Lipoprotein lipase regulation in the cyclophosphamide-treated rabbit: dependence on nutritional status

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Abstract Cyclophosphamide injection into the fasted rabbit induces a hypertriglyceridemia (4.6 mM vs. 0.8 mM in controls) and a defect of lipoprotein lipase (LPL), as measured in postheparin plasma (PHP). In contrast, administration of the drug into fed animals tends to increase PHP-LPL. The effects of cyclophosphamide on LPL activity and synthesis, depending on the nutritional state, were thus studied in two sites: periepididymal adipose tissue and heart. In adipose tissue, fasting decreased LPL activity to 45.2 mIU/g ($P < 0.001$) compared to 667.9 mIU/g in fed animals. PHP-LPL activity was also decreased by 45% upon starvation. These modulations appeared to be related to plasma insulin levels. The relative rate of synthesis of fat tissue LPL was decreased from 0.32% total protein synthesis in fed animals to 0.10% in fasted rabbits, concordant with a reduction in the expression of LPL specific mRNA. Cyclophosphamide administration to the fed rabbit led to decreases of LPL activity and synthesis in the adipose tissue, similar to those observed upon starvation. However, when injected into fasted animals, the drug did not further depress fat tissue LPL. Fasting did not change heart LPL activity (288.3 mIU/g vs. 239.3 in fed animals) nor its relative rate of synthesis (0.21% of total protein synthesis). However, cyclophosphamide induced opposite effects, depending on the nutritional state: after injection into fed animals, heart LPL activity increased up to 477.2 mIU/g ($P < 0.01$) with a concomitant increase in the LPL synthesis rate. Conversely, drug administration into fasted rabbits led to a decrease of heart LPL activity to 133.9 mIU/g. Similar qualitative variations were recorded in postheparin plasma. Hence, although insensitive to nutritional modulations, heart LPL responded differently to cyclophosphamide, depending on the nutritional state. In spite of those different modulations of heart and adipose tissue LPL, the enzyme isolated from these two sources displayed similar molecular mass, immunoreactivity, and catalytic properties. The effects of cyclophosphamide injection on very low density lipoprotein (VLDL)-triacylglycerol (TG) synthesis were also investigated, as a possible determinant of hypertriglyceridemia. The drug stimulated TG synthesis in both nutritional states, and maximally by 45% in fed animals. Hence, a defect of heart and postheparin plasma LPL appears as a major determinant of hypertriglyceridemia in cyclophosphamide-treated fasted rabbits. The opposite variations of heart LPL towards cyclophosphamide suggest that the drug may unveil some effects of insulin on heart LPL.

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Supplementary key words heart LPL • adipose tissue LPL • insulin • postheparin plasma

Lipoprotein lipase (LPL) plays a major role in the hydrolysis of triacylglycerol in VLDL and chylomicrons, thus providing free fatty acids for energy metabolism or storage (1, 2). The enzyme is present in a variety of tissue, notably in adipose tissue, heart, and skeletal muscle (3–5), where it has been detected both in terms of activity and as immunoreactive protein (5, 6). Isolation of the LPL cDNA has provided the primary sequence of the enzyme and of its functional domains in various species, and has enabled investigators to follow its synthesis, mostly in adipose tissue (7–10), heart, adrenals, and in the mammary gland (10–12).

Lipoprotein lipase activity and synthesis are under hormonal control. Insulin can induce LPL activity and synthesis of LPL mRNA in cultured adipocytes (13, 14), and this may explain the nutritional modulations of adipose tissue LPL observed in humans (15), guinea pigs, or rats (16). By contrast, conflicting observations have been reported concerning the modulation of heart LPL by in-

Abbreviations: A.U., arbitrary units; BSA, bovine serum albumin; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propane-sulfonate dihydrate; EDTA, [ethylene-bis(oxyethylenenitrilo)]-tetraacetic acid; FFA, free fatty acids; IDL, intermediate density lipoprotein; IgG, immunoglobulin G; LPL, lipoprotein lipase; PAGE, polyacrylamide gel electrophoresis; PHP, postheparin plasma; mRNA, messenger RNA; SDS, sodium dodecyl sulfate; SE, standard error; TG, triacylglycerol; TNF, tumor necrosis factor; VLDL, very low density lipoprotein.

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sulin, depending on the animal of investigation (16). Further differences between heart and adipose LPL come from the observation that, in the rat, the two enzyme sources display different kinetic parameters and molecular mass (17). Little is known, so far, about LPL regulation in the rabbit, an animal model widely used in lipoprotein metabolism.

Massive hypertriglyceridemia is observed in animals developing severe infectious or invasive processes (18). Various mediators secreted by the activated macrophages appear to be involved in this process, such as interferon, tumor necrosis factor (TNF), and interleukins (19). Experimentally, injection of TNF or endotoxin treatment can induce a defect in LPL activity and synthesis, either in the adipose tissue or in the heart, depending on the animal model studied (5, 20–22). These effects were reproduced in vitro on preadipocytes (23), and in mesenchymal heart cells (24).

We have previously reported that irradiation or antimitotic administration to the rabbit leads to an accumulation of large, triacylglycerol-rich VLDL and to a defect in LPL activity, as measured in postheparin plasma (25–27). The abnormal VLDL also contain new apolipoprotein determinants of low molecular mass called apolipoprotein X (25–27). In spite of these structural peculiarities, those abnormal VLDL undergo the normal catabolic cascade in the presence of added LPL (27). This prompted us to focus on the LPL defect itself. Because cyclophosphamide has to be cleaved in the liver to release active antimetabolites, only an in vivo investigation could be emphasized. Furthermore, as the hypertriglyceridemia induced by cyclophosphamide was observed only in fasted animals, we suspected that the drug could interfere with the nutritional modulations of LPL or exert different effects, depending on the levels of insulin.

In the present study, we have investigated the effects of cyclophosphamide on LPL activity and synthesis in the rabbit major productor sites, heart and adipose tissue, and on its expression in the vascular compartment. This was done in both fed and fasted animals. Moreover, the effects of the drug on VLDL secretion were followed, as another possible determinant of antimitotic-induced hyperlipemia.

MATERIALS AND METHODS

Materials

Cyclophosphamide, Endoxan Asta 500, Paris, France, was kindly provided by Professeur M. Carton, Centre Claudius Régaud, Toulouse, France. Heparin was from Roche, Basel, Switzerland and heparin-Sepharose CL-6B was from Pharmacia-LKB, Bromma, Sweden. The scintillation cocktails, Aqualuma and Lumasolv, were from Packard. The following compounds were purchased from Sigma, St. Louis, MO: alkaline phosphatase-conjugated anti-chicken IgG, bovine serum albumin (essentially fatty acid-free), CHAPS, lysophosphatidylcholine from egg yolk, leupeptin, pepstatin, trasylol, and trioleoylglycerol. The Hank's medium and the antibiotics, penicillin and streptomycin, came from Seromed, Munich, Germany. The methionine-free EMEM medium was obtained from Flow Laboratories, Les Ulis, France. Glycerol tri[9-10(n)-^{3}H]oleate (18.5–37 GBq/mmol, 2–10 Ci/mmol), and deoxy-cytidine-tri-[5'-α-^{32}P]phosphate (110 TBq/mmol, 3000 Ci/mmol) were from the Radiochemical Center, Amersham, United Kingdom. L-[^{35}S]methionine was obtained from NEN-DuPont de Nemours, France.

Animals and treatments

Male New Zealand rabbits weighing between 2.5 and 3.0 kg received a control diet (Usine d'Alimentation Rationnelle, Epinay-sur-Orge, France). Fasted animals were starved 40 h before being killed. Cyclophosphamide-treated rabbits received a single dose, representing 1/2 of the half lethal dose (65 mg/kg) of Endoxan ASTA 500, into an ear marginal vein. The lethal dose has been determined by Brock (28). When fasted rabbits were treated, the drug injection was performed after 24 h fasting. No additional food was given to the animals, and they were killed 16 h thereafter. In order to estimate the secretion rate of triglyceride-rich lipoproteins, animals were injected intravenously with Triton WR-1339 4 h before the end of the experiment; this detergent blocks the catabolism of these lipoproteins (29). Blood samples were taken before injection and again after 2 and 4 h, and animals were killed thereafter. Triacylglycerols were measured in all plasma samples. Eventually, triglyceride-rich lipoproteins were separated as the $d < 1.006$ g/ml ultracentrifugal plasma fraction and the triacylglycerol measurements were performed in this fraction. Differences in the measurements before and after Triton WR-1339 administration were normalized as secretion rate per ml and per hour.

Tissue homogenates

Animals were killed by exsanguination and pieces of tissue were obtained from following sites: periepididymal fat pads, heart, and eventually from diaphragm, leg muscle, lung, and liver. The tissues were weighed and homogenized in 9 volumes of ice-cold 0.025 M ammonium buffer, pH 8.2, containing heparin (5 IU/ml), leupeptin (10 μg/ml), pepstatin (1 μg/ml), Trasylol (25 KIE/ml), EDTA (5 mM) and CHAPS (4 mM).

The samples were centrifuged for 10 min at 3,000 *g* and 20 μl of the clear phase containing the solubilized enzyme was assayed for LPL activity (16). Postheparin blood was collected onto 2.5 mM EDTA from the ear vein of other rabbits 10 min after injection of 175 IU heparin per kg body weight. Postheparin plasma was obtained by a 15-min centrifugation at 1,000 *g*. Preheparin plasma was also sampled.

