Hormonal and metabolic responses to maintained hyperglycemia during prolonged exercise

D. P. M. MacLaren, T. Reilly, I. T. Campbell, and C. Hopkin. Hormonal and metabolic responses to maintained hyperglycemia during prolonged exercise. J. Appl. Physiol. 87(1): 124–131, 1999.—We studied the effects of maintained hyperglycemia (12 mmol/l) on endurance exercise to determine the hormonal and metabolic responses, the maximal rate of glucose infusion (i.e., utilization), and the effects on muscle glycogen stores. Eight men undertook two trials during which they exercised on a cycle ergometer at an intensity of ~70% peak $O_2$ uptake for 120 min. In the first trial (trial A), subjects had their blood glucose concentration clamped at 12 mmol/l 30 min before exercise and throughout exercise. The same rate and volume of infusion of saline as had occurred for trial A were used in a placebo trial (trial B). Maintained hyperglycemia resulted in significantly lowered plasma concentrations of nonesterified fatty acid, glycerol, 3-hydroxybutyrate, epinephrine, norepinephrine, and growth hormone ($P < 0.001$) during exercise, whereas concentrations of plasma insulin were significantly elevated ($P < 0.001$). Calculations of the rates of total carbohydrate oxidation showed that trial A resulted in significantly higher values when compared with trial B ($P < 0.01$) and that the maximal rates of glucose infusion varied between 1.33 and 2.78 g/min at 100–120 min. Muscle glycogen concentrations were significantly depleted ($P < 0.01$) after both trials (trial A, 170.3 µmol/g dry wt decrease; trial B, 206 µmol/g dry wt decrease), although this apparent difference may be accounted for by storage of 22.6 g glucose during the 30-min prime infusion. The results from this study confirm that maintained hyperglycemia attenuates the hormonal response and promotes carbohydrate oxidation and utilization and that muscle glycogen may not be spared.

Methods

Subjects. Eight healthy, well-trained men (7 club-level cyclists and 1 club-level cross-country runner) gave their informed consent in accordance with the procedures approved by the Ethics Committees of the Royal Liverpool Hospital and of Liverpool John Moores University. The age, body mass, and peak $O_2$ intake $(V_{O2\text{peak}})$ were (mean ± SD) 37.4 ± 11.6 yr, 68.9 ± 6.7 kg, and 3,994 ± 540 ml/min, respectively.

Experimental design. To determine $V_{O2\text{peak}}$, each subject first underwent a test on an electrically braked cycle ergometer, using a continuous incremental test to volitional exhaustion. During subsequent visits to the laboratory (after an overnight fast), subjects exercised for 120 min at an intensity corresponding to 70% $V_{O2\text{peak}}$ while under a condition of maintained hyperglycemia by using glucose infusion (trial A) or saline infusion (trial B). The glucose-infusion trial took place first, because the rate of saline infusion needed to match that for glucose infusion. Subjects had similar dietary intakes and refrained from strenuous exercise for 48 h before each trial.

On the day of the trials, subjects arrived after an overnight fast and without having engaged in physical activity during the previous 24 h. After each subject voided urine, a 16-gauge iv cannula was inserted into a forearm vein of the left hand of each subject, while the right hand was placed in a box heated to 65°C to “arterialize” the blood (1). After 20 min, another cannula was inserted retrogradely into a dorsal vein of the
corresponding to 70% \( \dot{V}O_2 \)peak. During the prime infusion and before a 120-min bout of exercise began at an intensity of 95% \( \dot{V}O_2 \)peak, plasma glucose concentration to 12 mmol/l by variation of the rate of infusion every 5 min, according to the arterialized plasma glucose concentration, and measured by using an Analox GM7 analyzer (Analox Instruments, London, UK). Changes in infusion rate were calculated by using a Sharp MZ-80B computer.

