercise, approximately 20 ml of blood was drawn and processed as described for later measurements of U-^{14}C plasma glucose specific activity and for measurement of G, INS, GG, E, NE, FFA and GH.

VO_2, VCO_2, and $^{14}CO_2$ measurements during exercise

At the same time as the blood samples were drawn, VO_2 and VCO_2 were determined on line as previously described and expired air was trapped for the later determination of $^{14}CO_2$ specific activity as described in Chapter 3.

LABORATORY ANALYSES

Plasma glucose and lactate concentrations

Plasma glucose concentrations were determined by the glucose oxidase method using a glucose analyser (Glucose Analyser 2, Beckman Instruments Inc., Fullerton, Ca, USA). Lactate concentrations were measured onan aliquot the same plasma samples by spectrophotometric (Beckman Model 35, Beckman Instruments Inc., Fullerton, Ca, USA) enzymatic assays (Lactate PAP, bioMérieux, Lyon, France).

Plasma glucose and lactate specific activity

Samples for determination of plasma glucose and lactate specific activities were processed as described in Chapter 3. After scintillation counting it was found that the dpm of the lactate sample was too low to be reliable when calculating the specific activity. Thus these data were not used to calculate lactate oxidation. Rate of glucose oxidation (R_{ox}) was calculated as previously described in Chapter 3.

Plasma insulin and glucagon and serum growth hormone concentrations

INS, GG and GH were determined using radioimmunoassay techniques (Coat-A-Count Insulin; Double Antibody Glucagon, both Diagnostic Products, Los Angeles, USA; hGh RIA, Pharmacia, Uppsala, Sweden).

Plasma Catecholamine Concentrations

Catecholamine concentrations were determined by means of high performance liquid chromatography (HPLC) with electrochemical detection (690). To 1.5 ml of plasma in a polypropylene tube, an internal standard (10 pg/ml dehydrobenzylamine (DBHA) in 0.1N HCl), 0.4 ml of 2M Tris-HCl buffer (pH 8.7) and 10 mg of acid-washed aluminium oxide were added. The mixture was shaken for 15 minutes, after which the supernatant was discarded and the aluminium oxide precipitate washed 3 times with 1 ml of 0.02M Tris-HCl buffer (pH 8.1). The fluid was then carefully aspirated and 0.1 ml 1% v/v glacial acetic acid was added and the samples shaken and then centrifuged at 2000 rpm for 15 minutes. The clear supernatant was transferred to clean tubes and stored at -20°C for not longer than 2 days prior to chromatography. The final supernatant was injected onto the HPLC column (Resolve C18-Novapak 3.9 x 150 mm, particle size 5μl, temperature ambient; Waters 510 pump, Waters 712 Wisp injector) in a volume of 15 μl. The mobile phase included 50 mM sodium acetate, 20 mM citric acid, 3.75 mM sodium-1-octane-sulfonate, 1.0 mM di-n-butylamine, 0.135 mM NaEDTA in 95% methanol, pH 4.3. The flow rate was 1.0 ml/min (pressure <14000 kPa), the working potential was +0.6 V against Ag/AgCl reference electrode, 0.5 nA full scale sensitivity and at-time constant set on 2 sec (active filter switched on). The cell volume was 2.5 μl. The integration was performed using a Spectra-Physics SP4400 Chrom Jet Recording Integrator using the following parameters: attenuation: 64, chart speed: 0.5 cm/min, peak width: 6, peak threshold: 2000.

Serum Free Fatty Acid Concentrations

Serum free fatty acid concentrations were measured using an enzymatic colorimetric assay (601) (Half-micro test, Boehringer Mannheim, Mannheim, Germany).

Total carbohydrate and fat oxidation

Rates of CHO and fat oxidation were calculated from the VO_2 and VCO_2 values using the formulae of Consolazio et al. (122) and updated by Frayn (205). Because protein oxidation has been shown to be negligable at 70% of $VO_{2\,max}$ (65), protein oxidation was not included in the calculations.

Urine specimens

Urine specimens collected before and at the end of exercise were examined for the presence of ketones and glucose using urinary dipsticks (Keto-Diabur-Test 5000; Boehringer Mannheim, Bell Lane, Lewes, East Sussex, United Kingdom).

STATISTICAL TREATMENT

All results are presented as means ± SEMs. Statistical significance ($p < 0.05$) of between-group differences were assessed by a two way ANOVA with repeated measures over time, followed by a Tukey's Honest Significance test. For some measurements where convergence of data in the second half of the trial resulted in masking of significant differences on the ANOVA, an unpaired t-test was used to compare Area-Under-the Curve (AUC) measurements.

CHAPTER 5

INFLUENCE OF MUSCLE GLYCOGEN CONTENT ON METABOLIC REGULATION.

INTRODUCTION

A decrease in carbohydrate (CHO) oxidation, as indicated by a decreased respiratory exchange ratio (RER) after a low CHO diet (215, 231, 257, 267, 268, 310, 312, 424, 501, 527) has been described at rest (215, 231, 312), and during exercise at various intensities (49, 215, 230, 231, 257, 267, 311, 312, 397, 421, 424, 501). Other effects on substrate metabolism and hormonal changes during exercise that have been demonstrated as a result of a low-CHO diet or fasting before exercise are a decrease in plasma insulin (215, 257, 268) and lactate (257) concentrations, and an increase in plasma norepinephrine (215, 268), epinephrine (215, 268), glucagon (215, 268), growth hormone (GH) (215), cortisol (215), glycerol (215, 312), ketone (215, 268) and free fatty acid (FFA) (215, 257, 268, 312) concentrations. However, it is not clear whether the effects seen in these studies on substrate metabolism and hormonal responses were due to muscle or liver glycogen depletion or, except in cases where glucose was infused during exercise (215, 268), due to a general "carbohydrate deficiency", i.e., a shortage of total carbohydrate available for oxidation. In some studies, subjects were overnight fasted (257) or became hypoglycaemic despite ingestion of a small pre-exercise meal (312). In an attempt to exclude the effects of an overall carbohydrate deficiency, glucose has been infused during exercise in some studies (215, 268), but in one of these (268), subjects who ingested a low carbohydrate diet were overnight fasted, while control subjects on a normal diet ate a 60% carbohydrate meal 3 hours before exercise; while in the other (215), subjects were overnight fasted and glucose infusion was begun only after subjects had reached exhaustion.

When euglycaemia is maintained by glucose infusion during exercise, glucose which disappears from circulation is replaced by infused glucose. This should prevent any metabolic or hormonal effects as a consequence of inadequate extramuscular and extrahepatic carbohydrate availability. Thus if the metabolic effects of glycogen depletion described in the studies cited (215, 257, 268, 312) persist in subjects with low muscle glycogen content despite maintenance of euglycaemia, this would indicate that the responses are related specifically to reduced intramuscular or intrahepatic carbohydrate availability.

Since hyperinsulinaemia has minimal effects on hepatic glucose uptake in the absence of hyperglycaemia (107), the predominant effect of insulin infusion during exercise in which euglycaemia is maintained is an increase in **muscle** glucose uptake (145). Thus if the metabolic effects of glycogen depletion are counteracted by insulin infusion during euglycaemic exercise, the effects of

intramuscular and intrahepatic carbohydrate availability will be differentiated and provide evidence that the metabolic effects described (215, 257, 268, 312) are related specifically to intramuscular carbohydrate availability.

Finally, in subjects with normal glycogen content, maintenance of hyperglycaemia results in an increase in glucose oxidation during exercise compared to maintenance of euglycaemia (265). However, unlike euglycaemia, the rate of glucose oxidation is less than the rate of glucose infusion when hyperglycaemia is maintained during exercise (265). It was therefore hypothesised that the "excess" glucose that was not oxidised in subjects with normal muscle glycogen content during hyperglycaemia (265), would be oxidised when muscle glycogen content is low.

Therefore, the first aim of this study was to investigate the effect of low muscle glycogen content, without concomitant fatigue, on fuel substrate utilisation during prolonged, moderate intensity exercise by maintaining euglycaemia and manipulating plasma insulin concentrations in order to provide an alternate source of intramuscular carbohydrate. The second aim was to determine whether the metabolic and hormonal effects of low muscle glycogen content could be overcome by providing an excess of oxidizable carbohydrate by intravenous glucose infusion.

METHODS

Subjects

Twenty-seven endurance-trained male cyclists participated in the study. Subject characteristics are given in Table 5.1. Following the procedure described in Chapter 4 to deplete muscle glycogen, subjects were randomly assigned to one of 4 groups: low glycogen, euglycaemic (LGE) (n=8), normal glycogen, euglycaemic (NGE) (n=6), low glycogen, euglycaemic, hyperinsulinaemic (LGEI) (n=5) and low glycogen, hyperglycaemic (LGH) (n=8).

Subjects in the low glycogen groups (LGE, LGEI and LGH) ingested a low CHO diet for the 36 hours following the depletion regimen, while NGE subjects followed an unrestricted diet. All subjects were instructed to do only light training on the second day (approximately 1 hour of low-intensity cycling). On the third day, subjects returned to the laboratory and followed the experimental protocol described in Chapter 4. Plasma glucose concentration was maintained at ~4.5 mmol/l (euglycaemic subjects) or ~9 mmol/l (hyperglycaemic subjects) by infusing a sterile 25% m/v glucose solution using the euglycaemic (LGE, NGE), euglycaemic, hyperinsulinaemic (LGEI), and hyperglycaemic (LGH) glucose clamp techniques (146), modified as described in Chapter 3. In LGEI, insulin was infused in normal saline at a rate of 0.2 mU/kg/min. Blood, muscle biopsy and urine samples were taken and processed as described in Chapter 4.

STATISTICAL ANALYSES

All results are presented as means ± SEMs. Statistical significance ($p < 0.05$) of between-group differences were assessed by a two way ANOVA with repeated measures over time, followed by a Tukey's Honest Significance test for unequal n. An unpaired t-test was used for single data.

RESULTS

Subject characteristics are given in Table 5.1. There were no significant differences between groups in any of the parameters shown.

Table 5.1. Characteristics of subjects

	NGE (n=6)	LGE (n=8)	LGEI (n=5)	LGH (n=8)
Age (yr)	26±3	24±2	22±4	29±3
Mass (kg)	76±2	75±3	70±1	73±3
FFM (kg)	66±2	65±3	61±1	62±2
% Body Fat	14±1	13±1	13±1	15±1
Peak Work Rate (W)	382±23	363±19	344±9	364±18
$VO_{2\,max}$ (l/min)	4.40±0.30	4.57±0.15	4.46±0.13	4.45±0.15

Values are means ± SEM. LGE, low glycogen, euglycaemic; NGE, normal glycogen, euglycaemic; LGEI, low glycogen, euglycaemic, hyperinsulinaemic; LGH, low glycogen, hyperglycaemic; FFM is fat free mass; $VO_{2\,max}$, maximal O_2 consumption. No significant differences between groups.

Pre-Exercise

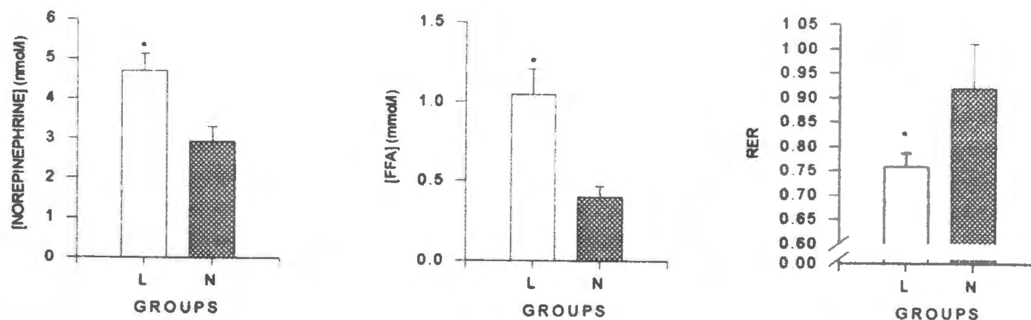


Figure 5.1. Plasma norepinephrine, serum free fatty acid concentrations and respiratory exchange ratio at rest in subjects with low muscle glycogen content (L) and in subjects with normal muscle glycogen content (N). *Significantly different ($p<0.05$).

Pre-exercise muscle glycogen concentrations were significantly higher in NGE than in LGE, LGEI or LGH ($p<0.05$) (Table 5.2). Resting serum free fatty acid (FFA) and plasma norepinephrine concentrations before exercise were higher (1.05±0.16 vs 0.38±0.08 mmol/l and 4.71±0.43 vs 2.92±0.37 nmol/l, respectively) and respiratory exchange ratios (RER) lower (0.76±0.03 vs 0.88±0.03) (Figure 5.1) ($p<0.05$) in subjects with low muscle glycogen content (L) than in subjects with normal muscle glycogen content (N). In contrast, no significant differences were found in plasma glucose (4.48±0.12 v 5.22±0.41 mmol/l, L v N respectively), lactate (1.31±0.10 v 1.52±0.16 mmol/l), insulin (4.29±0.63 v 5.03±0.37 μU/ml), glucagon (92±4 v 105±8 pg/ml), epinephrine (0.16±0.06 v 0.06±0.01 nmol/l) or growth hormone (GH) (14±4 v 23±12 mU/l) concentrations at rest between L and N groups. Pre- and post-exercise urine specimens were negative on dipstick examination for both ketones and glucose.

Table 5.2. Total carbohydrate and fat oxidation for 145 min of cycling and pre- and post-exercise muscle glycogen content in NGE, LGE, LGEI and LGH subjects

	NGE	LGE	LGEI	LGH
F_{tot} (g)	76±12*	126±6	88±18$^{\theta}$	72±7$^{\psi}$
CHO_{tot} (g)	387±32*	257±42	347±50$^{\theta}$	361±23$^{\psi}$
Pre-exercise Muscle Glycogen (mmol/kg ww)	134±5*	80±8	63±11	80±5
Post-exercise Muscle Glycogen (mmol/kg ww)	45±11	31±6	35±7	39±6

Values are means ± SEM. LGE, low glycogen, euglycaemic; NGE, normal glycogen, euglycaemic; LGEI, low glycogen, euglycaemic, hyperinsulinaemic, LGH, low glycogen, hyperglycaemic; CHO_{tot}, total carbohydrate oxidation calculated from gas exchange data; F_{tot}, total fat oxidation from gas exchange data. *Significantly different between NGE and LGE ($p<0.05$); $^{\theta}$Significantly different between LGE and LGEI ($p<0.05$); $^{\psi}$Significantly different between LGE and LGH ($p < 0.05$).

Exercise trial

Values for rate of oxygen consumption during exercise (VO_2) are given in Table 5.3. VO_2 did not differ significantly between groups and did not change significantly over the duration of the trial as the workload was maintained at 70% of $VO_{2\,max}$.

