Exercise-Induced Fall in Insulin and Increase in Fat Metabolism During Prolonged Muscular Work

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The role of the exercise-induced fall in insulin in fat metabolism was studied in dogs during 150 min of treadmill exercise alone (controls) or with insulin clamped at basal levels by an intraportal infusion to prevent the normal fall in insulin concentration (ICs). To counteract the suppressive effect of insulin on glucagon release, glucagon was supplemented by an intraportal infusion in ICs. In all dogs, catheters were placed in a carotid artery and in the portal and hepatic veins for sampling and in the vena cava and the splenic vein for infusion purposes. Glucose levels were clamped in ICs to recreate the glycemic response evident in controls. In controls, insulin fell by 7 ± 1 μU/ml but was unchanged from basal levels in ICs (0 ± 2 μU/ml). Glucagon, norepinephrine, epinephrine, and cortisol rose similarly in controls and ICs. Arterial free–fatty acid (FFA) levels rose by 644 ± 126 μeq/L in controls but did not increase in ICs (−12 ± 148 μeq/L). Arterial glycerol levels rose by 337 ± 43 and 183 ± 19 μM in controls and ICs. Hepatic FFA delivery and fractional extraction increased by 17 ± 3 and 0.06 ± 0.02 μmol · kg^{-1} · min^{-1}, respectively, in controls. In ICs, hepatic FFA delivery increased by only 1 ± 2 μmol · kg^{-1} · min^{-1}, whereas hepatic fractional extraction fell slightly (−0.03 ± 0.03). Consequently, net hepatic FFA uptake rose by 4.8 ± 1.5 μmol · kg^{-1} · min^{-1} in controls but decreased slightly in ICs (−0.5 ± 1.1 μmol · kg^{-1} · min^{-1}). At least partly because of the differences in hepatic FFA uptake, arterial β-hydroxybutyrate (50 ± 14 vs. 23 ± 8 μM) and net hepatic β-hydroxybutyrate output (2.2 ± 0.7 vs. 0.4 ± 0.3 μmol · kg^{-1} · min^{-1}) rose more in controls than in ICs. In summary, the exercise-induced fall in insulin *1*) is essential to the increase in FFA levels during prolonged muscular work, *2*) facilitates hepatic FFA uptake by enhancing the delivery of FFAs to the liver and their extraction by the liver, and *3*) enhances the net β-hydroxybutyrate output by the liver, at least in part through the effects described in *1* and 2. Hence, the exercise-induced fall in insulin is essential for the transition to the increased rate of FFA metabolism that occurs as work duration progresses. *Diabetes* 38:484–90, 1989

Cortisol	1 nm	= 0.360 μg/dl
Epinephrine	1 pM	= 0.183 pg/ml
Free fatty acid	1 μM	= 1 μeq/L
Glucagon	1 ng/L	= 1 pg/ml
Glucose	1 mM	= 18 mg/dl
Glycerol	1 mM	= 9.21 mg/dl
β-Hydroxybutyrate	1 μM	= 0.01 mg/dl
Insulin	1 pM	= 0.139 μU/ml
Lactate	1 mM	= 1 meq/L
Norepinephrine	1 nM	= 169 pg/ml

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Received for publication 27 May 1988 and accepted in revised form 1 November 1988.

The most important change in substrate utilization that occurs as exercise duration progresses is the transition to an increased rate of free–fatty acid (FFA) metabolism. This increase in FFA utilization delays glycogen depletion and provides the muscle with a virtually inexhaustible supply of substrate for energy production. Despite the importance of FFA usage during prolonged exercise, little is known about the signals that control the mobilization and metabolism of this substrate during muscular work. It is evident from studies in which β-adrenergic blockade has been administered during exercise that the mobilization of this quantitatively important fuel source requires the presence of β-adrenergic effects of the catecholamines (1–4). However, the role the fall in insulin may play in regulating exercise-induced changes in fat metabolism is less clear. When insulin is reduced below normal exercise levels by somatostatin (5), fasting (6), or diabetes (7), the exercise-induced increment in FFA levels is exaggerated. Conversely, hyperinsulinemia leads to a reduction in FFA levels during exercise (8), presumably because of a decrease in lipolytic rate. Nevertheless, the role of insulin in

fat metabolism under normal exercise conditions remains to be established.

As with FFA mobilization, the regulation of FFA oxidation and conversion into ketone bodies at the liver during muscular work is not fully understood. During the latter stages of prolonged exercise, ketone body output from the liver is accelerated, and concentrations rise. The role of the fall in insulin in this increase in ketogenesis is unclear, because rises in glucagon and the catecholamines are also evident during exercise and may stimulate this process (9–11). Studies designed to elucidate the specific roles of insulin and the counterregulatory hormones would provide important information about the regulation of hepatic fat metabolism and ketogenesis during exercise.

