Epinephrine Stimulates Human Muscle Lipoprotein Lipase Activity In Vivo

Steen B. Pedersen, Jens F. Bak, Palle Holck, Ole Schmitz, and Bjørn Richelsen

Lipoprotein lipase (LPL) is involved in lipoprotein metabolism and nutrient partitioning in both adipose tissue and skeletal muscle, and LPL activity is regulated by various hormones and the nutritional state. However, the action of catecholamines has not been thoroughly investigated in humans. Therefore, the effects of exogenous epinephrine on skeletal muscle LPL (SM-LPL) activity and whole-body lipid oxidation were studied. Muscle biopsies were obtained from eight healthy subjects before, during, and after epinephrine infusion. Somatostatin was infused to suppress endogenous insulin production and insulin was infused at a constant rate to maintain basal insulin levels throughout the study. After an equilibrium period (120 minutes), epinephrine (0.05 μg/kg/min) was infused for another 120 minutes. Epinephrine stimulated SM-LPL activity by 21.8% ± 6.8% above basal levels from 1.44 ± 0.25 to 1.69 ± 0.28 μmol free fatty acid (FFA)/h/g muscle ($P < .02$), increased plasma FFA 270% from 0.147 to 0.544 mmol/L ($P < .05$), and increased lipid oxidation 45% from 4.37 to 6.36 mg/kg/min ($P < .05$). The increase in SM-LPL activity was positively correlated with the increase in whole-body lipid oxidation ($R = .75$, $P < .05$). Finally, lipid oxidation and SM-LPL activity were negatively correlated with whole-body glucose oxidation. Overall, the results demonstrate that epinephrine is able to stimulate SM-LPL activity in humans, and thus may have opposite effects on adipose tissue and SM-LPL activity.

THE REGULATION OF human skeletal muscle lipoprotein lipase (SM-LPL) activity is complex and not fully understood.[1] Insulin increases LPL activity in adipose tissue, whereas insulin in short-term infusion studies seems to decrease LPL activity in skeletal muscle.[2,3] Thus, an inverse regulation of LPL by insulin seems to exist between adipose tissue and muscle, thereby promoting fat uptake in adipose tissue after a meal (during high insulinemia) and simultaneously reducing fat uptake in muscle tissue.[2] During exercise, both LPL activity and LPL mRNA are increased in muscle tissue[4] concomitantly with a reduction in adipose tissue.[5] Thus, the regulation of LPL in muscle and adipose tissue has important implications for fuel partitioning depending on both the nutritional state and degree of exercise. In addition, LPL activity is of major importance for the regulation of plasma very–low-density lipoprotein triglycerides and high-density lipoprotein cholesterol.[6] Previously, our group and others have found that muscle LPL in the fasting state is the main determinant for the removal of plasma triglycerides.[3,7]

Previous studies in rats have suggested that epinephrine may stimulate LPL activity in muscle,[8-12] and very recently, it has been demonstrated that the synthetic catecholamine isoproterenol increases muscle LPL activity in humans in vivo.[13] The present study was performed to study the effects of the endogenous catecholamine epinephrine on SM-LPL activity and on substrate oxidation. To maintain constant insulin levels during the epinephrine infusion, somatostatin was used to block endogenous insulin secretion and insulin was constantly infused throughout the study to maintain basal insulin values. Muscle biopsies were obtained before, during, and after epinephrine infusion to study the influence of epinephrine on SM-LPL activity.

SUBJECTS AND METHODS

Subjects

Eight lean healthy male subjects without a family history of endocrine or metabolic disease participated in the study. The mean age was 24.6 ± 1.1 years, and the mean body mass index was 23.3 ± 0.9 kg/m^2 (mean ± SEM). The subjects were not engaged in any sort of regular exercise and did not receive any medication. The experimental protocol was approved by the local ethics committee. All participants provided written informed consent.

