Regulation of fat-carbohydrate interaction in skeletal muscle during intense aerobic cycling

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Dyck, D. J., C. T. Putman, G. J. F. Heigenhauser, E. Hultman, and L. L. Spriet. Regulation of fat-carbohydrate interaction in skeletal muscle during intense aerobic cycling. *Am. J. Physiol.* 265 (*Endocrinol. Metab.* 28): E852–E859, 1993. —Six male subjects received either a saline (control) or Intralipid infusion during 30 min rest and 15 min cycling at 85% maximal O_2 uptake ($\dot{V}O_{2\,max}$) to examine the regulation of fat-carbohydrate interaction (glucose-fatty acid cycle) in skeletal muscle. Muscle biopsies were sampled immediately before and at 3 and 15 min of exercise in both trials. A muscle biopsy was also taken at −30 min rest in the Intralipid trial. Intralipid infusion significantly elevated plasma free fatty acids above control during rest (0.21 ± 0.04 to 0.94 ± 0.09 mM) and exercise (5 min: 1.27 ± 0.15 mM; 15 min: 1.42 ± 0.13 mM). Muscle glycogen degradation was significantly lower in the Intralipid trial (109.7 ± 29.3 vs. 194.7 ± 32.1 mmol/kg dry muscle). Muscle lactate accumulation after 15 min was similar in both trials (control, 60.7 ± 12.2 and Intralipid, 60.9 ± 12.4 mmol/kg dry muscle). Muscle citrate increased at rest during Intralipid (0.32 ± 0.06 to 0.58 ± 0.06 mmol/kg dry muscle) but was not different between trials at 3 min (control, 0.73 ± 0.07 and Intralipid, 0.68 ± 0.06 mmol/kg dry muscle) and 15 min of cycling. Resting acetyl-CoA was unaffected by Intralipid and increased similarly in both trials at 3 min of cycling (control, 59.0 ± 10.3 and Intralipid, 50.7 ± 13.6 μmol/kg dry muscle) and remained unchanged at 15 min. Pyruvate dehydrogenase activity increased five- to sevenfold during exercise and was similar in both trials (15 min: control, 2.42 ± 0.30 and Intralipid, 2.79 ± 0.41 $mmol \cdot min^{-1} \cdot kg\ wet\ wt^{-1}$). The results suggest that, during 15 min of cycling at 85% $\dot{V}O_{2\,max}$, muscle citrate and acetyl-CoA are not responsible for reduced glycogenolysis in the Intralipid trial as proposed in the classic glucose-fatty acid cycle. It is suggested that regulation exists at the level of muscle glycogen phosphorylase.

Intralipid; muscle glycogenolysis; pyruvate dehydrogenase; phosphofructokinase; citrate; acetyl-coenzyme A; glucose-fatty acid cycle

THE CONCEPT OF the glucose-fatty acid cycle was proposed in the 1960s by Randle and co-workers (16, 17, 31) while studying rat heart and diaphragm muscles. It was postulated that enhanced fat oxidation elevates muscle citrate and acetyl-CoA contents, resulting in the down-regulation of carbohydrate metabolism due to inhibition of the enzymes phosphofructokinase (PFK) and pyruvate dehydrogenase (PDH). This concept has been supported by in vitro studies (1, 13, 15) demonstrating regulation of these enzymes as classically proposed. However, since the studies by Randle and co-workers (16, 17, 31), there has been controversy regarding the existence of the glucose-fatty acid cycle in human skeletal muscle during exercise. The majority of studies have been concerned with the regulation of glucose uptake in the face of enhanced fat oxidation, whereas fewer studies have examined the aspect of glycogen utilization. Furthermore, there is a lack of information regarding the mechanisms by which fat-carbohydrate interaction occurs in skeletal muscle and whether the regulation is consistent with that proposed in the classic glucose-fatty acid cycle.

Several studies have investigated the effects of elevated plasma free fatty acids (FFA) on carbohydrate utilization in humans during fasting, fat feeding, and infusion of triglyceride emulsion at rest (2, 14, 27, 38, 40) and exercise (12, 19, 25, 27, 32). Studies using fasting or high-fat meals to elevate plasma FFA (12, 25, 27) have the disadvantage of altering substrate and hormone levels and possibly confounding the results. Infusion of a triglyceride emulsion (Intralipid) has the advantage of acutely elevating arterial FFA levels without additional substrate or hormonal changes. Intralipid infusion studies in resting humans (2, 14, 38, 40) have clearly demonstrated a reduced glucose utilization subsequent to elevated plasma FFA. However, few studies have investigated the effects of Intralipid infusion during exercise, and these studies have yielded inconsistent results. Ravussin et al. (32) were unable to demonstrate a lower respiratory exchange ratio with Intralipid during prolonged low-intensity exercise. Hargreaves et al. (19) were unable to demonstrate reduced muscle glycogen utilization during dynamic knee extensions with Intralipid infusion but demonstrated a significant reduction in thigh glucose uptake during rest, exercise, and recovery.