Measurement of LPL activity

The samples (20–100 μl) were incubated 20 min at 37°C with 100 μl substrate (2.67 mM triacylglycerol). LPL was assayed according to Nilsson-Ehle and Eckman (30) using a sonicated [^{3}H]trioleoylglycerol emulsion stabilized with lysophosphatidylcholine, as a substrate, and heat-inactivated rabbit serum, as a source of apoC-II. Incubations were carried out in the presence of BSA 2% (w/v). When LPL was assayed in postheparin plasma, duplicate measurements were run in the presence of a specific anti-LPL IgG, and results were corrected accordingly. In the case of tissue homogenates, the antibody inhibited more than 90% of the measured lipase activity, and a comparable inhibition was found in the absence of apoC-II. Free fatty acids were extracted according to Belfrage and Vaughan (31). One mU is defined as one nanomole of free fatty acid released per min.

Labeling with [^{35}S]methionine and LPL immunoprecipitation

Periepidydimal fat pad pieces, 2 g, were incubated for 90 min at 37°C with [^{35}S]methionine, 40 μCi/ml, in 4 ml methionine-free EMEM, containing 1.25 mM HEPES, 1 mM glutamine, and 4% (w/v) BSA. When the tissue was taken from normally fed animals, the labeling was performed in presence of 10^{-7} M insulin. The radiolabeled medium was then removed, and the tissue was homogenized in 4 ml 0.025 M ammonium buffer, pH 8.2, containing heparin (5 IU/ml), leupeptin (10 μg/ml), pepstatin (1 μg/ml), Trasylol (25 KIE/ml), EDTA (5 mM), Triton X-100 (0.8%, v/v), and SDS (0.04%, w/v).

In order to label heart LPL, the heart was pulse-labeled during a recycling perfusion of 20 min with 50–80 μCi of [^{35}S]methionine per ml of methionine-free EMEM supplemented as above. The organ was then perfused with an EMEM chase-medium containing 1.42 mg/ml methionine for another 20 min. Finally, this medium was renewed and heparin, 5 IU/ml, was added for an additional recycling perfusion of 30 min. The heart was then dissected and pieces (1–2 g) were homogenized as described above for adipose tissue.

Tissue homogenates were centrifuged 20 min at 1,500 *g* and the supernatant was used for LPL immunoprecipitation. Radiolabeled proteins were quantified by trichloroacetic acid (TCA) precipitation. Briefly, 10 μl sample was spotted onto a Whatman GF/C filter. The filter was then incubated alternatively at 4°C, then at 100°C, and again at 4°C in a 10% (w/v) TCA solution. After washing with methanol and acetone, the filter was counted for radioactivity in 4 ml of a scintillation mixture, made of Aqualuma–Lumasolve–water 80:18:2. Under these conditions, between 5 and 13% of the initial radioactivity was recovered in tissue proteins.

For the immunoprecipitation of tissue LPL, the supernatant obtained after centrifugation of the homogentate was brought to an SDS concentration of 1% (w/v) and heated at 100°C for 5 min. Aliquots corresponding to 10^6 dpm were diluted to 4 ml with 0.02 M sodium phosphate, pH 7.4, containing 0.15 M NaCl, 0.02 M EDTA, 10% Triton X-100 (v/v), 0.1% SDS (w/v), 0.1 mM PMSF, and 0.73 mM NaN_3. Incubation with 4 μl anti-LPL IgG was carried out at 4°C for 18 h. Preliminary experiments with variable concentrations of antibody had shown that immunoprecipitation was maximal under those conditions. This anti-LPL antibody, kindly provided by Prof. G. Bengtsson-Olivecrona, was raised in chicken against bovine milk LPL (32), and was shown to react with the rabbit enzyme. IgG were purified from egg yolk. A second incubation was performed for 2 h at room temperature with goat anti-chicken IgG coupled to Sepharose beads. The complex formed was then precipitated by 1,500 *g* centrifugation, and washed three times in the incubation medium. The immunoprecipitate was then electrophoresed on 12% (w/v) polyacrylamide/ 0.2% (w/v) SDS. Radiolabeled LPL was located by autoradiography on a Kodak XAR-5n X-ray film and was quantified by cutting the gel and counting, using the same scintillation cocktail as above. Negative controls of immunoprecipitation were performed using a nonimmune chicken IgG.

RNA analysis and quantification: the cDNA probe

The cDNA coding for human lipoprotein lipase was obtained from Genentech (San Francisco, CA), and was carried out in the Department of Biophysics and Biochemistry (Prof. T. Olivecrona), University of Umea, Sweden. The cDNA spanned from nucleotide 1 to nucleotide 2413, including the sequence coding for the 448 amino acid LPL and a 3' flanking region. The cDNA was provided as an insert in the *E. coli* pUC 18 plasmid. The liberated probe was labeled with [^{32}P]dCTP by the random primed labeling technique using a commercial kit (Boehringer-Mannheim, Germany).

Total RNA was extracted from adipose tissue and heart following the method of Chirgwinn et al. (33). Briefly, the tissues were homogenized in 4 M guanidium thiocyanate and were layered onto a 5.7 M CsCl cushion. RNA was pelleted by ultracentrifugation at 94,000 *g* for 18 h, and denatured in glyoxal for 1 h at 50°C.

For Northern blot analysis, 20 μg total RNA was separated by electrophoresis on agarose 1% (w/v), in 0.01 M sodium phosphate buffer, pH 7.0, and then transferred onto nylon membranes.

For slot-blot quantification, serial dilutions of denatured RNA (between 10 and 0.612 μg) were spotted onto nitrocellulose filters, using a Bio-Rad dot-blot apparatus.

Prehybridization was performed for 18 h at 42°C in 50% (w/v) formamide. Hybridization was performed under similar conditions using 2×10^6 dpm/ml ^{32}P-labeled cDNA probe. Positive hybridization was revealed by autoradiography, and relative intensity of the spots was assessed by optical integration.

Partial purification of LPL, kinetic studies, and enzyme stability

Postheparin plasma or tissue homogenates obtained as described above from fed rabbits were diluted 2:1 with 0.2 M Tris-HCl, pH 7.4, 0.65 M NaCl, in presence of 20% glycerol (v/v) and Trasylol (25 K.I.U./ml). The mixture was incubated for 2 h at 4°C with heparin-Sepharose. The gel was then washed with buffers of increasing molarity and LPL was eluted at 1.5 M NaCl in presence of 20% glycerol (v/v). The enzyme was stabilized by addition of BSA 4% (w/v) and heparin (5 IU/ml). Heart perfusates containing heparin were used as another source of enzyme and treated identically.

In kinetic studies, increasing concentrations of [^{3}H]TG-labeled VLDL were incubated in Tris-HCl buffer, 0.2 M, pH 8.2, containing BSA 4% (w/v), and with or without an LPL preparation (12.5 mU/ml). Incubations were carried out at 37°C for 30 min and lipids were extracted.

To follow the enzyme stability, LPL preparations were kept at 37°C for 0–180 min in a medium containing heparin (5 IU/ml) and BSA 4% (w/v).

Immunoinhibition and immunoblotting

The IgG antibody was kindly provided by T. Olivecrona (Umea, Sweden). It was raised in chicken against LPL purified from guinea pig milk (32). This IgG cross-reacted with the partially purified LPL from rabbit postheparin plasma in immunoblot assays. Half inhibition of the LPL activity in postheparin plasma occurred at a 1:64 dilution.

To follow the immunoinhibition of the enzymatic activity, 50 μl of LPL source was preincubated with 50 μl of serial dilutions of IgG for 2 h at 4°C. Then 50-μl duplicate samples of this mixture were assayed for LPL activity, as above.

To determine the enzyme molecular mass, partially purified enzyme from different sources was run on SDS/PAGE (0.01%/12.5%). Proteins were transferred to nitrocellulose and LPL was revealed using the specific IgG against LPL. An anti-chicken IgG from goat, conjugated to alkaline phosphatase, was used as a second antibody.

Analytical procedures

Cholesterol was measured with the cholesterol esterase/cholesterol oxidase technique (34) using commercial kits (Boehringer, Mannheim, Germany). Triacylglycerols were assayed enzymatically (35) in an automatic analyzer (Boehringer, as above). Proteins were estimated according to Lowry et al. (36) using BSA as a standard. ApoB-100 was determined by immunoelectrodiffusion with an anti-human apoB antibody, cross-reacting with rabbit apoB. Insulin was measured using a specific radioimmunoassay (CIS, Paris, France). Creatine kinase and lactate dehydrogenase were measured using routine standard methods (37, 38). Tumor necrosis factor (TNF) was measured in rabbit plasma by an immunoradiometric assay using antibodies raised against human recombinant TNF (Medgenix, Brussels, Belgium). Cross-reactivity with rabbit TNF was indicated by the measurement of substantial immunoreactive material in the media recovered from rabbit cultured (monocyte-derived) macrophages.

VLDL or heart lipids were extracted by the Bligh and Dyer procedure (39) after acidification with 0.014 ml formic acid per ml medium. Neutral lipids were separated by thin-layer chromatography using petroleum ether–diethyl ether–acetic acid 165:35:2 (v/v/v). Radioactivity was measured in a Packard Tricarb apparatus (Zurich, Switzerland) with automatic quenching correction. Data were expressed as means ± SE. Statistical significance was assessed by the Student's t test for unpaired samples.