RER (Figure 5.2), total fat oxidation for 145 minutes of exercise, total carbohydrate oxidation (Table 5.2), rates of carbohydrate oxidation during exercise and rates of fat oxidation were similar in LGEI, LGH and NGE, but RER, total carbohydrate oxidation and rate of carbohydrate oxidation (Table 5.3) were significantly lower and total fat oxidation (Table 5.2) and rate of fat oxidation (Table 5.3) significantly higher in LGE ($p<0.05$). In LGEI and LGH, RER (Figure 5.2) at the start of exercise was the same as in LGE, but did not change significantly over the duration of exercise, and thus at the end of exercise was higher than in LGE and not significantly different from NGE. In LGE and NGE, but not in LGEI or LGH, rate of carbohydrate oxidation decreased significantly over the duration of the trial and rate of fat oxidation increased ($p<0.05$). FFA (Figure 5.3A) were significantly higher in LGE than NGE and LGH over the first 45 minutes of exercise ($p<0.05$) and increased significantly over the duration of exercise in both these groups. In LGEI, FFA decreased significantly over the duration of exercise ($p<0.05$) and were significantly ($p<0.05$) lower than in LGE. FFA were significantly higher in LGH than in NGE, during the first 25 minutes of exercise, but thereafter declined and were not significantly different from NGE.

Table 5.3. Steady state gas exchange data and rate of carbohydrate and fat oxidation during 145 min of cycling in NGE, LGE, LGEI and LGH subjects.

	Time (minutes)							
	5	25	45	65	85	105	125	145
VO_2 (l/min)								
NGE	3.21	3.21	3.20	3.14	3.10	3.14	3.19	3.22
	±0.10	±0.14	±0.11	±0.11	±0.10	±0.13	±0.12	±0.14
LGE	3.29	3.30	3.22	3.21	3.18	3.18	3.15	3.22
	±0.16	±0.16	±0.17	±0.13	±0.17	±0.15	±0.16	±0.21
LGEI	3.12	3.15	3.09	3.11	3.12	3.08	3.17	3.21
	±0.09	±0.10	±0.08	±0.11	±0.06	±0.09	±0.10	±0.10
LGH	2.87	2.86	2.97	2.93	2.94	2.94	2.94	3.04
	±0.16	±0.16	±0.12	±0.10	±0.10	±0.10	±0.11	±0.11
CHO_{ox} (g/min)								
NGE#	3.10*	2.99*	3.13*	2.78*	2.61*	2.60*	2.48*	2.44*
	±0.32	±0.21	±0.29	±0.29	±0.28	±0.25	±0.23	±0.21
LGE#	2.55	2.25	2.10	1.77	1.92	1.84	1.92	1.22
	±0.28	±0.27	±0.28	±0.27	±0.26	±0.23	±0.29	±0.31
LGEI	2.37	2.25	2.14	2.40	2.67	2.43	2.75^{θ}	3.10^{θ}
	±0.45	±0.37	±0.35	±0.36	±0.41	±0.46	±0.34	±0.34
LGH	2.46	2.40	2.51	2.55^{Ψ}	2.62^{Ψ}	2.62^{Ψ}	2.62^{Ψ}	2.65^{Ψ}
	±0.16	±0.15	±0.14	±0.18	±0.17	±0.16	±0.14	±0.19
F_{ox} (g/min)								
NGE#	0.44*	0.48*	0.42*	0.53*	0.57*	0.59*	0.66*	0.69*
	±0.12	±0.08	±0.09	±0.12	±0.11	±0.08	±0.10	±0.07
LGE#	0.69	0.80	0.81	0.93	0.86	0.89	0.85	0.99
	±0.04	±0.05	±0.06	±0.04	±0.07	±0.05	±0.07	±0.05
LGEI	0.67	0.73	0.73	0.65	0.55	0.62	0.55^{θ}	0.44^{θ}
	±0.17	±0.15	±0.14	±0.13	±0.14	±0.15	±0.10	±0.10
LGH	0.51^{Ψ}	0.53^{Ψ}	0.54^{Ψ}	0.51^{Ψ}	0.48^{Ψ}	0.50^{Ψ}	0.49^{Ψ}	0.53^{Ψ}
	±0.05	±0.06	±0.04	±0.06	±0.06	±0.06	±0.05	±0.09

Values are means ± SEM. LGE, low glycogen, euglycaemic; NGE, normal glycogen, euglycaemic; LGEI, low glycogen, euglycaemic, hyperinsulinaemic; LGH, low glycogen, hyperglycaemic; VO_2, rate of oxygen uptake; CHO_{ox}, rate of carbohydrate oxidation calculated from gas exchange data; F_{ox}, rate of fat oxidation from gas exchange data. *Significantly different between NGE and LGE ($p<0.05$); $^{\theta}$Significantly different between LGE and LGEI ($p < 0.05$); $^{\Psi}$Significantly different between LGE and LGH ($p < 0.05$); #Significant change over time ($p<0.05$).

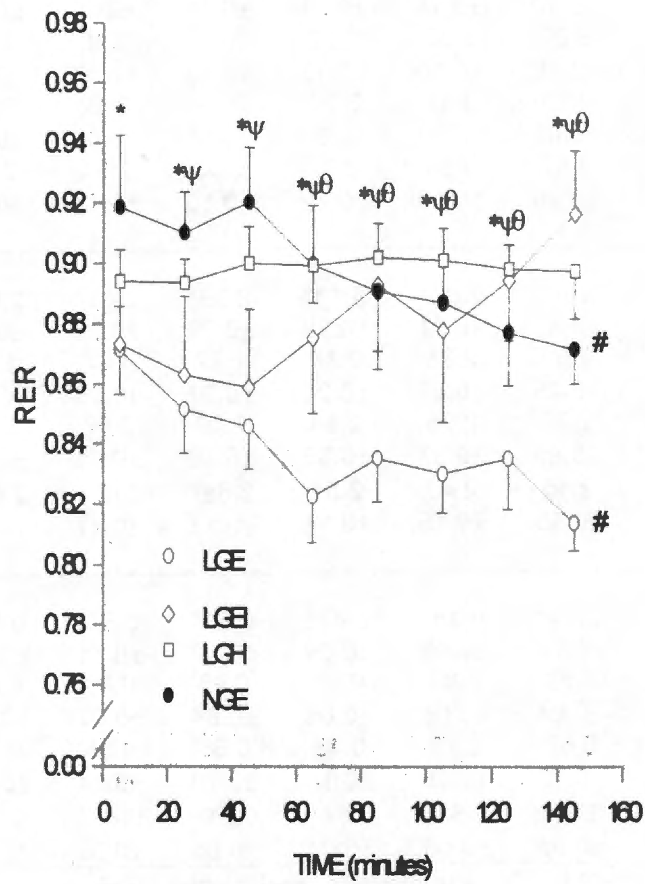


Figure 5.2. Respiratory exchange ratio in LGE, NGE, LGEI, and LGH. [ψ]Significant difference between LGE and LGH ($p<0.05$); [θ]significant difference between LGE and LGEI ($p<0.05$); [*]significant difference between LGE and NGE; [#]significant decrease over time ($p<0.05$).

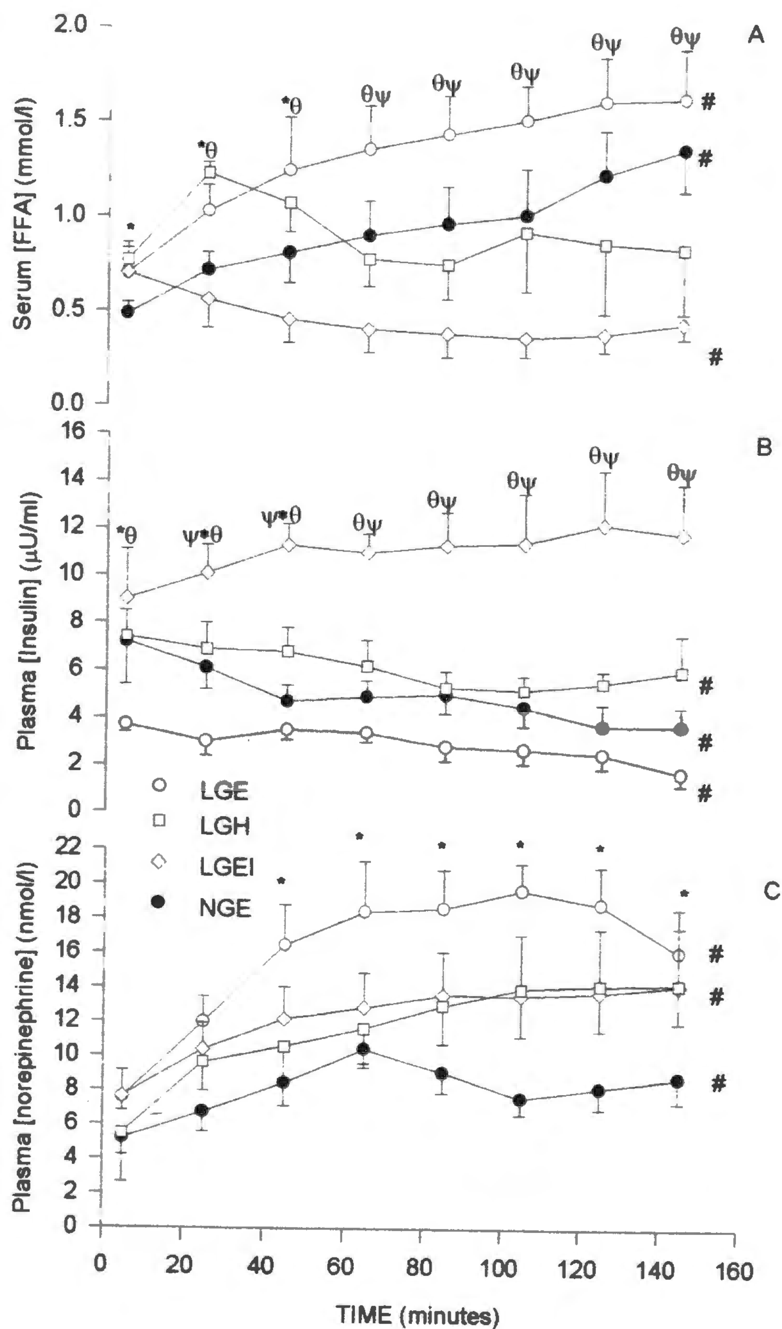


Figure 5.3 Serum free fatty acid (A); plasma insulin (B) and plasma norepinephrine (C) concentrations in LGE, LGEI, LGH and NGE subjects. *Significant difference between LGE and NGE ($p<0.05$); [Ψ]significant difference between LGE and LGH ($p<0.05$); [θ]significant difference between LGE and LGEI ($p<0.05$); [#]significant change over time ($p<0.05$).

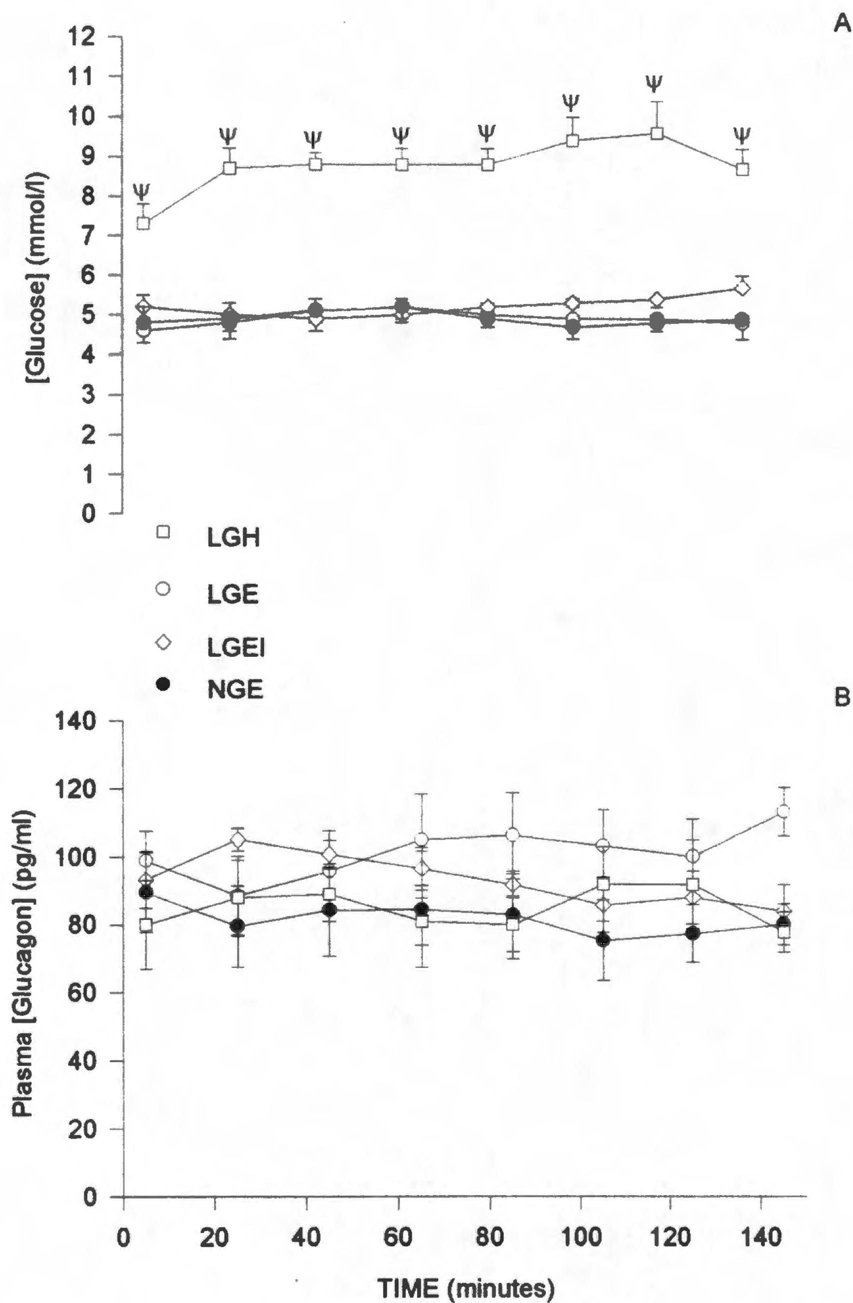


Figure 5.4. Plasma glucose (A) and glucagon (B) concentrations in LGE, LGEI, LGH and NGE subjects. $^{\Psi}$Significantly higher in LGH than in LGE, LGEI and NGE There were no significant changes over time.

Mean plasma glucose concentrations (Figure 5.4A) after 5 minutes of exercise were 4.9±0.1, 5.0±0.1, 5.2±0.1 and 9.0±0.1 mmol/l for LGE, NGE, LGEI and LGH, respectively, with a coefficient of

variation within groups of 3, 6, 6 and 3% respectively. No glucose was found in the urine of any subject at the end of the trial. Plasma lactate concentrations after 5 minutes of exercise were 2.37±0.44, 2.78±0.78, 2.19±0.25, and 1.70±0.26 mmol/l and declined over the duration of exercise to 2.23±0.48, 1.34±0.31, 1.76±0.34 and 1.56±0.29 mmol/l, in LGE, NGE, LGEI and LGH, respectively. Only the decline in NGE was statistically significant ($p<0.05$).