Two 10-ml blood samples were taken in lithium-heparin syringes (Monovette, Sarstedt, Leicester, UK) at rest (−30 min), after the prime infusion (0 min), and every 20 min during the exercise. The blood was centrifuged at 3,000 rpm and then divided into aliquots. Plasma was stored at −20°C before later analysis for lactate, NEFA, glycerol, 3-hydroxybutyrate (3-OHB), cortisol, insulin and GH. Plasma samples for the determination of catecholamines were stored at −70°C, while blood for glucagon assays was collected in syringes containing Trasylol.

An on-line gas-analysis system (PK Morgan) was used for the determination of \( \dot{V}O_2 \) and respiratory exchange ratio over a 5-min period before the prime infusion, in the last 5 min of the prime infusion, and at 15, 30, 60, 90, and 120 min of exercise. These values were subsequently used for the calculation of carbohydrate and fat oxidation rates.

Immediately after the exercise bout, subjects were requested to lie supine while the infusion rate was slowly decreased, so as to prevent rebound hypoglycemia. Furthermore, a muscle biopsy sample was taken from the anterior quadriceps by using a conchotome, after administration of a local anesthetic and after an incision of the skin and muscle fascia. This process occurred within 5 min of the completion of exercise. The muscle sample was placed in a sterile Eppendorf tube before being plunged into liquid nitrogen. The sample was then stored at −70°C until it was analyzed later for glycogen content. To minimize trauma, we did not take a resting muscle sample on the same day as the exercise; rather, the sample was taken at the same time of day 3 wk after the second trial. Subjects conformed to their previous eating and exercise patterns for the 48-h period before the sample was taken.

Subjects repeated the same procedure 3 wk after the first trial, but they received a 0.9% saline infusion instead of the dextrose. The rate of infusion of the saline was identical to that for the glucose-infusion trial, so that the total volume given in both trials was identical.

Analyses. Plasma NEFA values were determined by an enzymatic spectrophotometric method, while a portion of the plasma was deproteinated with perchloric acid (7% wt/vol) before assay for lactate, glycerol, and 3-OHB by using enzymatic methods. All these analyses were performed on a Cobas-Bio centrifugal analyzer (Roche Products, Welwyn Garden City, Herts, UK). Plasma insulin was determined by using RIA with an insulin RIA kit (IM.78, Amersham International, Amersham, UK), and plasma glucagon was determined by using an \( ^{125}I \)-glucagon RIA kit (IDS, Bournemouth, UK). Both plasma GH and cortisol were assayed by using in-house RIA methods; the former was accomplished with reagents supplied by the Supra Regional Assay Laboratory (Royal Infirmary, Edinburgh, UK), and the \( ^{125}I \)-label was supplied by NETRIA (St. Bartholomew's Hospital, London, UK), and the latter was accomplished by reagents supplied by Bioanalysis (Cardiff, UK). The two in-house assays showed a bias of <10% on the National External Quality Assessment Scheme. Plasma epinephrine and norepinephrine concentrations were analyzed by using high-performance liquid chromatography with electrochemical detection via an in-house method (Department of Clinical Chemistry, Royal Liverpool University Hospital, Liverpool, UK).

Freeze-dried muscle biopsy samples were prepared and analyzed for glycogen concentration according to the method of Edwards et al. (8).

Statistics. ANOVA with repeated measures was used to determine whether there were any significant differences between the trials and the time points for the plasma metabolites and hormones. Significant \( F \) values were followed up by using a Tukey post hoc test. The carbohydrate oxidation rates were subjected to the determination of the area under the curve before a t-test was applied. The rate of glucose utilization was analyzed by employing a one-way ANOVA to determine whether significant differences accrued with time. Paired t-tests were performed on the muscle glycogen concentrations. Significance was accepted at \( P < 0.05 \).

RESULTS

Blood metabolites. Glucose infusion maintained plasma glucose concentrations at −12 mmol/l, whereas saline infusion resulted in a relatively constant value of 5 mmol/l (Fig. 1). Plasma glucose in the exercise period ranged from 11.5 ± 1.0 to 12.6 ± 0.6 mmol/l for the glucose infusion; this reflects the stability of the clamp.