RESULTS

Lipids, lipoprotein lipase, insulin, and TNF levels in rabbit plasma

Occurrence of hypertriglyceridemia in the rabbit upon antimitotic treatment has already been reported in our laboratory. To better define the conditions under which the animals develop the increase in triacylglycerol, different protocols were checked (**Table 1**). The effects of cyclophosphamide treatment were studied in normally fed or in 40-h fasted animals. The levels of triacylglycerol and cholesterol were not significantly changed after a 40-h fast, although a 45% decrease of LPL activity was noted in postheparin plasma. Those nutritional modulations of LPL activity appear correlated with the insulin variations (13.6 vs. 0.42, $P < 0.01$, Table 1), and this hormone is known to regulate LPL synthesis. Indeed, in control animals, a linear relationship ($r = 0.85$, $P < 0.01$) was observed between the levels of (preheparin) plasma insulin and the lipoprotein lipase activity measured in postheparin plasma (**Fig. 1**).

Injection of cyclophosphamide to fasted animals led to a massive accumulation of plasma triacylglycerol (4.6 mmol/l) while LPL activity was found to be further depressed (24.4 vs. 51.2, $P < 0.05$). An inverse linear relationship ($r = 0.64$, $P < 0.05$) was observed between the levels of plasma TG and the LPL activity in postheparin plasma of the fasted rabbits (data not shown). The hypertriglyceridemia was associated with a slight increase in plasma cholesterol, which might reflect the impaired catabolism of cholesterol-enriched VLDL.

When cyclophosphamide was administered to normally fed rabbits, no hypertriglyceridemia occurred, yet a slight increase in plasma cholesterol was again observed. LPL activity in postheparin plasma was somewhat increased, although not significantly, because of large interexperiment variations (Table 1).

Measurements of TNF in rabbit plasma gave the following average values: 8.6 ± 1.6 pg/ml in control fed rab-

TABLE 1. Lipid, insulin levels, and lipoprotein lipase activity in rabbit plasma

Variable	Fed		Fasted	
	Control	Treated	Control	Treated
Triacylglycerol (mM)	0.91 ± 0.07	0.96 ± 0.11	0.82 ± 0.07	4.56 ± 0.93[b]
Cholesterol (mM)	1.20 ± 0.06	1.69 ± 0.18[a]	1.54 ± 0.20	2.20 ± 0.19[a]
Insulin (μU/ml)	13.60 ± 2.70[d]	9.10 ± 3.82	0.42 ± 0.40	1.05 ± 0.61
LPL activity in PHP (mU/ml)	93.7 ± 15.4[c]	146.5 ± 30.2	51.2 ± 6.2	24.4 ± 8.8[a]

Animals were either fed ad libitum or fasted for 40 h. Cyclophosphamide-treated rabbits received a single dose of the drug (65 mg/kg intravenously) 16 h before blood samples were taken. Blood was collected in EDTA (0.25 mM) and plasma was assayed for triacylglycerol, cholesterol, and insulin content. Animals were then injected intravenously with heparin (175 IU/kg) and postheparin plasma (PHP) was obtained 8 min thereafter to measure LPL activity. Statistical significance was assessed by Student's t test for unpaired samples (11–16 measurements).
[a], $P < 0.05$; [b], $P < 0.01$: treated versus control.
[c], $P < 0.05$; [d], $P < 0.01$: fasted control versus fed control.

bits and 9.0 ± 1.0 pg/ml in fed and cyclophosphamide-treated animals. In the fasted state, the values were 14.7 ± 3.3 pg/ml in control animals and 14.6 ± 4.0 pg/ml after treatment. The differences among the four groups were not significant, and all values were in the low range of the assay. Hence, cyclophosphamide administration was not associated with a higher level of TNF in plasma.

Lipoprotein lipase activity in adipose tissue and heart: nutritional and cyclophosphamide-induced modulations

LPL activity was assayed in the main production sites after enzyme solubilization and stabilization in presence of heparin plus detergents (**Table 2**). In normally fed control rabbits, the highest levels of LPL activity were found in adipose tissue (668 ± 66 mU/g) and then in heart (239 ± 41 mU/g), compared to other tissues such as lung (190 ± 45 mU/g), liver (24.7 ± 17.7 mU/g), diaphragm (133 ± 42 mU/g), or leg quadriceps (42.5 ± 5.7 mU/g) (data not shown). Adipose tissue LPL was reduced more than 90% in fasted rabbits compared to their fed counterparts ($P < 0.001$, Table 2). By contrast, the activity in heart remained constant. Thus, upon fasting, it was no longer the adipose tissue but the heart that displayed the highest level of LPL activity per gram.

The effects of cyclophosphamide treatment on the level of LPL activity in the two major production sites were found to be dependent on the nutritional status. In fed animals, antimitotic administration led to a 75% reduction in fat pad LPL, whereas heart LPL was doubled ($P < 0.01$). By contrast, when cyclophosphamide was administered to 40-h fasted animals, heart LPL activity declined by 55%, whereas adipose tissue LPL, already at a low level upon fasting, was not further depressed. The occurrence of hypertriglyceridemia under these conditions suggests that clearance of plasma triacylglycerol in fasted animals may depend on the levels of heart LPL activity. Indeed, an inverse exponential relationship ($r = 0.69$, $P < 0.01$) was observed between LPL activity in heart homogenates and triglyceridemia as measured in fasted rabbits (**Fig. 2**). All the variations in tissue LPL activity were reproducibly observed and reached statistical significance (Table 2).

Lipoprotein lipase synthesis in rabbit heart and adipose tissue

In order to investigate whether the changes in LPL activity, among the various conditions tested, reflected modulations in the rate of synthesis of the enzymatic protein, the incorporation of [^{35}S]methionine into immunoprecipitable LPL was followed in both adipose tissue and heart. In the first case, 1–2 g fat pad pieces were incubated in presence of 4.8–8.8 × 10^8 dpm radiolabeled methionine per g. For heart LPL, labeling was achieved through pulse/chase experiments during a perfusion, using 2.2–3.1 × 10^8 dpm of [^{35}S]methionine per g of organ, and

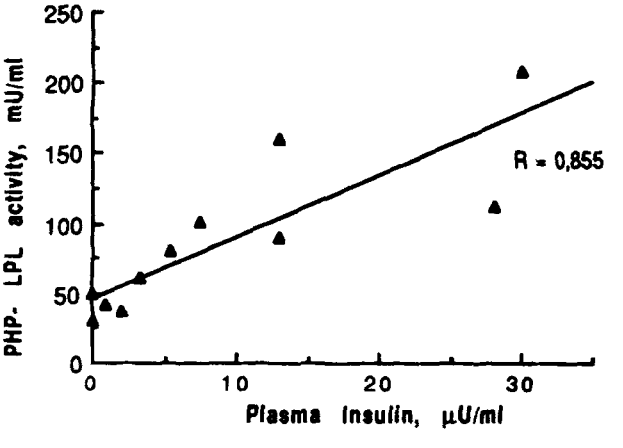

Fig. 1. Correlation between plasma insulin and lipoprotein lipase activity measured in postheparin plasma. LPL activity was measured in PHP from control rabbits, either fed normally or fasted for 40 h. Blood was taken 8 min after heparin injection. Insulin levels were measured with a specific radioimmunoassay in the preheparin plasma. Values are from 11 individual experiments. The linear regression factor between both parameters was 0.855 ($P < 0.01$).

TABLE 2. Lipoprotein lipase activity in rabbit adipose tissue and heart

Tissue	Fed Rabbits		Fasted Rabbits	
	Untreated	Treated	Untreated	Treated
	mU/g tissue			
Adipose tissue	667.9 ± 66.0	146.8 ± 17.6[a]	45.2 ± 9.5[a]	48.9 ± 15.0
Heart	239.3 ± 41.1	477.2 ± 48.4[b]	288.3 ± 56.3	133.9 ± 29.8[c]

Rabbits (2.5–3.2 kg) were either fed ad libitum or fasted for 40 h prior to being killed. Cyclophosphamide treatment was performed 16 h before killing as described in Table 1. Tissue pieces were homogenized in 0.025 M ammonium buffer, pH 8.2, containing 4 mM CHAPS, heparin (5 U/ml), and protease inhibitors. LPL activity was measured and is expressed as mU/g tissue. Data from 10 experiments.

[a], $P < 0.001$; [b], $P < 0.01$: statistical significance referred to fed untreated controls.

[c], $P < 0.05$: referred to fasted untreated animals.

excess unlabeled methionine during the chase period. Immunoprecipitation of ^{35}S-labeled LPL was performed with a chicken antibody against human LPL, cross-reacting with the rabbit enzyme. The ^{35}S radioactivity incorporated into LPL is expressed as both percent of tissue radiolabeled total protein, and as dpm in immunoprecipitated LPL, normalized to 5×10^8 dpm initial radioactivity incubated per g tissue. As shown in **Fig. 3**, a single radioactive band with the expected molecular weight of LPL was obtained after polyacrylamide electrophoresis of the immunoprecipitated adipose tissue. LPL accounted for 0.32% of the newly synthesized proteins in adipocytes from fed animals. Upon fasting, incorporation of [^{35}S]methionine into adipose tissue total protein was slightly decreased compared to the fed state (5.9% vs. 8.7%, NS), and the relative rate of synthesis of LPL was twice as low (**Table 3**). The combination of both effects resulted in a threefold reduction in the radioactivity recovered with the immunoprecipitated [^{35}S]LPL. Administration of cyclophosphamide to fed animals also significantly decreased—and in similar proportions—the amount of newly synthesized LPL. However, the drug did not further reduce the rate of synthesis of the enzyme when given to fasted animals.