Although several studies have examined various aspects of the glucose-fatty acid cycle during rest and exercise, there has not been a detailed examination in exercising humans. A previous experiment in our laboratory demonstrated significant sparing of muscle glycogen during the initial 15 min of intense aerobic exercise subsequent to caffeine ingestion (37). Caffeine may enhance FFA mobilization via increases in plasma epinephrine concentration. The measurements of citrate, acetyl-CoA, and acetyl-CoA-to-CoA ratio in muscle were not consistent with the classic regulation of the cycle. However, the muscle measurements were not made before 15 min, which was the period during which the glycogen sparing occurred. PDH activity was also not measured. Therefore, the initial purpose of the present study was to determine whether enhanced exogenous FFA availability (Intralipid and heparin infusion) would spare muscle glycogen during 15 min of intense aerobic cycling, independent of hormonal and substrate changes. Second, if muscle glycogen was spared in the presence of elevated FFA during exercise, we measured muscle citrate, acetyl-CoA, and PDH activity to determine if the regulation of fat-carbohydrate interaction was consistent with the classically proposed theory.

METHODS

Subjects. Six healthy males of varied training status, mean age 27.0 (range, 21-31) yr, and weight 74.9 (range, 60.2-84.2) kg volunteered for the experiment. Two subjects were classified as well trained, two as moderately trained, and two as untrained (see Table 4). Subjects were informed of the experimental procedure and potential risks both verbally and in writing and gave consent. The experiment was approved by the University Ethics Committee.

Preexperimental protocol. All participants reported to the laboratory before the onset of experiments to perform an incremental maximal O_2 uptake ($\dot{V}O_{2\,max}$) test on a cycle ergometer. The mean $\dot{V}O_{2\,max}$ for the group was 3.97 ± 0.31 (SE) l/min. Subjects reported to the laboratory on a second occasion before the experiment for a practice trial at a power output designed to elicit ~85% $\dot{V}O_{2\,max}$.

For the actual experiment, each subject reported at the same time of day on two occasions, separated by 7-10 days. Subjects cycled for 15 min at ~85% $\dot{V}O_{2\,max}$, while receiving, in randomized order, an infusion of either saline (control) or a triglyceride emulsion of soybean oil (20% Intralipid). Identical pan balance weights, cadence, and seat height were used to ensure that the power outputs were identical between trials.

Subjects were instructed to maintain normal training and dietary patterns during the study. Analysis of the diets indicated a moderately high percentage of carbohydrate intake (57% of total caloric consumption), ensuring adequate liver, muscle, and blood carbohydrate stores before each trial. Subjects refrained from caffeine consumption and intense physical activity 48 h before each trial and reported to the laboratory having eaten a meal high in carbohydrates 2-4 h before the experiment.

Experimental protocol. Subjects rested in the supine position, and a catheter was placed percutaneously in an antecubital vein. Catheter patency was maintained with a saline drip (~100-175 ml/h), and a resting blood sample was obtained at −30 min (Fig. 1). In the control trial, subjects remained resting for 30 min while receiving a saline infusion. At the end of the rest period, a final blood sample and a muscle biopsy from vastus lateralis were taken. In the Intralipid trial, a second catheter was placed in the contralateral antecubital vein for infusion of Intralipid at a rate of 102 ml/h. An initial resting muscle biopsy and blood sample were taken at −30 min just before the initiation of Intralipid infusion. Intralipid infusion was started, and 1,000 U heparin (1-ml bolus) were immediately administered. Blood samples were taken at −20, −10, and 0 min at rest. Additional heparin (500 U) was administered at −15 and 0 min during rest, and a second resting muscle biopsy was taken at 0 min (Fig. 1).

Subjects then cycled at ~85% $\dot{V}O_{2\,max}$ for 15 min (Fig. 1). The Intralipid infusion rate was increased to 180 ml/h during cycling. Muscle biopsies were taken at 3 and 15 min of cycling in both trials. Blood samples were taken while the subject was exercising at 1.5, 5, 10, and 14 min of exercise. Expired gas samples were collected for 1-min periods in Douglas bags starting at 6 and 11 min of exercise. A total of 2,000 U heparin was administered during the Intralipid trial. Although this is less than the dose given to promote anticoagulation, we guarded against the possibility of excessive bleeding during biopsy sampling in several ways; subjects refrained from using aspirin during the week preceding the trial, pressure was maintained on the resting biopsy site during the 30-min resting infusion, the exercise component of the experiment was limited to 15 min, and the leg was bandaged for several hours after the trial.

Analyses. Expired gas samples were analyzed for O_2 and CO_2 content with an Applied Electrochemical S-3A O_2 analyzer and a Sensor Medics LB-2 CO_2 detector. Expired volumes were measured with a Parkinson-Cowan volumeter calibrated with a Tissot spirometer.

Muscle biopsies were immediately frozen (5-7 s from the insertion of the needle) in liquid N_2. A small piece of each biopsy (15-20 mg) was removed under liquid N_2 and used for the determination of PDH activity (9). The remainder of the sample was freeze-dried, dissected free of nonmuscular elements, and powdered. Two aliquots (2-3 mg each) were used for the enzymatic determination of muscle glycogen (21) on the resting (0 min) and 15-min exercise biopsies. Approximately 10 mg of each sample were extracted in 0.5 M $HClO_4$ (1.0 mM EDTA) and neutralized with 2.2 M $KHCO_3$ for the determination of ATP, phosphocreatine (PCr), creatine, lactate, and citrate (3). The remaining muscle was extracted in identical fashion and analyzed for acetyl-CoA and acetylcarnitine, as outlined by Cederblad et al. (6).