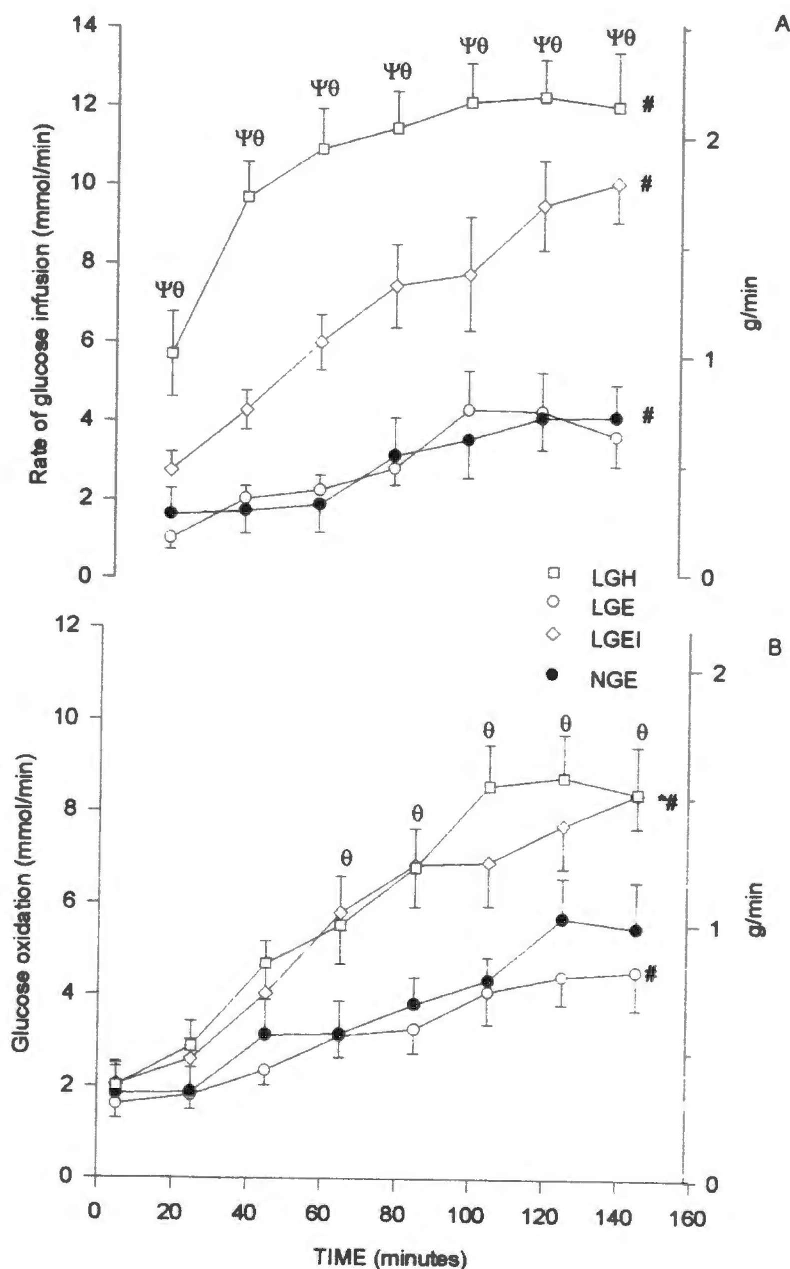


Figure 5.5. Rate of glucose infusion (A) and rate of glucose oxidation (B) in LGE, LGEI, LGH and NGE subjects. [Ψ]Significantly higher in LGH than in LGEI; [θ]significantly higher in LGEI than in LGE or NGE; *significantly higher in LGH than in LGE, NGE or LGEI; [#]significant increase over time ($p<0.05$).

The rate of glucose infusion required to maintain blood glucose concentrations at ~ 5 mmol/l (LGE, NGE, LGEI) or ~ 9 mmol/l (LGH) (Figure 5.5A) was significantly greater in LGH than in LGEI and in both these groups was significantly higher than in LGE and NGE ($p<0.05$). The glucose infusion rate did not differ between the latter two groups. In all groups, rate of glucose infusion increased progressively over the duration of exercise, with a marked increase in LGE between 80 and 100 minutes of exercise. The total amount of glucose infused during the 145 min of exercise (Figure 5.6) was higher in LGH than in LGEI (1484±125 vs 956 ±121 mmol ($p<0.05$)) and was higher in both these groups than in NGE or LGE (403±107 and 342±69 mmol, respectively).

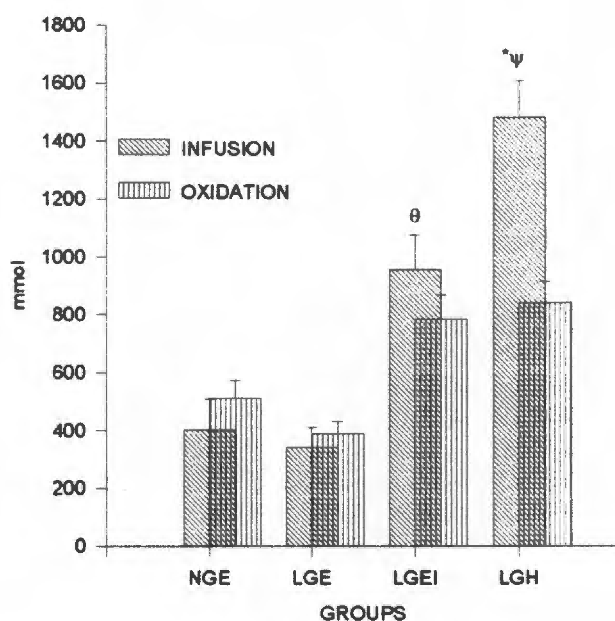


Figure 5.6 Comparison of total amount of glucose infused over 145 minutes of exercise with total glucose oxidation over the same period. *Glucose infusion significantly higher in LGH than in LGEI ($p<0.05$); $^{\theta}$glucose infusion and oxidation significantly higher in LGEI than in NGE or LGE ($p<0.05$); $^{\psi}$glucose oxidation in LGH significantly different from infusion, not significantly different from oxidation in LGEI and significantly higher than in NGE or LGE ($p<0.05$).

Rates of glucose oxidation (Figure 5.5B) increased progressively over the duration of exercise in all groups and peak rates in LGH and LGEI were 8.3±0.11 and 8.4±0.7 mmol/min (1.51±0.13 and

1.51±0.19 g/min), respectively, compared with 5.6±0.9 and 4.5±0.8 mmol/min (1.03±0.16 and 0.82±0.15 g/min) in NGE and LGE, respectively ($p<0.05$). Total glucose oxidation (Figure 5.6) did not differ significantly between LGE and NGE (389±44 vs 513±61 mmol; 70±8 vs 92±11 g) but both were lower than LGH and LGEI (840±74 and 785±83 mmol; 151 ±15 and 141±15 g, respectively) ($p<0.05$). In LGE, LGEI and NGE, total glucose oxidation matched the total amount of glucose infused (Figure 5.6) and showed a corresponding increase over the duration of exercise (Figure 5.5A and B). However, in LGH, only 66% of the total amount of glucose infused, was oxidised. The contribution of glucose to total carbohydrate oxidation increased significantly ($p<0.05$) over the duration of the trial in all groups to 51±10, 43±7, 56±8 and 58±5% in LGE, NGE, LGEI and LGH, respectively ($p<0.05$ for NGE vs LGH and LGEI).

The contribution of glucose oxidation to total energy was significantly ($p<0.05$) higher in LGH and LGEI than in NGE or LGE, reaching peaks of 41±4, 37±3, 26±3 and 21±2%, respectively.

Muscle glycogen concentrations (Table 5.2) were significantly higher at the start of exercise in NGE than LGE, LGEI or LGH. Muscle glycogen disappearance (MGD) (Figure 5.7) was greater ($p<0.05$) in NGE than LGE, LGEI or LGH (90±11 vs 49±9, 28±14, and 41±4 mmol/kg ww, respectively) but the latter three groups did not differ significantly. There were no significant differences in muscle glycogen concentrations between groups at the end of exercise (Table 5.2).

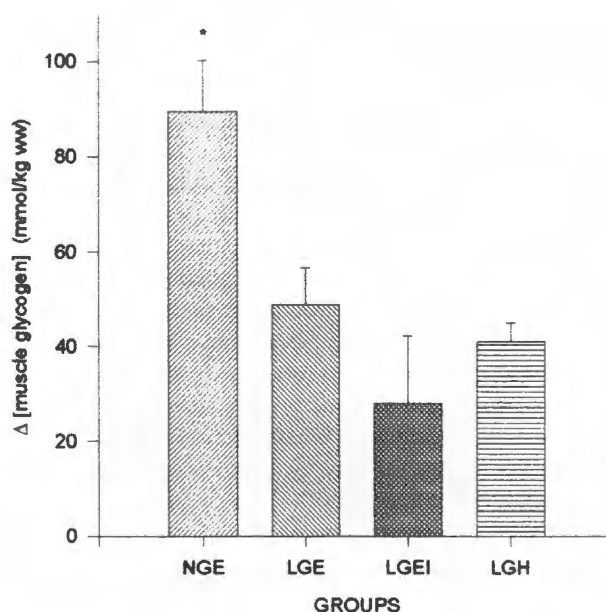


Figure 5.7. Muscle glycogen disappearance in LGE, LGEI, LGH and NGE subjects. *Significantly ($p<0.05$) higher in NGE than LGE, LGEI and LGH.

Plasma insulin concentrations (Figure 5.3B) were significantly ($p<0.05$) higher in LGEI than in NGE, LGH or LGE throughout exercise and in NGE were significantly higher ($p<0.05$) than in LGE until 45 minutes. There was a significant ($p<0.05$) increase in plasma insulin concentrations over the duration of exercise in LGEI and a significant ($p<0.05$) decrease in LGE and NGE. In contrast, there were no significant differences between groups in plasma glucagon concentrations (Figure 5.4B), which did not change significantly over the duration of exercise.

Plasma norepinephrine concentrations (Figure 5.3C) increased significantly in LGE until 125 minutes of exercise and in NGE until 65 minutes of exercise and did not change significantly thereafter, whereas in LGEI and LGH, norepinephrine increased throughout exercise ($p<0.05$). Plasma norepinephrine concentrations were significantly ($p<0.05$) higher in LGE than in NGE throughout exercise and were intermediate in LGEI and LGH between LGE and NGE, but not significantly different from them. Plasma epinephrine concentrations (E) in LGE, NGE, LGEI and LGH at 5 minutes of exercise were 0.06±0.001, 0.07 ±0.01, 0.68 ±0.07 and 0.06±0.01 nmol/l respectively ($p<0.05$ for LGEI vs LGE, LGH and NGE) and did not change significantly from this 5 minute value over the duration of exercise in LGE, LGH and NGE. In LGEI, epinephrine remained higher throughout exercise than in LGE or NGE ($p<0.05$), but did not change significantly over time. Mean

serum growth hormone concentrations were 58±16, 40±23, 67±12 and 12±4 pg/ml in LGE, NGE, LGEI and LGH, respectively but there were no significant differences between groups and concentrations did not change significantly over time.

DISCUSSION

The most significant findings in this study were that the rate of glucose oxidation was not influenced by muscle glycogen levels, i.e. was not different between NGE and LGE, that lowered muscle glycogen content at the start of exercise resulted in a shift toward lipid oxidation and that both insulin infusion and hyperglycaemia counteracted the metabolic effects of low glycogen content (Figure 5.8).

As found in other studies (215, 312), RER was lower and serum FFA and plasma norepinephrine concentrations were higher at rest in glycogen-depleted subjects (Figure 5.1). Although the effect of low muscle glycogen content on RER (312) and FFA (215) concentrations at rest have been reported previously, in these studies (215, 312) resting plasma glucose concentrations were lower in the glycogen-depleted state and therefore it cannot be said with certainty that it was the low glycogen as opposed to the low blood glucose concetration that caused the decrease in RER and increase in FFA. In the current study, however, resting plasma glucose concentrations were normal and not different between groups, probably since the subjects were ingesting approximately 64 g of CHO daily, 30 g of which was in a mixed meal each morning and three hours before the trial. Thus the lower RER and increased FFA can be attributed to low muscle or liver glycogen content, or both. Liver glycogen content would be low as it has been shown to decrease to 22-55 mmol/kg ww after 3 days on a low-carbohydrate diet (484). If 29% (351) of the carbohydrate ingested in the morning meal was taken up by splanchnic tissues, with a liver mass of 1.8 kg, liver glycogen content would remain very low after the meal at 49-82 mmol/kg ww. In addition, only moderate liver glycogen repletion takes place with glucose infusion even in resting subjects (485). This was shown in the study of Nilsson and Hultman (485), in which infusion of glucose at a mean rate of 5.8 mmol/min in resting subjects for 4 hours resulted in an increase in liver glycogen content of only 76 mmol/kg ww, even though plasma glucose concentrations remained above 10 mmol/l and rose to a peak of ~20 mmol/l after 90 minutes. Thus it is unlikely that much liver glycogen repletion took place during the trials. However, the low RER at the start of exercise in subjects with low glycogen content was restored during exercise in LGEI and LGH to that of subjects with normal muscle glycogen content (NGE) as a result of insulin and glucose infusion, respectively (Figure 5.2). Since insulin in the absence of hyperglycaemia (as in LGEI) increases muscle (710) but not liver glucose uptake (107), the higher RER in LGEI and LGH than in LGE would most likely be the result of an increase in glucose oxidation (Figure 5.5B) as a consequence of an increase in muscle glucose uptake (145, 710).

