Response of Adipose Tissue Lipoprotein Lipase to the Cephalic Phase of Insulin Secretion

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Modulation of lipoprotein lipase (LPL) allows a tissue-specific partitioning of triglyceride-derived fatty acids, and insulin is a major modulator of its activity. The present studies were aimed to assess in rats the contribution of insulin to the response of adipose tissue and muscle LPL to food intake. Epididymal and retroperitoneal adipose LPL rose 65% above fasting values as early as 1 h after the onset of a 30-min high-carbohydrate meal, with a second activity peak 1 h later that was maintained for an additional 2 h. Soleus muscle LPL was decreased by 25% between 0.5 and 4 h after meal intake. The essential contribution of insulin to the LPL response to food intake was determined by preventing the full insulin response to meal intake by administration of diazoxide (150 mg/kg body wt, in the meal). The usual postprandial changes in adipose and muscle LPL did not occur in the absence of an increase in insulinemia. However, the early (60 min) increase in adipose tissue LPL was not prevented by the drug, likely because of the maintenance of the early centrally mediated phase of insulin secretion. In a subsequent study, rats chronically implanted with a gastric cannula were used to demonstrate that the postprandial rise in adipose LPL is independent of nutrient absorption and can be elicited by the cephalic (preabsorptive) phase of insulin secretion. Obese Zucker rats were used because of their strong cephalic insulin response. After an 8-h fast, rats were fed a liquid diet ad libitum (orally, cannula closed), sham fed (orally, cannula opened), or fed directly into the stomach via the cannula during 4 h. Insulinemia increased 10-fold over fasting levels in ad libitum– and intragastric-fed rats and threefold in sham-fed rats. Changes in adipose tissue LPL were proportional to the elevation in plasma insulin levels, demonstrating that the cephalic-mediated rise in insulinemia, in the absence of nutrient absorption, stimulates adipose LPL. These results demonstrate the central role of insulin in the postprandial response of tissue LPL, and they show that cephalically mediated insulin secretion is able to stimulate adipose LPL. *Diabetes* 48:452–459, 1999

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BAT, brown adipose tissue; LPL, lipoprotein lipase; NEFA, nonesterified fatty acid; STZ, streptozotocin; WAT, white adipose tissue.

Lipoprotein lipase (LPL) (EC 3.1.1.34) is a glycoprotein produced by several extrahepatic tissues, notably adipose tissue and muscle. Anchored at the luminal surface of capillary endothelial cells, the enzyme hydrolyzes the triglyceride component of circulating lipoproteins, releasing nonesterified fatty acids (NEFAs), which are then stored or oxidized by tissues. By virtue of its tissue-specific modulation (1), LPL takes part in the energy-partitioning processes, and the enzyme has been suggested as a candidate gene implicated in the etiology of obesity (2,3) and NIDDM (4). The activity of the enzyme is modulated by several endocrine factors in congruence with the state of energy balance of the organism. For instance, LPL activity is chronically elevated in adipose tissue (5–7) and low in skeletal muscle (8) of obese humans. On the other hand, overexpression of LPL in skeletal muscle was shown to prevent diet-induced obesity in mice (9).

In addition to long-term changes in basal LPL activity observed in conditions such as obesity and NIDDM, the enzyme is subject to acute modulation. Specifically, LPL is strongly influenced by the nutritional status. In the postprandial state, adipose tissue LPL is increased relative to fasting levels, whereas muscle LPL tends to be reduced (10). Alterations in these acute tissue-specific changes in LPL may impact upon the long-term distribution of lipid substrates among storage and oxidizing tissues and, therefore, influence the development of obesity.

Insulin is a major modulator of LPL. Studies in isolated and cultured adipocytes have determined that the hormone acutely increases LPL activity (11,12) and secretion (12,13), which is mostly attributable to positive effects of insulin on mRNA stability (14) and changes at the posttranslational level (15) but not due to an acute increase in LPL gene expression (14,16). In vivo, adipose tissue LPL is markedly decreased in streptozotocin (STZ)-induced diabetes (17,18), an effect that is reversed by insulin treatment (18,19), and it is increased during a euglycemic-hyperinsulinemic clamp in rats (20) and humans (21). The modulatory effects of insulin on LPL activity in muscle tissues are less well characterized. A short-term lowering of insulinemia, such as that after fasting (22) or physical exercise (23,24), is usually accompanied by an increase in muscle enzyme activity, whereas acute insulin administration to normal rats (25) and humans (21,26) has the opposite effect. Long-term (4 days) insulin depletion decreases LPL in both heart and skeletal muscle (19), suggesting that maintenance of normal muscle LPL requires the presence of the insulin-mediated modulation of protein turnover. Finally, previous studies have suggested

that skeletal muscle LPL activity may be more closely related to insulin action on glucose uptake (27) or lipid metabolism (28) than to insulinemia per se, and that the presence of other factors is needed for insulin to alter LPL in isolated cardiomyocytes (29). These studies suggest that, unlike adipose LPL, muscle LPL may be modulated by insulin through indirect pathways.