The aim of our experiment was to assess the role of the normal exercise-induced fall in insulin on fat mobilization and FFA conversion into the ketone β-hydroxybutyrate during prolonged exercise. For this purpose, dogs were exercised on a treadmill for 150 min with an intraportal infusion of insulin, which was designed to prevent the normal exercise-induced fall in this hormone without creating hyperinsulinemia. To counteract the suppressive effect of insulin on glucagon release, intraportal supplementation of glucagon was administered to restore normal exercising levels. In addition, glucose was infused peripherally to prevent a fall in plasma glucose. This model allows the fall in insulin to be assessed during exercise while the normal counterregulatory response is maintained.

MATERIALS AND METHODS

Animals and surgical procedures. Experiments were performed on a total of 11 mongrel dogs (mean wt 20.5 ± 0.6 kg) of either sex that had been fed a standard diet (Kal Kan beef dinner and Wayne Lab Blox: 51% carbohydrate, 31% protein, 11% fat, and 7% fiber, based on dry wt). Sixteen days before each experiment, a laparotomy was performed under general anesthesia (25 mg/kg pentobarbital sodium), during which Silastic catheters (0.04 ID) were inserted into the portal vein, the left common hepatic vein, and a carotid artery for sampling. In addition, Silastic catheters (0.03 ID) were inserted into the vena cava for infusion of indocyanine green and glucose and into a splenic vein for hormone infusions. After insertion, the catheters were filled with saline containing 200 U/ml heparin (Abbott, North Chicago, IL), their free ends were knotted, and they were placed in a subcutaneous pocket so complete closure of skin incisions was possible.

Starting 1 wk after surgery, dogs were trained to run on a motorized treadmill. Training consisted of 3–5 sessions in which the duration and intensity of exercise were increased progressively. Dogs were not exercised 48 h before an experiment. Three days before each experiment, blood was drawn to determine the leukocyte count and the hematocrit of the animal. Only animals that had *1*) a leukocyte count $<18{,}000/mm^3$, *2*) a hematocrit >38%, *3*) a good appetite (consuming all of the daily ration), and *4*) normal stools were used.

On the day of the experiment, after an 18-h fast, the subcutaneous ends of the catheters were freed through a small skin incision made under local anesthesia (2% lidocaine, Astra, Worcester, MA). The contents of each catheter were aspirated, and the catheters were flushed with saline. Silastic tubing was connected to the exposed catheters, and they were then brought onto the back of the dog where they were secured with quick-drying glue, allowing for convenient sampling. Saline was infused through the arterial catheter at a slow rate (0.1 ml/min) throughout the experiment.

Experimental procedures. Experiments consisted of a dye-equilibration period (−120 to −40 min), a control period (−40–0 min), and an exercise period (0–150 min). A constant-rate infusion of indocyanine green (0.1 mg · m^{-2} · min^{-1}) was started at −120 min and continued for the duration of the experiment. The work rate used in these experiments was 100 m/min, 12% grade. This work rate elicits a fivefold increase in oxygen uptake (12). Dogs were studied as normal controls (n = 6) and with insulin levels clamped at basal levels (n = 5). In insulin-clamp experiments, it was necessary to infuse glucagon to normal exercise levels to counteract the suppressive effect of insulin on glucagon release. The insulin levels were clamped by an intraportal infusion of the hormone at a rate of 125 μU · kg^{-1} · min^{-1} 5 min after the onset of exercise and then increased to 250 μU · kg · min^{-1} at 30 min, 300 μU · kg^{-1} · min^{-1} at 60 min, and 325 μU · kg^{-1} · min^{-1} at 90 min. Glucagon was incremented by 0.65 ng · kg^{-1} · min^{-1}, at 30 min of exercise and increased by 0.30 ng · kg^{-1} · min^{-1} every 30 min thereafter. The glycemic response evident in control experiments was simulated during insulin-clamp experiments by a peripheral glucose infusion. The glucose infusion rates used in these experiments were derived from measurements of plasma glucose levels, provided every 5 min during the exercise period by 0.3-ml arterial blood samples. To avoid the possible effects of a glucose infusion on aspects of fat metabolism, glucose was not infused until a fall in circulating glucose levels could be detected. In no experiment in which glucose levels were clamped was an increase in glycemia evident.