With regard to the heart tissue (**Table 4**), the incorporation of [^{35}S]methionine into both total protein and immunoprecipitable LPL was not different in fed or fasted animals. LPL accounted for 0.22% of the newly synthesized proteins. As observed earlier for the enzymatic activity, the effects of cyclophosphamide on LPL synthesis appeared dependent on the nutritional state. With ad libitum-fed rabbits, the radioactivity recovered in newly synthesized LPL was found to be significantly higher in antimitotic-treated animals due to an increase in both the incorporation of [^{35}S]methionine in tissue total protein and in the relative rate of synthesis of LPL (0.39%). When administered into 40-h-fasted animals, cyclophosphamide induced no significant change in the amount of immunoprecipitated [^{35}S]LPL, despite a tendency towards a lower relative rate of synthesis.

In order to follow the modulations of LPL synthesis at the transcriptional level, the variations in mRNA specific for LPL were estimated by slot-blot hybridization (**Fig. 4**), using a cDNA probe for human LPL, cross-hybridizing with rabbit RNA. The intensity of mRNA hybridization, as followed by optical integration of the autoradiograms, appeared to vary in parallel to changes in the synthesis of new [^{35}S]LPL. Indeed, in the adipose tissue from fed and cyclophosphamide-treated animals, the signal of mRNA-cDNA hybridization was only 19% ± 2% of that recorded in the tissue from control animals. Fasting induced a similar decrease. However, the drug was still effective in reducing the levels of LPL-mRNA in the fasted state, leading to a further 70% decrease in the intensity of the hybridization signal. In heart, LPL-mRNA was higher after cyclophosphamide treatment of the fed animal (signal ratio of 240% ± 18%

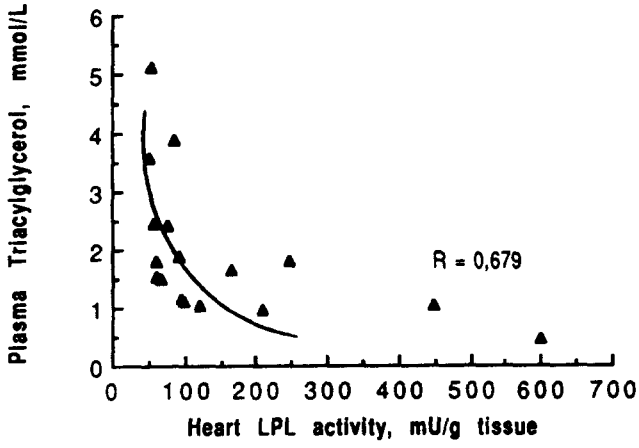

Fig. 2. Correlation between plasma triacylglycerol and heart lipoprotein lipase activity in fasted rabbits. Heart LPL activity was measured after solubilization by detergents, as described in Table 2. Data are from fasted rabbits either treated or not treated with 65 mg/kg cyclophosphamide. Plasma was taken from the same animals before they were killed and triacylglycerol was measured. Values are from 17 individual experiments. An inverse relationship between both parameters was observed with an exponential regression factor of 0.687 ($P < 0.01$).

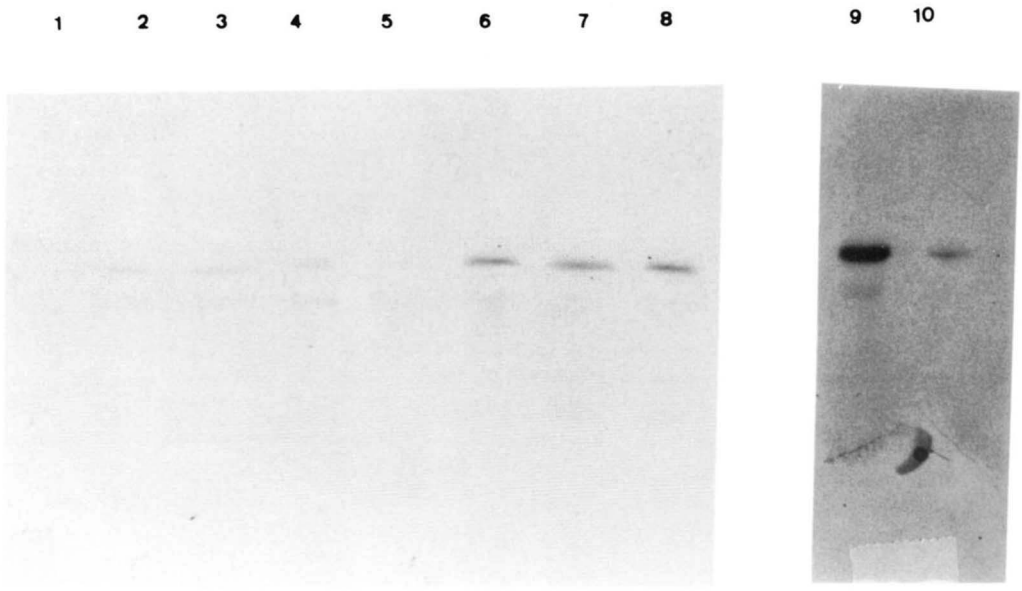

Fig. 3. Immunoprecipitation of lipoprotein lipase from rabbit adipose tissue. Pieces of periepididymal fat pads (1–2 g) were incubated with 20–40 μCi [^{35}S]methionine for 90 min. Adipose tissue was then homogenized with detergents and then incubated with either a specific chicken anti-LPL IgG or with a nonimmune IgG (lane 1). Immunoprecipitated LPL was then separated by SDS-polyacrylamide gel electrophoresis and detected by autoradiography. Lane 1: fed rabbit (nonimmune IgG); lanes 2–5: fasted rabbits (anti-LPL IgG); lanes 6–9: fed rabbits; lane 10: fed rabbit treated with cyclophosphamide.

compared to control fed animals). Conversely, fasting did not regulate the amount of heart LPL-mRNA, and the antimitotic treatment in fasted animals had no effect on the intensity of hybridization (signal ratio of 99% ± 6%, comparing treated hearts and controls).

Effects of cyclophosphamide on VLDL-triacylglycerol secretion

From the experiments described above, it was concluded that down-regulation of adipose tissue LPL upon fasting, combined with the effect of cyclophosphamide on heart LPL, result in a significant decrease of lipoprotein lipase, as expressed in the vascular compartment. However, this decrease may not be the sole determinant of the hypertriglyceridemia observed under those conditions. We wondered whether the drug might affect the rate of secretion of triglyceride-rich lipoproteins. To test this hypothesis, four groups of animals were studied as described above, i.e., with or without cyclophosphamide, in fed and fasted states. Four hours before the end of the experiment, blood samples were taken and animals were then injected intravenously with Triton WR-1339, 350 mg/kg, in order to block the catabolism of triglyceride-rich lipoproteins. Blood samples were taken again after 2 and 4 h, triacylglycerols were measured, and the differ-

TABLE 3. Synthesis of lipoprotein lipase in fat pad adipose tissue

Variable	Fed Rabbits		Fasted Rabbits	
	Untreated	Cyclophosphamide-Treated	Untreated	Cyclophosphamide-Treated
[^{35}S]methionine incorporation in tissue protein (%)	8.7 ± 1.7	13.5 ± 6.5	5.9 ± 1.1	7.3 ± 1.5
LPL relative synthesis rate (%)	0.32 ± 0.06	0.10 ± 0.02[a]	0.15 ± 0.04[b]	0.17 ± 0.06
LPL-associated ^{35}S radioactivity (dpm)	158000 ± 46500	69500 ± 13500[b]	52900 ± 19000[b]	75000 ± 36000

Fat pad pieces (1–2 g) were incubated with 20–40 μCi [^{35}S]methionine and were then homogenized. Incorporation of the radiolabel into tissue total protein was determined by TCA precipitation and is expressed as percent of the incubated [^{35}S]methionine. Relative rate of LPL synthesis (as % total ^{35}S-labeled protein) was assessed after immunoprecipitation of 10^6 dpm of incorporated protein radioactivity with 4 μl of a specific chicken anti-LPL antibody. Detection of radioactive LPL was performed by polyacrylamide gel electrophoresis, and the band was cut and counted for radioactivity. Radioactivity recovered in immunoprecipitated LPL was normalized to 5 × 10^8 dpm [^{35}S]methionine initially added per g tissue. Data are from four experiments and are means ± SE.

[a], $P < 0.01$; [b], $P < 0.05$: statistical significance referred to fed untreated animals.

TABLE 4. Synthesis of lipoprotein lipase in the perfused rabbit heart

Variable	Fed Rabbits		Fasted Rabbits	
	Untreated	Cyclophosphamide-Treated	Untreated	Cyclophosphamide-Treated
[^{35}S]methionine incorporation in tissue protein (%)	5.6 ± 2.0	10.6 ± 3.9	6.3 ± 0.2	10.5 ± 4.5
LPL relative synthesis rate (%)	0.21 ± 0.04	0.39 ± 0.28	0.22 ± 0.05	0.12 ± 0.01
LPL-associated ^{35}S radioactivity (dpm)	77500 ± 46500	261000 ± 173000[a]	68500 ± 17000	73500 ± 47000

Hearts were pulse-labeled for 10 min with 50–70 μCi [^{35}S]methionine per ml, and then were chased with excess unlabeled methionine and further perfused in the presence of heparin. After homogenization, incorporation of the radiolabel into total tissue protein was determined by TCA precipitation and is expressed as percent of the incubated [^{35}S]methionine. Relative rate of LPL synthesis (as % total ^{35}S-labeled protein) was assessed after immunoprecipitation of 10^6 dpm incorporated protein radioactivity with 4 μl of a specific chicken anti-LPL antibody. Detection of radioactive LPL was performed by polyacrylamide gel electrophoresis, and the band was cut and counted. Radioactivity recovered in immunoprecipitated LPL was normalized to 5×10^8 dpm [^{35}S]methionine initially added per g tissue. Data are from four experiments and are means ± SE.