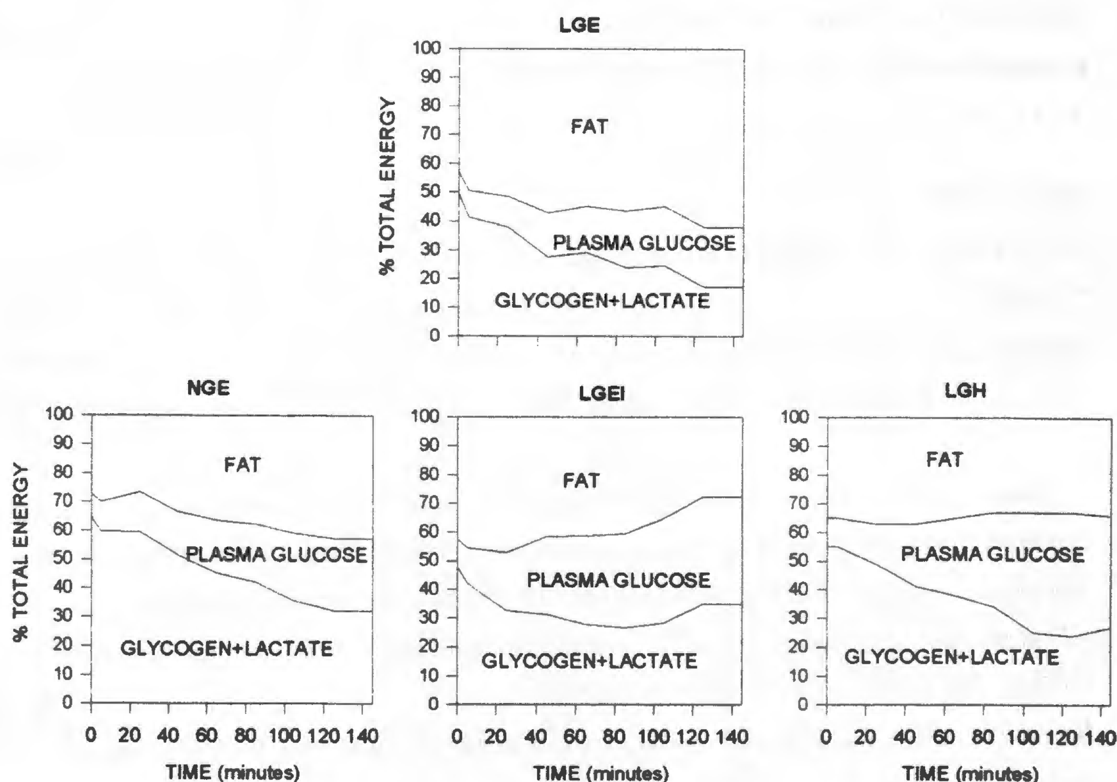


Figure 5.8. Overall metabolic effects of: i) glycogen depletion during euglycaemia (LGE vs NGE; ii) plasma glucose concentrations if muscle glycogen is low (LGE vs LGH) at the start of exercise and iii) insulin infusion on glycogen depletion (LGE vs LGEI vs NGE). FAT, fat oxidation as a % of total energy expenditure; GLUCOSE, glucose oxidation as a % of total energy expenditure; GLYCOGEN+LACTATE, oxidation of carbohydrate other than glucose (glycogen and 3-carbon products) as a % of total energy expenditure.

The significant increase in rate of glucose oxidation over the duration of exercise in all groups, but particularly the similarity in increase between LGE and NGE (Figure 5.5B) is in agreement with two recent studies in which there was no difference in glucose uptake between subjects with low or normal glycogen content (257), and no difference in glucose oxidation between subjects with high or normal glycogen content (65). This indicates that it is not the gradual decline in muscle glycogen content that drives the progressive increase in glucose oxidation during exercise.

When the entire exercise duration is considered, mean RER was not significantly different between NGE and LGH. However, there was a difference between these groups in the source of carbohydrate utilised. In LGH, the contribution of glucose to total carbohydrate oxidation rose rapidly, reaching

58±5% at 105 minutes, but reached only 31±4% in NGE at that time. The latter increased to 43±7% during the last 45 minutes of exercise. Since glycogen content does not exert an influence on the rate of glucose oxidation, as discussed above, the higher rate of glucose oxidation in LGH can be attributed to either the high prevailing glucose concentration or the concomitant hyperinsulinaemia, or both.

The greater disappearance of muscle glycogen in NGE than in LGE, LGEI or LGH (Figure 5.7) is consistent with a number of studies which have found that higher muscle glycogen levels at the start of exercise result in a greater rate of muscle glycogen utilisation during exercise (65, 257). Though there were no significant differences in muscle glycogen utilisation between any of the low-glycogen groups (LGE, LGEI and LGH), there appeared to be a tendency toward a glycogen-sparing effect with insulin infusion (LGEI).

A possible control mechanism for the fuel substrate interactions observed in this study is the "glucose-fatty acid cycle" (526), which proposes that an increase in FFA oxidation leads to an inhibition of pyruvate dehydrogenase and thereby inhibits carbohydrate oxidation. However, when FFA were infused (652) into isolated, perfused rat hindquarters at a concentration which is saturating for FFA uptake and oxidation in this preparation, unlike the finding in the current study, FFA uptake did not differ during stimulation between muscle that was low and muscle that was high in glycogen, but glucose uptake was higher in glycogen-depleted muscle. Thus in the isolated, perfused muscle preparation, where neural and hormonal control is absent, FFA uptake is not stimulated, but glucose uptake is increased in glycogen depleted muscle. Thus by exclusion, the increased lipid oxidation in the current study was probably due to stimulation of lipolysis as a result of neural or hormonal control factors outside the muscle which increased availability of FFA to the muscle. Similarly, since glucose uptake is increased in isolated, glycogen-depleted muscle, the lack of increased glucose oxidation in the current study indicates neural or hormonal inhibition of glucose uptake. Therefore, under normal physiological conditions, glucose oxidation is not increased with glycogen depletion but instead a switch takes place toward lipid oxidation due to neural or hormonal factors. High insulin concentrations, however, negate this. Teleologically, this may be a mechanism to compensate for a reduced intramuscular carbohydrate availability without predisposing to hypoglycaemia.

The significant difference in plasma insulin concentrations (Figure 5.3B) between LGE and NGE during exercise (but not at rest) suggests that when euglycaemia is maintained, plasma insulin concentrations are influenced by muscle glycogen content. Since the most dominant effects of insulin infusion in LGEI compared to LGE were an increase in glucose oxidation (Figure 5.5B) and a decrease in FFA (Figure 5.3A), muscle glucose uptake in LGE was probably limited by the lower insulin (710) and higher norepinephrine (89) concentrations (Figure 5.3B, C). This probably also occurred in NGE after the first hour of exercise when muscle glycogen concentrations of NGE would be approaching those of LGE (65).

Although the increased insulin in LGEI (Figure 5.3B) decreased the norepinephrine response to glycogen depletion slightly (Figure 5.3C), norepinephrine in LGEI was not significantly different from LGE. However, it was significantly different between subjects with low and normal glycogen content (LGE and NGE). Because the difference in norepinephrine persisted, with only some attenuation with insulin infusion in subjects with low muscle glycogen content, it, together with insulin, as discussed in the preceding paragraph, are the most likely controllers of the metabolic changes due to muscle glycogen depletion observed in this and other studies (312). However, the lower insulin concentrations in LGE could also be explained by the inhibition of insulin release by norepinephrine (567). Catecholamines also oppose the effects of insulin on lipid metabolism and glucose uptake (89), which would result in an increase in FFA and lipid oxidation and a lower rate of blood glucose oxidation in subjects with low muscle glycogen content than would be expected considering the availability of glucose and the low availability of endogenous glycogen. However, lower rate of blood glucose oxidation was not observed in LGE subjects.

The similarity in RER (Figure 5.2), serum FFA and plasma norepinephrine concentrations (Figure 5.3A and C) and insulin (Figure 5.3B) and glucagon (Figure 5.4B) concentrations during exercise in LGH and NGE subjects suggests that these parameters are controlled in a reciprocal manner both by plasma glucose concentrations during exercise and by muscle glycogen content at the start of exercise.

An interesting observation is that the lower RER (Figure 5.2) and higher FFA and norepinephrine concentrations (Figure 5.3A and C) during exercise in glycogen-depleted subjects (LGE) in this study are similar to those found in patients with muscle phosphorylase deficiency (McArdle's disease) (McAD) (402) who have impaired utilisation of muscle glycogen. Neural feedback from chemoreceptors in contracting muscle is involved in cardiovascular control (463) and likewise, studies of McArdle's disease strongly link the metabolic and cardiovascular defects of this disease with neural feedback from chemoreceptors in contracting muscle (402, 515, 669). Thus, given the striking similarities in the metabolic and hormonal differences between LGE subjects and McAD patients (253, 669) and their respective controls, there are strong indications in the current study of similar direct metabolic signalling of low glycogen status from muscle, which results in an increase in norepinephrine and FFA concentrations and lipid oxidation, and a decrease in insulin concentrations.

In conclusion, in this study: i) muscle glycogen depletion before exercise resulted in an increase in fat oxidation both at rest and during exercise, but had no effect on the rate of oxidation of glucose when euglycaemia was maintained by infusion of glucose alone; ii) insulin infusion in glycogen depleted subjects increased glucose oxidation and decreased FFA concentrations and fat oxidation; and iii) glucose oxidation was not higher in glycogen depleted subjects compared with subjects with normal muscle glycogen content when euglycaemia was maintained without hyperinsulinaemia.

CHAPTER 6

PRE-EXERCISE MUSCLE GLYCOGEN CONTENT AFFECTS METABOLIC REGULATION DESPITE MAINTENANCE OF HYPERGLYCAEMIA.

INTRODUCTION

Compared with maintenance of euglycaemia, maintenance of hyperglycaemia by glucose infusion results in an increase in glucose oxidation during exercise in subjects with normal glycogen content (265). However, there is a discrepancy between the rate of glucose oxidation and the rate of glucose infusion. Similarly, in Chapter 5, a discrepancy was noted during exercise with hyperglycaemia. However, RER in the hyperglycaemic, glycogen-depleted subjects was higher than that in euglycaemic, glycogen-depleted subjects but not different from that of subjects with normal glycogen content in whom euglycaemia was maintained during the same exercise protocol. However, despite the significantly lower RER in glycogen-depleted, euglycaemic subjects than in subjects with normal muscle glycogen content, the rate of glucose oxidation did not differ.

The object of the current study was therefore to investigate whether RER in glycogen depleted subjects would be the same as, or lower and glucose oxidation similar to, or higher than in subjects with normal glycogen content if hyperglycaemia were maintained in both groups during exercise.

METHODS

Thirteen endurance-trained male cyclists participated in the study. After following the glycogen-depletion protocol described in Chapter 4, subjects were randomly assigned to one of 2 groups: low glycogen, hyperglycaemic (LGH) (n=8) and normal glycogen, hyperglycaemic, (NGH) (n=5).

Subjects in the low glycogen group (LGH) ingested a low CHO diet as described in Chapter 4, while subjects in the NGH group followed their normal diet. All subjects were instructed to do only light training on the second day (approximately 1 hour of low-intensity cycling). On the third day, subjects returned to the laboratory and followed the experimental protocol described in Chapter 4. Plasma glucose concentration was maintained at ~9 mmol/l by infusing a 25% m/v glucose solution using the hyperglycaemic glucose clamp technique (146) modified as described in Chapter 3, during 145 minutes of exercise at 70% of $VO_{2\ max}$. Blood, muscle biopsy and urine samples were taken and processed as described in Chapter 4.

Statistical treatment

All results are presented as means ± SEMs. Statistical significance ($p < 0.05$) of between-group differences were assessed by a two way ANOVA with repeated measures over time, followed by a Tukey's Honest Significance test for unequal n. An unpaired t-test was used for single data. For some measurements where convergence of data in the second half of the trial resulted in masking of significant differences on the ANOVA, an unpaired t-test was used to compare Area-Under-the Curve (AUC) measurements.

RESULTS

Subject characteristics are given in Table 6.1. There were no significant differences between groups in any of these parameters.

Table 6.1. Characteristics of subjects

	LGH (n=8)	NGH (n=5)
Age (yr)	29±3	24±3
Mass (kg)	73±3	73±5
FFM (kg)	62±2	68±7
% Body Fat	15±1	12±1
Training (km/week)	256±20	280±9
Peak Work Rate (W)	364±18	367±32
$VO_{2\ max}$ (l/min)	4.45±0.15	4.61±0.23

Values are means ± SEM. LGH, low glycogen, hyperglycaemic; NGH, normal glycogen, hyperglycaemic; FFM is fat free mass; $VO_{2\ max}$, maximal O_2 consumption.

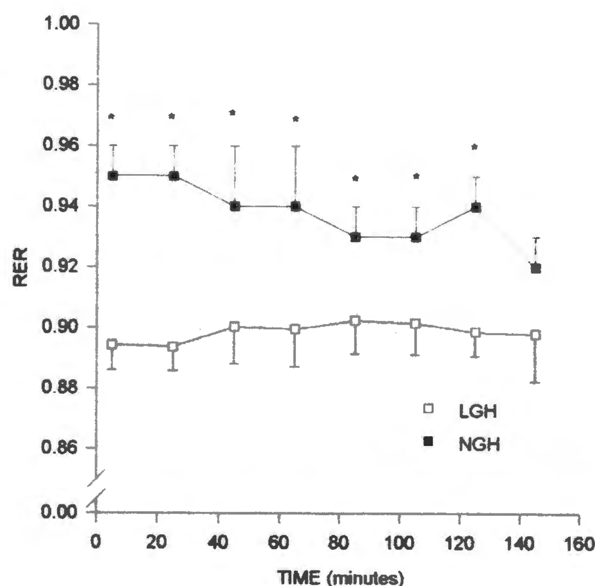


Figure 6.1. Respiratory exchange ratio in LGH and NGH subjects during 145 minutes of exercise. *Significantly lower in LGH than NGH ($p<0.05$). No significant changes over the duration of exercise.

Values for rate of oxygen consumption (VO_2), and rates (g/min) of fat and carbohydrate oxidation during exercise are given in Table 6.2 and total (g) fat oxidation and total carbohydrate oxidation for 145 minutes of exercise in Table 6.3. VO_2 did not differ significantly between groups and did not change significantly over the duration of the trial as the workload was maintained at 70% of $VO_{2\,max}$. Respiratory exchange ratio (RER) (Figure 6.1) and total carbohydrate oxidation (Table 6.3) were significantly lower and total fat oxidation (Table 6.3) significantly higher in LGH than in NGH ($p<0.05$). Rate of fat oxidation (Table 6.2) was significantly higher and rate of carbohydrate oxidation (Table 6.2) was lower in LGH than in NGH until 85 min and 125 min respectively ($p<0.05$), but the change in RER, rate of carbohydrate oxidation and rate of fat oxidation over the duration of the trial in NGH was not statistically significant. The free fatty acid concentrations (FFA) (Figure 6.2A) were significantly greater in LGH than in NGH ($p<0.05$).

Table 6.2. Steady state gas exchange data and rate of carbohydrate and fat oxidation during 145 min of cycling in LGH and NGH subjects

	Time (mins)							
	5	25	45	65	85	105	125	145
VO_2, l/min								
LGH	2.87	2.86	2.97	2.93	2.94	2.94	2.94	3.04
	±0.16	±0.16	±0.12	±0.10	±0.10	±0.10	±0.11	±0.11
NGH	2.99	3.06	3.08	3.06	3.12	3.11	3.14	3.16
	±0.14	±0.16	±0.14	±0.18	±0.15	±0.13	±0.15	±0.13
CHO_{ox}, g/min								
LGH	2.46*	2.40*	2.51*	2.55*	2.62*	2.62*	2.62*	2.65
	±0.16	±0.15	±0.14	±0.18	±0.17	±0.16	±0.14	±0.19
NGH	3.39	3.49	3.38	3.39	3.22	3.24	3.34	3.16
	±0.32	±0.34	±0.40	±0.45	±0.33	±0.34	±0.28	±0.19
F_{ox}, g/min								
LGH	0.51*	0.53*	0.54*	0.51*	0.48	0.50	0.49	0.53
	±0.05	±0.06	±0.04	±0.06	±0.06	±0.06	±0.05	±0.09
NGH	0.23	0.23	0.28	0.29	0.36	0.36	0.33	0.40
	±0.07	±0.07	±0.09	±0.11	±0.06	±0.06	±0.05	±0.02

Values are means ± SEM. LGH, low glycogen, hyperglycaemic; NGH, normal glycogen, hyperglycaemic; VO_2, rate of oxygen uptake; CHO_{ox}, rate of carbohydrate oxidation calculated from respiratory gas exchange data; F_{ox}, rate of fat oxidation from respiratory exchange data. *Significantly different between NGH and LGH ($p < 0.05$). There were no significant changes over time.