Given the nature of the tissue-specific modulation of LPL by food intake and insulin, it is likely that insulinemia participates in the response of LPL to changes in the nutritional status. However, factors other than insulin that are affected by food intake may conceivably influence LPL. Although the response of LPL to food intake as well as the acute and long-term modulation of the enzyme by insulin have been studied extensively, the quantitative contribution of insulin to the acute consequences of food intake on tissue LPL activity has not yet been evaluated systematically. The present studies were therefore designed to better characterize such a contribution. The first aim of the studies was to determine the time course of the tissue-specific response of LPL to acute food intake under well-defined feeding conditions and to relate enzyme activity to postprandial insulinemia. The second aim was to test the hypothesis that postprandial changes in LPL are dependent upon the insulin response to food intake. This was achieved by administering diazoxide, an inhibitor of insulin secretion, along with the food. Finally, since the onset of feeding elicits a very rapid preabsorptive (cephalic) phase of insulin secretion, which results from oropharyngeal and other stimuli (30–34), a third aim was to verify whether, in a feeding paradigm, the cephalic phase of insulin secretion could influence tissue LPL activity. To this end, rats were fitted with gastric cannulas and fed either orally, with or without nutrient absorption, or intragastrically to compare the consequences of cephalic- and nutrient-mediated insulin secretion on tissue LPL activity.

RESEARCH DESIGN AND METHODS

Study 1: postprandial time course of insulin and LPL. A total of 64 male Sprague-Dawley rats, initially weighing 225–250 g, were purchased from Charles River Laboratories (Saint-Constant, Canada) and housed individually in stainless steel cages in a room kept at 23 ± 1°C with a 12:12-h light-dark cycle (lights on at 2000). Animals were cared for and handled in conformance with the *Canadian Guide for the Care and Use of Laboratory Animals*, and the protocols were approved by our institutional animal care committee. Rats initially had ad libitum access to tap water and to a purified high-carbohydrate diet consisting of 65% energy as carbohydrate (equal proportions of cornstarch and dextrose), 14% as corn oil, and 21% as protein (casein). The high-carbohydrate diet was chosen to intensify postprandial insulin secretion. After the initial 1-week period of acclimation, rats were subjected for a total of 5 days to a meal intake protocol with the same diet, in which food was removed during the lighted period (2200) and a 30-min meal was provided 30 min after the onset of the dark period. Ad libitum access to food was restored 3 h after meal intake except on the day of the experiment. On that day, rats were divided into eight groups of eight rats each. Groups were killed by decapitation either before (fasted) or 30, 60, 90, 120, 180, 240, or 360 min after the beginning of the meal. To delineate the postprandial time course of tissue LPL and plasma insulin, blood, three white adipose tissue (WAT) depots (retroperitoneal, epididymal, inguinal), interscapular brown adipose tissue (BAT), and the soleus muscle were harvested.

Study 2: food intake with inhibition of insulin secretion on LPL. A pilot study was first conducted to evaluate the ability of diazoxide to inhibit insulin secretion (35) after a feeding protocol similar to that used in study 1 described above. A total of 12 lean *(Fa/?)* and 12 obese *(fa/fa)* Zucker rats, 5–6 weeks of age, were handled in the same conditions as in the time-course experiment (study 1). Rats were fitted with a permanent polyethylene cannula in the left jugular vein under isoflurane anesthesia, and they were allowed to recover from surgery for 3 days. Thereafter, after an overnight fast, rats were fed a high-carbohydrate meal for 30 min. Powdered diazoxide was mixed into the meal of half of the rats in order to provide a final concentration of 150 mg diazoxide/kg body wt. Diazoxide could not be given before the meal because the drug reduces subsequent food intake (data not shown). Plasma insulin was quantitated in serial blood samples taken through the venous catheter in the fasted state at the earliest time after meal intake (30 min) and 30 and 90 min later. For the main protocol, a cohort of 40 male Sprague-Dawley rats, initially weighing 225–250 g, were accustomed to the high-carbohydrate diet and to the meal intake protocol described in study 1. On the day of the experiment, rats were divided into five groups of seven rats each. Four groups were fed the 30-min high-carbohydrate meal, with (two groups) or without (two groups) diazoxide (150 mg/kg body wt), and they were compared with a fasted control group that was killed before the meal. Rats were killed by decapitation at 60 and 180 min after the beginning of the meal, according to the two postprandial peaks in adipose tissue LPL activity that have been observed in the time-course experiment.

Study 3: cephalic- versus nutrient-mediated insulin secretion on LPL. A total of 16 female obese *(fa/fa)* Zucker rats, aged 5–6 weeks (Canadian Breeding Laboratories, Saint-Constant, Canada), were housed individually under controlled temperature (23 ± 1°C) and lighting (lights off between 0300 and 1700), and they had ad libitum access to food (14.4 kJ/g pelleted stock diet, Charles River Rodent Animal Diet, distributed by Ralston Products, Woodstock, Canada) and tap water. Genetically obese Zucker rats were used because they exhibit a strong cephalic phase of insulin secretion (36). At 1 week after their arrival, rats were chronically implanted with a gastric cannula and allowed to recover for 1 week. During the last 4 days of the recovery period, rats were fed ad libitum the stock diet along with a high-energy (420 kJ/100 ml) high-carbohydrate liquid diet (Boost, Mead Johnson, Belleville, Canada) consisting of 15.57 g carbohydrate, 2.30 g fat, and 4.25 g protein per 100 ml. On the day of experiment, all rats were fasted for 8 h (from 0500), and four animals were killed after that period. The remaining rats were then refed for 4 h with the liquid diet alone under one of the following conditions: ad libitum oral feeding (gastric cannula closed), sham oral feeding (gastric cannula opened), or gastric feeding (via the gastric cannula). A 4-h feeding period was chosen to allow for the evaluation of cephalically mediated insulin secretion on maximal postprandial LPL activity (end of the 2- to 4-h plateau determined in study 1, taking into account a possible delay in adipose LPL activation in obese rats). Sham- and gastric-fed rats were pair-fed to the ad libitum group, which ingested ~40 ml of the liquid diet during the 4-h period. Sham- and gastric-fed rats were given the food with an infusion pump, which allowed a constant delivery rate to both groups. The liquid diet was drip-added to the drinking bottle of the sham-fed animals to avoid overconsumption of the liquid diet and to expose the animals to food for the same period of time as the other groups. At the end of the feeding period, rats were anesthetized with an intraperitoneal injection of 0.4 ml/100 g body wt of a mixture containing 20 mg/ml ketamine and 2.5 mg/ml xylazine, and blood and tissues were harvested immediately thereafter.