The mean muscle wet-to-dry ratio (±SE) at rest was 4.04 ±

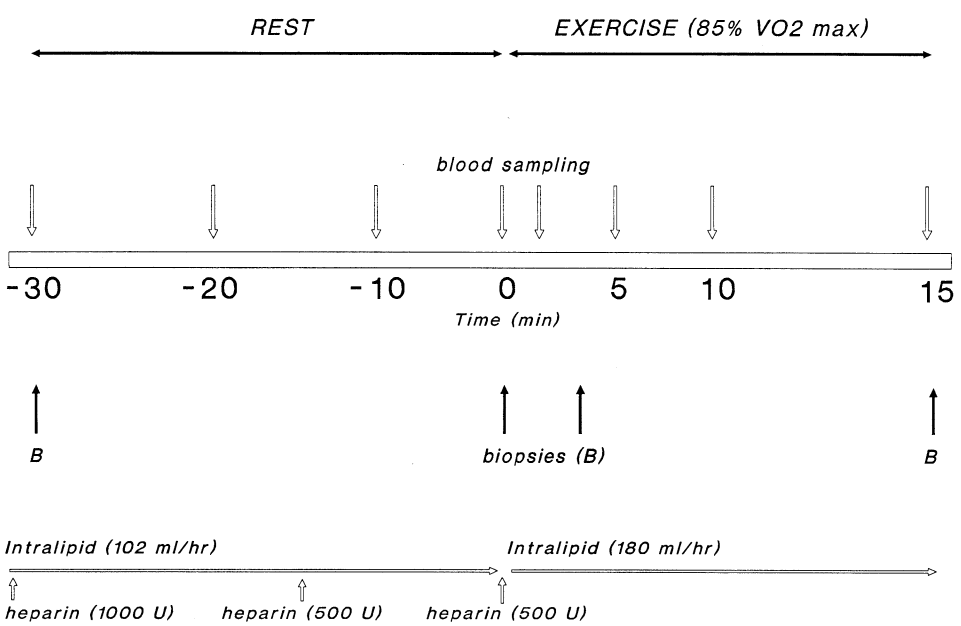

Fig. 1. Experimental protocol. $\dot{V}O_{2\,max}$, maximal O_2 uptake.

0.05 and increased to 4.50 ± 0.05 after 15 min of cycling. All muscle metabolites were expressed per kilogram dry muscle and were corrected to the highest total creatine content for an individual's biopsies. The mean total creatine was 110.2 ± 3.5 mmol/kg dry muscle.

PDH activity in the transformed or active form (PDHa) was measured in muscle homogenates, as outlined by Constantin-Teodosiu et al. (9), with the modification of increasing the concentration of NAD, coenzyme A (CoASH), and thiamine pyrophosphate to 3.0, 1.0, and 1.0 mM, respectively. Briefly, the reaction was initiated by the addition of pyruvate, and 200 μl homogenate aliquots were sampled at 1, 2, and 3 min. The reaction was terminated in each sample by adding 40 μl of 0.5 M $HClO_4$ and subsequently neutralized with 1.0 M K_2CO_3. Acetyl-CoA production was measured in each aliquot, as outlined by Cederblad et al. (6), and regressed against time to determine the reaction rate. PDHa was expressed as millimoles acetyl-CoA produced per minute per kilogram wet muscle. Total creatine was measured on the muscle homogenate to correct for variations in nonmuscular elements of the biopsies.

Blood samples were divided into two aliquots (5 ml into a sodium-heparinized tube for metabolite and hemoglobin measurements and 7 ml into a second sodium-heparinized tube for catecholamine determination). A 200-μl aliquot of heparinized blood was added to 1.0 ml of 0.6 N $HClO_4$, and the supernatant was used for fluorometric determination of whole blood glycerol, lactate, and glucose (3). A 1-ml aliquot of heparinized blood was analyzed for hemoglobin content and O_2 saturation with a hemoximeter IL 482 (Instrument Laboratory, Lexington, MA). Heparinized blood collected for catecholamine determination was mixed with 120 μl 0.24 M ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA)-reduced glutathione and analyzed by high-performance liquid chromatography (Waters), as described by Weiker et al. (41).

For valid plasma FFA determination after heparin administration, in vitro lipolysis due to the presence of lipoprotein lipase (LPL) must be prevented. A 1.5-ml aliquot of heparinized blood was added to an Eppendorf tube containing 30 μl 0.2 M EGTA to prevent in vitro lipolysis and was centrifuged. Plasma was stored at −20°C and analyzed with an enzymatic colorimetric technique (Wako NEFA C kit; Wako Chemicals, Dallas, TX). EGTA inactivation of LPL was reported to be as effective as an alternative method of LPL inactivation involving the addition of 200 μl 5 M NaCl to 800 μl plasma and heating at 56°C for 30 min (19). Our own comparison of the two techniques demonstrated that initial FFA concentrations were similar but that repeated bouts of freezing and thawing resulted in elevation of FFA in the EGTA-treated samples only. Therefore, all FFA concentrations reported in the present study were determined after the initial thaw.

Statistics. All blood and muscle metabolites were compared between trials at a given time point using a paired *t* test. Statistical significance was accepted at $P \leq 0.05$. All data are expressed as means ± SE.

RESULTS

Oxygen uptake and heart rates were not significantly different between the control and Intralipid trials at 6 and 11 min of exercise (Table 1).

