Effects of elevated plasma FFA and insulin on muscle glycogen usage during exercise

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COSTILL, D. L., E. COYLE, G. DALSKY, W. EVANS, W. FINK, AND D. HOOPES. *Effects of elevated plasma FFA and insulin on muscle glycogen usage during exercise*. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 43(4): 695–699, 1977. – Seven men were studied during 30 min of treadmill exercise (~70% $\dot{V}O_{2\ max}$) to determine the effects of increased availability of plasma free fatty acids (FFA) and elevated plasma insulin on the utilization of muscle glycogen. This elevation of plasma FFA (1.01 mmol/l) with heparin (2,000 units) decreased the rate of muscle glycogen depletion by 40% as compared to the control experiment (FFA = 0.21 mmol/l). The ingestion of 75 g of glucose 45 min before exercise produced a 3.3-fold increase in plasma insulin and a 38% rise in plasma glucose at 0 min of exercise. Subsequent exercise increased muscle glycogen utilization and total carbohydrate (CHO) oxidation 17 and 13%, respectively, when compared to the control trial. This elevation of plasma insulin produced hypoglycemia (<3.5 mmol/l) in most subjects throughout the exercise. These data illustrate the regulatory influence of both plasma insulin and FFA on the rate of CHO usage during prolonged severe muscular activity.

fat metabolism; carbohydrate metabolism; blood glucose; lactic acid; plasma glycerol

PREVIOUS STUDIES HAVE SHOWN the importance of carbohydrate (CHO) metabolism during prolonged exercise (1, 5, 16). Depletion of muscle glycogen and/or exercise-induced hypoglycemia have both been acknowledged as causes for exhaustion during severe exercise lasting an hour or longer (6, 16, 24). Despite a progessive increase in fatty acid mobilization and utilization during long-term activity, CHO remains a necessary carbon source in muscle metabolism. There is evidence to suggest, however, that an increasing rate of free fatty acid (FFA) oxidation in prolonged submaximal exercise serves to inhibit muscle glycogen utilization (23). If, on the other hand, FFA mobilization is inhibited by the action of insulin and glucose, the rate of muscle glycogen utilization might well be accelerated during prolonged muscular activity.

In the present study we have assessed the regulatory influence of increased plasma free fatty acids (FFA) and glucose-elevated insulin on the rate of muscle glycogen utilization during 30 min of submaximal exercise.

METHODS

Subjects and experimental procedure. Six endurance-trained (running) males and one untrained male served as subjects in this investigation. Their age, height, weight, and maximal oxygen uptake averaged (mean ± SE) 27.7 ± 2.7 yr, 183 ± 2.5 cm, 71.5 ± 2.5 kg, and 58.4 ± 5.9 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively. Based on histological studies, tissue taken from the subjects' gastrocnemius muscle were found to contain 58 ± 5.9% type I fibers (high oxidative, slow twitch). On the average, each participant performed 30 min of treadmill running at 68% of his maximal oxygen uptake on three separate occasions. One trial was conducted 45 min after the men had ingested 75 g of glucose in 300 ml of water in order to establish high plasma insulin and glucose levels at the onset of exercise (*trial G*).

A second trial required the subjects to eat a fatty meal 4.5–5 h before the exercise (*trial F*). Thirty minutes prior to the exercise, 2,000 units of heparin were administered into a forearm vein to promote the breakdown of plasma triglyceride, thereby elevating plasma FFA and glycerol. The third exercise session was conducted after a 5- to 6-h fasting period and served as a controlled condition (*trial C*). The order of testing was randomized with a 7-day interval separating the trials.

Prior to giving their written consent to participate, the men were fully informed of all risks and discomforts associated with this investigation. A medical history, including previous bleeding abnormalities, was completed by each subject before initiating these studies. In addition, venous blood (50 ml) was obtained from the participants before and 30 min after the heparin administration (*trial F*). These samples were subsequently used to determine platelet counts (19), platelet aggregations (25), and activated partial thromboplastin (Thrombokinetograph). The anticoagulating influence of the heparin was found to be abated within 30–60 min following the injection. All participants were free of any abnormal clotting characteristics. It should be noted that no complications were associated with either the biopsy or heparin procedures.

Analytical procedures. Venous blood was sampled from a forearm vein immediately before and at 10, 20, and 30 min of exercise. In *trials G* and *F*, blood was also taken before glucose ingestion and heparin administration, respectively. These samples were obtained without stasis, allowed to clot, and the serum frozen and stored at −20°C. All blood was subsequently assayed for serum glucose, free fatty acids (FFA), glycerol, triglyceride (TG), lactic acid, and insulin as previously described (4, 15, 18, 21, 26, 29).