Processing of blood samples. The collection and immediate processing of blood samples have been described (13). Plasma glucose concentrations were determined by the glucose oxidase method on a Beckman glucose analyzer (Fullerton, CA). Whole-blood glycerol and β-hydroxybutyrate concentrations were determined in samples deproteinized in 4% perchloric acid (0.5 ml whole blood in 1.5 ml perchloric acid) according to the method developed by Lloyd et al. (14) for the Technicon AutoAnalyzer (Tarrytown, NY). Plasma concentrations of indocyanine green were determined spectrophotometrically (805 nm) in arterial and hepatic vein plasma samples immediately on completion of the study. Immunoreactive glucagon concentrations were measured in plasma samples containing 50 μl of 500 KIU/ml Trasylol (FBA Pharmaceuticals, New York) by radioimmunoassay with Unger's 30K antiserum (15). Interassay coefficient of variation (C.V.) for glucagon was 8%. Immunoreactive insulin was measured with the Sephadex-bound antibody procedure (C.V. of 11%; 16). The lower limit of this assay is 2 μU/ml. Plasma epinephrine (EPI) and norepinephrine (NE) levels were determined with a radioenzymatic assay (C.V. of 11 and 13%, respectively; 17). Plasma cortisol was measured with the Clinical Assays Gamma Coat™ radioimmunoassay kit (Clinical Assays, Travenol-Genetech Diagnos-

tics, Cambridge, MA) with an interassay C.V. of 6%. FFAs were determined by the method described by Ho (18).

Materials. Indocyanine green was purchased from Hynson, Westcott, and Dunning (Baltimore, MD). Phadebas insulin radioimmunoassay kits were purchased from Pharmacia (Piscataway, NJ). Glucagon 30K antiserum was obtained from the University of Texas Southwestern Medical School (Dallas, TX), and the standard glucagon and ^{125}I-labeled glucagon were obtained from Novo (Copenhagen). Catecholamine assay kits (Cat-A-Kit) were obtained from Upjohn (Kalamazoo, MI). Insulin and glucagon were obtained from Squibb-Novo (Princeton, NJ) and Lilly (Indianapolis, IN), respectively. Nickel-63, used in the determination of FFAs, was obtained from New England Nuclear (Boston, MA).

Calculations. The dye-extraction technique used measures total hepatic blood flow but cannot differentiate between separate inputs from the portal vein and hepatic artery (19). The proportion of the hepatic blood supply provided by the hepatic artery was assumed to be 28%, based on a compilation of data by Greenway and Stark (20). Furthermore, we have determined, with Doppler flow probes chronically implanted on the portal vein and the hepatic artery, that the arterial-to-portal blood flow ratio does not change appreciably during exercise (unpublished observations). Net hepatic FFA uptake was determined by the formula [(0.28A + 0.72P) − H]HF, where A, P, and H are plasma FFA concentrations in the artery, portal vein, and hepatic vein, respectively, and HF is the hepatic plasma flow as determined by indocyanine green measurements. Net hepatic glycerol uptake was calculated similarly except that whole-blood glycerol concentrations and hepatic blood flow were used. Hepatic fractional extraction of FFA equaled the net hepatic FFA uptake divided by its load reaching the liver [(0.28A + 0.72P)HF]. Net hepatic β-hydroxybutyrate output was calculated by the equation [H − (0.28A + 0.72P)]HF. Whole-blood β-hydroxybutyrate levels and hepatic blood flow were used in these calculations. Net gut outputs of FFA and glycerol were calculated by the equation [(P − A) × 0.72]HF. Concentrations and flows were derived from plasma and whole-blood measurements for FFA and glycerol, respectively. Statistical comparisons were made by analysis of variance followed by unpaired *t* tests for each intergroup comparison, according to Snedecor and Cochran (21). Data are expressed as means ± SE.