Intralipid infusion during 30 min of rest increased plasma FFA concentration from 0.21 ± 0.04 to 0.94 ± 0.09 mM (Fig. 2*A*). No change was observed at rest in the control trial. During exercise, FFA levels increased in the Intralipid trial to 1.27 ± 0.15 and 1.42 ± 0.13 mM at 5 and 15 min, respectively, and were unchanged in the control trial (Fig. 2*A*). Plasma FFA were significantly higher at all time points beyond −30 min during the Intralipid trial compared with control.

Table 1. *Oxygen uptake and heart rate during cycling*

	Time, min	
	6	11
Percentage of $\dot{V}O_{2\,max}$		
Control	86.9±2.5	90.8±2.6
Intralipid	85.9±2.0	89.0±3.7
Heart rate, beats/min		
Control	168±2	174±3
Intralipid	167±4	176±4

Values are means ± SE. $\dot{V}O_{2\,max}$, maximal O_2 uptake.

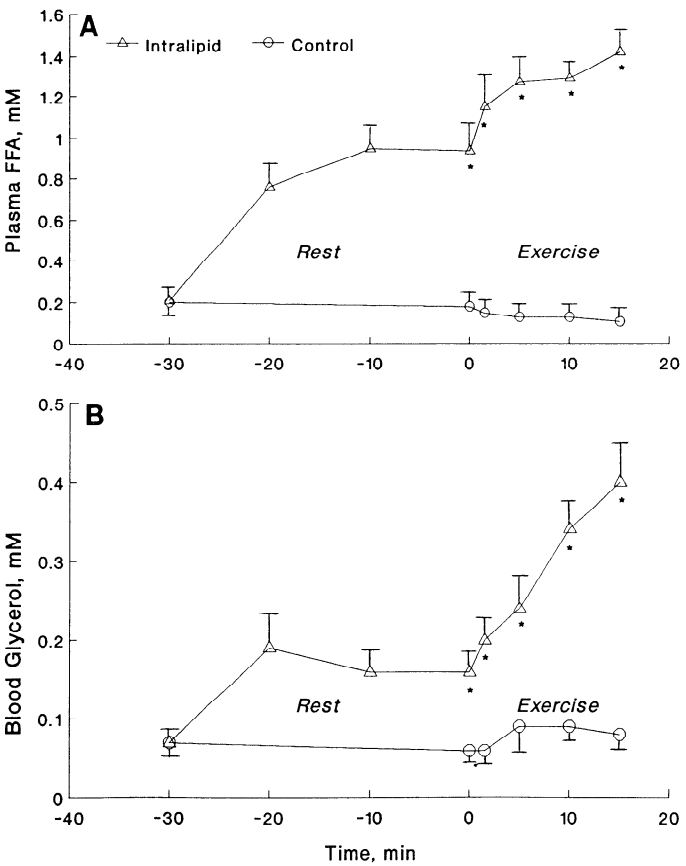

Fig. 2. Plasma free fatty acid (FFA) and blood glycerol concentrations at rest and during intense aerobic cycling with saline (control) or Intralipid infusion. *Significantly different from control.

Changes in blood glycerol were similar to the observed changes in plasma FFA (Fig. 2*B*). Intralipid infusion resulted in a significant elevation of resting blood glycerol in comparison with control from 0.07 ± 0.01 to 0.16 ± 0.02 mM and nearly a sixfold increase by 15 min of exercise (Fig. 2*B*).

There were no significant differences in blood lactate at rest or during exercise as a result of Intralipid infusion (Fig. 3). Blood glucose concentration was similar at −30 min in both trials but was significantly lower in the Intralipid trial just before exercise (3.5 ± 0.1 vs. 4.2 ± 0.3 mM; Fig. 3*B*).

Intralipid infusion had no effect on blood catecholamine levels during rest or exercise (Table 2). Epineph-

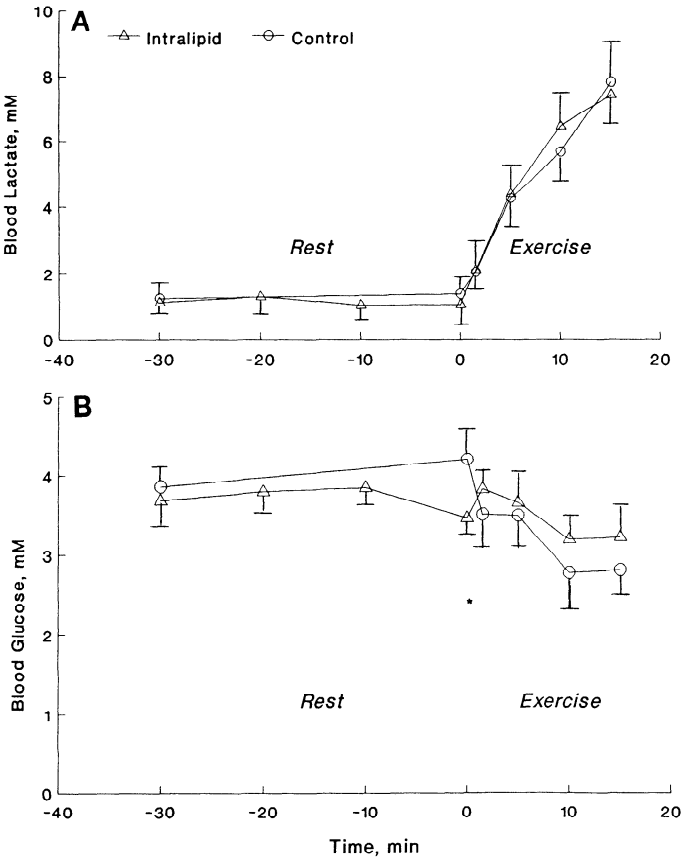

Fig. 3. Blood lactate and glucose concentrations at rest and during intense aerobic cycling with saline (control) or Intralipid infusion. *Significantly different from control.