[a], $P < 0.05$: statistical significance referred to fed untreated animals.

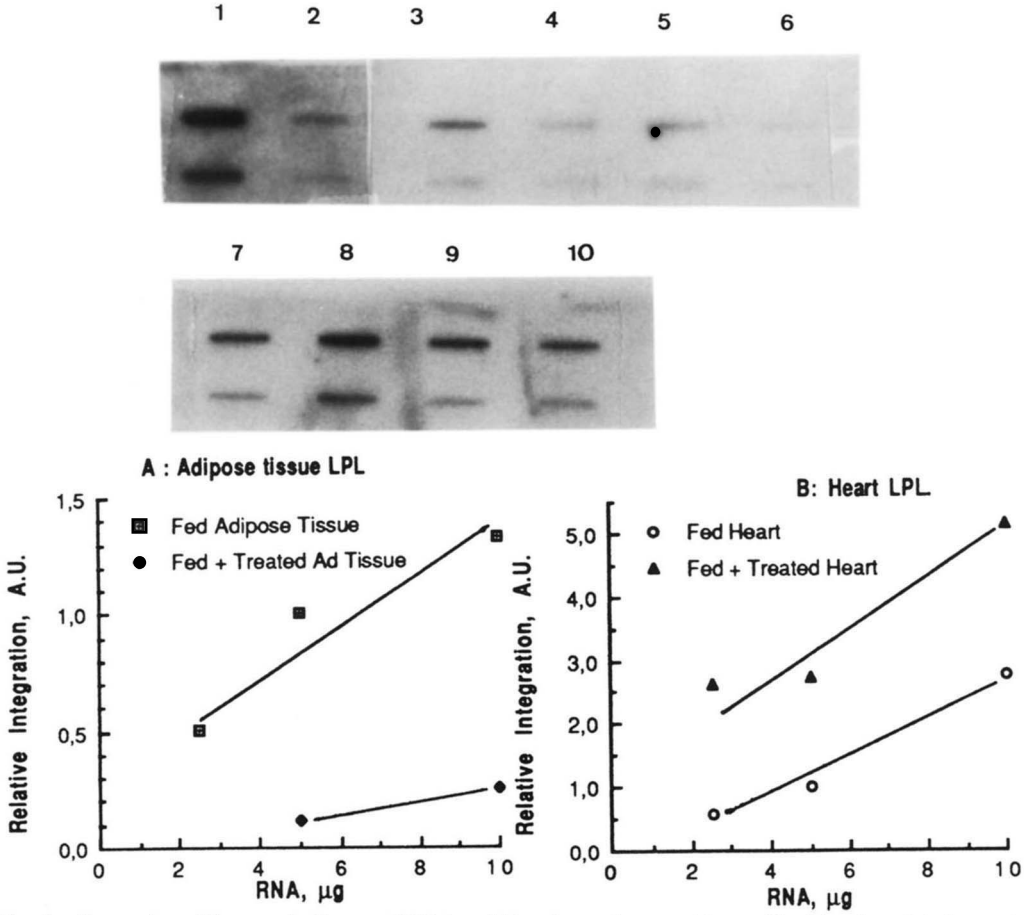

Fig. 4. Expression of lipoprotein lipase mRNA in rabbit adipose tissue and heart. Nutritional modulation and effects of cyclophosphamide. Rabbits were either fed ad libitum or fasted for 40 h and treated or not treated with cyclophosphamide, as described in Table 1. Total RNA was extracted from heart and adipose tissue. Various concentrations of total RNA were spotted onto a nitrocellulose film and hybridized with a [^{32}P]dCTP-labeled cDNA probe coding for (human) lipoprotein lipase. Positive hybridization was detected by autoradiography. The spots obtained for the concentrations of 10 and 5 μg are shown in the upper part of the figure. Lanes 1–6: slot-blot hybridization of adipose tissue RNA; lane 1: control fed rabbit; lane 2: fed and cyclophosphamide-treated rabbit; lanes 3 and 5: fasted control rabbits; lanes 4 and 6: fasted and cyclophosphamide-treated rabbits; lanes 7–10: slot-blot hybridization of heart RNA; lane 7: control fed rabbit; lane 8: control and cyclophosphamide-treated rabbit; lane 9: control fasted rabbit; and lane 10: fasted and cyclophosphamide-treated rabbit. Lower part of figure: A: relative optical integration of the slot-blot for adipose tissue LPL-mRNA as a function of RNA concentrations under two conditions, fed controls and fed + cyclophosphamide-treated animals. B: relative optical integration of the slot-blot for heart LPL-mRNA. Legends as in A.

ences observed after Triton administration were normalized as secretion rate per hour (**Table 5**). The values obtained in two experiments were quite different, yet similar qualitative variations were observed between the different treatments. Secretion rates of triacylglycerol were higher in fed than in fasted control animals. Cyclophosphamide induced an increase in TG secretion (36%, on average) in both nutritional states. When triglyceride-rich lipoproteins were isolated as the d < 1.006 g/ml ultracentrifugal fraction, the same differences were registered (data not shown). The TG secretion rate in this fraction was 57 nmol/ml per h in fasted controls and 138 nmol/ml per h in treated animals. In normally fed rabbits, the corresponding values were 112 nmol/ml per h and 181 nmol/ml per h for control and cyclophosphamide-treated rabbits, respectively.

Hence, cyclophosphamide appears to increase moderately the rate of triacylglycerol secretion, an effect that may contribute to the occurrence of hypertriglyceridemia in fasted and cyclophosphamide-treated animals.

TABLE 5. Effects of cyclophosphamide on the secretion of triglyceride-rich lipoproteins

Nutritional State	Cyclophosphamide	Triglyceride Secretion Rate Exp. 1	Exp. 2
		nmol/ml/h	
Fasted	–	280	170
Fasted	+		200
Fed	–	1100	220
Fed	+	1600	320

Rabbits either fed normally or fasted 40 h were treated with 65 mg/kg cyclophosphamide (+) or with vehicle only (–) during the last 16 h. Four h before the end of the experiment, blood samples were taken and animals were then injected intravenously with Triton WR-1339, 350 mg/kg, in order to block the catabolism of triglyceride-rich lipoproteins. Blood samples were taken again after 2 and 4 h and the animals were then killed. Triacylglycerol was measured in all plasma samples and the secretion rate was calculated per h and per ml plasma. Data are from two experiments.

Characteristics of lipoprotein lipase isolated from rabbit adipose tissue, heart, and postheparin plasma

The experiments described above have shown that, in the rabbit, adipose LPL and heart LPL respond differently to nutritional modulation or to antimitotic administration. To check whether LPL originating from those two organs have similar or different properties, the enzyme was isolated from postheparin plasma, adipose tissue, or heart homogenates by heparin affinity chromatography, and also from heart perfusates after heparin infusion. The enzymes from heart, adipose tissue, and postheparin plasma were identically inhibited by a specific chicken antiserum against LPL. Half inhibition was observed at a dilution of 1:64 (**Fig. 5**). The LPL activity from both fat tissue and heart proved to be stable up to 3 h at 37°C in a medium containing albumin and heparin. The same behavior was observed with LPL present in adipocyte culture media or in heart perfusates (data not shown).

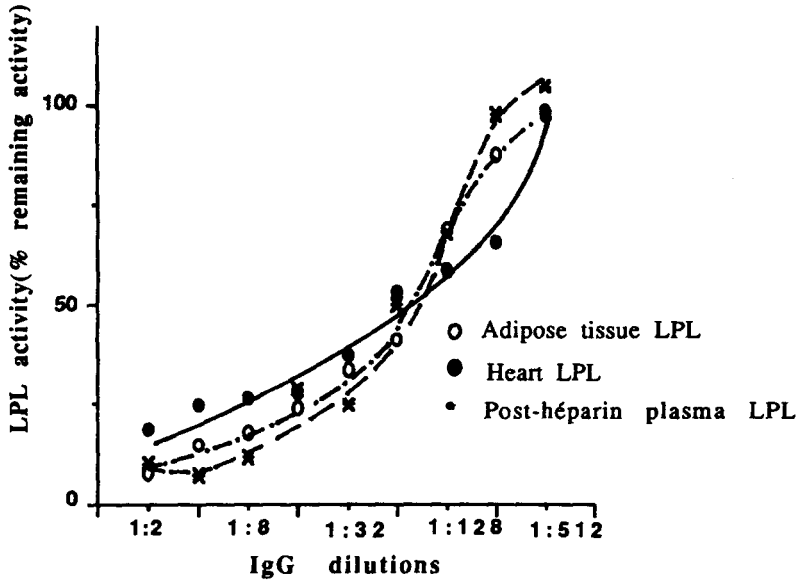

Fig. 5. Immunoinhibition of the activity of LPL isolated from different rabbit sources. Lipoprotein lipase isolated from heart, adipose tissue, or postheparin plasma was preincubated for 60 min at 4°C with an identical volume of a dilution of IgG, directed against LPL. Then, 25 μl of the mixture was assayed for LPL activity. Control preincubations were performed with nonimmune chicken IgG. Results are expressed as percent remaining activity, relative to samples preincubated with nonimmune IgG. The 100% activity represented 47 mIU/ml for adipose tissue LPL (○), 33 mIU/ml for heart LPL (●), and 70 mIU/ml for the enzyme isolated from postheparin plasma (★).