Implantation of gastric cannula. The technique for implanting the gastric cannula has been described in detail elsewhere (37). Before the surgery, rats were food deprived for 24 h to ensure a complete gastric emptying. Rats were anesthetized with an intraperitoneal injection of 0.4 ml/100 g body wt of the ketamine-xylazine solution mentioned above. A small incision was made in the abdomen starting 4–5 mm lateral to the mid-line and 3–4 mm caudal to the sternum. The stomach was exposed through the incision and gently pulled out of the abdominal cavity while being kept moist with isotonic saline. A purse-string suture, ~2 cm in diameter, was secured to the anterior part of the stomach without completely perforating its wall. An incision was made, and the stainless steel cannula was implanted in the anterior wall. The cannula was positioned with the flange inside the stomach, and a polypropylene mesh was used on the outside surface of the stomach. This knitted polypropylene mesh, by promoting growth of scar tissue, allowed a firm and leakproof seal around the cannula. The stomach was then returned to its normal position. Incisions of both abdominal muscles and skin were closed with interrupted sutures. The gastric cannula was plugged with a screw, which allowed for easy ad libitum, sham, or gastric feeding.

Plasma and tissue sampling. Immediately after death, blood was collected, centrifuged (1,500*g* for 15 min at 4°C), and the separated plasma was stored at –70°C until later biochemical measurements. In study 3, a piece of liver was also harvested, quickly frozen on dry ice, and stored at –70°C. Inguinal, epididymal (or parametrial), retroperitoneal WAT, interscapular BAT, and soleus and heart (in studies 2 and 3) muscles were excised, and ~50 mg from each tissue was homogenized with all-glass tissue grinders (Kontes, Vineland, NJ). Adipose tissue samples were homogenized in 1 ml of a solution containing 0.25 mol/l sucrose, 1 mmol/l EDTA, 10 mmol/l Tris-HCl, and 12 mmol/l deoxycholate, pH 7.4. Muscle samples were homogenized in 1 ml of a solution containing 1 mol/l ethylene glycol, 50 mmol/l Tris-HCl, 3 mmol/l deoxycholate, 10 IU/ml heparin, and 5% (vol/vol) aprotinin (Trasylol; Miles Pharmaceuticals, Rexdale, Canada), pH 7.4. Muscle homogenates were quickly frozen at –70°C. Adipose tissue homogenates were centrifuged (12,000*g* for 20 min at 4°C), and the fraction between the upper fat layer and the bottom sediment was removed, diluted with 4 vol of the homogenization solution without deoxycholate, and stored at –70°C until LPL activity measurement.

Plasma determinations. Plasma glucose concentrations were measured with a Beckman glucose analyzer (Fullerton, CA). Insulin was determined by radioimmunoassay using a reagent kit from Linco Research (St. Charles, MO) with rat insulin as standard. Plasma triglycerides were measured by an enzymatic method using a reagent kit from Boehringer Mannheim (Montréal, Canada) that allowed correction for free glycerol. Plasma NEFAs were also determined by an enzymatic colorimetric technique (Wako Pure Chemical Industries, Richmond, VA).

Tissue measurements. Frozen liver samples were thawed, and total lipids were extracted according to the method of Folch et al. (38) and solubilized in isopropanol. Triglycerides in the lipid extracts were then quantitated using the reagent kit mentioned above. For the measurement of LPL activity, 100 µl of thawed tissue homogenates were incubated under gentle agitation for 1 h at 28°C with 100 µl of a substrate mixture consisting of 0.2 mol/l Tris-HCl buffer, pH 8.6, which contained 10 MBq/l [*carboxyl*-^{14}C]triolein (Amersham, Oakville, Canada) and 2.52 mmol/l cold triolein emulsified in 50 g/l gum arabic, as well as 20 g/l fatty acid–free bovine serum albumin, 10% human serum as a source of apolipoprotein C-II, and either 0.2 or 2 mol/l NaCl. Free oleate released by LPL was then separated from intact triolein and mixed with Universol (Du Pont-NEN, Montréal, Canada), and sample radioactivity was determined in a scintillation counter. LPL activity was calculated by subtracting lipolytic activity determined in a final NaCl concentration of 1 mol/l (non-LPL activity) from total lipolytic activity measured in a final NaCl concentration of 0.1 mol/l. LPL activity was expressed as microunits (1 µU = 1 µmol NEFAs released per hour of incubation at 28°C). The interassay coefficient of variation was 3.2%, and it was determined using bovine skim milk as a standard source of LPL. Protein content of the tissue extracts was measured by the method of Lowry et al. (39). Data are expressed as specific activity of LPL (microunits per gram tissue protein).

Statistical analysis. Data are presented as means ± SE. Differences between individual group means were analyzed by Fisher's protected least squares difference test. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Study 1: postprandial time course of insulin and LPL. Rats voluntarily ingested ~5 g of the high-carbohydrate diet during the 30-min meal. The meal resulted in a significant increase in glycemia, which began 60 min after the onset of the meal (Fig. 1). Glycemia was then briefly reduced at 90 min, increased thereafter until 240 min, and had returned to fasting levels at 360 min. Insulin levels were elevated above fasting levels 30 min after the onset of the meal, remained high until 180 min, and had returned to baseline values at 360 min. Plasma NEFA concentrations decreased by half as early as 30 min after the onset of the meal, remained low until 240 min, and had returned to fasting levels at 360 min. Plasma triglycerides were increased (from 1.2 to up to 2.0 mmol/l) from 90 to 180 min after the beginning of the meal (data not shown).