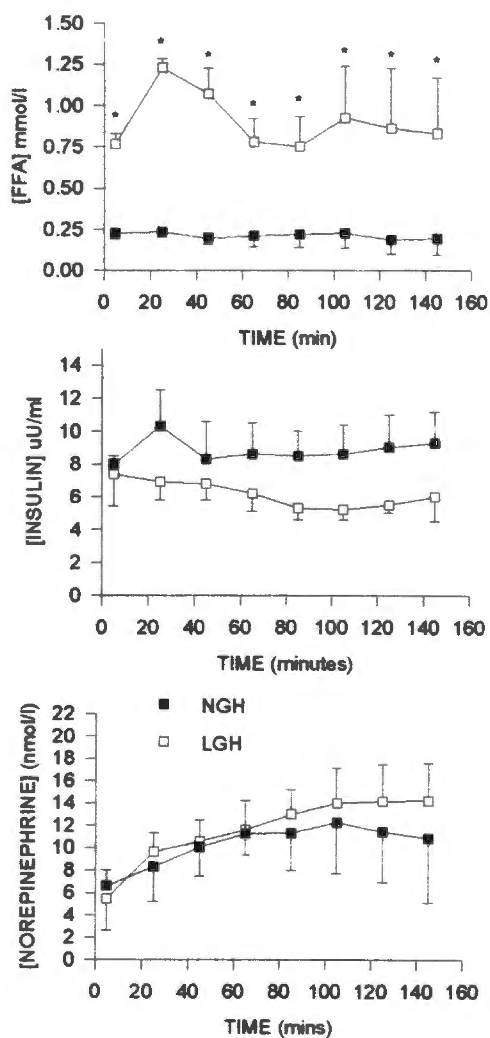


Figure 6.2. Free fatty acids (A); insulin (B) and norepinephrine (C). *Significantly different ($p<0.05$).

Mean plasma glucose concentrations (Figure 6.3A) after 5 minutes of exercise were 9.0±0.1, and 9.5±0.1 mmol/l for LGH and NGH respectively, with a coefficient of variation within groups of 3 and 4% respectively. Plasma insulin concentrations did not change significantly over the duration of exercise in either group but the AUC for insulin (Figure 6.2B) was significantly ($p<0.05$) less in LGH than in NGH. There were no significant differences between groups in concentrations of plasma glucagon (Figure 6.3B), norepinephrine concentrations (Figure 6.2C) or growth hormone. The latter showed great variability between subjects, especially in NGH (AUC 1507±342 vs 4930±2419 mU/l.min for LGH vs NGH, respectively).

The rate of glucose infusion required to maintain blood glucose concentrations at ~ 9 mmol/l is shown in Figure 6.4A and increased significantly throughout the trial in both groups ($p<0.05$). The total amount of glucose infused during the 145 min of exercise (Figure 6.5) was 1484±125 and 1529±86 mmol in LGH and NGH, respectively. This was not significantly different.

Rates of glucose oxidation (Figure 6.4B) increased progressively ($p<0.05$) in both groups until 85 minutes when a plateau was reached in NGH. In LGH a plateau was also reached 20 minutes later. Peak rates of glucose oxidation were 8.3 ±1.1 and 9.2±1.7 mmol/min (1.51±0.19 and 1.66±0.31 g/min) in LGH and NGH, respectively. Total glucose oxidation (Figure 6.5) did not differ significantly between LGH and NGH (840±74 vs 987±111 mmol; 151±13 vs 177±20 g). In both groups, the total amount of glucose oxidised was significantly lower than total amount of glucose infused (66% vs 65% in LGH and NGH, respectively) (Figure 6.5).

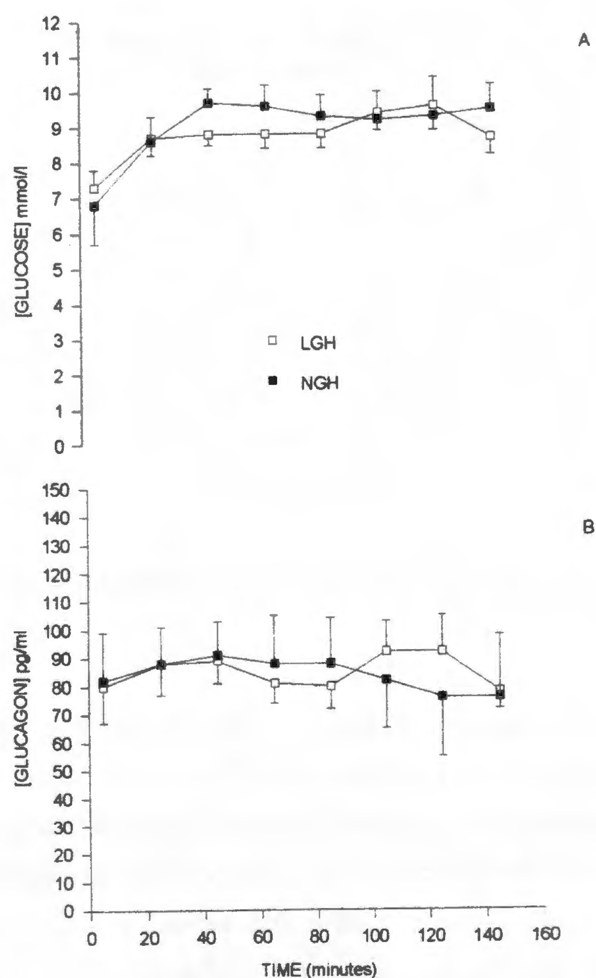


Figure 6.3. Plasma glucose (A) and glucagon (B) concentrations in LGH and NGH subjects. There were no significant differences between groups and no significant changes over time.

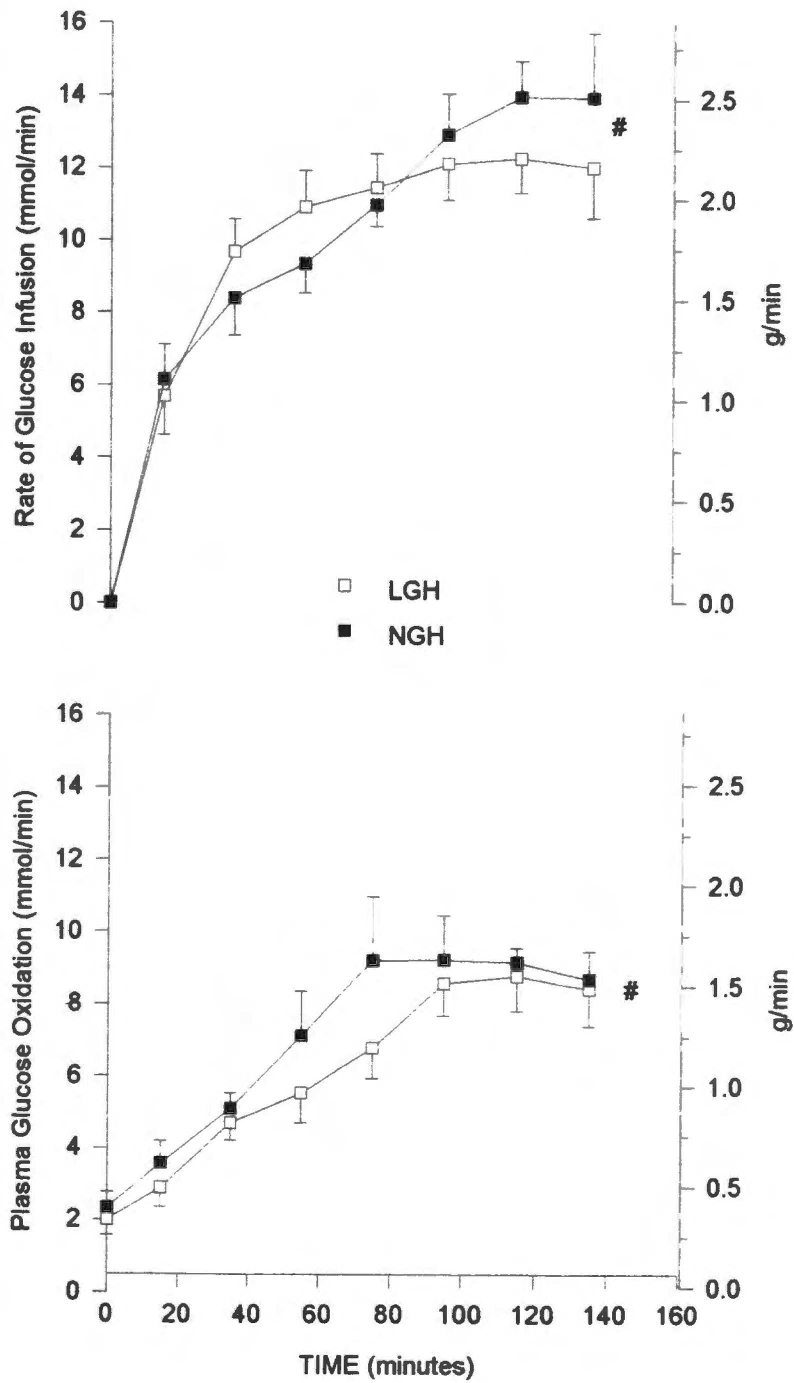


Figure 6.4. Rate of glucose infusion (A) and rate of glucose oxidation (B) in LGH and NGH. No significant differences between groups. #Significant increase over time ($p<0.05$).

The contribution of glucose to total carbohydrate oxidation did not differ between groups and increased significantly ($p<0.05$) until 125 min to 53±5% in NGH and until 105 min to 58±5% in LGH, whereafter it remained relatively constant. There was also no significant difference between groups in the contribution of glucose oxidation to total energy, which reached peaks of 41±4% and 44±5% in LGH and NGH respectively, after 105 min.

Muscle glycogen concentrations (Table 6.3) were significantly higher at the start of exercise in NGH than LGH. Muscle glycogen disappearance (Figure 6.6) was greater ($p<0.05$) in NGH than LGH (78±22 and 41±4 mmol/kg ww, respectively). There were no significant differences in muscle glycogen concentrations between groups at the end of exercise (Table 6.3). Plasma lactate concentrations (Figure 6.7) were significantly ($p<0.05$) lower throughout exercise in LGH than in NGH.

Table 6.3. Total carbohydrate and fat oxidation for 145 min of cycling and pre- and post-exercise muscle glycogen content in LGH and NGH subjects

	LGH	NGH
F_{tot} (g)	72±7*	43±8
CHO_{tot} (g)	361±23*	464±44
Pre-exercise Muscle Glycogen (mmol/kg ww)	80±5	134±12*
Post-exercise Muscle Glycogen (mmol/kg ww)	39±6	57±20

Values are means ± SEM. LGH, low glycogen, hyperglycaemic; NGH, normal glycogen, hyperglycaemic; CHO_{tot}, total carbohydrate oxidation calculated from gas exchange data; F_{tot}, total fat oxidation from gas exchange data. *Significantly different between NGH and LGH ($p < 0.05$).

DISCUSSION

The most significant findings in this study are that, despite differences in muscle glycogen content, glucose oxidation was not different between NGH and LGH (Figures 6.4 and 6.8) and that muscle glycogen depletion resulted in a shift toward lipid oxidation (Figures 6.1 and 6.8) even under conditions of hyperglycaemia (Figure 6.5).

Respiratory exchange ratio was lower during exercise in LGH subjects than in NGH (Figure 6.1). Since glucose oxidation did not differ between groups during exercise (Figure 6.3A), it is apparent that the lower RER in LGH must have been as a result of glycogen depletion. In addition, the effect of glycogen depletion resulting in a shift toward lipid oxidation cannot be fully overcome by glucose

infusion, even when the rate of infusion exceeds the rate of oxidation (Figures 6.5 and 6.6) and blood glucose concentration is twice normal. The slight, although not significant decrease in RER in NGH during exercise can be attributed to the decline in muscle glycogen content toward the end of exercise.

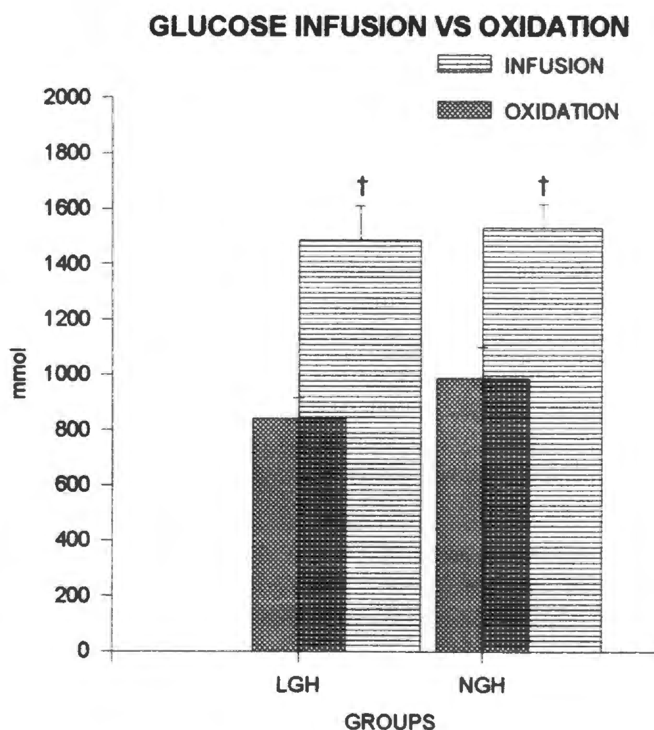


Figure 6.5. Comparison of total amount of glucose infused over 145 minutes of exercise with total glucose oxidation over the same period. [†]Rate of glucose infusion significantly higher than rate of glucose oxidation ($p<0.05$).

As found in the study discussed in Chapter 5, there was no difference in rates of glucose oxidation between these hyperglycaemic subjects with either low (LGH) or normal (NGH) muscle glycogen content. Similar to the findings of Hawley et al. (265) in subjects with normal muscle glycogen content, total glucose oxidation was significantly lower than the total amount of glucose infused (Figure 6.5) in both NGH and LGH. The reason for this apparent upper limit in glucose oxidation is probably that the exercise intensity was not high enough to elicit a greater increase in the rate of carbohydrate oxidation (563). Thus glucose oxidation is not increased with glycogen depletion but instead a switch takes place toward lipid oxidation even when plasma glucose concentrations are raised to the upper physiological limit. This strengthens the previous argument (Chapter 5) that this

may be a teleological mechanism to compensate for a reduced availability of intramuscular carbohydrate availability without predisposing to hypoglycaemia.