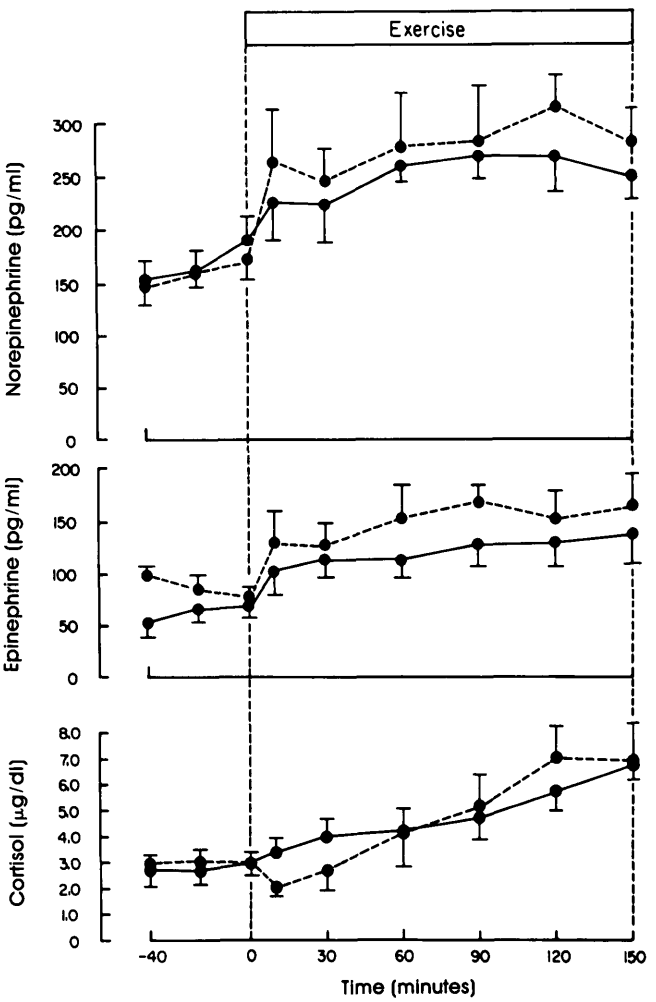

FIG. 2. Effect of exercise alone (*solid lines; n* = 6) or with insulin levels clamped at basal level (*dashed lines; n* = 5) on arterial plasma norepinephrine, epinephrine, and cortisol. Data are means ± SE.

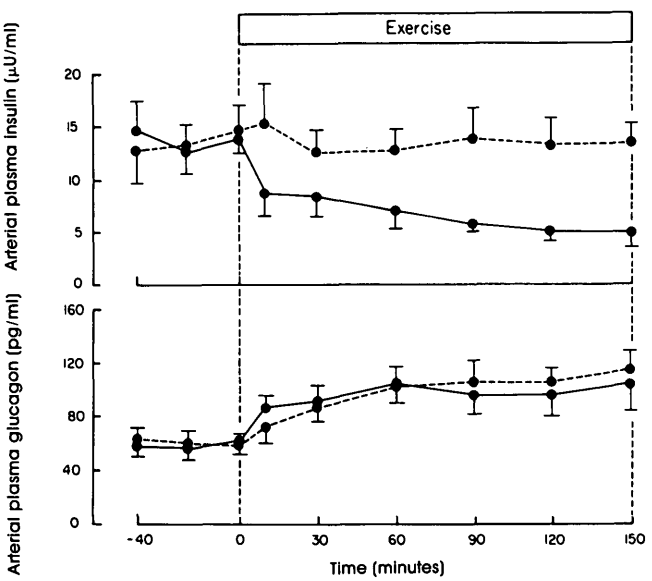

FIG. 1. Effect of exercise alone (*solid lines; n* = 6) or with insulin levels clamped at basal level (*dashed lines; n* = 5) on arterial plasma insulin and arterial plasma glucagon. Data are means ± SE.

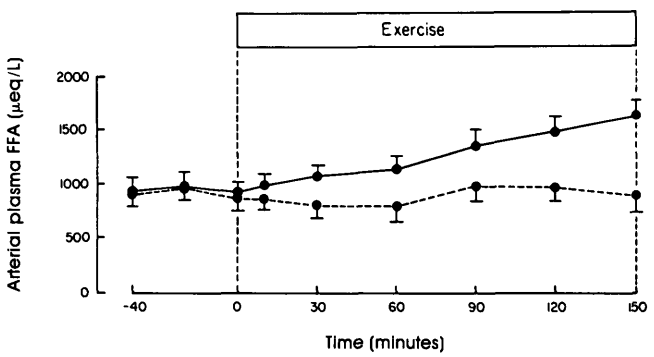

FIG. 3. Effect of exercise alone (*solid line; n* = 6) or with insulin levels clamped at basal level (*dashed line; n* = 5) on arterial plasma free fatty acids (FFA). Data are means ± SE.