The postprandial time course of LPL specific activity in three WAT depots, interscapular BAT, and soleus muscle is depicted in Fig. 2. In WAT, a first peak was measured as early as 60 min after the onset of the meal, and a second peak was observed between 120 and 240 min. At 90 min after the beginning of the meal, LPL specific activity was reduced to levels that were not significantly different from fasting values. Although LPL activity in all three WAT depots responded to meal ingestion in a similar pattern, the inguinal response was somewhat less marked compared with the other two studies reported below and did not reach statistical significance (P = 0.17 between times 0 and 60 min). In BAT, the first peak of LPL activity was delayed to 90 min, but the second peak remained at 180 min. After ingestion of the meal, LPL activity in soleus muscle decreased significantly by an average of 19% from 30 until 240 min, and it had returned to fasting values at 360 min.

Study 2: food intake with inhibition of insulin secretion on LPL. The findings of the pilot study aimed at verifying the efficacy of diazoxide to blunt the postprandial rise in insulinemia in lean and obese Zucker rats are depicted in Fig. 3. Insulinemia increased at least as early as 30 min after the onset of the meal in both lean (fourfold) and obese (2.5-fold) animals ($P < 0.05$ compared with fasting), regardless of the

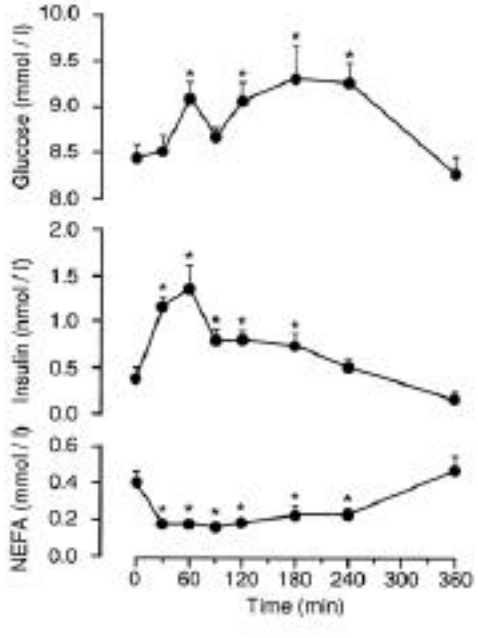

FIG. 1. Time course of plasma glucose, insulin, and NEFAs in rats fed a 30-min high-carbohydrate meal. The meal was started at 0 min after an overnight fast. Symbols represent means ± SE of eight animals. *$P < 0.05$, different from time 0.

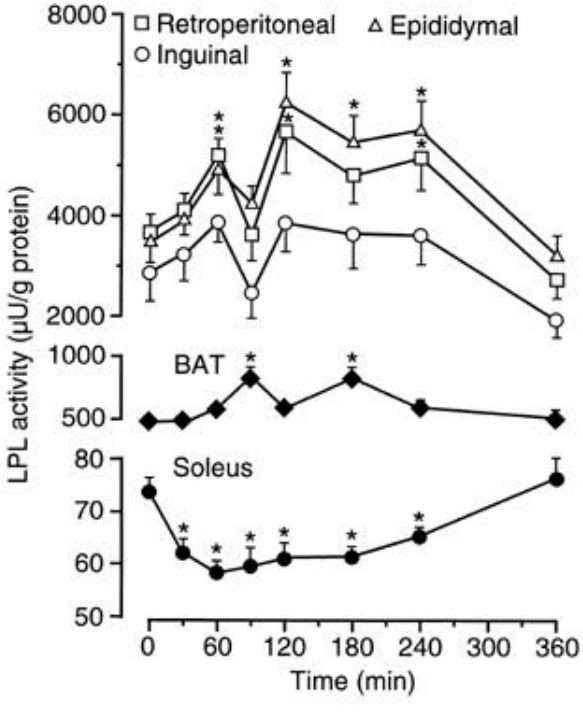

FIG. 2. Time course of LPL specific activity in three WAT (retroperitoneal, epididymal, inguinal), one BAT (interscapular), and soleus muscle in normal rats fed a 30-min high-carbohydrate meal. The meal was started at 0 min after an overnight fast. Symbols represent means ± SE of eight animals. *$P < 0.05$, different from time 0.

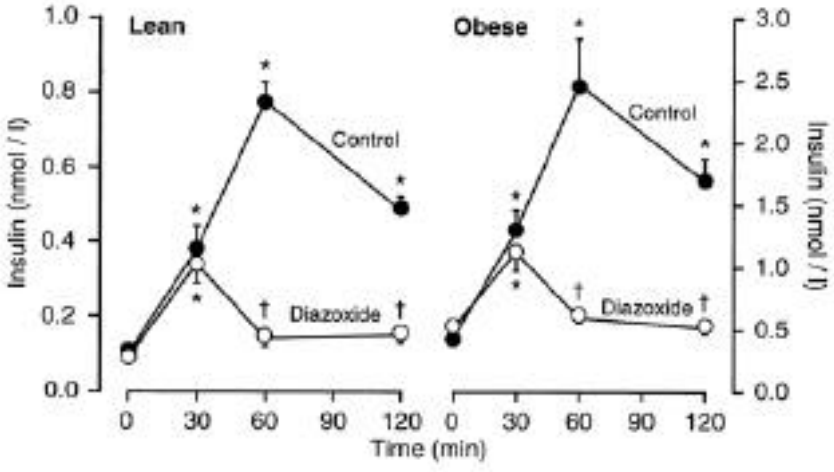

FIG. 3. Postprandial time course of insulinemia in lean *(Fa/?)* and obese *(fa/fa)* Zucker rats fed a 30-min high-carbohydrate meal, with (○) or without (●) diazoxide (150 mg/kg body wt) mixed with the meal. The meal was started at 0 min after an overnight fast. Symbols represent means ± SE of six animals. Note that the right scale (obese rats) is threefold that of the left scale (lean rats). *$P < 0.05$, different from time 0; †$P < 0.05$, different from group without diazoxide at the same time point.