The significant difference between LGH and NGH during exercise in AUC for insulin (Figure 6.2B) suggests that even during hyperglycaemia, plasma insulin concentrations are influenced by muscle glycogen content. In the current study, glucose uptake in the muscle in LGH was possibly limited by the lower insulin (339, 710) and higher FFA (55) concentrations (Figure 6.2B and A) compared to NGH. Hyperinsulinaemia increases glucose uptake during hyperglycaemia at rest (180, 647), thus even though both groups were hyperglycaemic, the lower plasma insulin concentrations in LGH may explain why total glucose oxidation was not increased in LGH relative NGH to compensate for the reduced availability of muscle glycogen. In contrast to the previous study in euglycaemic subjects with either normal or low muscle glycogen content (Chapter 5), norepinephrine concentrations were not significantly different between groups (Figure 6.2C).

As discussed in Chapter 5, the lower RER and higher FFA in the current study (Figure 6.1 and 6.2C) during exercise in glycogen-depleted subjects (LGH) are similar to those found in patients with muscle phosphorylase deficiency (McArdle's disease) (402, 669). Since studies of McArdle's disease link the metabolic and cardiovascular defects of this disease with neural feedback from chemoreceptors in contracting muscle (402, 515, 669), the failure to restore RER in glycogen-depleted subjects with a glucose infusion to those found in similarly hyperglycaemic subjects with normal muscle glycogen content suggests that there is direct metabolic signalling from the muscle.

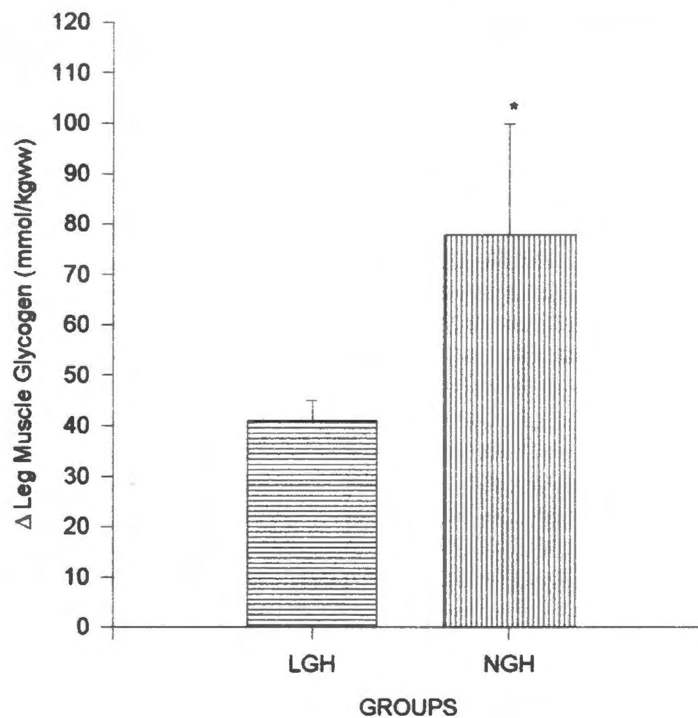


Figure 6.6. Rate of muscle glycogen disappearance in LGH and NGH subjects. *Significantly higher in NGH than LGH ($p<0.05$).

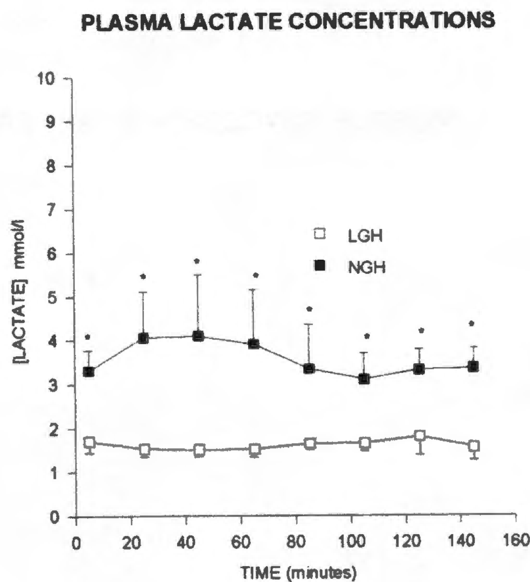


Figure 6.7 Plasma lactate concentrations during 145 minutes of exercise in NGH and LGH. *Significantly higher in NGH than LGH throughout exercise ($p<0.05$).

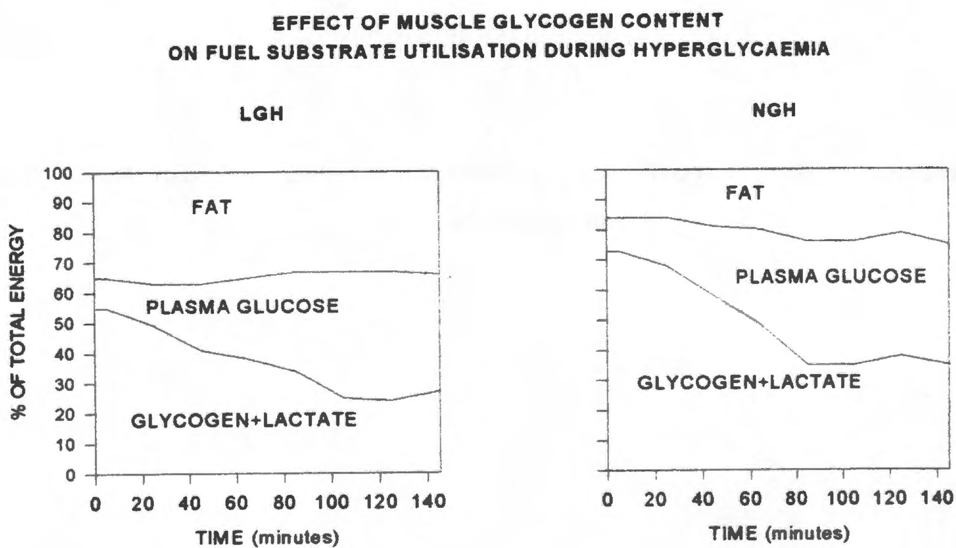


Figure 6.8 Comparison of overall metabolic effects of glycogen depletion during hyperglycaemia (LGH vs. NGH).

The greater muscle glycogen disappearance in subjects with a higher muscle glycogen content at the start of exercise (Figure 6.6) was reflected in higher plasma lactate concentrations in NGH than in LGH (Figure 6.7). This is similar to a number of studies which have found that higher muscle glycogen content at the start of exercise results in a greater rate of muscle glycogen utilisation during exercise (230, 259, 544) which does not appear to be influenced by the availability of plasma glucose.

In conclusion, in this study it has been shown that: i) when exercise is started with muscle glycogen depletion but without concomitant fatigue, exogenous glucose provided to maintain hyperglycaemia is not used to any greater extent than when muscle glycogen content is normal, but instead the energy deficit is made up by an increase in fat oxidation; ii) there is an upper limit to the rate of glucose oxidation during exercise at 70% of $VO_{2\ max}$ with hyperglycaemia irrespective of muscle glycogen status; and iii) net muscle glycogen utilisation is determined by the muscle glycogen content at the start of exercise even when hyperglycaemia is maintained during exercise.

CHAPTER 7

CONCLUSIONS AND SUMMARY - A Synthesis of the Studies

The aim of this study was to investigate the effect of low muscle glycogen content on fuel substrate utilisation during prolonged, moderate intensity exercise while either euglycaemia with or without hyperinsulinaemia, or hyperglycaemia was maintained to provide an alternative source of carbohydrate. Subjects in these studies started exercise with low muscle glycogen content so that the fatigue which is normally concomitant with low muscle glycogen content as a result of prolonged exercise, did not influence either the results or the subjects' ability to continue exercise for a number of hours in a glycogen depleted state.

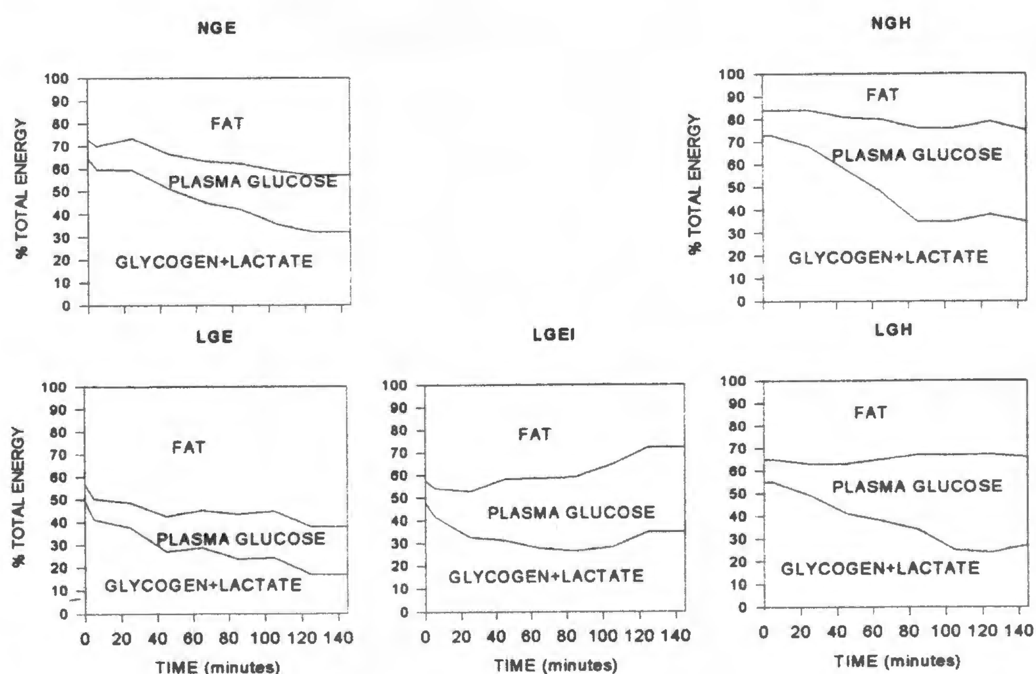


Figure 7.1. Overall metabolic effects of: i) glycogen depletion during euglycaemia (LGE vs NGE) and hyperglycaemia (LGH vs NGH); ii) plasma glucose concentrations if muscle glycogen is low (LGE vs LGH) or normal (NGE vs NGH) at the start of exercise; iii) insulin infusion on glycogen depletion (LGE vs LGEI vs NGE) and iv) insulin infusion on glucose oxidation (LGE vs LGEI vs LGH).

The most significant findings in this study are that firstly, despite ingestion of a small meal 3 hours before exercise and maintenance of euglycaemia or even hyperglycaemia, glucose oxidation was not different between subjects with normal or low glycogen content (NGE and LGE, and NGH and LGH). Secondly, low muscle glycogen content resulted in a shift toward lipid oxidation despite glucose infusion that matched or was in excess of the demands of the muscle (Figure 7.1).

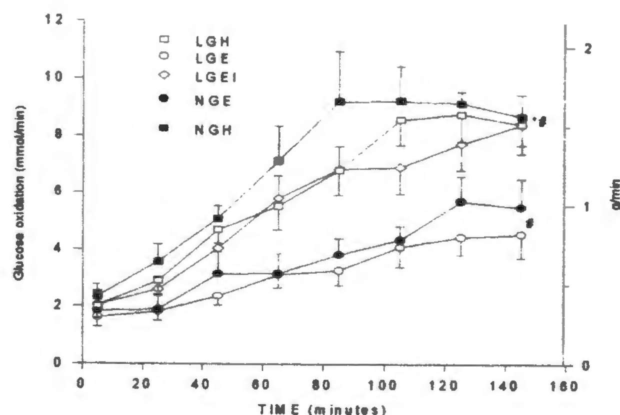


Figure 7.2 Rate of glucose oxidation in LGE, NGE, LGEI, LGH and NGH subjects. *Significantly higher in LGH, NGH and LGEI than in LGE or NGE; #significant increase over time ($p<0.05$).

In subjects with low glycogen content, the maintenance of hyperinsulinaemia with euglycaemia (LGEI) and hyperglycaemia with its associated hyperinsulinaemia (LGH), resulted in similar increases in glucose oxidation (Figure 7.2) compared with maintenance of euglycaemia alone (LGE), despite the fact that the rate of glucose infusion was significantly higher in LGH than in LGEI. Thus the rate of glucose oxidation was increased as much during hyperinsulinaemia with euglycaemia as during hyperglycaemia, even though glucose uptake (indicated by the higher glucose infusion rate required to maintain hyperglycaemia) was stimulated more with hyperglycaemia than hyperinsulinaemia, indicating that increased insulin concentrations during exercise increase the rate of glucose oxidation and could be the reason for increased glucose oxidation during hyperglycaemia. However, it would require an experiment in which somatostatin was infused to prevent an increase in endogenous insulin concentrations, to determine definitively whether the increase in glucose oxidation was due to an increase in plasma glucose or the increase in insulin concentration.

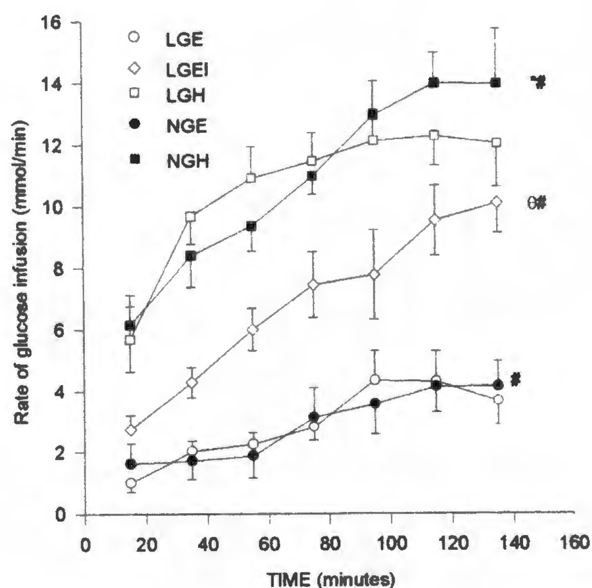


Figure 7.3. Rate of glucose infusion in LGE, NGE, LGEI, LGH and NGH subjects. *Significantly higher in LGH and NGH than in LGE, NGE or LGEI; $^{\theta}$significantly higher in LGEI than in LGE or NGE; $^{\#}$significant increase over time ($p<0.05$).