![Fig. 1. Plasma glucose concentrations (in mmol/l) at rest (−30 min), after prime infusion of glucose (0 min), and during 120 min of exercise. Values are means ± SE; \( n = 8 \) subjects in each trial.](http://jap.physiology.org/Downloaded_fromhttp://jap.physiology.org/)
procedure. Hypoglycemia was not displayed by any of the subjects with saline infusion, where plasma glucose concentrations at 100 and 120 min were 5.1 ± 0.4 and 4.7 ± 0.7 mmol/l, respectively. ANOVA found a significant difference between trials (P < 0.01). Differences were also apparent with time (P < 0.01), although this was mainly because of the changes in concentration from resting values to values after the prime infusion of glucose (P < 0.01).

Plasma lactate concentrations became elevated during exercise in both trials (Fig. 2). These levels remained significantly augmented throughout the exercise period (P < 0.01); glucose infusion produced a significantly higher response than did saline infusion (P < 0.05).

The plasma NEFA, glycerol, and 3-OHB responses to saline and glucose infusion were similar in their pattern (Fig. 2), although significant differences were found between the trials for NEFA, glycerol, and 3-OHB (P < 0.01). The glucose infusion resulted in lower levels compared with saline infusion. During exercise with saline infusion, concentrations of these metabolites increased significantly (P < 0.01); however, with glucose infusion, NEFA and 3-OHB remained depressed. Plasma levels of glycerol continued to increase marginally, but not significantly, during exercise with glucose infusion.

Plasma hormones. Elevated plasma insulin concentrations resulting from glucose infusion were apparent (Fig. 3). Mean values increased from 7.0 ± 2.5 mU/l at rest to 25.9 ± 6.3 mU/l after the prime infusion. During subsequent exercise by the subjects, the insulin concentrations became elevated up to 60 min (33.7 ± 15.9 mU/l) before falling to 20.9 ± 6.1 mU/l at 120 min. During exercise under conditions of saline infusion, a typical response to exercise occurred, with a decrease in insulin levels from 7.2 ± 2.4 mU/l at the start to 3.3 ± 0.5 mU/l at 120 min. ANOVA revealed a significant difference between the trials (P < 0.01).

Plasma glucagon concentrations were significantly depressed under glucose infusion (P < 0.05), with the values remaining relatively unchanged (368 ± 116 pg/ml at rest; 347 ± 128 pg/ml at 120 min). With saline infusion, however, the concentrations increased significantly during exercise (371 ± 101 pg/ml at rest; 571 ± 179 pg/ml at 120 min). Figure 3 illustrates these changes.

Plasma epinephrine concentrations increased significantly during exercise with both treatments (P < 0.01), although the response was attenuated with glucose infusion (Fig. 4). Plasma norepinephrine showed a similar pattern (Fig. 4), in that there was a significant increase with duration of exercise (P < 0.01) and a significant attenuation with glucose infusion compared with saline infusion (P < 0.01).

Significant differences were found between treatments for plasma GH (Fig. 4); glucose infusion resulted in lower concentrations (P < 0.05). Exercise led to a significant rise in GH levels, particularly with saline infusion (P < 0.01). The highest mean values were obtained at 60 min for glucose infusion (14.5 ± 11.2 mU/l) and at 80 min for saline infusion (24.6 ± 12.9 mU/l).

No significant differences were found for plasma cortisol concentrations between the trials (P > 0.05), although a significant increase was found with the

![Fig. 2. Plasma concentrations of lactate, nonesterified fatty acids (NEFA), 3-hydroxybutyrate (3-OHB), and glycerol at rest (−30 min), after prime infusion (0 min), and during 120 min of exercise. Values are means ± SE; n = 8 subjects in each trial.](image-url)
duration of exercise (P < 0.05). The latter was essentially due to a significant increase with saline infusion after 80 min (Fig. 4).