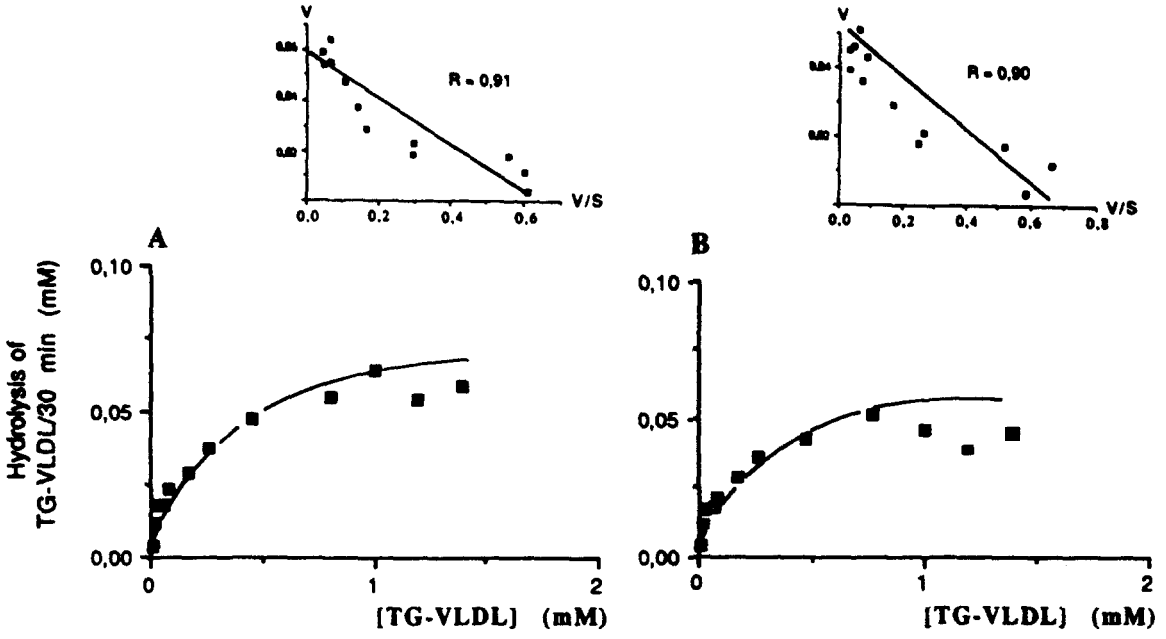

Fig. 6. Concentration kinetics for the hydrolysis of VLDL-[^{3}H]TG, as induced by adipose tissue LPL (A) and heart LPL (B). Lipoprotein lipase was partially purified from adipose tissue and heart homogenates by heparin-Sepharose chromatography. All enzyme preparations were kept in a Tris-HCl buffer, 0.2 M, pH 7.4, containing 0.15 M NaCl, 4% (w/v) BSA, 20% (v/v) glycerol, and heparin (5 IU/ml). Increasing concentrations of [^{3}H]trioleoylglycerol-labeled VLDL were incubated with adipose tissue LPL (12.5 mIU/ml) or heart LPL (13.3 mIU/ml) in Tris-HCl, 0.2 M, pH 7.4, containing 0.15 M NaCl and BSA 4% (w/v). Incubations were carried out for 45 min at 37°C. Lipids were extracted, analyzed by thin-layer chromatography, and the TG hydrolysis was calculated (μM/30 min). One experiment representative of two identical studies. Inserts show the V/S = f(V) expression.

The affinity and catalytic efficiency of the different LPL preparations towards VLDL-triacylglycerol were compared in hydrolysis kinetic experiments. Saturable profiles were evident in all cases (see **Fig. 6A and 6B,** for adipose tissue and heart LPL, respectively). The estimated kinetic parameters were almost similar for the lipoprotein lipase isolated from either fat or heart tissues (K_m around 0.07 mM). Values of the same order of magnitude were also obtained with the LPL partially purified from postheparin plasma or present in heparin-containing heart perfusates (**Table 6**).

Heart and adipose LPL were also analyzed by SDS-PAGE, transferred, and immunoblotted using the specific anti-LPL IgG. In both cases one major band with an apparent molecular mass of 54.7 ± 1.8 and 52.4 ± 0.9 kDa, respectively, was detected (Table 6) with no appearance of smaller fragments.

In order to confirm the identity of the LPL isolated from heart and adipose tissue, the extracted RNAs were separated by agarose electrophoresis, transferred, and hybridized with a cDNA probe coding for human LPL. For comparison, the Northern blot analysis of guinea pig tissue RNAs was run in parallel, and indicated the presence of three different sizes of mRNA for LPL, according to previous reports: 3.8, 3.3, and 2.1 kB. In rabbit tissues, a single transcript of 3.8 kB was detected in both heart and adipose tissue (not shown).

Hence, the different LPL preparations likely derive from the same transcript and display similar molecular mass and catalytic properties.

TABLE 6. Kinetic parameters and molecular mass of LPL purified from heart, adipose tissue, and postheparin plasma

Variable	K_m	V_{max}	Molecular Mass
	mM	*mM/30 min*	*kDa*
Postheparin plasma	0.046	0.032	
Tissue homogenate			
Adipose tissue	0.069 (0.056–0.081)	0.038 (0.032–0.043)	54.7 ± 1.8
Heart	0.070 (0.055–0.085)	0.038 (0.035–0.041)	52.4 ± 0.9
Heart perfusate	0.085 (0.075–0.096)	0.056 (0.065–0.047)	

LPL, partially purified from different sources as described in Materials and Methods, was incubated with increasing concentrations of [^{3}H]-TG-VLDL as in Fig. 5. Kinetic parameters were assessed by the two reciprocal and the V = f(V/S) methods. V_{max} is expressed for 12.5 mIU per ml. Results are from one or two experiments. The molecular mass of LPL partially purified from adipose tissue and heart was estimated after polyacrylamide gel electrophoresis followed by immunoblotting with a specific antibody against LPL (three determinations).

DISCUSSION

Lipoprotein lipase activity in rabbit tissues appears to be differentially modulated by the nutritional status or by antimitotic administration. In agreement with previous reports from this laboratory, cyclophosphamide injection into fasted rabbits induced a massive hypertriglyceridemia associated with a defect of LPL as measured in postheparin plasma (25). In the present study, heart LPL activity was found to be most sensitive to the depressing effect of the drug in fasted animals. On the other hand, starvation down-regulated the level of LPL synthesis and activity in the adipose tissue. The combination of both effects may probably account for the enzyme deficiency in the vascular compartment, as observed in fasted and cyclophosphamide-treated animals. Moreover, the increased VLDL-TG secretion induced by the drug might also contribute to the observed hyperlipemia. Conversely, administration of cyclophosphamide to normally fed animals resulted in a stimulation of LPL synthesis and activity in the heart, associated with an increase in the activity measured in postheparin plasma.

The present observations emphasize the role of LPL deficiency in triggering hypertriglyceridemia in cyclophosphamide-treated fasted rabbits. The arguments in favor of this evidence are the following. The drug treatment leads to an accumulation of plasma triacylglycerol only in the fasted state, a situation where adipose tissue LPL is at its lowest level and where the drug depresses heart LPL activity. This results in a 75% decrease in the LPL activity measured in the vascular compartment and, as illustrated in Fig. 2, there might be a threshold of heart LPL activity under which hypertriglyceridemia will occur. The stimulation of (VLDL) triacylglycerol synthesis by cyclophosphamide is unlikely to play a major role since it did not exceed 50%, whereas accumulation of plasma triacylglycerol was increased more than fourfold in fasted and cyclophosphamide-treated rabbits. Furthermore, stimulation of triglyceride synthesis with the drug was observed in both fed and fasted animals, whereas the effects on heart LPL were dependent on the nutritional state, as was the occurrence of hypertriglyceridemia. This strengthens the hypothesis that lowering LPL activity in two major sites of its synthesis is a major determinant of the observed hypertriglyceridemia.

This experimental model allows a study of the respective contributions of heart and periepididymal adipose tissue to postheparin plasma LPL activity (PHP-LPL), and emphasizes the role of the heart LPL in the fasted state. For instance, in fed animals, 3 g of periepididymal fat pad tissue and 8 g of heart tissue would contribute about 40% of the PHP-LPL, considering 100 ml as the plasma volume per rabbit. Upon starvation, heart LPL itself would contribute more than 45%, and thus is likely to be of importance, together with LPL in skeletal muscle, in the clearance of plasma triacylglycerol.

The nutritional regulations of adipose LPL in the rabbit are concordant with similar observations in other species and particularly in humans (15), guinea pigs (5, 10, 16), and chickens (40). These nutritional modulations are likely explained by the long-term regulation by insulin on LPL synthesis in adipocytes (13). Indeed, in isolated human adipocytes, insulin was reported to increase by two- to threefold the relative synthesis of LPL and the expression of specific LPL mRNA (13). Conflicting observations have been reported on the regulation of rat adipose LPL (41). Fasting would little affect the rate of synthesis but would rather channel LPL to a degradation pathway and not towards secretion in an active form. In our experiments with the rabbit, the parallel decreases in LPL activity, rate of synthesis, and specific mRNA do not support this scheme, and species differences may be invoked regarding the trigger points of insulin regulation of adipose tissue LPL.