Needle biopsies were obtained from the lateral aspect of the gastrocnemius muscle before and immediately after exercise (10). The specimen was quickly cleaned of all connective tissue, divided into four or five pieces and each part weighed on an electrobalance at timed intervals to determine the sample's wet weight at the time it was removed from the needle. After weighing, the samples were frozen and stored in liquid nitrogen. Three to four pieces of tissue were subsequently analyzed for glycogen (17) and the values averaged to represent the muscle glycogen content. The reliability coefficient for 56 repeated analyses was 0.98, with the error of the method less than 5%.

After exercise, one piece of the muscle sample was placed in a mounting medium (OCT) and frozen in isopentane cooled with liquid nitrogen for histochemical analysis. Sections (10 μm thick) were cut in a cryostat at −20°C and stained for myosin adenosine triphosphatase (ATP-ase) at pH 4.3 (24). The distribution of glycogen in one of the serial sections was estimated from the periodic acid-Schiff (PAS) reaction as described by Pearse (20). Respiratory exchange was measured at rest before exercise and each minute during the treadmill run using a semiautomatic gas sampling system (28). Gas samples were analyzed for oxygen and carbon dioxide with Beckman E-2 and LB-2 analyzers, respectively. These instruments were calibrated with known gases before the exercise and were reexamined immediately after the trial to determine if any electrical drift had occurred. Neither instrument showed any change in calibration as a result of the 30-min testing.

Respiratory exchange was used to estimate the total carbohydrate (CHO) and lipid utilization during exercise (7). Although caution must be used in such calculations, CHO utilization and muscle glycogen depletion in this study were found to be closely related (r = 0.64), thereby supporting the contention that the respiratory exchange ratio (R) can be used as a valid indicator of substrate metabolism during these exercise conditions. Statistical comparisons were made for mean differences using a t-test for paired observations.

RESULTS

Oxygen consumption during the 30-min exercise bouts represented roughly 68% of the subject's $\dot{V}O_{2\ max}$. As shown in Fig. 1, the men consumed more oxygen during exercise in the high FFA (F) trial than during the high glucose (G) treatment ($P < 0.05$).

The ratio of exchanged carbon dioxide and oxygen (R) was significantly ($P < 0.05$) less during *trial F* and greatest during *trial* G (Fig. 1). Based on these respiratory exchange data we have estimated the total carbohydrate (CHO) and lipid oxidized during the exercise bouts. These data for muscle glycogen utilization and blood lactate concentrations are presented in Table 1. Our estimates of total CHO utilization show that significantly less CHO was used when plasma FFA was elevated by the diet-heparin procedure than in the other two treatments. This constituted reductions of 17 and 26% when compared to the control and *trial G*, respectively. Similar estimates of lipid oxidation show that the subjects used 32 and 68% more fat during *trial F* than during the control and *trial G*, respectively.

On the average, muscle glycogen usage was 40 and 49% less ($P < 0.05$) during *trial F* than during the control and *trial G*, respectively (Table 1). Both estimated CHO and muscle glycogen utilization were significantly greater ($P < 0.05$) in *trial G* than during the control treatment. Thus, both the muscle biopsy and respiratory exchange data reflect a sparing of glycogen during exercise following the elevation of plasma FFA.

Since many of the muscle samples obtained after exercise in the present study contained over 100 mmol/kg wet tissue, it was often difficult to determine from histological sections if a selective fiber recruitment pattern existed. Nevertheless, the type I fibers frequently showed a lighter PAS stain than the type IIa and IIb fibers. Two subjects, however, showed lighter staining in the type IIa and IIb fibers than in the type I fibers after exercise. These two subjects showed little

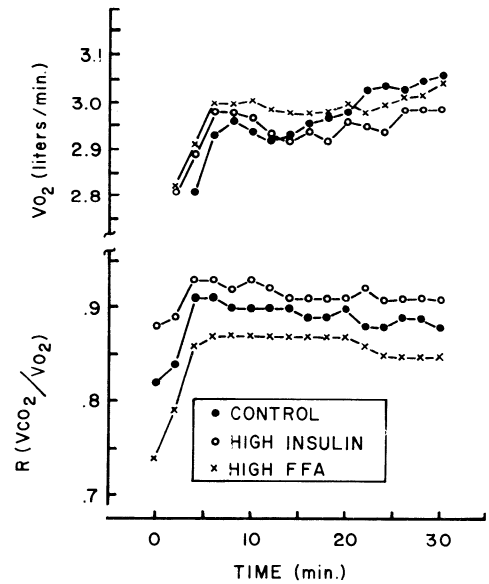

FIG. 1. Mean oxygen consumption ($\dot{V}O_2$) and respiratory exchange ratios during exercise in control, hyperglycemic (High Insulin), and elevated plasma free fatty acid (High FFA) trials.