Table 2. *Plasma epinephrine and norepinephrine concentrations at rest and during intense cycling*

Time, min	Epinephrine		Norepinephrine	
	Intralipid	Control	Intralipid	Control
−30	0.39±0.05	0.44±0.09	1.72±0.15	1.55±0.09
0	0.36±0.02	0.41±0.09	1.50±0.12	1.58±0.11
5	0.85±0.07	1.09±0.20	8.23±0.85	11.11±2.54
10	1.76±0.34	2.09±0.45	20.99±2.94	24.87±2.57

Values are means ± SE in nmol/l.

rine and norepinephrine concentrations progressively increased throughout exercise in both trials.

Muscle ATP content was unaffected by exercise and Intralipid infusion (Table 3). Muscle PCr decreased by 50–60% at 3 min of exercise in both trials. After 15 min

Table 3. *Muscle ATP and PCr contents at rest and during intense cycling*

Time, min	ATP		PCr	
	Intralipid	Control	Intralipid	Control
−30	26.5±0.6		79.0±2.0	
0	27.0±1.2	26.1±1.0	79.4±2.0	79.4±3.7
3	26.4±1.2	25.6±0.9	37.2±6.1	31.5±4.0
15	26.4±0.6	24.9±1.1	37.5±4.7*	24.9±3.6

Values are means ± SE in mmol/kg dry muscle. PCr, phosphocreatine. * Significantly different from control.

Table 4. *Individual muscle glycogen data before and after 15 min of intense cycling*

Subject	Intralipid			Control		
	0 min	15 min	Δ	0 min	15 min	Δ
1	793.3	669.7	123.6	840.8	548.9	291.9
2	429.1	348.7	80.4	425.4	301.7	123.7
3	340.9	255.5	85.4	260.4	129.9	130.5
4	829.6	582.3	247.3	635.4	341.5	293.9
5	250.3	175.0	75.3	287.4	107.0	180.4
6	367.5	321.5	46.0	326.5	178.6	147.9
Means	501.8	392.1*	109.7*	462.7	267.9	194.7
±SE	100.8	78.7	29.3	93.9	67.9	32.1

Values are expressed as mmol glucosyl units/kg dry muscle. Training status as follows: *subjects 1* and *4*, well trained; *subjects 2* and *5*, moderately trained; *subjects 3* and *6*, untrained. * Significantly different from control.

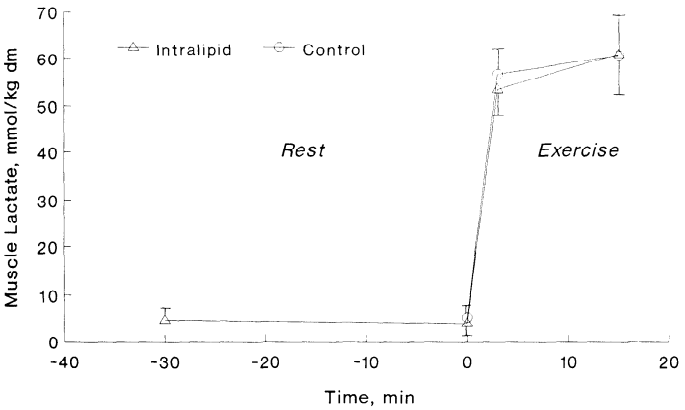

Fig. 4. Muscle lactate content at rest and accumulation during intense aerobic cycling with saline (control) and Intralipid infusion. dm, dry muscle.

of cycling, PCr content was significantly higher in the Intralipid trial vs. control.

Resting muscle glycogen contents were not significantly different between trials (Table 4). Muscle glycogen degradation during the Intralipid trial (109.7 ± 29.3 mmol/kg dry muscle) was significantly less than during the control trial (194.7 ± 32.1 mmol/kg dry muscle) and represented a 44% sparing of muscle glycogen. Muscle glycogen was also measured in three subjects in the −30-min resting biopsy to establish that there was no change in resting glycogen as a result of Intralipid infusion. The mean glycogen contents of the −30-min and 0-min resting biopsies were 545.9 ± 127.2 and 552.0 ± 141.2 mmol/kg dry muscle, respectively.

Intralipid infusion had no effect on resting muscle lactate or accumulation during exercise, despite the magnitude of glycogen sparing (Fig. 4). Muscle citrate content increased from 0.32 ± 0.06 to 0.58 mmol/kg dry muscle at rest during Intralipid infusion, a value significantly greater than the control resting citrate content (0.37 ± 0.08 mmol/kg dry muscle; Fig. 5). Muscle citrate continued to increase during exercise, but at 3 and 15 min there was no difference between trials.