However, some discrepancy is noted if one compares the amplitude of nutritional modulation in terms of activity (−94% upon fasting) and in terms of rate of synthesis (−66%) suggesting that insulin would also be involved in LPL activation. Insulin, by stimulating the entry of glucose into the cell may stimulate the glycosylation steps of the enzymatic protein during the intracellular maturation of LPL (42, 43).

Another target of insulin regulation might concern the secretion processes. As shown in 3T3-L1 cells, addition of insulin promotes some release of cellular LPL by a process independent of protein synthesis but which may involve phospholipase C activation (44, 45). In turn, the generated diacylglycerol would activate the cellular protein kinase C. In this context, recent studies carried out on both a macrophage cell line and on preadipocytes have demonstrated that phospholipase C and protein kinase C activation can cause induction of LPL mRNA (45, 46). In complementary experiments carried out in ex vivo isolated adipocytes, we observed a proportional decrease in LPL activity among the secretory, heparin-releasable, and intracellular compartments, in cells isolated from fasted rabbits, suggesting that in this species, lack of insulin has little effect on LPL secretion from adipose tissue (A. Lespine, unpublished observation).

The effects of cyclophosphamide on adipose tissue LPL were essentially similar to those of fasting. The drug induced a 60% reduction in the newly synthesized [^{35}S]LPL and a corresponding decrease in the LPL-mRNA, associated with a 78% decrease in activity. The effects of cyclophosphamide administration and of fasting on LPL activity were not cumulative, suggesting that they are directed towards common mechanisms. Although purely speculative at this time, cyclophosphamide may inhibit the syn-

thesis of specific proteins, LPL for example, or may affect the synthesis of an insulin-responsive transcription factor.

In the rabbit, heart LPL appeared insensitive to nutritional modulation in terms of activity, synthesis, and expression of specific mRNA. Depending on the animal model studied, various changes of heart LPL upon feeding and fasting have been reported (5, 47). Nevertheless, no clear relationship has been found between the levels of insulin and heart LPL activity. In guinea pigs for instance, no change is recorded in LPL activity upon starvation or in the level of LPL mRNA in the heart (10). However, in alloxan-treated diabetic rats, heart LPL is lowered, but it can be restored by perfusing the organ in presence of insulin (48), suggesting that certain conditions may unveil a stimulatory role of the hormone. In our model, although nutritional variations failed to display any effect on heart LPL in control animals, cyclophosphamide stimulated LPL synthesis and activity only in fed animals, i.e., in presence of insulin but not in its absence. Several hypotheses may be open to future research regarding this observation. First, cyclophosphamide might affect the synthesis of an inhibitor of the insulin response of heart LPL. Alternatively, cyclophosphamide or its metabolites (4-cetocyclophosphamide and carboxyphosphamide) might interfere with (increase) the cell levels of cyclic AMP, a potent modulator of heart LPL (49, 50), either directly through the adenylcyclase/phosphodiesterase balance or indirectly. It may be recalled that the effects of cAMP on adipose tissue LPL and heart LPL are just opposite (50), as were the effects of cyclophosphamide in fed animals.

Cyclophosphamide administration in fasted animals did not significantly change the rate of synthesis of heart LPL or the level of specific mRNA. However, the LPL activity recovered in heart under these conditions was reduced by 50%, and this decline was critical for the occurrence of the LPL defect in the vascular compartment. Hence, cyclophosphamide metabolites may have side effects on LPL activity. Current perfusion studies carried out in our laboratory indicate that the reduction in heart LPL activity observed in fasted/treated rabbits mostly concerns the secretory and heparin-releasable pools of the enzyme (A. Lespine, unpublished studies). Hence a defective secretion pathway may target LPL to the degradation pathway more than in the control situation (51).

Experimental hypertriglyceridemias, developing in a comparable time scale, have been observed in animals receiving an in vivo injection of TNF or interferon (5, 19, 22). Those cytokines are secreted by the activated macrophages in response to invasive or severe infectious processes (52). In the rat, adipose LPL appears specifically lowered by TNF, while the activity in heart is either unchanged or even increased (5, 22). Furthermore, the fatty acid uptake from chylomicrons was enhanced in hearts from TNF-treated rats (22). In this species, when adipose LPL was first depressed by induction of diabetes, TNF did not further decrease the heart enzyme (53).

The picture obtained with cyclophosphamide has some common features with the modulation by TNF. In the fed state, antimitotic treatment reduced LPL synthesis and activity in adipose tissue but had opposed effects on heart LPL. However, the hyperlipidemia became patent only in the fasted state, when both sources of LPL were repressed. In this case, cyclophosphamide treatment has little influence on heart LPL synthesis, but reduces heart LPL activity. Finally, measurements of the immunodetectable TNF showed no accumulation of the mediator following antimitotic treatment. However, considering the very rapid catabolism of TNF and the fact that the TNF-induced hypertriglyceridemias reported in the literature occur in a time interval comparable to that observed with cyclophosphamide administration, one cannot rule out a transient accumulation of the cytokine during the hours preceding the occurrence of an LPL defect.

VLDL from our irradiated or cyclophosphamide-treated rabbits contained new apolipoproteins called apoX (25–27). Their presence did not impair the normal conversion of VLDL to intermediate density lipoproteins and LDL (27), but apoX could eventually modulate the synthesis of LPL. In the present model, high doses of cyclophosphamide were used to induce a strong defect in lipoprotein lipase. Hence, the observations may have little relevance with regard to regular antimitotic therapy. This question was recently addressed in our laboratory by analyzing the lipoprotein profile of six patients receiving therapeutic doses of cyclophosphamide (500 mg/m^2). Variations in the levels of triglyceride and cholesterol were not significant in the course of the perfusion (24 h). However, VLDL isolated from the patients accumulated apoX. Hence, occurrence of the latter apoX and lipoprotein lipase defect may not be related.

In conclusion, adipose tissue LPL and heart LPL are differentially regulated in the rabbit in response to nutritional modulations and to antimitotic administration. Occurrence of hypertriglyceridemia and LPL deficiency in the vascular compartment occur when both adipose tissue LPL is at its lowest level upon insulin deprivation, and when heart LPL is depressed by the drug. Furthermore, cyclophosphamide may be used as a tool to unveil normally nonexpressed reglation of heart LPL by insulin.

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REFERENCES

1. Nilsson-Ehle, P., A. S. Garfinkel, and M. Schotz. 1980. Lipolytic enzymes and plasma lipoprotein metabolism. *Annu. Rev. Biochem.* **49:** 667–693.