TABLE 1. *Muscle glycogen utilization, carbohydrate and lipid oxidation, and blood lactic acid concentrations*

Trial	Muscle Glycogen		Δ, mmol/kg	CHO_{est}, g	$Lipid_{est}$, g	LA, mmol/l
	Before	After				
C	137.0	102.9	34.1* †	67.7* †	16.5* †	3.0*
	±19.8	±18.5	±3.2	±5.0	±1.7	±0.6
F	135.5	115.1	20.4‡	56.1‡	21.7‡	1.9‡
	±13.6	±17.0	±5.1	±4.2	±1.4	±0.3
G	146.3	106.4	39.9	76.3	12.9	2.6
	±16.0	±18.9	±4.6	±3.7	±1.6	±0.5

Values are mean ± SE. Δ = muscle glycogen utilization, CHO_{est} = estimated total carbohydrate oxidation, $Lipid_{est}$ = estimated lipid oxidation, and LA = mean blood lactic acid concentration during 30 min of exercise in control (C), high FFA (F), and high insulin and glucose (G) conditions. * High FFA vs. control, $P < 0.05$ (t-test). † High insulin vs. control, $P < 0.05$ (t-test). ‡ High FFA vs. high insulin, $P < 0.05$ (t-test).

sparing of muscle glycogen (−12 to −16%) when plasma FFA were elevated. Such individual variations in muscle fiber recruitment and fiber dependence on glycogen utilization may, in part, explain the varied degrees of glycogen sparing that was observed (range = 12–100% reductions in glycogen used) following the diet-heparin treatment. Little relationship, however, was found between the percentage of type I fibers and the degree of glycogen sparing ($r = 0.30$).

Since the exercise intensity was limited to roughly 68% of $\dot{V}O_{2\ max}$, the blood lactic acid accumulation was small (Table 1). Nevertheless, the lactate values were significantly ($P < 0.05$) lower during the *trial F* than in either of the other exercise bouts, thereby supporting the observation that less carbohydrate was used in the *trial F*.

Figure 2 illustrates the changes in plasma triglyceride (TG), free fatty acids (FFA), and glycerol during the three exercise bouts. These blood constituents remained unchanged during the control and *trial G*. As anticipated, the fatty meal ingested 4.5–5.0 h before the exercise elevated the plasma TG approximately 24% above the control, fasting value. The administration of heparin produced a precipitous decline in plasma TG (−61%), with a concomitant rise in plasma FFA (+323%) and glycerol (+679%) (Fig. 2).

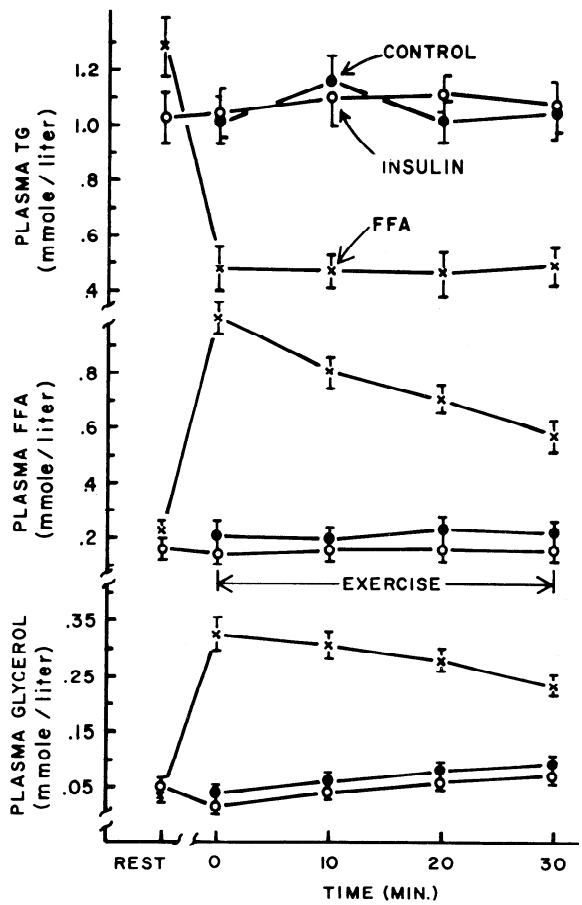

FIG. 2. Mean ± SE values for plasma triglyceride (TG), free fatty acids (FFA), and glycerol at rest and during exercise in control, hyperglycemic (Insulin), and elevated free fatty acids (FFA) trials.