presence or absence of diazoxide. In contrast, the presence of diazoxide in the meal resulted in a marked decrease in plasma insulin concentrations in both lean and obese rats, at 60 and 120 min after the onset of the meal, to levels that were no longer different from that measured in the fasted state.

As determined in the main diazoxide protocol aimed at quantitating the LPL response to a meal, rats ingested ~5 g of the high-carbohydrate diet, and no significant difference was observed between groups (Table 1). Confirming the observations of study 1, plasma glucose and insulin concentrations in the control group were increased 1 h after the onset of the meal and remained high at least for an additional 2 h. Inhibition of insulin secretion by diazoxide (postprandial insulinemia similar to fasting values) resulted in a marked elevation in glycemia at both time points. Plasma triglyceride levels were increased in both control and diazoxide-treated rats 3 h after the beginning of meal intake, whereas plasma NEFA concentrations were reduced 1 and 3 h after the meal only in the control group. Diazoxide blocked the basal insulin-mediated inhibition of lipolysis, and postprandial NEFA levels in treated rats were even higher than those in fasted animals.

As was the case in study 1, LPL specific activity in the retroperitoneal ($P = 0.03$), epididymal ($P = 0.005$), and inguinal ($P = 0.002$) WAT of control animals was significantly increased as early as 1 h after the onset of the meal (Fig. 4*A*) and was further elevated 2 h later ($P < 0.05$ compared with 1-h values for each depot). In contrast, LPL activity in diazoxide-treated rats was also increased 1 h after the meal in the three depots, but then it decreased 2 h later to levels that were not significantly different from those of fasted animals. In BAT, the first postprandial peak of LPL activity was not observed, probably because it was delayed to 90 min after the onset of the meal, as observed in study 1. However, a significant increase ($P = 0.001$) in LPL activity was measured 2 h later in the control group, whereas diazoxide blocked this elevation, and enzyme activity levels were similar to those of fasted animals.

In the control group, food intake resulted in a significant reduction in soleus muscle LPL activity at 1 ($P = 0.001$) and 3 ($P = 0.003$) h after the beginning of the meal (Fig. 4*B*). In the same group, cardiac LPL specific activity was decreased after 3 h only ($P = 0.01$). In all cases, diazoxide prevented the reduction in LPL activity.

Study 3: cephalic- versus nutrient-mediated insulin secretion on LPL. After a 4-h refeeding subsequent to an 8-h fasting, plasma glucose and triglycerides as well as liver triglyceride content were significantly higher than fasting levels in ad libitum–and intragastric-fed animals only (Table 2). At that time, plasma NEFA concentrations were three- to sixfold lower than fasting levels in these two groups. In sham-fed rats, no significant change in these variables occurred after feeding, confirming that the nutrients of the liquid diet did not reach the intestinal tract and were not absorbed. Whereas insulin levels increased 10-fold in ad libitum– and intragastric-fed rats, insulinemia in sham-fed animals was threefold higher than that of fasted rats at the end of the feeding period.

TABLE 1
Food intake during a 30-min high-carbohydrate meal with or without diazoxide (150 mg/kg body wt) and plasma variables measured before, 1 h after, and 3 h after the onset of the meal

		1 h after meal		3 h after meal	
	Fasting	Control	Diazoxide	Control	Diazoxide
Food intake (g)		5.3 ± 1.0	5.03 ± 0.6	6.2 ± 1.0	4.9 ± 0.3
Glucose (mmol/l)	8.1 ± 0.3	9.1 ± 0.2	15.4 ± 0.7*	9.8 ± 0.4†	13.2 ± 0.6*†
Insulin (nmol/l)	0.31 ± 0.04	0.95 ± 0.10†	0.22 ± 0.02*	0.68 ± 0.08†	0.25 ± 0.02*
NEFAs (mmol/l)	0.35 ± 0.07	0.10 ± 0.01†	0.60 ± 0.06*†	0.21 ± 0.05	0.84 ± 0.04*†
Triglycerides (mmol/l)	0.96 ± 0.15	1.14 ± 0.13	1.13 ± 0.12	1.77 ± 0.14†	1.77 ± 0.16†

Data are means ± SE of seven rats. *$P < 0.05$, different from control group at that time point; †$P < 0.05$, different from fasting.