There was no difference between LGE and NGE in the rate of glucose infusion required to maintain euglycaemia, or between LGH and NGH in the rate of glucose infusion required to maintain hyperglycaemia (Figure 7.3). Thus muscle glycogen content does not influence the rate of infusion needed to maintain either eu- or hyperglycaemia. Although the rate of glucose oxidation and total glucose oxidation over the exercise period in the hyperglycaemic groups (LGH and NGH) was similar to that in LGEI (Figure 7.2 and 7.4), only about 65% of the total glucose infused was oxidised in the hyperglycaemic groups. However, in LGEI, the higher total glucose infusion was mirrored by an increased rate of (Figure 7.2), and total (Figure 7.4) glucose oxidation, with the total amount of glucose oxidised matching the total infused (Figure 7.4). Thus the discrepancy between the rate of infusion and oxidation and apparent upper limit in glucose oxidation is determined neither directly by muscle glycogen content nor by plasma glucose concentrations. Since the availability of exogenous carbohydrate in the current studies was not limited, it is possible that the exercise intensity was not high enough to elicit a further increase in the rate of carbohydrate oxidation (326, 563).

Since insulin infusion counteracted the metabolic effects of glycogen depletion in euglycaemic subjects with low glycogen content (LGEI), it is suggested that the decrease in RER and high FFA concentrations previously described (215, 268, 310, 312) are related specifically to intramuscular

carbohydrate availability, as the hyperinsulinaemia in the LGEI subjects would have had the effect of increasing glucose available to the muscle.

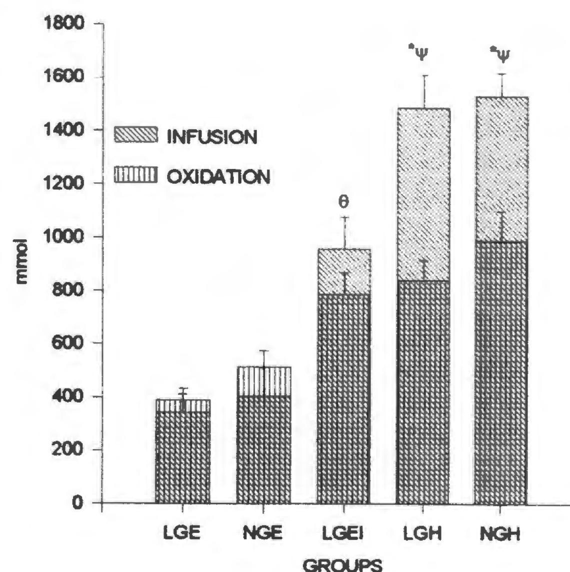


Figure 7.4. Comparison of total amount of glucose infused over 145 minutes of exercise with total glucose oxidation over the same period. *Glucose infusion significantly higher in LGH and NGH than in LGEI ($p<0.05$); $^{\theta}$glucose infusion and oxidation significantly higher in LGEI than in NGE or LGE ($p<0.05$); $^{\Psi}$glucose oxidation in LGH and NGH significantly different from infusion, not significantly different from oxidation in LGEI and significantly higher than in NGE or LGE ($p<0.05$).

Although glycogen content did not affect rates of glucose oxidation in either eu-or hyperglycaemia (LGE and NGE or LGH and NGH), the rate of glucose oxidation increased significantly over the duration of exercise in all groups (Figure 7.2). The progressive increase in glucose oxidation which occurs during exercise (65, 421) has been ascribed to the progressive decline in muscle glycogen content based on the findings of Gollnick et al. (230) and the effects observed in isolated, perfused rat hindquarters (195, 274, 510, 544, 652, 730) that glucose uptake is inversely related to muscle glycogen depletion. However, in the current study, G_{ox} was not significantly different between subjects with hyperglycaemia or hyperinsulinaemia or both (LGH, NGH and LGEI), but was higher in these three groups than in euglycaemic (LGE and NGE) subjects. This is in agreement with the results of a recent study by Hargreaves et al. (257), showing that glucose oxidation is not increased as a result of muscle glycogen depletion in vivo in humans. Thus the progressive increase in glucose oxidation cannot be ascribed to the progressive decline in glycogen content.

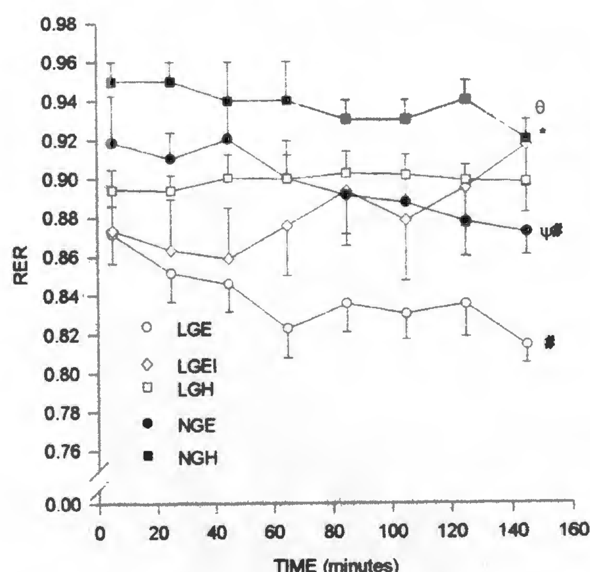


Figure 7.5. Respiratory exchange ratio in LGE, NGE, LGEI, LGH and NGH subjects. *Significant difference between LGH and NGH ($p<0.05$); $^{\theta}$significant difference between LGE and LGEI ($p<0.05$); $^{\psi}$significant difference between LGE and NGE; #significant decrease over time ($p<0.05$).

Respiratory exchange ratio (RER) (Figure 7.5) was lower at rest and throughout exercise in subjects with low muscle glycogen content than in the corresponding group with normal muscle glycogen content and with the same plasma glucose concentrations (i.e. LGE vs NGE and LGH vs NGE). The exception was in LGEI, where infusion of insulin during exercise resulted in an increase in RER compared with LGE. The reason that RER in LGEI was higher than in LGE was the increase in glucose oxidation (Figures 3 and 4), which must have been as a result of an increase in muscle glucose uptake (145, 710). Since RER was not significantly different between NGE, LGEI and LGH and all were higher than LGE, and since glucose oxidation was not increased with glycogen depletion, the effect of glycogen depletion was a shift toward lipid oxidation that could only be overcome by increasing intramuscular carbohydrate availability by glucose or insulin infusion.

A shift to lipid oxidation rather than increased glucose oxidation as glycogen becomes limited, may be a mechanism to compensate for a reduced availability of intramuscular carbohydrate without predisposing to hypoglycaemia. This makes teleological sense since the brain is entirely dependent on blood glucose for its energy supply and since it contains few insulin receptors, it is also dependent on the absolute blood glucose concentration for its glucose uptake (222, 338, 608, 649, 694), whereas

skeletal and cardiac muscle have a wider choice of fuel (287). Thus if intramuscular carbohydrate is low and lipid oxidation is increased, blood glucose can be preserved for use by the brain.

Although muscle glycogen content decreased in all groups during exercise (Figure 7.6), the rate of decline was greater in subjects who started exercise with normal muscle glycogen content, since at the end of exercise their muscle glycogen concentrations did not differ significantly from that of the subjects who started exercise with low muscle glycogen content. Thus it is not surprising that as exercise progressed, RER in NGE decreased toward that of LGE. This also tended be the case if NGH and LGH are compared, but the decline in RER in NGH was not significant most likely because of the high glucose concentration that was maintained. However, although RER in LGEI was similar to LGE at the start of exercise (as both groups started exercise with low muscle glycogen concentrations), by 45 minutes of exercise, RER in LGEI was similar to that of NGE and by the end of exercise was significantly different from LGE (Figure 7.5). This was because RER decreased significantly in NGE and LGE but actually tended to increase slightly (but not significantly), in LGEI (Figure 7.5) as a result of the gradual but progressive increase in plasma insulin concentrations (Figure 7.7). The latter caused an increase in glucose oxidation (Figure 7.2) and a decrease in FFA (Figure 7.8), probably due to an inhibition of lipolysis.

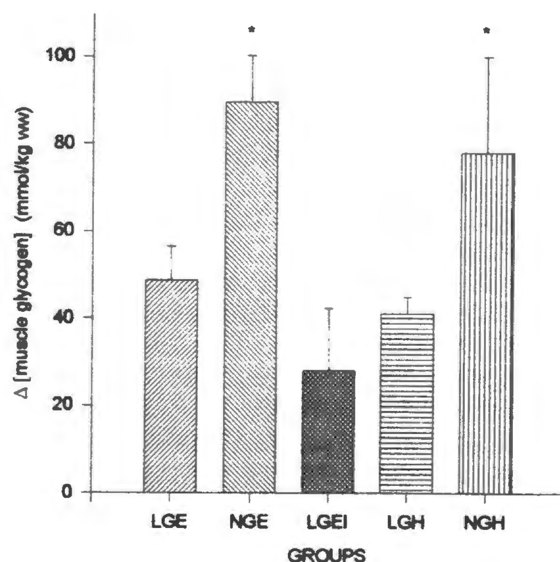


Figure 7.6. Muscle glycogen disappearance in LGE, NGE, LGEI, LGH and NGH subjects. *Significantly ($p<0.05$) higher in NGE and NGH than LGH, LGE or LGEI. LGE, LGEI and LGH not significantly different; NGE and NGH not significantly different.

Glycogen content influenced free fatty acid concentrations both at rest and during exercise, since these were significantly higher at rest in glycogen-depleted subjects than in subjects with normal muscle glycogen concentrations (Figure 7.8), higher throughout exercise in LGH than in NGH and higher in LGE than in NGE in the first hour of exercise. After the first hour, FFA remained higher in LGE than in NGE but the difference was no longer significant, again probably because of a decline in muscle glycogen content in NGE after the first hour of exercise (65) so that the muscle glycogen concentrations of NGE were approaching those of LGE. Superimposed on the effect of glycogen concentrations, FFA were also influenced by plasma glucose concentrations, since in LGH, FFA followed the same rise as in LGE during the first 25 minutes of exercise, then decreased sharply as plasma glucose concentrations rose toward 9 mmol/l. Insulin also influenced FFA, since in LGEI, FFA at the start of exercise were high as in the other glycogen-depleted subjects, but decreased rapidly as soon as plasma insulin concentrations rose (Figure 7.7 and 7.8). In NGE, FFA started low and remained low throughout exercise. The fact that FFA were significantly higher in subjects who started exercise with low muscle glycogen content than in those who started with normal muscle glycogen content at the same plasma glucose concentrations, but that FFA were also strongly influenced both by plasma glucose concentrations in subjects who started exercise with similar muscle glycogen content and by insulin infusion, indicates a great sensitivity in mobilisation of FFA to the availability of intramuscular carbohydrate. Although glucose supply was not limited in the euglycaemic clamp, glucose oxidation did not increase and FFA were not decreased unless glucose uptake was increased by artificially raising plasma insulin concentrations.

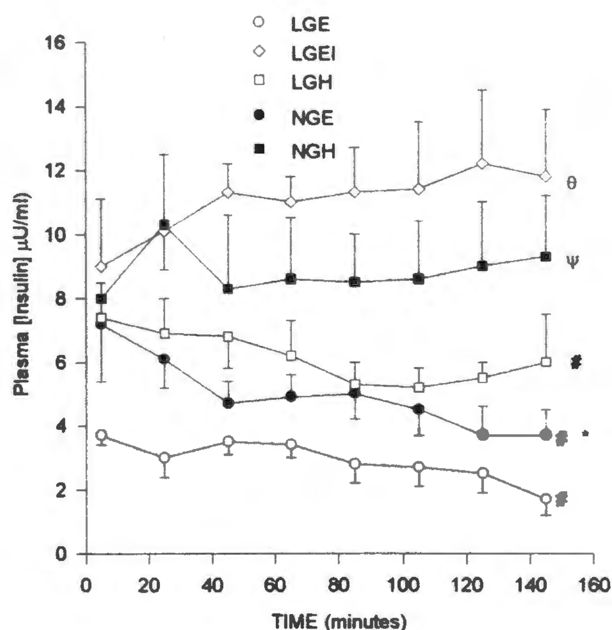


Figure 7.7. Plasma insulin concentrations in LGE, NGE, LGEI, LGH and NGH subjects. *Significant difference between LGE and NGE ($p<0.05$); $^{\theta}$significant difference between LGE and LGEI ($p<0.05$); $^{\Psi}$significant difference between LGH and NGH ($p<0.05$); #significant change over time ($p<0.05$).

As discussed in Chapter 5, because FFA are raised with glycogen depletion, a possible local control mechanism for the metabolic interactions observed in this study is the "glucose-fatty acid cycle" (526). This proposes that an increase in FFA oxidation leads to an inhibition of pyruvate dehydrogenase and thereby inhibits carbohydrate oxidation. This interaction has been described in human skeletal muscle at rest (353), but its existence has been questioned (582), especially during exercise in humans (179, 256). Most studies on this mechanism in exercising humans have used heparin and Intralipid to increase plasma FFA (179, 256), but Li et al. (408) suggested that where adequate endogenous substrates are available, the muscle preferentially oxidises those endogenous fuels and the existence of the glucose-fatty acid interaction is only unmasked when utilisation of endogenous fuels is blocked. The increased lipid oxidation in LGE and LGH was probably as a result of stimulation of lipolysis and increased availability of FFA due to control factors outside the muscle, namely the lower plasma insulin concentrations and higher plasma norepinephrine concentrations in LGE compared with NGE and lower plasma insulin concentrations in LGH compared with NGH. The glucose-fatty acid cycle does not explain the increased mobilisation of FFA or the reason for the low insulin concentrations.

The effect of low muscle glycogen content on RER (215, 231, 268, 310, 312, 424, 501, 527) and FFA concentrations (215, 230, 268, 310, 312, 424, 527) at rest and during exercise have been reported previously, but in many of these studies (215, 268, 424), the subjects were overnight fasted, and their resting plasma glucose concentrations were lower in the glycogen-depleted state. This raised the question of whether these effects were due to muscle glycogen depletion, liver glycogen depletion, or hypoglycaemia. As discussed in Chapter 5, liver glycogen content at the start of exercise in the studies in this thesis would have been only about 49-82 mmol/kg ww and with the glucose or insulin infusion protocols followed in LGH or LGEI respectively, it is unlikely that liver glycogen repletion took place during the trials (107, 143, 485), since in the study of DeFronzo et al. (143) even when plasma glucose concentrations were raised in humans to ~13 mmol/l with plasma insulin at ~40μU/ml, little splanchnic glucose uptake took place.

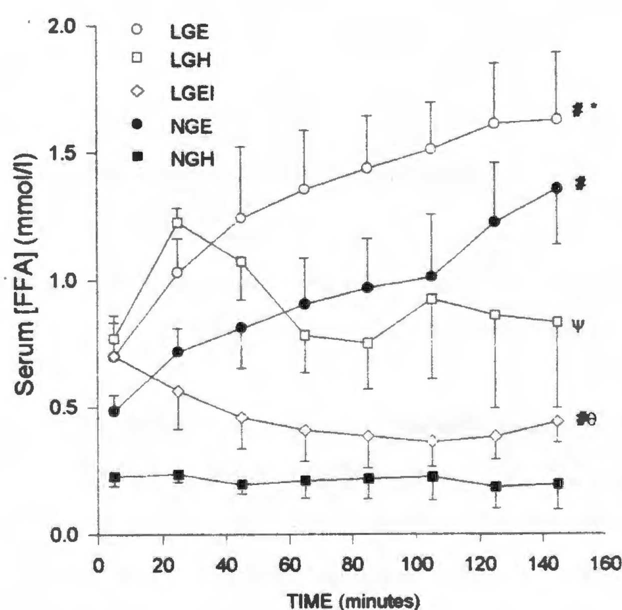


Figure 7.8. Serum free fatty acid concentrations in LGE, NGE, LGEI, LGH and NGH subjects. *Significant difference between LGE and NGE ($p<0.05$); $^{\theta}$significant difference between LGE and LGEI ($p<0.05$); $^{\psi}$significant difference between LGH and NGH ($p<0.05$); #significant change over time ($p<0.05$).