Both muscle acetyl-CoA and acetylcarnitine were unaffected by Intralipid infusion at all time points (Fig. 6). Acetyl-CoA increased during the first 3 min of cycling (control, 59.0 ± 13.5 mmol/kg dry muscle; Intralipid, 50.7

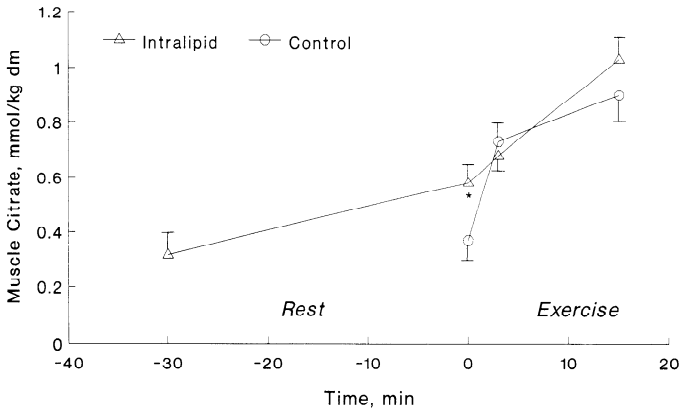

Fig. 5. Muscle citrate content at rest and accumulation during intense aerobic cycling with saline (control) and Intralipid infusion. *Significantly different from control.

± 13.6 mmol/kg dry muscle) and was unchanged thereafter. After 3 and 15 min of exercise, acetylcarnitine increased to 14.9 ± 0.8 and 12.6 ± 0.7 mmol/kg dry muscle and 25.0 ± 3.5 and 22.8 ± 2.5 mmol/kg dry muscle in the control and Intralipid trials, respectively. PDHa was similar at 0 min rest in the Intralipid trial compared with control (0.37 ± 0.05 vs. 0.49 ± 0.08 mmol acetyl-CoA units·min^{-1}·kg wet wt^{-1}; Fig. 6). There were dramatic increases in PDHa by 3 min of exercise and no further changes in activity at 15 min. PDHa values were not statistically different between trials at 3 and 15 min.

DISCUSSION

The present study demonstrated that Intralipid and heparin infusion, resulting in elevated plasma FFA, produced a significant sparing of muscle glycogen during 15 min of intense aerobic cycling. The glycogen sparing that occurred with Intralipid infusion was unrelated to changes in muscle citrate and acetyl-CoA contents. These results demonstrate that the downregulation of glycogenolysis at 85% $\dot{V}O_{2\,max}$ in the presence of high FFA was not mediated as proposed in the classic glucose-fatty acid cycle. These findings were observed in all subjects, regardless of their training status and resting glycogen contents.

Regulation of fat-carbohydrate interaction. Several studies have used Intralipid infusion both at rest (2, 14, 38, 40) and exercise (19, 32) to investigate the effect of elevated plasma FFA on carbohydrate metabolism. In addition, fat feeding (12, 25) and fasting (27) have been used to elevate plasma FFA before exercise. Studies at rest have unequivocally demonstrated a significant reduction in glucose utilization subsequent to elevated plasma FFA. Relatively few studies have measured glucose flux in exercising humans, although Hargreaves et al. (19) and Knapik et al. (27) demonstrated a reduced glucose uptake during exercise subsequent to Intralipid infusion and fasting, respectively.

There is also little direct information regarding muscle metabolism during exercise when FFA availability is enhanced, and the information is controversial. Costill et al. (12) and Knapik et al. (27) demonstrated a reduced glycogen utilization during cycling at 70 and 45% $\dot{V}O_{2\,max}$, respectively, but Hargreaves et al. (19) reported no sparing during knee extensor exercise. Jansson and Kaijser (25) reported significantly lower muscle lactate during exercise after a fat meal but did not measure glycogen. However, in a related series of experiments, Jansson and