As can be observed in Fig. 2, plasma TG remained low ($\bar{x} = 0.48$ mmol/l) throughout the exercise in *trial F*. Plasma FFA and glycerol, on the other hand, declined steadily during this exercise bout. On the average, plasma FFA dropped from 1.01 mmol/l at 0 min of exercise, to 0.57 mmol/l at the 30th min of work (%Δ = −44%), while glycerol decreased from 0.32 to 0.23 mmol/l (%Δ = −28%).

The ingestion of 75 g of glucose produced a 38% increase in plasma glucose, which was accompanied by a 3.3-fold increase in plasma insulin (Fig. 3). In the first 10–20 min of exercise in *trial G*, plasma glucose dropped dramatically to a low of 3.5 mmol/l, which was significantly ($P < 0.05$) below the resting and preexercise values in the control and *trial F*. This hypoglycemic state persisted through the 30th min of exercise. Although plasma insulin decreased during exercise in *trial G*, the values were still significantly elevated at 30 min of exercise.

During the control and F exercises, plasma glucose showed a steady, significant increase ($P < 0.05$) above the resting and preexercise levels (Fig. 3). This increase was significantly greater ($P < 0.05$) in the F than in the control trial. Plasma insulin, on the other hand, remained unchanged during exercise in these exercise sessions.

Using the Borg scale (3), the participants rated the three exercise bouts to be equally difficult. This scale consists of numbers from 6 to 20 in a quarto format with descriptive words printed beside every other number, ranging from "very, very light" at 7 to "very, very hard" at 19. In the present study the subject ratings averaged 11.1, 11.6, and 12.1 for the F, G, and control exercise bouts, respectively. Exercise heart rates and energy expenditures did not differ in the three treatments, thereby providing physiological evidence of the similarities in the efforts required during the treadmill tasks.

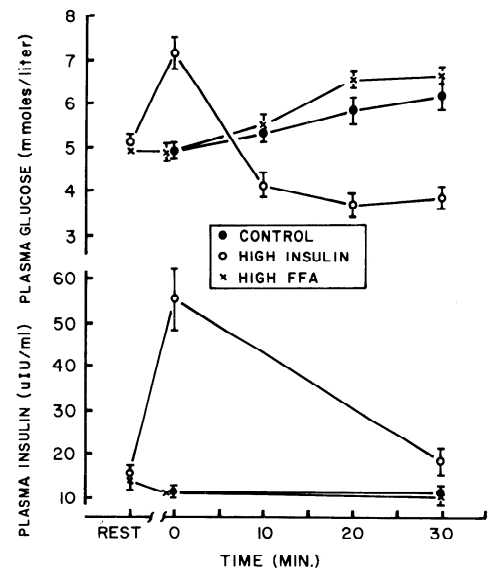

FIG. 3. Mean ± SE plasma glucose and insulin values at rest and during exercise in control, hyperglycemic (High Insulin), and elevated free fatty acid (High FFA) trials.

DISCUSSION

Glycogen sparing. The degree of glycogen utilization from the gastrocnemius muscle was found to be closely related to the total CHO utilization estimated from respiratory exchange. The data presented in Table 1 confirm this relationship and suggest that calculations of substrate utilization from O_2 consumption and CO_2 production are valid. The present study clearly demonstrates a glycogen sparing effect when plasma FFA are elevated following lipase activation with heparin. Recent studies using rats have shown similar reductions in muscle glycogen utilization with the increased plasma FFA concentrations (23). The magnitude of the muscle glycogen sparing effect was similar in the two investigations; 40–50% less glycogen was used with increased plasma FFA.

The reduction in muscle glycogen utilization of the diet/heparin treated subjects is apparently due to the inhibitory effect of citrate on phosphofructokinase (13, 22). There is substantial evidence that increased FFA oxidation produces an accumulation of citrate in heart and skeletal muscle, thereby limiting the rate of glycolysis (12, 23).