Experimental Protocol

After a 10-hour overnight fast, the subjects were admitted to the laboratory. They were instructed not to perform any type of major exercise for the preceding 24 hours. Somatostatin (Ferring, Malmö, Sweden) was infused (400 μg/h) to inhibit endogenous insulin secretion. Insulin (Actrapid; Novo-Nordisk, Copenhagen, Denmark) was infused at a rate of 0.1 mU/kg/min to maintain basal insulin levels throughout the study. After a 120-minute equilibrium period (somatostatin and insulin infusion), a muscle biopsy was taken (basal value) and epinephrine was then infused (0.05 μg/kg/min) for 120 minutes, and a second muscle biopsy was obtained at the end of this period (epinephrine value). Sixty minutes after the epinephrine infusion was completed, the last muscle biopsy was taken (recovery value). The muscle biopsies were obtained from the vastus lateralis with a Bergstrom biopsy needle using local anesthesia (lidocaine 1%). All biopsies were obtained from the same leg at a minimum distance of 5 cm from the previous site. The biopsies were immediately cleaned of blood and frozen in liquid nitrogen for later analysis.

Indirect calorimetry (Deltatrac monitor: Datex Instrumentarium, Helsinki, Finland) was performed for 20 minutes at the end of the basal condition, the end of the epinephrine infusion, and the end of the recovery phase to allow measurement of the respiratory exchange ratio.[14] The initial 5 minutes of calorimetry at every phase were used for acclimatization, and no data were collected during this process. Estimated rates of lipid and glucose oxidation and total energy expenditure were calculated after correction for protein oxidation, which was estimated after the measurement of urinary excretion of

From the Department of Endocrinology and Metabolism, Aarhus Amtssygehus, and Medical Department M, Aarhus Kommunehospital, Aarhus University Hospital, Aarhus, Denmark.

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Address reprint requests to Steen B. Pedersen, MD, PhD, Department of Endocrinology and Metabolism, Aarhus Amtssygehus, Aarhus University Hospital, DK-8000 Aarhus C, Denmark.

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Table 1. SM-LPL Activity and Plasma Parameters in the Basal Period, During Epinephrine Infusion, and in the Recovery Period (mean ± SEM, N = 8)

Parameter	Basal	Epinephrine	Recovery
SM-LPL activity (μmol FFA/h/g muscle)	1.44 ± 0.25	1.69 ± 0.28†	1.66 ± 0.23
Plasma parameters			
Glucose (mmol/L)	5.3 ± 0.1	10.4 ± 0.9*	8.9 ± 0.5*
Epinephrine (pg/mL)	62 ± 10	598 ± 51*	52 ± 8
FFA (mmol/L)	0.147 ± 0.02	0.544 ± 0.03*	0.084 ± 0.05*
Insulin (μU/mL)	8.1 ± 4	8.4 ± 3	9.7 ± 1.2*
Lipid oxidation (mg/kg/min)	4.37 ± 0.68	6.36 ± 1.09*	6.45 ± 0.66*

*$P < .05$ *v* basal.
†$P < .02$ *v* basal.

urea.[15] Urine for determination of urea was collected from the beginning of the basal period to the end of the study.

An intravenous cannula was placed in a heated dorsal hand vein for sampling arterialized blood. The criterion for a satisfactory position was an oxygen saturation greater than 90%. Another intravenous cannula was positioned in an antecubital vein. Blood samples were drawn at −10, 0, +60, and +90 minutes and then every 15 minutes during the experimental period for analysis of glucose, insulin, epinephrine, norepinephrine, and nonesterified free fatty acids (FFAs).

LPL Assay

The frozen muscle tissue (50 mg) was cut into small pieces and preincubated for 30 minutes on an ice bath in 500 μL Tris hydrochloride buffer containing heparin (200 IU/mL), L-α-Lecitin (0.2 mg/mL), L-α-phosphatidylcholine (Sigma, St Louis, MO), 1% human albumin (FFA-free), human fasted serum, and ^{14}C-triolein as previously described.[3] The incubation was continued for 120 minutes at 37°C in a shaking water bath, and the heparin-releasable LPL activity was determined as previously described.[3] Results are presented as micromoles of FFA per hour per gram of muscle.