2. Scow, R. O., E. J. Blanchette-Mackie, and L. C. Smith. 1980. Transport of lipid across capillary endothelium. *Fed. Proc.* **39:** 2610–2617.
3. Peterson, J., T. Olivecrona, and G. Bengtsson. 1985. Distribution of lipoprotein lipase and hepatic lipase between plasma and tissues: effect of hypertriglyceridemia. *Biochim. Biophys. Acta.* **837:** 262–270.
4. Wallinder, L., G. Bengtsson, and T. Olivecrona. 1982. Purification and properties of lipoprotein lipase in guinea pig milk. *Biochim. Biophys. Acta.* **711:** 107–113.
5. Semb, H., J. Peterson, J. Tavernier, and T. Olivecrona. 1987. Multiple effects of Tumor Necrosis Factor on lipoprotein lipase in vivo. *J. Biol. Chem.* **262:** 8390–8396.
6. Vilarò, S., M. Llobera, G. Bengtsson-Olivecrona, and T. Olivecrona. 1988. Synthesis of lipoprotein lipase in the liver of newborn rats and localization of the enzyme by immunofluorescence. *Biochem. J.:* **249:** 549–556.
7. Senda, M., K. Oka, W. V. Brown, P. K. Qasba, and Y. Furuichi. 1987. Molecular cloning and sequence of a cDNA coding for bovine lipoprotein lipase. *Biochemistry.* **84:** 4369–4373.
8. Kirchgessner, T. G., K. L. Svenson, A. J. Lusis, M. C. Schotz, and R. Lawn. 1987. The sequence of cDNA encoding lipoprotein lipase: a member of a lipase gene family. *J. Biol. Chem.* **262:** 8463–8466.
9. Wion, K., T. Kirchgessner, A. Lusis, M. Schotz, and R. Lawn. 1987. Human lipoprotein lipase complementary DNA sequence. *Science.* **235:** 1638–1641.
10. Enerbäck, S., H. Semb, G. Bengtsson-Olivecrona, P. Carlsson, M. Hermansson, T. Olivecrona, and G. Bjursell. 1987. Molecular cloning and sequence analysis of cDNA encoding lipoprotein lipase of guinea pig. *Gene.* **58:** 1–12.
11. Kirchgessner, T. G., R. C. LeBoeuf, C. A. Langner, S. Zollman, C. H. Chang, B. A. Taylor, M. C. Schotz, J. I. Gordon, and A. J. Lusis. 1989. Genetic and developmental regulation of the lipoprotein lipase gene: loci both distal and proximal to the lipoprotein lipase structural gene control enzyme expression. *J. Biol. Chem.* **264:** 1473–1482.
12. Semenkovich, C. F., S. H. Chen, M. Wims, C. C. Luo, W. H. Li, and L. Chan. 1989. Lipoprotein lipase and hepatic lipase mRNA tissue specific expression, developmental regulation, and evolution. *J. Lipid Res.* **30:** 423–431.
13. Ong, J. M., T. J. Kirchgessner, M. C. Schotz, and P. A. Kern. 1988. Insulin increases the synthetic rate and messenger RNA level of lipoprotein lipase in isolated adipocytes. *J. Biol. Chem.* **263:** 12933–12938.
14. Kern, P. A., S. Marshall, and R. H. Eckel. 1985. Regulation of lipoprotein lipase in primary cultures of isolated human adipocytes. *J. Clin. Invest.* **75:** 199–208.
15. Ong, J. M., and P. A. Kern. 1989. Effect of feeding and obesity on lipoprotein lipase activity, immunoreactive protein, and messenger RNA levels in human adipose tissue. *J. Clin. Invest.* **84:** 305–311.
16. Semb, H., and T. Olivecrona. 1986. Nutritional regulation of lipoprotein lipase in guinea pig tissues. *Biochim. Biophys. Acta.* **876:** 249–255.
17. Fielding, C. J. 1976. Lipoprotein lipase: evidence for high- and low-affinity enzyme sites. *Biochemistry.* **15:** 879–884.
18. Kaufmann, R. L., C. F. Matson, and W. R. Beisel. 1976. Hypertriglyceridemia produced by endotoxin: role of impaired triglyceride disposal mechanisms. *J. Infec. Dis.* **133:** 448–555.
19. Kurzrock, R., M. F. Rhode, J. R. Quesada, S. H. Gianturco, W. A. Bradley, S. A. Sherwin, and J. U. Gutterman. 1986. Recombinant interferon induces hypertriglyceridemia and inhibit post-heparin lipase activity in cancer patients. *J. Exp. Med.* **164:** 1093–1101.
20. Bagby, G. J., and J. A. Spitzer. 1980. Lipoprotein lipase activity in rat heart and adipose tissue during endotoxic shock. *Am. J. Physiol.* **238:** 325–329.
21. Bagby, G. J., C. B. Corll, and R. R. Martinez. 1987. Triacylglycerol kinetics in endotoxic rats with suppressed lipoprotein lipase activity. *Am. J. Physiol.* **253:** 59–64.
22. Chajek-shaul, T., G. Friedman, O. Stein, E. Shiloni, J. Etienne, and Y. Stein. 1989. Mechanism of hypertriglyceridemia induced by Tumor Necrosis Factor administration to rats. *Biochim. Biophys. Acta.* **1001:** 316–324.
23. Cornelius, P., S. Enerback, G. Bjursell, T. Olivecrona, and P. Pekala. 1988. Regulation of lipoprotein lipase mRNA content in 3T3-L1 cells by tumor necrosis factor. *Biochem. J.* **249:** 765–769.
24. Friedman, G., R. Gallily, T. Chajek-Shaul, O. Stein, E. Shiloni, J. Etienne, and Y. Stein. 1988. Lipoprotein lipase in heart cell cultures is suppressed by bacterial lipopolysaccharide: an effect mediated by production of tumor necrosis factor. *Biochim. Biophys. Acta.* **960:** 220–228.
25. Dousset, N., R. Feliste, M. Carton, and L. Douste-Blazy. 1984. Irradiation-induced free cholesterol accumulation in very low density lipoproteins. *Biochim. Biophys. Acta.* **794:** 444–453.
26. Loudet, A. M., N. Dousset, B. Perret, M. Ierides, M. Carton, and L. Douste-Blazy. 1985. Triacylglycerol increase in plasma very low density lipoproteins in cyclophosphamide-treated rabbit: relationship with cholesteryl ester transfer activity. *Biochim. Biophys. Acta.* **836:** 376–384.
27. Lespine, A., N. Dousset, B. Perret, M. De Forni, H. Chap, and L. Douste-Blazy. 1988. Accumulation of large VLDL in cyclophosphamide-treated rabbits. Relationship with lipoprotein lipase deficiency. *Biochem. Biophys. Res. Commun.* **154:** 633–640.
28. Brock, N. 1967. Pharmacologic characterization of cyclophosphamide (NSC 26271) and cyclophosphamide metabolites. *Cancer Chemother. Res.* **51:** 315–325.
29. Reaven, E. P., O. G. Kolterman, and G. M. Reaven. 1974. Ultrastructural and physiological evidence for corticosteroid-induced alterations in hepatic production of very low density lipoprotein particles. *J. Lipid Res.* **15:** 74–83.
30. Nilsson-Elhe, P., and R. Eckman. 1977. Rapid simple and specific assays for lipoprotein lipase and hepatic lipase. *Artery.* **3:** 194–209.
31. Belfrage, P., and M. Vaughan. 1969. Simple liquid-liquid partition system for the isolation of labeled oleic acid from mixtures with glycerides. *J. Lipid Res.* **10:** 341–344.
32. Olivecrona, T., and G. Bengtsson. 1983. Immunochemical properties of lipoprotein lipase. Development of an immunoassay applicable to several mammalian species. *Biochim. Biophys. Acta.* **752:** 38–46.
33. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* **18:** 5294–5299.
34. Röschlau, P., E. Bernt, and W. Gruber. 1974. Enzymatische Bestimmung des Gesamt-Cholesterins im Serum. *Z. Klin. Chem. Klin. Biochem.* **12:** 403–407.
35. Wahefeld, A. 1974. Triglyceride determination after enzymatic hydrolysis. *In* Methods of Enzymatic Analysis. Vol. 4. H. U. Bergmeyer, editor. Verlag Chimie, Weinheim. 1131–1135.
36. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol re-

agent. *J. Biol. Chem.* **193:** 265–275.
37. Gruber, W. 1978. Inhibition of creatine kinase activity by Ca^{++} and reversing effect of EDTA. *Clin. Chem.* **24:** 177–178.
38. Wroblevsky, F., and J. La Due. 1955. Lactic dehydrogenase activity in blood. *Proc. Soc. Exp. Biol.* **90:** 210–215.
39. Bligh, E. G., and W. J. Dyer. 1959. A rapid method for total lipid extraction and purification of phospholipids. *Can. J. Biochem. Physiol.* **37:** 911–918.
40. Cooper, D. A., J. C. Stein, P. J. Strielman, and A. Bensadoun. 1989. Avian adipose lipoprotein lipase: cDNA sequence and reciprocal regulations of mRNA levels in adipose and heart. *Biochim. Biophys. Acta.* **1008:** 92–101.
41. Doolittle, M. H., O. Ben-Zeev, J. Elovson, D. Martin, and T. G. Kirchgessner. 1990. The response of lipoprotein lipase to feeding and fasting. Evidence for posttranslational regulations. *J. Biol. Chem.* **265:** 6291–6295.
42. Parkin, S. M., K. Walker, P. Ashby, and D. S. Robinson. 1980. Effects of glucose and insulin on the activation of lipoprotein lipase and on protein synthesis in rat adipose tissue. *Biochem. J.* **188:** 195–199.
43. Semb, H., and T. Olivecrona. 1989. The relation between glycosylation and activity of guinea-pig lipoprotein lipase. *J. Biol. Chem.* **264:** 4195–41200.
44. Pradines-Figueres, A., C. Vannier, and G. Ailhaud. 1988. Short-term regulation by insulin of lipoprotein lipase secretion in adipose cells. *Biochem. Biophys. Res. Commun.* **154:** 982–990.
45. Chan, B. L., M. P. Lisanti, E. Rodriguez-Boulan, and A. R. Saltiel. 1989. Insulin-stimulated release of lipoprotein lipase by metabolism of its phosphatidylinositol anchor. *Science.* **241:** 1670–1672.
46. Auwerx, J. H., S. Deeb, J. D. Brunzell, G. Wolfbauer, and A. Chait. 1989. Lipoprotein lipase gene expression in THP-1 cells. *Biochemistry.* **28:** 4563–4567.
47. Cryer, A. 1981. Tissue lipoprotein lipase activity and its action in lipoprotein metabolism. *Int. J. Biochem.* **13:** 525–541.
48. O'Looney, P., M. V. Maten, and G. V. Vahouny. 1983. Insulin-mediated modifications of myocardial lipoprotein lipase and lipoprotein metabolism. *J. Biol. Chem.* **258:** 12994–13001.
49. Stam, H., and W. C. Hülsmann. 1984. Effects of hormones, aminoacids and specific inhibitors on rat heart lipoprotein lipase and tissue neutral lipase activities during long term perfusion. *Biochim. Biophys. Acta.* **794:** 72–82.
50. Friedmann, G., T. Chajek-Shaul, O. Stein, and Y. Stein. 1983. Modulation of lipoprotein lipase activity in cultured rat mesenchymal heart cells and preadipocytes by dibutyryl cyclic AMP, cholera toxin and 3-isobutyl methyl xanthine. *Biochim. Biophys. Acta.* **752:** 106–117.
51. Bensadoun, A. 1991. Lipoprotein Lipase. *Annu. Rev. Nutr.* **11:** 217–237.
52. Oliff, A. 1988. Role of TNF in cachexia. *Cell.* **54:** 141–142.
53. Feingold, K. R., M. Soued, I. Staprans, L. A. Gavin, M. E. Donahue, B. J. Huang, A. H. Moser, R. Gulli, and C. Grunfeld. 1989. Effect of Tumor Necrosis Factor (TNF) on lipid metabolism in diabetic rat. Evidence that inhibition of adipose tissue lipoprotein lipase activity is not required for TNF-induced hyperlipemia. *J. Clin. Invest.* **83:** 1116–1121.