The significant difference in plasma insulin concentrations between LGE and NGE and in the AUC for plasma insulin concentrations between LGH and NGH during exercise (Figure 7.7), suggests that

when euglycaemia or even hyperglycaemia is maintained, plasma insulin concentrations are influenced by muscle glycogen content and confirms that the demonstration of lower plasma insulin concentrations in glycogen-depleted subjects in previous studies (49, 213, 215, 257, 268, 397, 421, 622, 717) was not only due to the relative hypoglycaemia which occurred due to overnight fasting, but was indeed due to glycogen depletion. It was expected that glucose uptake by the muscle in LGE and LGH would be higher than in NGE and NGH in order to restore the rate of carbohydrate oxidation to that of NGE and NGH respectively. However, this did not occur, since uptake was probably limited by the lower insulin concentrations (38, 88, 89, 108, 145, 191, 198, 307, 399, 531, 586, 614, 685, 688, 710) in the subjects with low glycogen content. Since glucose oxidation can only increase during exercise if glucose uptake increases (710), the lower glucose uptake may explain why glucose oxidation was not increased in LGE relative to NGE and in LGH relative to NGH respectively, to compensate for the reduced availability of muscle glycogen.

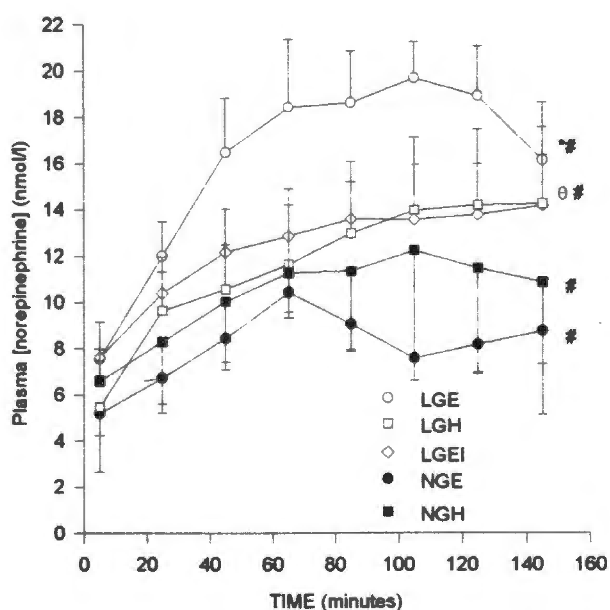


Figure 7.9. Plasma norepinephrine concentrations in LGE, NGE, LGEI, LGH and NGH subjects. *Significant difference between LGE and NGE ($p<0.05$); θsignificant difference between NGE and LGEI ($p<0.05$); #significant change over time ($p<0.05$).

A decrease in plasma insulin concentrations results in an increase in lipolysis and a decrease in glucose uptake (89). This led to an increase in FFA and lipid oxidation and was probably the reason for the lower rate of glucose oxidation in glycogen-depleted subjects than would be expected considering the availability of glucose and the low availability of endogenous carbohydrate. Thus it is not surprising that the most dominant effects of insulin infusion in LGEI were an increase in glucose oxidation (Figure 7.4) and a decrease in FFA (Figure 7.8). Despite the higher plasma glucose concentrations in LGH than in NGE, plasma insulin concentrations (Figure 7.7) were not significantly different between these two groups. However, plasma insulin concentrations did not decrease as much in LGH as in LGE relative to NGE and may explain why glucose oxidation in the muscle in LGH was higher. Thus the decrease in plasma insulin concentration with glycogen depletion seems to play a key role in regulating the switch to lipid oxidation and preventing an increase in glucose uptake by the exercising muscle.

However, the flaw in the argument for insulin as the major regulator in these studies is that plasma insulin concentrations were not different at rest between subjects with low glycogen content and subjects who started exercise with normal muscle glycogen concentrations, and yet RER at rest was lower and FFA concentrations higher in the former groups than in the latter. However, norepinephrine concentrations (NE) at rest were significantly higher in the subjects with low glycogen content than in subjects with normal muscle glycogen concentrations, and although the increased INS in LGEI decreased the NE response to glycogen depletion slightly (Figure 7.9), NE was not significantly different from LGE, whereas it was significantly different between LGE and NGE. Because the difference in NE concentrations persisted with only some attenuation with insulin infusion in subjects with low glycogen content, NE could also be a controller of the metabolic changes due to muscle glycogen depletion observed in this and other studies (312). The lower insulin concentrations in LGE could be explained by the inhibition by NE of INS release (567). Catecholamines also oppose the effects of insulin on lipid metabolism and glucose uptake (89). The argument against NE as the key regulator, however, is that plasma NE was not significantly different during exercise between LGH and NGH (Figure 7.9), although mixed venous blood samples were used, which may not accurately reflect small differences in sympathetic discharge. Sympathetic discharge was the most likely source of NE, since plasma epinephrine concentrations did not differ between groups on the basis of muscle glycogen content. Thus it appears that NE and INS play a dual role in mediating the lower RER due to low glycogen content, with NE predominant at rest and INS predominant during exercise.

Respiratory exchange ratio is low relative to workload in patients with McArdle's disease (McAd) compared with normal subjects and is increased during glucose infusion, but remains lower than in normal subjects. Similarly, in the current study, an infusion of glucose did not restore RER in subjects with low glycogen content (LGE and LGH) to that seen in subjects who started exercise with normal

muscle glycogen content (NGE and NGH) (Figure 7.5). However, maintenance of hyperglycaemia in subjects with low glycogen content (LGH) during exercise resulted in a normalisation of the balance of lipid and carbohydrate oxidation to that found in subjects with normal muscle glycogen content during maintenance of euglycaemia (NGE). This was accomplished by an increased rate of glucose oxidation (Figure 7.2).

In a study by Vissing et al. (669), glucose utilisation, FFA, growth hormone and cortisol concentrations were higher and insulin lower in saline-infused McAD patients than in saline infused, normal, matched control subjects at both the same relative and absolute workloads. NE and heart rate were higher in patients than in the control subjects at the same absolute workload. When the McAD patients were infused with glucose during exercise at a rate that caused plasma glucose concentrations to rise to ~8 mmol/l with a corresponding increase in insulin concentrations, glucose utilisation in patients rose above that in control subjects while FFA, NE, growth hormone, cortisol and heart rate decreased to values similar to those in controls. The differences found in FFA, INS, and NE between subjects with normal and low glycogen content (NGE and LGE) are very similar to the differences between saline-infused McAD patients and control subjects in the study of Vissing et al. (669) just described. Similarly the higher rate of glucose oxidation in LGH in the current study compared with NGE is similar to the response of McAD patients to a glucose infusion (669) compared with saline-infused controls. The only notable difference is that INS in the current study were not different between LGH and NGE, while insulin concentrations were higher in glucose-infused McAD patients than in controls.

Neural feedback from chemoreceptors in contracting muscle are involved in cardiovascular control (216, 303, 347, 423, 426, 428, 463, 682). Likewise, the metabolic and cardiovascular defects associated with McArdle's disease have been linked with neural feedback from chemoreceptors in contracting muscle (402, 515, 669). In addition, patients with McArdle's disease have been shown to have reduced muscle sympathetic nerve activity during static exercise (515) in addition to their metabolic abnormalities. It is therefore interesing that there are striking similarities between the metabolic and hormonal differences between euglycaemic subjects with normal and low muscle glycogen concentrations (LGE and NGE) and the differences between saline-infused McAD patients and controls. Similarly the response of glycogen-depleted subjects to hyperglycaemia in the current study and findings in the studies on patients with McArdle's disease (253, 669) parallel each other. Since there is direct metabolic signalling of low glycogen availability from muscle in McAD, the similarity in findings in the current series of studies suggests similar direct signalling of low glycogen content, which results in an increase in norepinephrine and FFA concentrations and lipid oxidation, and a decrease in insulin concentrations. However, this response can be overridden if glucose uptake into the muscle is increased by an increase in plasma glucose or insulin concentrations, or both. The most likely signalling pathway is via Group III and IV muscle afferents.

Stimulation of Group III and IV muscle afferents results in an increase in glucose production and influences concentrations of certain regulatory hormones, including insulin in cats (667). Patients with McAD or PFK deficiency have a greater than normal change in heart rate for a change in oxygen uptake ($\Delta Q/\Delta VO_2$) during exercise, which has been linked to a deficiency of oxidisable fuel (404, 405, 407). Since the $\Delta Q/\Delta VO_2$ decreases when the enzymatic defect is bypassed by infusing glucose in McArdle's disease patients (402, 405) or lactate or FFA in PFK deficiency (407) and increases when nicotinic acid is used to block mobilisation of FFA in McArdle's disease, it is interesting to speculate that a similar afferent feedback mechanism from contracting muscle may occur in humans. In the current study, insulin concentrations were significantly different between subjects with low and normal muscle glycogen content during both eu- and hyperglycaemia (LGE and NGE and between LGH and NGH) (Figure 7.7). NE (Figure 7.9) and FFA (Figure 7.8) were higher in LGE than in NGE and FFA were higher in LGH than NGH. Thus together, the data from previous studies (361, 515, 667) and the current data suggest a reflex pathway from skeletal muscle which controls carbohydrate and fat metabolism.

Muscle afferents and the hypothalamic locomotor region (which is involved in the initiation of locomotion), both project to the nucleus reticularis gigantocellularis (NGC). Bilateral electrolytic lesion of the NGC at the pontomedullary border in cats causes greater responses in tidal volume, respiratory frequency and heart rate to stimulation of the hypothalamic locomotor region than the responses recorded prior to lesioning. In decorticate cats, stimulation of the posterior hypothalamic motor region, with simultaneous neuromuscular blockade to prevent feedback from muscle afferents, results in increases in hepatic glucose appearance, plasma glucose, epinephrine, norepinephrine and glucagon concentrations and decreases in plasma insulin concentrations (668). In rats, anaesthesia of the ventromedial hypothalamus (VMH) results in increased resting plasma concentrations of glucose, lactate, glycerol, epinephrine and norepinephrine. During exercise, initial hepatic glucose production, plasma catecholamines, subsequent plasma glucose concentrations and overall hepatic glycogenolysis were lower in VMH-anaesthetised rats compared with control rats. In another study (658), anaesthesia of the paraventricular nucleus (PVN) resulted in attenuation of the exercised-induced response of plasma epinephrine and corticosterone. Thus, since i) muscle afferents are connected to the hypothalamic locomotor region via the NGC (543), ii) blood glucose appearance and plasma glucose and glucoregulatory hormones concentrations can all be controlled from the hypothalamus (673) and iii) there are interconnections between the various hypothalamic nuclei and between hypothalamic nuclei and the sympathetic nervous system and hypophysis (673), integration of the results of the current and previous studies (361, 515, 658, 667, 668, 673) may explain the co-ordinated metabolic control via central command, neuromuscular reflexes and humoral metabolic feedback.

When the consequences of muscle glycogen depletion (77) or an inability to utilise muscle glycogen as in McArdle's disease (574) are considered, namely poor exercise tolerance and an increase in inosine 5'-monophospate (IMP) (77, 574) and ammonia (77, 574) in the muscle, and taking into account the dependence of the central nervous system on maintenance of blood glucose concentrations, there is good reason for the existence of a pathway to increase fat metabolism when muscle glycogen content is low, in order to preserve both muscle glycogen and blood glucose. The actual stimulant (or inhibitor) for the muscle afferents is not known but various possibilities are discussed in Chapter 5. Glycogen itself as a signal molecule is unlikely, as patients with McArdle's disease do not have a deficiency of muscle glycogen, but they are unable to utilise their glycogen stores. Since only G-1,6-P_2 is lower in both glycogen depleted muscle before and after exercise (621) and in muscle of patients with McArdle's disease (715), if this molecule has an effect on group III and IV muscle afferents, the differences in RER, NE and FFA at rest and during exercise between LGE and NGE and between LGH and NGH in this study would be explained. It has been shown to be an important molecule in metabolic control (34) and has been shown to increase in muscle with insulin infusion (324) and to decrease with training in rats (462), which is noteworthy because training results in a shift to lipid oxidation (81).

In summary:

i) Low muscle glycogen content in non-fatigued subjects results in an increase in fat oxidation compared with subjects with normal muscle glycogen content both at rest and during exercise, but has no effect on the rate of oxidation of glucose when euglycaemia is maintained by infusion of glucose alone.

ii) When hyperglycaemia is maintained in subjects with low muscle glycogen content, glucose oxidation is higher than when euglycaemia is maintained in subjects with normal glycogen content during exercise at 70% of VO_{2max}, but RER is not different.

iii) Insulin infusion (to increase glucose uptake by muscle) in in subjects with low muscle glycogen content increases glucose oxidation and decreases FFA concentrations and fat oxidation with no significant attenuation of plasma NE concentrations compared with subjects not receiving insulin, suggesting that the metabolic effects of a low carbohydrate diet are specifically due to a decrease in intramuscular carbohydrate availability.

iv) Although rates of glucose infusion and oxidation are matched under euglycaemic conditions, with hyperglycaemia 43% of the glucose infused is not oxidised. Thus there is an upper limit to the rate of glucose oxidation during exercise with hyperglycaemia irrespective of muscle glycogen status.

v) The progressive increase in glucose oxidation during prolonged exercise is not due to progressive glycogen depletion.

vi) Net muscle glycogen utilisation is determined by the muscle glycogen content at the start of exercise even when hyperglycaemia is maintained during exercise, and despite the increased rate of glucose oxidation in LGH and LGEI, there is no sparing of muscle glycogen compared with LGE.

vii) The rate of glucose oxidation does not match the rate of glucose infusion during hyperglycaemia suggesting that the maximum rate of carbohydrate oxidation may be partly controlled by the exercise intensity.

viii) The metabolic response to exercise in subjects with low muscle glycogen content is very similar to the metabolic response to exercise in patients with muscle phosphorylase deficiency and likewise, the response to hyperglycaemia in in subjects with low muscle glycogen content is very similar to the metabolic response to hyperglycaemia in patients with muscle phosphorylase deficiency. Therefore, similar control mechanisms may be operative regarding substrate oxidation.

ix) The shift towards lipid metabolism both at rest and during exercise with low muscle glycogen content may be mediated by a muscle afferent pathway via NE or insulin.

CHAPTER 8

References

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