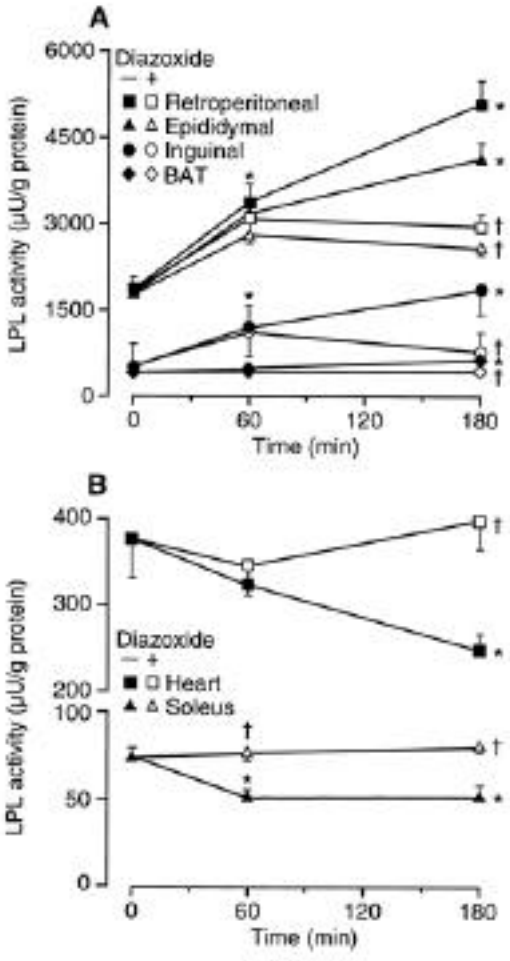

FIG. 4. Specific activity of adipose (*A*) and muscle (*B*) LPL in normal rats fed a 30-min high-carbohydrate meal, with (open symbols) or without (solid symbols) diazoxide (150 mg/kg body wt) mixed with the meal. The meal was started at 0 min after an overnight fast. Symbols represent means ± SE of seven animals. *$P < 0.05$, different from time 0, which is the case for all WAT depots in both groups at 60 min; †$P < 0.05$, different from group without diazoxide.

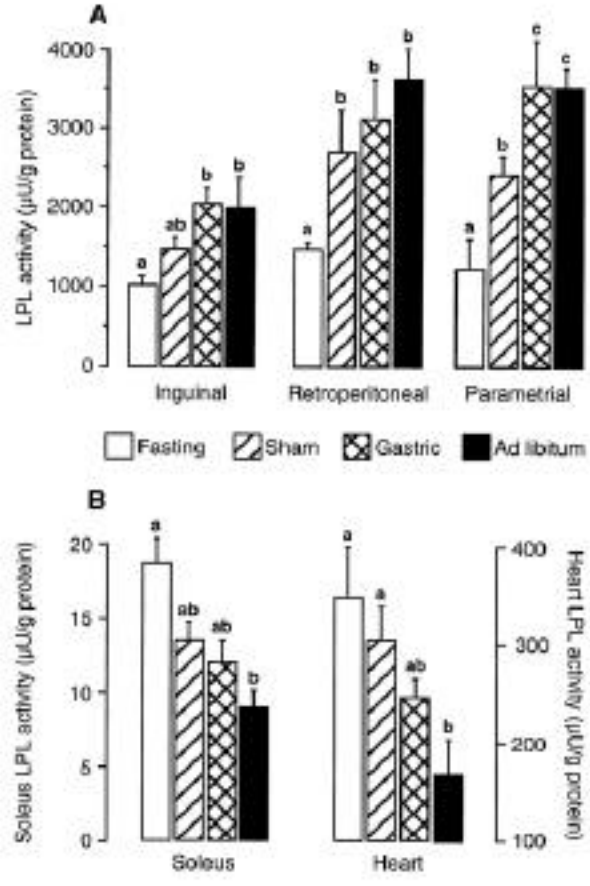

FIG. 5. Specific activity of adipose (*A*) and muscle (*B*) LPL in obese (*fa/fa*) Zucker rats in the fasted state or after 4 h of refeeding with a high-energy liquid diet in the following conditions: sham (orally, gastric cannula opened), intragastrically (into the stomach via the cannula), or ad libitum (orally, gastric cannula closed). Refeeding occurred after 8 h of fasting. Symbols represent means ± SE of four animals. Means not sharing a common letter designation are different from each other ($P < 0.05$).

Ad libitum and intragastric feeding of rats resulted in a marked increase in LPL activity ($P < 0.01$ in each of the three depots) in all WAT harvested (Fig. 5*A*). In sham-fed rats, LPL activity was higher in parametrial ($P = 0.04$) and retroperitoneal ($P = 0.05$) tissues compared with that of fasted animals, but parametrial LPL was slightly lower than that of the ad libitum– and intragastric-fed groups. Although changes in LPL activity in sham-fed rats did not reach significance in the inguinal depot ($P = 0.14$), a similar tendency was observed.

In skeletal and cardiac muscles, refeeding led to a decrease in LPL activity ($P < 0.01$ in both muscles; Fig. 5*B*) in the ad libitum group only. Rats fed intragastrically had lower LPL activity than fasted rats, but means of the two groups were not significantly different from each other ($P < 0.10$ in both muscles). Soleus and heart LPL activity in the sham-fed group remained unaltered.

DISCUSSION

The studies described herein were designed to characterize the contribution of insulin to the response of tissue LPL to acute food intake. The results show that acute food intake brings about a synchronized increase in LPL activity of several WAT depots and BAT as well as a decrease in muscle LPL, both of which quickly follow the postprandial increase in insulinemia. The studies also demonstrate that an increase in insulinemia is a necessary condition for the full expression of the tissue-specific response of LPL to acute food intake. The present findings further show that the centrally mediated secretion of insulin can be of sufficient magnitude to elicit an LPL response to food intake, and that this action is independent of nutrient absorption.