Since the capacity for FFA metabolism is dependent on a high respiratory potential, it is reasonable to assume that the influence of elevating the plasma FFA is confined to the more oxidative muscle fibers. Human skeletal muscle fibers are generally classified as type I (slow twitch, high oxidative), type IIa (fast twitch, moderately oxidative), and IIb (fast twitch, low oxidative) (11). Gollnick et al. (14), and others (8, 9) have demonstrated that during prolonged exercise at 60–70% of $\dot{V}o_{2\,max}$ the type I muscle fibers show the highest rates of glycogen depletion. This has been interpreted to suggest that these fibers are most frequently recruited during this type of exercise (14).

Blood glucose. While the elevation of plasma FFA effectively reduced the rate of muscle glycogen utilization, it was also associated with a rise in blood glucose concentration (Fig. 3). This response was possibly induced by the inhibitory effect of accumulated glucose 6-phosphate on hexokinase, thereby limiting the entry of glucose into the cell (22, 27). Previous studies performed under similar circumstances (elevated plasma FFA) have shown a decline in blood glucose during 30 min of exercise in rats (23). This apparent species and/or experimental difference may partially be explained by the fact that the elevation of plasma FFA in rats produced a rise in plasma insulin, thereby increasing the cellular removal of glucose. Our subjects, however, showed no change in plasma insulin as a result of the diet/heparin treatment. Hepatic glucose release is closely matched to the rate of glucose uptake-utilization by muscle during exercise (27). This results in a relatively constant blood glucose concentration until liver glycogen is depleted. Under fasting conditions, the increased production of glucose by the liver during exercise is, at least in part, mediated by an increase in plasma glucagon, with a concomitant decrease in plasma insulin (23). These controls on blood glucose concentrations were markedly upset by the ingestion of glucose 45 min before exercise (*trial G*). The subsequent elevation of blood glucose was accompanied by a 3.4-fold increase in plasma insulin (Fig. 3) and a probable decrease in plasma glucagon concentration. At the onset of exercise, blood glucose concentration dropped well below the fasting levels as a result of the combined effects of insulin and exercise on the uptake of glucose by the active tissues. With plasma insulin elevated and hepatic-glucose release depressed, plasma glucose concentrations remained low throughout the 30 min exercise bout in the *trial G*.

The importance of blood glucose in providing a source of fuel for exercising muscle and nervous tissue is common knowledge (18, 27). Early investigations demonstrated that blood glucose concentrations may decrease during long-term exercise, thereby suggesting that exhaustion in such work may be associated with the lack of available blood glucose (2, 6). This contribution of hypoglycemia to muscular exhaustion was not supported by the current data. During *trial G*, several of the subjects were found to have blood glucose concentrations as low as 2.3 mmol/l. Despite their state of hypoglycemia, these men experienced no greater fatigue than during the trials when their blood glucose averaged 4.6–5.8 mmol/l. It should be remembered that the exercise-hypoglycemia observed in *trial G* was not accompanied by marked depletion of muscle glycogen and, thus, may not be comparable to the hypoglycemia-exhaustion reported by Christensen and Hansen (6). It is interesting to note, however, that two of the subjects, who were found to have the lowest blood glucose values during *trial G*, also utilized 71 and 100% more muscle glycogen, respectively, than during the control exercise bout. This would suggest that when blood glucose concentrations are very low, the exercising muscle may adjust the rate of glycogen utilization to compensate for the lack of available glucose, provided muscle glycogen stores are high. In any event, the present research design does not permit us to determine the effects of the hypoglycemia observed during *trial G* on endurance performance.

Plasmid lipids. As anticipated, the diet/heparin treatment resulted in a marked elevation of plasma glycerol and FFA, with a concomitant decline in plasma triglycerides. Subsequently, exercise produced a marked decline in plasma glycerol and FFA. The present data do not permit us to determine whether the decrease in glycerol was associated solely with intracellular re-esterification of triglycerides. A more likely explanation for this decline is the fact that the splanchnic uptake of glycerol may be markedly increased. Wahren et al. (27), have demonstrated a 10-fold increment in glycerol uptake during prolonged exercise, which was attributed to its greater availability.

Conclusion. These studies demonstrate that increased availability of plasma FFA slows the rate of carbohydrate utilization in exercising human skeletal muscle. Elevation of plasma glucose and insulin prior to exercise, on the other hand, increased the rate of carbohydrate metabolism. While the elevation of plasma FFA produced a muscle glycogen sparing effect, the ingestion of carbohydrates 45 min before exercise

reduced the availability of blood borne glucose without a compensatory increase in plasma FFA. As a result, a greater dependence was placed on muscle glycogen for energy during the exercise.

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