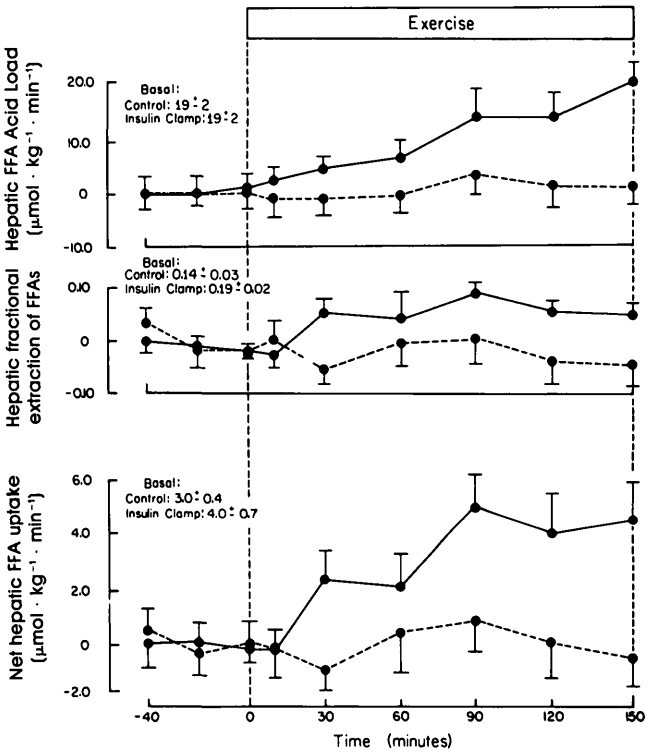

FIG. 4. Effect of exercise alone (*solid lines; n* = 6) or with insulin levels clamped at basal level (*dashed lines; n* = 5) on changes in hepatic free–fatty acid (FFA) delivery, fractional extraction, and net uptake. Changes from baseline as opposed to absolute values were used because basal FFA fractional extraction and net hepatic uptake were different in the 2 protocols. Data are means ± SE.

RESULTS

Arterial plasma glucagon and insulin concentrations. Plasma insulin levels fell from 12 ± 2 to 5 ± 1 μU/ml in control experiments ($P < .05$), whereas insulin levels were maintained at basal levels (13 ± 2 μU/ml) during insulin-clamp experiments (Fig. 1). Plasma glucagon levels rose similarly in control (from 62 ± 5 to 105 ± 20 pg/ml by 150 min of exercise) and insulin-clamp (from 63 ± 5 to 114 ± 12 pg/ml by 150 min of exercise) studies.

Arterial plasma NE, EPI, and cortisol concentrations. Plasma NE levels rose similarly in control (from 166 ± 12 to 252 ± 15 pg/ml by 150 min of exercise) and insulin-clamp (from 160 ± 12 to 278 ± 40 pg/ml by 150 min of exercise) experiments (Fig. 2). In addition, there was no significant difference in the exercise-induced increments in EPI in control (62 ± 7 to 143 ± 36 pg/ml by 150 min of exercise) and insulin-clamp (88 ± 7 to 165 ± 37 pg/ml by 150 min of exercise) experiments. Cortisol rose similarly from 2.7 ± 0.3 to 6.7 ± 0.4 μg/dl and from 3.0 ± 0.3 to 6.8 ± 1.9 μg/dl by the end of exercise in control and insulin-clamp studies, respectively.

Plasma FFA concentration, net hepatic uptake, and fractional extraction and net gut output. The arterial FFA concentration rose significantly from 941 ± 81 to 1615 ± 149 μeq/L ($P < .01$) by the end of exercise when insulin levels fell but did not change appreciably from its resting value during exercise when the insulin level was clamped (Fig. 3). FFA concentrations were significantly lower between 60 and 150 min of exercise when insulin was clamped ($P < .05$). These concentration differences resulted in a greater ($P < .01$) hepatic FFA load during exercise in control (19 ± 2 at rest and 36 ± 4 μmol · kg^{-1} · min^{-1} after 150 min of exercise) compared with insulin-clamp (19 ± 2 at rest and 20 ± 3 μmol · kg^{-1} · min^{-1} after 150 min of exercise) experiments (Fig. 4). Hepatic FFA fractional extraction rose by 0.06 ± 0.02 in control experiments ($P < .05$) but decreased insignificantly by 0.03 ± 0.03 when insulin levels were clamped at basal. Net hepatic FFA uptake rose from a resting rate of 3.0 ± 0.4 to 7.8 ± 1.7 μmol · kg^{-1} · min^{-1} in control dogs ($P < .01$) but fell slightly from 4.0 ± 0.7 to 3.5 ± 1.2 μmol · kg^{-1} · min^{-1} ($P > .05$) by the end of exercise in insulin-clamp experiments. In control dogs, net gut FFA output rose from 0.0 ± 0.1 to 6.3 ± 1.1 μmol · kg^{-1} · min^{-1} ($P < .01$), whereas in insulin-clamped dogs, this variable increased from 0.2 ± 0.1 to only 1.8 ± 0.7 μmol · kg^{-1} · min^{-1} ($P < .05$) (Table 1). Net gut FFA output was significantly higher at 120 and 150 min of exercise ($P < .05$) in control dogs compared with those in which insulin levels were clamped.