Meal intake was followed by a rapid increase in plasma insulin levels that reached a maximum at the earliest (30 min) investigated time point after the onset of food intake. The present protocol did not allow for blood sampling during the meal itself, since pilot studies have shown that the procedure disturbs the ingestive behavior of the animals. Previous studies using different feeding methods have, however, demonstrated that the increase in plasma insulin occurs at the very beginning of food ingestion (30,34). This early phase of insulin secretion, termed the cephalic phase, is independent from nutrient absorption and is elicited by olfactory, visual, gustatory, and oropharyngeal reflexes that act centrally to

TABLE 2
Plasma and tissue variables in Zucker rats after 8 h of fasting or after 4 h of sham, intragastric, or ad libitum refeeding with a high-energy liquid diet after 8 h of fasting

	Fasting	Refeeding		
		Sham	Intragastric	Ad libitum
Glucose (mmol/l)	8.5 ± 0.7*	8.8 ± 1.0*	14.9 ± 2.4†	11.5 ± 0.4*†
Insulin (nmol/l)	0.40 ± 0.07*	1.23 ± 0.22†	5.02 ± 0.94‡	4.04 ± 0.39‡
NEFAs (mmol/l)	0.68 ± 0.06*	0.51 ± 0.05†	0.15 ± 0.06‡	0.05 ± 0.05‡
Triglycerides (mmol/l)	1.73 ± 0.05*	1.23 ± 0.17†	2.35 ± 0.16‡	2.91 ± 0.09§
Liver triglycerides (mmol/g)	1.22 ± 0.22*	1.07 ± 0.34*	1.72 ± 0.27*	1.75 ± 0.31*

Data are means ± SE of four animals. Means not sharing the same symbol are significantly different from each other ($P < 0.05$).

stimulate insulin secretion via the vagus nerve (30). Because of the methodological constraints of the present feeding protocol, it was not possible in the first study to distinguish the cephalic phase of insulin secretion from that elicited by nutrient absorption, and the overall response of insulin to food intake appeared as a single prolonged peak that lasted from 30 to 240 min after the onset of the 30-min meal. As for nutrient absorption, glycemia displayed a two-phase increase after meal intake. After a short elevation between 30 and 60 min, which may correspond to an initial rapid absorptive phase, glucose decreased at 90 min, probably in response to the high levels of insulin present at that time. Subsequent stabilization of insulinemia at levels lower than the peak 60-min concentration allowed for a gradual increase in plasma glucose, which corresponded to the late absorptive phase. Finally, the strong antilipolytic action of insulin was reflected by a very rapid fall in plasma NEFA levels, which was maintained as long as insulin remained above fasting levels.

Determination of the time course of the acute adipose LPL response to food intake revealed two peaks of activity: the first 1 h (delayed to 1.5 h in BAT) and the other 3 h after the onset of the meal. Earlier studies also described increased LPL activity in rat epididymal fat depot (40) and isolated adipocytes (41) after 1 h of high-carbohydrate feeding and incubation with insulin, respectively. The slight delay between the insulin and the LPL response to food intake probably reflects the time needed for the hormone to activate LPL intracellularly. Studies conducted in rats (40,42) and humans (43) have shown that the elevation in adipose LPL activity after food intake is modulated by as yet poorly defined posttranslational mechanisms, which remain independent of transcriptional events (42,44) for at least several hours (40). This modulation probably involves the release of active LPL as well as the activation of an inactive pool of enzyme present in the fasted state (45). Similar mechanisms may operate in BAT, since refeeding and insulin increase LPL in this tissue (46). However, modulation of BAT LPL activity apparently involves pathways that require some changes in mRNA levels (46), possibly mediated by the adrenergic nervous system (47–49), which may explain the slightly longer delay for the appearance of the first peak in BAT LPL. The reason for the presence of two activity peaks in adipose LPL is unknown, but it may conceivably reflect a first activation of LPL by the early cephalic release of insulin, followed by a more prolonged activation by insulin secreted in response to nutrient absorption. Alternatively, the peaks may correspond to the early release of active LPL, followed by a later secretion of additional LPL after its activation. In contrast to adipose tissues, soleus LPL activity was decreased after food intake, confirming earlier findings (49,50). Muscle LPL did not display two activity waves as did WAT and BAT, suggesting distinct pathways for enzyme inactivation in muscle. It is, however, noteworthy that changes in LPL after the onset of the meal were quite rapid (within 1 h of onset of the meal) and remarkably well synchronized in all WAT depots as well as in muscle.

The study in which the inhibitor of insulin secretion diazoxide was given along with the meal demonstrates that insulin secretion is necessary for the full expression of the response of LPL to food intake. Diazoxide inhibits insulin secretion by opening ATP-dependent K^+ channels in β-cell membranes (35) and by lowering intracellular Ca^{2+} concentration. A previous study has shown that STZ-induced insulin withdrawal prevented the normal elevation in adipose LPL activity during the fasted-fed transition (18). However, STZ must be administered several days in advance and has effects other than the destruction of pancreatic islets of Langerhans (51). Therefore, the present study confirms in more physiological and acute conditions the critical role played by insulin in the response of adipose LPL to food intake, and it extends the concept to muscle tissues. In addition, the fact that plasma glucose and NEFAs were much higher in the diazoxide-treated rats than in the control animals, as was expected under low insulinemia, indicates that the absolute circulating levels of the substrates are not determinants of the LPL response to food intake in normal rats.