Oxidation rates. Calculations of the rate of whole body carbohydrate and fat oxidation from the V\(\dot{O}_2\) and RER data are displayed in Figs. 5 and 6. The mean area under the curve, followed by a paired t-test, revealed a significantly higher carbohydrate oxidation rate (P < 0.01) for glucose infusion than for saline infusion (320.8 ± 56.3 vs. 229.7 ± 46.1 g/120 min, respectively). The rate of carbohydrate oxidation during exercise did not vary significantly with time for glucose infusion (P > 0.05) but decreased significantly for saline infusion (P < 0.01).

The findings for fat oxidation were the inverse of those for carbohydrate oxidation (Fig. 6), in that glucose infusion resulted in a significantly attenuated response compared with saline infusion (P < 0.01). As exercise progressed, there was a significant shift toward fat oxidation with saline infusion (P < 0.01) that was not apparent with glucose infusion.

Glucose infusion. The rate of glucose infusion, as measured from the glucose-clamp procedure, increased significantly during exercise (P < 0.01). Figure 7 shows that this increase occurred up to 80–100 min before a plateau was established. At this stage, the mean value of 1.8 g/min represents a 61% increase from the start of the exercise period. When expressed as a percentage of the mean total carbohydrate oxidation rate, the mean glucose utilization rate (i.e., the rate of glucose infusion) was 40.8, 53.7, 57.2, 68.9, and 68.3% at 15, 30, 60, 90, and 120 min, respectively.

Muscle glycogen. Muscle glycogen concentrations were significantly lower at the end of both trials, as a result of the exercise, when compared with a resting value taken on a subsequent occasion (P < 0.01). The resting concentration was 308.5 ± 64.8 µmol/g dry wt, whereas the postexercise values were 138.2 ± 33.3 and 102.5 ± 27.5 µmol/g dry wt after conditions of glucose or saline infusion, respectively. Differences between the trials were also significant (P < 0.01).

Glucose storage during rest. The mean total glucose infused was 29.2 ± 7.7 g over the 30-min priming period when subjects were resting. The total carbohydrate oxidized during this 30-min period, calculated from the respiratory measures at -30 min and 0 min, was 6.6 ± 1.3 g. The difference between the glucose infused and that oxidized is assumed to result in storage of glucose. A value of 22.6 g was estimated to be stored during this period.

DISCUSSION

Hyperglycemia was maintained with little variation throughout the exercise period; this emphasizes the suitability of the clamp procedure to circumstances other than rest. This finding is in agreement with two other studies in which the glucose clamp has been used during exercise (4, 15), although those studies maintained hyperglycemia at 10 mmol/l. Because little variation occurred in the present investigation, the rate of glucose infusion appears to be reliable as a measure of whole body glucose utilization.

The hyperinsulinemia as a consequence of glucose infusion was expected, whereas the decrease after 60 min of exercise was similar to that observed previously (4) and was probably caused by the elevation in epinephrine that occurs during exercise. After 60 min of exercise, the plasma epinephrine concentration was 0.55 nmol/l, an increase of 50% above resting levels. Because insulin began to decrease after this time point, it is possible to speculate that a threshold exists at which insulin is inhibited by epinephrine. This is supported by findings that the insulin response to glucose infusion at rest in healthy subjects results in a continuous elevation of plasma insulin, whereas the
epinephrine concentration did not rise to >0.38 nmol/l (13). The response with saline infusion was similar to that normally observed during exercise, i.e., a gradual decline in insulin concentration caused by the sympa-thoadrenal inhibition of the beta cells of the pancreas via an increase in epinephrine levels (3).

Maintained hyperglycemia had a pronounced attenuating effect on the plasma glucagon concentration. This is an expected response to hyperglycemia, probably

**Fig. 4.** Plasma epinephrine (nmol/l), cortisol (nmol/l), norepinephrine (nmol/l), and growth hormone (GH; mU/l) at rest (-30 min), after prime infusion (0 min), and during 120 min of exercise. Values are means ± SE; n = 8 subjects in each trial.