Blood glycerol concentration, net hepatic uptake, and net gut output. Arterial blood glycerol levels rose from resting levels of 68 ± 7 to 405 ± 47 μM after 150 min of exercise (Fig. 5). In insulin-clamped dogs, glycerol rose from 75 ± 4 to only 258 ± 22 μM by the end of exercise. Although glycerol levels tended to be higher in control animals throughout the exercise period, differences were significant only at 150 min of exercise ($P < .05$). Net hepatic glycerol uptake had increased from resting levels of 1.3 ± 0.2 to 9.2 ± 1.7 μmol · kg^{-1} · min^{-1} by 150 min of exercise in controls but was reduced significantly in insulin-clamp experiments as levels rose from 1.8 ± 0.1 to only 6.4 ± 0.5 μmol · kg^{-1} · min^{-1} ($P < .05$) (Table 2). Net gut glycerol out-

TABLE 1
Net gut free–fatty acid (FFA) and glycerol output

		Exercise (min)					
	Resting	10	30	60	90	120	150
Net gut FFA output							
Control	0.0 ± 0.1	1.6 ± 0.6	3.0 ± 1.4	4.2 ± 1.4	5.9 ± 2.2	5.3 ± 1.4	6.3 ± 1.1
Insulin clamp	0.2 ± 0.1	1.7 ± 1.0	2.4 ± 0.7	3.5 ± 0.8	3.9 ± 1.0	1.9 ± 1.2*	1.8 ± 0.7*
Net gut glycerol output							
Control	0.1 ± 0.1	−0.2 ± 0.3	0.2 ± 0.4	0.4 ± 0.4	0.8 ± 0.3	0.7 ± 0.4	1.2 ± 0.4
Insulin clamp	0.0 ± 0.1	0.3 ± 0.3	0.3 ± 0.3	0.0 ± 0.3	0.4 ± 0.2	0.0 ± 0.3	0.2 ± 0.2*

Values are expressed in micromoles per kilogram per minute (means ± SE); n = 6 controls, 5 clamps.
*$P < .05$, vs. corresponding control point.

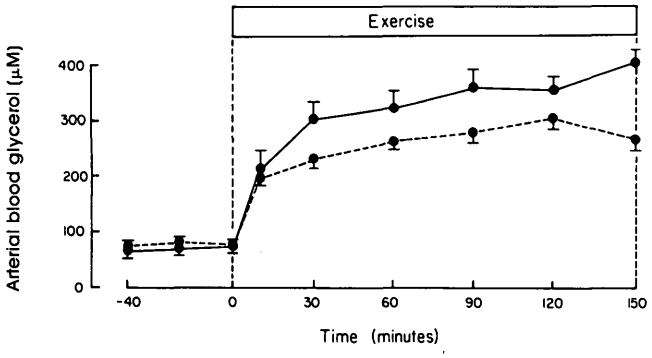

FIG. 5. Effect of exercise alone (*solid line; n* = 6) or with insulin levels clamped at basal level (*dashed line; n* = 5) on arterial blood glycerol. Data are means ± SE.

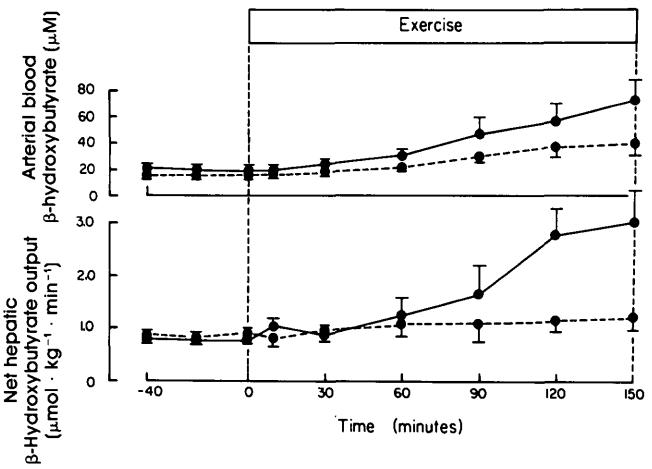

FIG. 6. Effect of exercise alone (*solid lines; n* = 6) or with insulin levels clamped at basal level (*dashed lines; n* = 5) on arterial β-hydroxybutyrate and net hepatic β-hydroxybutyrate output. Data are means ± SE.

put rose from 0.1 ± 0.1 at rest to 1.2 ± 0.4 μmol · kg^{-1} · min^{-1} after 150 min of exercise in control experiments (Table 1). In insulin-clamp experiments, net gut glycerol output increased to a lesser extent than in control studies ($P < .05$), rising from resting rates of 0.0 ± 0.1 to 0.2 ± 0.2 μmol · kg^{-1} · min^{-1} by the end of exercise.

Blood β-hydroxybutyrate concentration and net hepatic β-hydroxybutyrate output. Arterial blood β-hydroxybutyrate levels were 20 ± 3 and 16 ± 1 μM in the resting state in control and insulin-clamp experiments, respectively (Fig. 6). With exercise, β-hydroxybutyrate levels attained a level of 70 ± 15 μM after 150 min in control experiments compared to the significantly lower level of 39 ± 10 μM in insulin-clamp experiments ($P < .05$). In control experiments, net hepatic β-hydroxybutyrate output rose from 0.8 ± 0.1 to 3.0 ± 0.8 μmol · kg^{-1} · min^{-1} after 150 min of exercise, whereas in insulin-clamp experiments, it rose from 0.8 ± 0.1 to only 1.2 ± 0.3 μmol · kg^{-1} · min^{-1}. Net hepatic β-hydroxybutyrate output was significantly reduced in insulin-clamp experiments at 120 and 150 min of exercise ($P < .05$).