In WAT, LPL activity was increased 1 h after the onset of the meal whether diazoxide was present or not, but the further increase noted 3 h after the meal did not occur in the presence of diazoxide. In fact, LPL tended to return toward fasting values at that time point in diazoxide-treated animals. In preliminary experiments, it was shown that when the drug is administered at any time before the meal, subsequent food intake is significantly reduced. In addition, since the goal of the studies was to assess the contribution of insulin to the LPL response to voluntary food intake, gavage could obviously not be used. Thus, diazoxide had to be given mixed with the food. The procedure allowed for the fulfillment of the necessary condition of equal food intake in treated and untreated animals. However, it resulted in the maintenance of the cephalic phase of insulin secretion, which began within the first few minutes after meal presentation to rats (30) and thus before the intestinal absorption of the drug. As confirmed in the pilot study (Fig. 3), diazoxide was indeed unable to prevent the elevation in insulinemia measured 30 min after the onset of the meal. The lack of elevation in glycemia at that time (Fig. 1) strongly suggests the cephalic origin of this early increase in insulinemia. However, 1 h after the

onset of the meal, diazoxide had completely abolished insulin secretion and its hypoglycemic and antilipolytic actions, as shown in the main diazoxide study (Table 1). Taking into account the delay in the LPL response to food intake, it is therefore reasonable to suggest that the cephalic phase of insulin secretion, perhaps combined with the very beginning of the absorptive phase, was responsible for the increase in WAT LPL at that time point. This is consistent with a previous study in which acarbose, an intestinal glucosidase inhibitor that delays the absorption of glucose, when given along with a meal, lowered plasma insulin levels by 50% 1 h after meal intake but did not affect retroperitoneal adipose LPL activity compared with controls (52). Finally, it should be noted that the postprandial decrease in soleus and heart LPL activity was totally abolished by diazoxide. This finding demonstrates that the postprandial increase in insulinemia not only mediates the increase in adipose LPL but also is involved, either directly or indirectly through its action on muscle glucose (27) or fatty acid metabolism (28), in the response of muscle LPL to acute food intake. Lastly, it may be suggested that the maintenance of high postprandial LPL activity in muscle of diazoxide-treated rats may have compensated for its decreased activity in WAT and BAT, resulting in the maintenance of the global intravascular capacity to hydrolyze triglycerides, the levels of which remained unaffected by diazoxide treatment.

Earlier studies of the cephalic phase of insulin secretion in rats have used feeding stimuli of very short duration (30,34) and reported an insulin response lasting for a few minutes. In the present study, it was established that sham feeding of obese Zucker rats at a low rate to match the feeding pattern of ad libitum–fed animals resulted in an increase in insulinemia that lasted for at least 4 h. At the end of this period, insulinemia was threefold higher in sham-fed than in fasted rats but threefold lower in sham-fed than in ad libitum–fed rats. Although the experimental protocol did not allow for repeated blood sampling, the possibility that insulinemia may have been higher at the beginning than at the end of the sham-feeding period cannot be dismissed. In any case, sham feeding for several hours effectively resulted in a sustained insulin secretion in the obese Zucker rats. The latter are particularly sensitive to food deprivation (53), and whether a response comparable in magnitude and duration would be found in lean animals remains to be determined experimentally.

The observation of a significant stimulation of LPL activity in adipose tissue in animals fed orally with an open gastric cannula (sham-fed animals) constitutes the first report of an association between adipose LPL and the cephalic phase of insulin secretion. Because nutrient-related variables were not increased in sham-fed rats, confirming the effective prevention of nutrient absorption, the threefold elevation in plasma insulin levels observed 4 h into the feeding period could not have been elicited by nutrients. The findings rather indicate that ingestion of the diet induced a prolonged insulin response of cephalic origin, which led to an elevation in adipose LPL activity. It could be argued that the increase in adipose LPL observed in sham-fed rats might have been elicited by factors, other than insulin itself, that would have been altered as part of the reflex response to the initiation of food intake. However, the study with diazoxide, a compound that acts specifically on insulin secretion, strongly suggests that centrally mediated insulin secretion was indeed responsible for the modulation of tissue LPL in sham-fed animals. The roughly proportional response of LPL to insulinemia under the various means of feeding further underlines the key role played by insulin in the tissue-specific postprandial modulation of LPL. The 4 h of intragastric feeding resulted in an insulinemia comparable to that elicited by ad libitum feeding, and LPL activity in all tissues was comparable in ad libitum– and gastric-fed animals, whereas enzyme activity tended to be less stimulated by the smaller increase in insulinemia achieved in the sham-fed animals at that time. In fact, despite comparable trends, the latter appeared to be too weak to significantly increase LPL in the inguinal adipose depot or to decrease enzyme activity in muscle tissues. This may reflect a tissue-specific degree of sensitivity of LPL to insulin. Note also that the responsiveness of LPL to insulin might be affected by the state of resistance of glucose metabolism to the action of insulin that characterizes the Zucker rat (54). This is suggested by several human studies that have reported a shift in the dose-response curve of adipose LPL to insulin in massively obese subjects (7), a divergent response of muscle LPL to insulin infusion in normal and insulin-resistant subjects (21,26,55), and a closer relationship of muscle LPL with muscle glucose uptake than with insulinemia per se (8,27).

In summary, the present studies have demonstrated the key role of insulin in the response of adipose tissue and muscle LPL to food intake. Diazoxide administration and consequent inhibition of insulin secretion prevented the increase in LPL activity in all adipose depots studied, whereas a feeding-induced centrally mediated elevation in insulinemia without any nutrient absorption increased adipose tissue LPL activity. The feeding-induced decrease in muscle LPL was also abolished by inhibition of insulin secretion in normal rats, but cephalic-phase insulin release did not greatly alter muscle LPL in obese rats as it did in their adipose tissues, likely because of the insufficient magnitude of the insulin response. The present findings indicate that the postprandial increase in insulinemia is a requisite for a proper response of tissue LPL to food intake and may be a condition sufficient for such a response.

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