Statistical Analysis

The data are presented as the mean ± SEM. Student's paired *t* test was used for statistical analysis, and a *P* value less than .05 was considered significant.

RESULTS

LPL Activity

Epinephrine infusion for 120 minutes resulted in increased SM-LPL activity from 1.44 ± 0.25 to 1.69 ± 0.28 μmol FFA/h/g muscle ($P < .02$). During the recovery phase (60 minutes after cessation of epinephrine infusion) a third muscle biopsy was taken, and SM-LPL activity was only slightly lower than the level in the biopsy taken during epinephrine infusion (1.66 ± 0.23 μmol/h/g muscle). Thus, SM-LPL activity did not return to basal values 1 hour after the epinephrine infusion was completed (Table 1).

Hormones and Substrates

Plasma insulin was maintained at the basal level during epinephrine infusion, whereas plasma glucose increased from 5.3 ± 0.7 to 10.4 ± 2.3 mmol/L. After termination of the epinephrine infusion, plasma glucose decreased slowly, but the basal level was not reached by the end of the experiment (Fig 1).

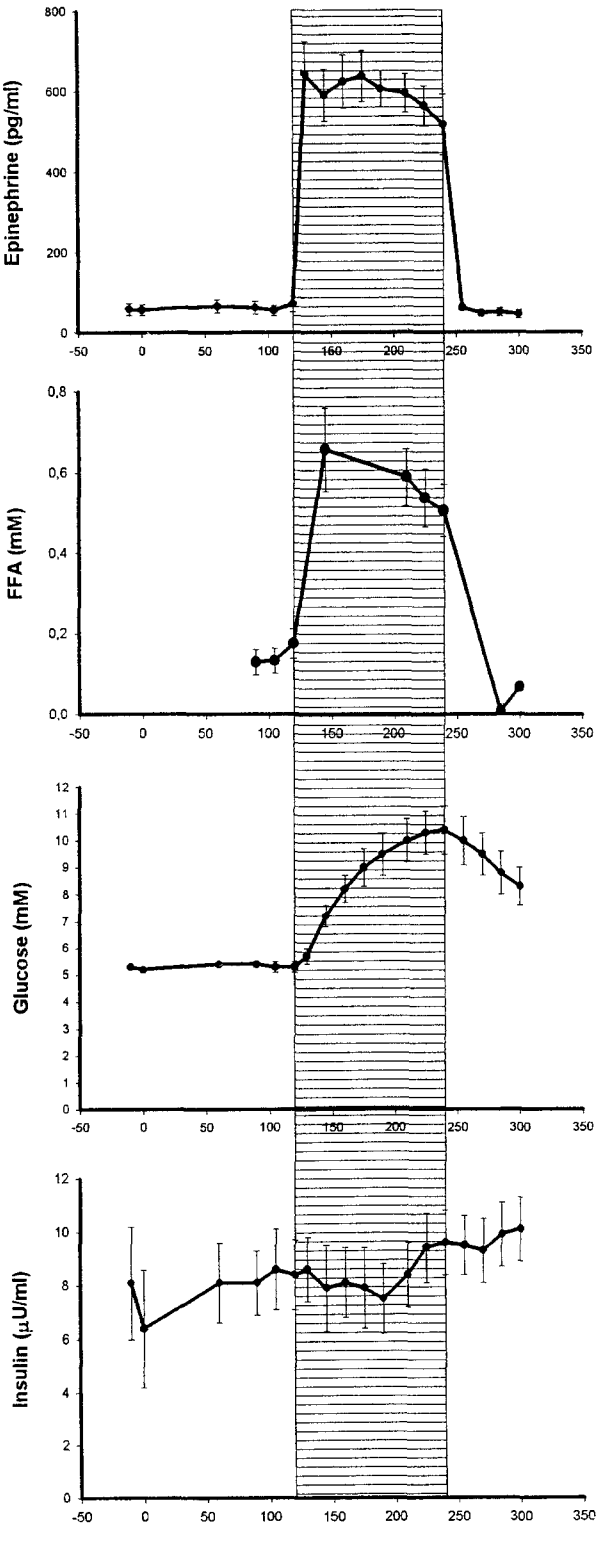

Fig 1. Changes in epinephrine, FFA, glucose, and insulin during the experiment (mean ± SEM). Epinephrine was infused from 120 to 240 minutes (shaded area).