Plasma glucose and blood lactate concentrations and exogenous glucose infusion rates. Arterial plasma glucose concentrations were 105 ± 1 and 109 ± 2 mg/dl at rest in control and insulin-clamp experiments, respectively (Table 3). Levels fell by <10% over the course of the exercise period. Arterial blood lactate levels rose transiently in controls from 768 ± 139 μM at rest to 1447 ± 316 μM after 60 min of exercise ($P < .01$), after which concentrations gradually returned to basal. In insulin-clamp experiments, the transient rise in lactate was diminished as levels rose from 530 ± 75 μM at rest to only 746 ± 241 μM after 60 min of exercise ($P < .05$). Lactate levels were significantly reduced in insulin-clamp experiments at 30 and 60 min of exercise. In every experiment in the insulin-clamp protocol, it was necessary to infuse glucose. The average glucose infusion rate during these experiments was 4.1 ± 0.4 mg · kg^{-1} · min^{-1}.

Estimated hepatic blood flow. Estimated hepatic blood flow was 32 ± 1 and 34 ± 1 ml · kg^{-1} · min^{-1} at rest in control and insulin-clamp experiments (Table 4). This rate did not change appreciably during exercise in either group (30 ± 2 and 33 ± 3 ml · kg^{-1} · min^{-1} at 150 min of exercise in control and insulin-clamp experiments, respectively).

DISCUSSION

Our studies demonstrate that the fall in insulin is essential to the increase in arterial FFA concentrations during exercise in normal dogs. This effect is consistent with the known antilipolytic actions of insulin (22). Furthermore, physiological changes in insulin appear to markedly affect the ketogenic response to exercise. This study, in combination with a previous report from our laboratory that showed the crucial role of the fall in insulin to glucose metabolism during exercise (23), demonstrates how the normal exercise-induced fall in insulin is critical for maintaining the optimal balance of substrate usage during exercise (24). Note that, in situations characterized by chronic insulin deficiency, the impact of exercise on fat metabolism may be different. For example, in depancreatized dogs, which have elevated FFA levels and turnover rates at rest, FFA levels do not rise further in response to exercise, even though the tracer-determined lipolytic rate increases (12).

Although FFA levels did not change in insulin-clamp experiments, glycerol levels rose by 4-fold (control dogs ex-

TABLE 2
Net hepatic glycerol uptake

		Exercise (min)					
	Resting	10	30	60	90	120	150
Control	1.3 ± 0.2	4.0 ± 0.6	5.8 ± 0.5	6.9 ± 1.2	8.2 ± 2.0	7.2 ± 1.0	9.2 ± 1.7
Insulin clamp	1.8 ± 0.1	4.4 ± 0.4	5.2 ± 0.5	5.8 ± 0.4	6.4 ± 0.4	6.5 ± 0.7	6.4 ± 0.5*

Values are expressed in micromoles per kilogram per minute (means ± SE); n = 6 controls, 5 clamps.
*$P < .05$ vs. corresponding control point.

TABLE 3
Arterial plasma glucose and blood lactate concentrations

		Exercise (min)					
	Resting	10	30	60	90	120	150
Arterial plasma glucose (mg/dl)							
Control	105 ± 1	102 ± 2	101 ± 2	103 ± 3	101 ± 2	100 ± 3	99 ± 2
Insulin clamp	109 ± 2	104 ± 3	102 ± 1	103 ± 3	102 ± 2	100 ± 4	97 ± 2
Arterial blood lactate (μM)							
Control	768 ± 139	859 ± 136	1277 ± 280	1447 ± 316	1113 ± 238	994 ± 232	754 ± 154
Insulin clamp	530 ± 75	530 ± 156	696 ± 124*	778 ± 131*	746 ± 241	848 ± 270	745 ± 162

Values are means ± SE; n = 6 controls, 5 clamps.
*$P < .05$ vs. corresponding control point.

hibited a 6.5-fold increase in glycerol), indicating that lipolysis was still increased even when insulin levels were clamped. That lipolysis was increased but FFA levels did not change implies that exercise, at least in the presence of an insulin clamp, increased either the clearance or the reesterification of FFAs. An increase in clearance is likely to occur because of increased blood flow, and consequently increased FFA inflow, to working muscle, the tissue that most readily uses FFAs (25). FFA reesterification may also be accelerated during exercise, depending on the glycolytic rate and redox state. The reesterification rate may be higher when the exercise-induced fall in this hormone is prevented, because whole-body glucose utilization has been shown to be elevated by ~20% under these conditions (23). This increased rate of glucose utilization could potentially enhance the rate of FFA reesterification by increasing the availability of α-glycerophosphate. However, because adipose tissue is not a quantitatively important site of glucose usage, this is not likely to be an important mechanism. It is unlikely that reesterification from lactate is enhanced in the insulin-clamp experiments, because, based on its arterial concentration, it is no more available (or even less so during the initial 60 min) for this process than it is in control experiments.