**Fig. 5.** Rate of carbohydrate (CHO) oxidation (g/min) at rest (-30 min), after prime infusion (0 min), and during 120 min of exercise. Values are means ± SE; n = 8 subjects in each trial.

**Fig. 6.** Rate of fat oxidation (g/min) at rest (-30 min), after prime infusion (0 min), and during 120 min of exercise. Values are means ± SE; n = 8 subjects in each trial.
caused by a direct effect of glucose on the pancreatic alpha cells rather than mediated by epinephrine, and the response illustrates the antagonistic roles of insulin and glucagon. The effect of a diminished glucagon concentration can be realized in a lowered splanchnic glucose output from glycogenolysis and gluconeogenesis. Therefore the infusion rate of glucose probably represents the total glucose uptake by tissues during exercise, because none is likely to accrue from the splanchnic beds.

The epinephrine response during saline infusion is typical of that to prolonged severe exercise, in which impulses from the motor centers in the brain and from exercising muscle elicit a work rate-dependent increase in sympathoadrenal activity. The increases in catecholamines in turn depress insulin secretion by α-receptor-mediated mechanisms (11). The diminished epinephrine levels under glucose infusion are caused by the availability of glucose in the ventromedial and ventrolateral cells of the hypothalamus which reduce sympathetic activity (9). The results of norepinephrine concentration in our study support previous findings of an increase during exercise, and further corroborate those findings that elevated plasma glucose concentrations attenuate the normal response to exercise.

At the onset of exercise, impulses from motor centers in the brain and from active muscles elicit an increase in sympathoadrenal activity and in the release of some pituitary hormones. Among these are increases in circulating levels of GH and ACTH. The latter leads to an increase in cortisol. Clearly, the results from the saline infusion in this study support the view that prolonged exercise elevate plasma GH and cortisol. Intensity and duration of exercise are the important factors governing secretion of these hormones (19). The fact that hyperglycemia suppresses GH has been demonstrated previously (14) and is probably a result of reduced activation of α-receptors by the cells of the hypothalamic ventromedial nuclei that are glucoreceptors. With saline infusion, the data for cortisol show an extended latent period (i.e., 80 min) compared with that for GH. Other authors (5, 12) have shown that cortisol is elevated after exercise, particularly if the exercise is prolonged. The 16% decrease in plasma cortisol concentration, as a result of the prime infusion of glucose and the subsequent reduction during exercise, gives credence to the view that glucose-sensitive receptors can modulate the cortisol response. A high-fat diet enhances the secretion of cortisol, whereas a high-carbohydrate diet attenuates this response (10). Nevertheless, plasma cortisol concentration may become elevated, despite an increase in blood glucose levels.

Elevated concentrations of insulin, together with diminished levels of catecholamines, cortisol, and GH that result from glucose infusion, favored a depression in plasma NEFA and 3-OHB throughout the exercise period. Insulin inhibits lipolysis and promotes lipogenesis, so any increase in glycerol noted during exercise demonstrates the occurrence of lipolysis, whereas continued depression of NEFA is suggestive of re-esterification. The prime glucose infusion significantly reduced concentrations of both glycerol and NEFA; this denotes impaired lipolysis. At no stage during exercise was the NEFA concentration elevated above the preexercise resting levels.

Saline infusion was accompanied by no significant change in NEFA, glycerol, or 3-OHB levels in plasma as a consequence of the prime infusion. Exercise, however, produced a significant increase in concentrations of these metabolites caused by enhanced hormonally stimulated lipolysis.

A notable effect of hyperglycemia was that total carbohydrate oxidation was maintained at a rate >2.5 g/min for the duration of the exercise. This is in contrast to a progressive reduction in carbohydrate oxidation to 1.5 g/min during saline infusion. The results compare favorably with those of Coyle et al. (4), but the values for carbohydrate oxidation during glucose infusion are somewhat lower than the 3.6 g/min observed by Hawley et al. (15). The maintenance of elevated carbohydrate oxidation with hyperglycemia and the decline during saline infusion meant that the difference between trials in relation to carbohydrate oxidation became progressively greater. At 120 min of exercise, the rate of carbohydrate oxidation was ~40% higher with glucose infusion compared with saline infusion.