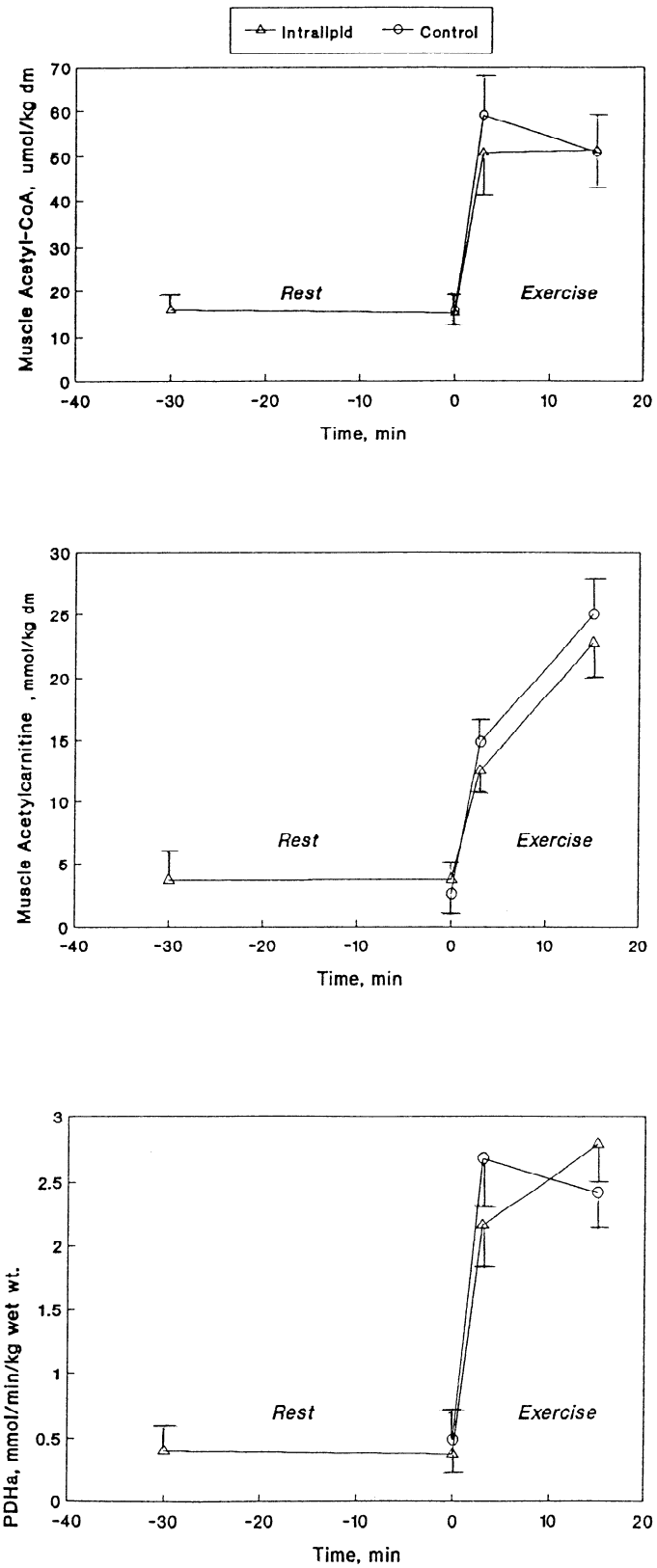

Fig. 6. Muscle acetyl-CoA and acetylcarnitine contents and transformation of pyruvate dehydrogenase (PDHa) at rest and during intense aerobic cycling with saline (control) and Intralipid infusion.

Kaijser reported less glycogen utilization after 6 min (24) and 25 min (23) of submaximal cycling (65% $\dot{V}O_{2\,max}$) after a high-fat diet. Ravussin et al. (32) were unable to demonstrate a reduction in carbohydrate utilization based on respiratory exchange measurements as a result of Intralipid infusion. From the previous studies, only Jansson and Kaijser (25) measured muscle citrate and reported higher citrate values at 5 min of exercise in the high-fat trial. No studies measured acetyl-CoA or PDH activity. Therefore, controversy exists whether carbohydrate is spared when fat availability is increased during exercise in humans, and there is a paucity of information regarding the mechanisms that regulate fat-carbohydrate interaction.

Increases in muscle citrate and acetyl-CoA content as a result of enhanced lipid oxidation may inhibit carbohydrate metabolism at the nonequilibrium reactions catalyzed by PFK and PDH, respectively, as proposed in the classic glucose-fatty acid cycle (16, 17, 31). This is supported by in vitro studies demonstrating inhibition of PFK and PDH by citrate and acetyl-CoA, respectively (1, 13, 15). In addition, several animal studies have demonstrated a relationship between reduced glycogen utilization in contracting oxidative fibers and elevated citrate, subsequent to enhanced fat availability (22, 33, 34). However, the present findings do not support this argument. Infusion of Intralipid at rest resulted in a significant elevation of muscle citrate above control, but this difference was abolished within 3 min of exercise. This extends the findings of Spriet et al. (37) in which muscle citrate was elevated at rest after caffeine ingestion but was similar to control at 15 min of cycling. Acetyl-CoA content was unaffected by Intralipid at all time points. In agreement with the similarity of acetyl-CoA content in the control and Intralipid trials, there was also no effect of Intralipid on PDHa during rest or exercise. Under the present experimental conditions, it is unlikely that either muscle citrate or acetyl-CoA are responsible for the downregulation of muscle glycogenolysis. However, there were no muscle measurements during the initial 3 min at this intensity, and it is possible that the glucose-fatty acid cycle may be regulated as originally proposed under conditions of rest or lower-intensity exercise.

The measurements of PDHa are made under optimal in vitro conditions and represent maximal flux through the transformed (active) form of the PDH complex. Therefore, it is possible that the actual flux through PDHa in the cell is less than what is measured in vitro. In the present study, the maximal activity through the transformed PDH was similar in the two trials, and, therefore, the total possible acetyl-CoA production from pyruvate was also similar. However, if glycogen utilization is corrected for muscle intermediate and lactate accumulation and estimated lactate release, it can be calculated that a lower than expected acetyl-CoA production must have occurred in the Intralipid trial. In the control trial, the calculated acetyl-CoA production was similar to that predicted from the in vitro measurements. This suggests that, depsite similar degrees of PDH transformation in the two trials, there existed a lower flux through this enzyme in the Intralipid trial. This reduced flux must have been due to the presence of intracellular variables. However, acetyl-CoA cannot be the regulator responsible for the reduced flux, as it was similar throughout exercise in the two trials. Recent evidence also suggests that elevated acetyl-CoA does not alter the activity of transformed (active) PDH during muscular contraction (10, 11). It is possible that muscle free CoASH content was lower during the Intralipid trial due to the formation of thioesters with coenzyme A, resulting in a reduced PDH flux (acetyl-CoA formation from pyruvate).