That preventing the fall in insulin abolishes the exercise-induced increment in arterial FFA levels is surprising when considering other results in dogs, which showed that β-adrenergic blockade also eliminates this response (2). It appears that both β-adrenergic stimulation and the fall in insulin are required for the full lipolytic response to exercise. Insulin can inhibit catecholamine-stimulated lipolysis in adipocytes by limiting the rise in cAMP (26). Furthermore, recent studies have shown that fast-acting lipolytic hormones such as the catecholamines act by phosphorylating a serine residue on hormone-sensitive lipase, and insulin can dephosphorylate this site (27). Thus, it is possible that a reduction in insulin levels may be necessary for the full expression of the catecholamine-mediated lipolytic response to exercise.

An interesting finding in these experiments is that the release of FFAs and glycerol from the gut is accelerated markedly during exercise. From our study and the data of Issekutz (2), in which whole-body lipolytic rate was estimated by tracer methods, it appears that the gut may make an ~10% contribution to the release of FFAs into the circulation during exercise. This lipolytic responsiveness of adipose tissue from the gut to exercise is surprising in light of previous studies showing that mesenteric adipose tissue is not influenced by infused NE (28,29) or other biogenic amines or by sympathetic nerve stimulation (28). Nevertheless, it appears that the full increase in gut lipolytic rate requires the fall in insulin. When insulin levels were clamped, FFA and glycerol release from the gut were attenuated.

The release of β-hydroxybutyrate from the liver was 2.5-fold less when insulin levels were clamped at basal compared with normal exercising controls. This decrease may be due to reduced FFA delivery to the liver and diminished FFA extraction by the liver. Although the impaired lipolytic rate caused by the failure of insulin to fall will diminish hepatic FFA delivery, it is less evident what mechanism is responsible for the reduced hepatic FFA fractional extraction. In adipocytes, insulin inhibits the bidirectional transport of catecholamine-stimulated FFA across the plasma membrane (30). Although the catecholamines are elevated during exercise and they appear to stimulate FFA extraction by the liver (9), it is not known whether this process can be inhibited by insulin.

We could not determine from our studies whether the fall in insulin also has a direct effect on ketogenesis within the liver. Previous studies performed in resting dogs have shown that when an isolated deficiency of insulin was created by somatostatin infusion with basal intraportal glucagon re-

TABLE 4
Estimated hepatic blood flow

		Exercise (min)					
	Resting	10	30	60	90	120	150
Control	32 ± 1	31 ± 2	30 ± 2	30 ± 2	31 ± 3	29 ± 3	30 ± 2
Insulin clamp	34 ± 1	31 ± 2	31 ± 2	30 ± 2	31 ± 3	30 ± 3	33 ± 3

Values are expressed in milliliters per kilogram per minute (means ± SE); n = 6 controls, 5 clamps.

placement, net hepatic ketone production increased despite the fact that FFA uptake by the liver remained constant (10). The increased rate of ketone body production by the liver in the face of a constant uptake of its precursor implies that a ketogenic mechanism within the liver is accelerated by the fall in insulin. Furthermore, when the FFA supply to the liver was increased in those studies (by infusions of a triglyceride solution and heparin) to levels similar to those seen during exercise, the effect of isolated insulin deficiency on ketogenesis was magnified substantially. The rise in ketogenesis observed under this condition was greater than the increases that were observed with hypoinsulinemia or hyperlipidemia alone, implying synergism. Thus, there is good evidence that the fall in insulin may stimulate ketogenesis not only by enhancing the FFA delivery to the liver and the FFA extraction by the liver but also by enhancing ketogenic processes within the liver.

In conclusion, the fall in insulin that occurs during exercise is essential for the transition to the increased rate of FFA mobilization that occurs as work duration progresses. Furthermore, the fall in insulin is essential for the exercise-induced increases in hepatic FFA delivery and extraction and, as a consequence, the rise in net hepatic FFA uptake. By providing the liver with FFAs for ketogenesis, the fall in insulin enhances the hepatic production of β-hydroxybutyrate. This study, in combination with other work from our laboratory (23), demonstrates the importance of physiological changes in insulin to substrate metabolism during exercise.

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