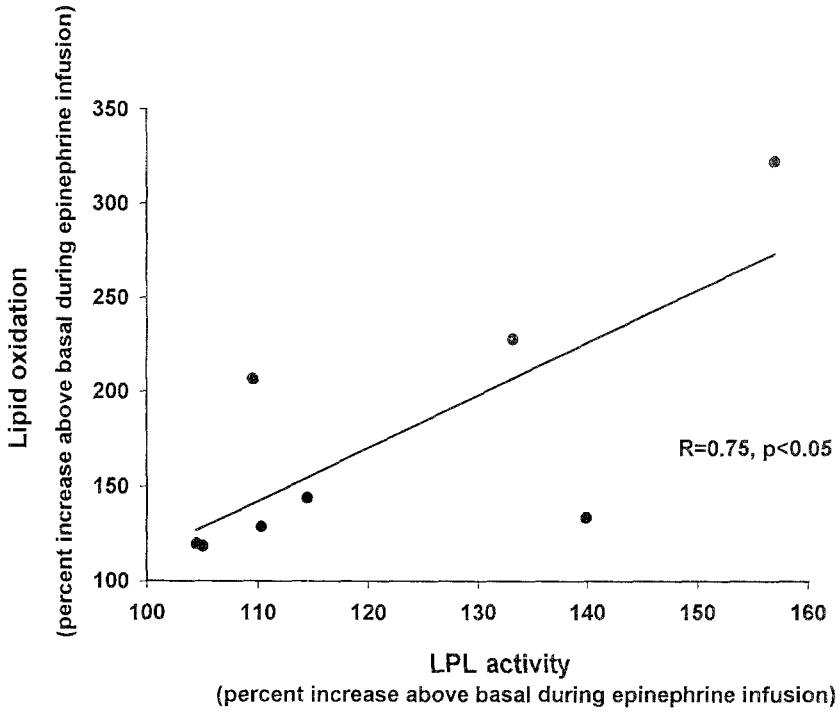

Fig 2. Association between the increase in SM-LPL activity and the increase in lipid oxidation during epinephrine infusion.

Plasma FFA concentrations were significantly elevated (270%) during epinephrine infusion, but returned rapidly to values even less than basal after the epinephrine infusion was stopped (Fig 1). Plasma epinephrine increased from 63 ± 42 to 599 ± 171 pg/mL during epinephrine infusion, but shortly after the epinephrine infusion was completed, plasma epinephrine returned to the basal level (Table 1 and Fig 1). Plasma norepinephrine did not change during the experiment (data not shown).

Associations Between SM-LPL Activity and Metabolic Variables

Indirect calorimetry showed that lipid oxidation increased significantly by 46% during epinephrine administration. Lipid oxidation was still elevated after the recovery period (Table 1). There was a significantly positive correlation between the increase in SM-LPL activity (percent above basal value) and the percent increase in lipid oxidation above basal values, both during and after epinephrine infusion ($r = .75$, $P < .05$ and $r = .74$, $P < .05$, respectively) (Fig 2). During all three periods, there was a negative association between SM-LPL activity and glucose oxidation ($r = -.70$, $P = .052$ for basal, $r = -.76$, $P < .05$ for epinephrine, and $r = -.94$, $P < .001$ for recovery). Finally, glucose oxidation and lipid oxidation were strongly negatively associated in all three periods ($r = -.80$, $P < .02$, $r = -.78$, $P < .03$, and $r = -.71$, $P = .05$).