Acetyl-CoA/acetylcarnitine relationship. Formation of acetylcarnitine during exercise provides a sink or temporary store for excess acetyl groups, maintaining a viable pool of free CoA (CoASH) during situations where the production of acetyl-CoA is greater than its condensation with oxaloacetate (5, 20). We are unaware of any previous reports of acetylcarnitine after 15 min of exercise at an intensity similar to that used in the present study. However, the acetylcarnitine value obtained at 3 min of exercise in the present study is similar to that reported after 4 min of intense electrical stimulation (20). Previous reports have demonstrated a linear relationship between muscle acetyl-CoA and acetylcarnitine formation in humans (8, 11), horses (5), and rats (36). However, the present study demonstrates a continuous increase in acetylcarnitine throughout exercise despite a lack of increase in acetyl-CoA beyond 3 min. It can be calculated that the Δacetylcarnitine-to-Δacetyl-CoA ratio from rest to 15 min is ~530 but from rest to 3 min is only ~250–280. This indicates that the buffering of acetyl units is not facilitated to its full extent during the initial stages of exercise. This is likely due to the rapid increase in turnover of the tricarboxylic acid (TCA) cycle at the onset of exercise and the resultant consumption of acetyl-CoA units to establish a new equilibrium with the TCA cycle. This is also supported in a study of prolonged moderate intensity (75% $\dot{V}O_{2\,max}$) where it can be calculated that the Δacetylcarnitine-to-Δacetyl-CoA ratio in the first 3 min was ~360 and thereafter increased to ~700 during the subsequent 37 min of exercise (10).

Elevation of muscle citrate. The present results have clearly indicated that there is no relationship between glycogen sparing in the Intralipid trial and the changes in muscle citrate and acetyl-CoA content. It has been proposed that elevation of citrate subsequent to oxidation of fatty acids or ketones may be the result of increases in oxaloacetate or acetyl-CoA-to-CoA ratio (17). It has also been suggested that oxaloacetate may be substrate limiting for the citrate synthase reaction (18, 29, 42). This is consistent with the findings of Sahlin et al. (35), who demonstrated a reduction in TCA cycle intermediates in exercising humans at fatigue despite adequate acetylcarnitine levels (reflecting availability of acetyl units). In the present study, muscle citrate increased significantly at rest during Intralipid infusion despite the absence of change in acetyl-CoA, suggesting that oxaloacetate may have been the limiting factor. It has been demonstrated in isolated liver mitochondria that oxidation of palmitoylcarnitine increases the carboxylation of pyruvate to oxaloacetate (30). Acetyl-CoA has been demonstrated to be a stimulator of liver pyruvate carboxylase (26, 30, 39), as

well as other acyl-CoA compounds such as propionyl-CoA (26). It is possible that an elevation of fatty acyl-CoA during the Intralipid trial may have stimulated pyruvate carboxylase, resulting in elevation of the oxaloacetate pool and subsequently citrate.

Glycogen sparing. A previous study in this laboratory demonstrated glycogen sparing (37) after caffeine ingestion during intense exercise (80% $\dot{V}O_{2\,max}$). It was postulated that enhanced lipid utilization may have been the cause of sparing, although this could not be proven. The present study was undertaken to determine whether elevation of plasma FFA would spare muscle glycogen during the initial 15 min of intense exercise when the rate of glycogen degradation would be the greatest (4). The degree of glycogen sparing in the present study (44%) is in close agreement with our previous finding (37). It is interesting to note that there was a strong correlation between resting glycogen content and the magnitude of glycogen degradation (control, $r = 0.81$ and Intralipid, $r = 0.87$), although this does not necessarily imply cause and effect. For example, well-trained subjects had the highest resting glycogen contents, and the large glycogen utilization in vastus lateralis in these subjects may have been due to preferential recruitment of this muscle in comparison with lesser trained individuals who were not experienced in cycling. There was no relationship between resting glycogen content or training status and the magnitude of glycogen sparing.

Regulation of glycogenolysis. It is not possible to discuss with any certainty the mechanism of glycogen sparing in the present study. However, regardless of the mechanism, there must ultimately be regulation at the level of glycogen phosphorylase. It is interesting to note that there was less PCr degradation during exercise in the Intralipid trial, which reached significance at 15 min. This suggests a closer match between ATP hydrolysis and phosphorylation of ADP to ATP in the Intralipid trial, resulting in a lower free ADP concentration. This should translate into a decreased free AMP concentration, which is a stimulator of glycogen phosphorylase *a* (7) and *b* (28). In addition, it may be possible that the formation of fatty acyl-CoA subsequent to entry of FFA into the cytoplasm may have a direct effect on glycolytic enzymes, including phosphorylase, resulting in the downregulation of glycogenolysis. Further study will be required to investigate these possibilities.

Summary. The present study has demonstrated a reduction in muscle glycogenolysis during intense aerobic cycling subsequent to an Intralipid-induced elevation of plasma FFA. The glycogen sparing could not be explained by citrate-induced inhibition of PFK or acetyl-CoA-induced inhibition of PDHa. Therefore, during 15 min of cycling at 85% $\dot{V}O_{2\,max}$, the interaction of fat-carbohydrate metabolism does not appear to be regulated as classically proposed in the glucose-fatty acid cycle. It is suggested that regulation exists at the level of glycogen phosphorylase.

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