In parallel with the widening in the rate of carbohydrate oxidation between trials, the rate of glucose infused (glucose utilization) increased steadily, from 1.1 g/min at the start of exercise to 1.8 g/min after 80–100 min and then remained at that level. This increase in the rate of glucose utilization during exercise was 40%. Since the rate of glucose uptake by muscle is mediated by GLUT-4 transporters, it is suggested that a combination of exercise, hyperglyc-
mia, and hyperinsulinemia resulted in the maximal rate of uptake being 1.8 g/min. Although this value is lower than the rate of 2.6 g/min presented by other authors (4, 15), the fact that our subjects were, on average, older and had a lower \( \text{VO}_2\text{peak} \) (and thereby exercised at a lower absolute exercise intensity) may account for the discrepancies. Indeed, two of the younger and fitter cyclists in our group produced peak glucose utilization rates of 2.57 and 2.78 g/min between 100 and 120 min, whereas two of our older cyclists produced peak values of only 1.33 and 1.51 g/min. Further investigations are warranted on the effects of age and exercise intensity on the rate of glucose utilization.

The differences between the rate of total carbohydrate oxidation and the rate of glucose utilization suggest that, despite hyperglycemia, exercising muscles use their own endogenous glycogen stores. This is supported by our findings of a significant depletion of muscle glycogen after glucose infusion. If we assume that the resting muscle glycogen concentration obtained on the subsequent visit reflects normal values, then the decrease in muscle glycogen after the glucose- and saline-infusion trials is 170.3 and 206 \( \mu \text{mol/g dry wt} \), respectively. Interpretation of these findings needs to be treated with caution, because the resting muscle glycogen value was achieved on a separate day from the postexercise value, albeit at the same time of day and after similar patterns of nutrition and activity over the previous 48 h. Furthermore, the 30-min prime infusion of glucose would most likely have resulted in elevated levels of muscle glycogen before exercise in that trial compared with saline infusion. We calculated a glucose storage of 22.6 g during the prime infusion. It is possible to speculate that the difference in postexercise muscle glycogen may be accounted for by elevated preexercise levels after glucose infusion, and, therefore, muscle glycogen was not spared. Indeed, this speculation is supported by the results of a study that established that the contribution of muscle glycogen to the total energy provision varied between 80 and 41% under conditions of maintained hyperglycemia, whereas under euglycemia the contribution varied between 50 and 21% (15). Clearly, hyperglycemia favors carbohydrate oxidation, and that includes use of muscle glycogen.

Previous studies on glucose infusion during exercise have demonstrated muscle glycogen sparing (2) or no sparing (4). Bergstrom and Hultman (2) infused glucose at a rate of \(~3 \text{ g/min iv during 1 h of one-legged exercise. They found that, as a result of an average blood glucose concentration of 21 mmol/l, muscle glycogen concentration was reduced compared with that of the controls. The fact that only one leg was exercised and that supraphysiological doses of glucose were infused could have resulted in this finding. More recently, Coyle et al. (4) elevated blood glucose to 10 mM in humans and maintained that level during 2 h of exercise. No muscle glycogen sparing was observed.

In summary, the results from this study confirm that maintained hyperglycemia promotes factors that enhance carbohydrate oxidation and utilization. The maximal rates of glucose utilization by tissue during endurance exercise at 70% \( \text{VO}_2\text{peak} \) varied between 2.78 g/min for fitter, young subjects to 1.33 g/min for the less fit, older subjects, although the relationship between age or exercise intensity with the rate of glucose utilization needs further investigation. Finally, whether muscle glycogen is spared under conditions of maintained hyperglycemia remains a matter of conjecture, although we suggest that it is not spared.

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REFERENCES