There was no association between FFA concentrations and SM-LPL activity during any of the three situations. Furthermore, plasma FFA concentrations were not correlated with lipid oxidation or glucose oxidation during any of the conditions. In addition, there was no correlation between arteriovenous differences in FFA concentrations (AV-FFA) and lipid oxidation or between AV-FFA and glucose oxidation (data not shown). Although FFA concentrations in plasma were elevated during epinephrine infusion, epinephrine did not affect AV-FFA, presumably indicating that the net FFA flux in the muscle compartment was unaltered by the epinephrine infusion.

DISCUSSION

The regulation of SM-LPL activity in humans is not fully characterized. In rodents, catecholamines stimulate LPL in skeletal muscle and in the heart. The present study shows that epinephrine during a 2-hour infusion can stimulate SM-LPL activity by 22% in humans, thus supporting a recent report demonstrating that the more potent synthetic β-adrenergic receptor agonist, isoproterenol, is able to stimulate human SM-LPL activity by about 45%.[13] Earlier studies indicate that adipose tissue LPL activity is reduced by catecholamines in rodents[16]; however, in a very recent study, isoproterenol was without any effect on human adipose tissue LPL activity in vivo.[13] On the other hand, insulin stimulates LPL in adipose tissue[2] but reduces LPL activity in skeletal muscle.[3] Therefore, to study the effects of epinephrine on SM-LPL, it was necessary to infuse somatostatin and insulin throughout the study to maintain basal insulin levels; a similar protocol was used in the study by Eckel et al.[13] Epinephrine also increased plasma FFA concentrations, an effect that is probably caused by increased adipose tissue lipolysis, as acipimox (a long-lasting nicotinic acid analog that blocks adipose tissue lipolysis) given together with epinephrine is completely able to prevent any change in the plasma FFA concentration.[17] Thus, the findings of increased plasma FFA, increased lipid oxidation, and increased SM-LPL activity support the notion that under situations with high catecholamine levels, ie, during stress or exercise, lipids are directed to the skeletal muscle as fuel for oxidation.

The fluctuation in plasma FFAs was very rapid. The increase (270%) occurred within 30 minutes after the epinephrine infusion started and decreased to values even below basal 30 minutes after completion of the infusion. Lipid oxidation was

also increased by epinephrine infusion (46%), and the increase was maintained at least 1 hour after the infusion was stopped, even at a time when plasma FFA was less than basal. In the present study, we were unable to demonstrate any correlation between plasma FFA and lipid oxidation; however, the percent increase in SM-LPL activity was positively correlated with the percent increase in lipid oxidation both during epinephrine infusion and during the recovery period. These findings contrast with previous studies in which plasma FFA was closely correlated with lipid oxidation during epinephrine infusion,[18] but tend to support data from other studies demonstrating that an isolated elevation of plasma FFA (by heparin and lipid infusion) was not associated with increased lipid oxidation, whereas infusion of triglyceride (without heparin) resulting mainly in an increase in plasma triglyceride without a change in plasma FFA was associated with an increase in total lipid oxidation.[19,20] Finally, Ferraro et al[21] demonstrated that SM-LPL activity was correlated with 24-hour lipid oxidation in sedentary Pima Indians. One possible explanation for these divergent findings regarding lipid oxidation and FFA was provided by Webber et al[22] in demonstrating that epinephrine infusion may favor oxidation of intratissue lipid rather than plasma FFA. The intramuscular lipolysis of TG is thought to be mediated by hormone-sensitive lipase (HSL), and there is evidence to suggest that muscle HSL and muscle LPL are simultaneously activated under a variety of conditions.[23] Thus, the increase in LPL activity might actually mirror an increase in muscle HSL activity,[24] so it is possible that the association between LPL and oxidation simply mirrors an association between HSL and lipid oxidation.

In conclusion, the present study demonstrates that infusion of the endogenous catecholamine epinephrine is able to stimulate LPL activity in skeletal muscle in humans, to increase plasma FFA, and to increase whole-body lipid oxidation. These findings support the notion that epinephrine is able to direct lipids to muscle tissue for oxidation, and increased SM-LPL might play a role in this respect.

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