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Changes in lipoprotein lipase modulate tissue energy supply during stress

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Ricart-Jané, David, Pilar Cejudo-Martín, Julia Peinado-Onsurbe, M. Dolores López-Tejero, and Miquel Llobera. Changes in lipoprotein lipase modulate tissue energy supply during stress. *J Appl Physiol* 99: 1343–1351, 2005. First published June 9, 2005; doi:10.1152/japplphysiol.00971.2004.—We studied the variations caused by stress in lipoprotein lipase (LPL) activity, LPL-mRNA, and local blood flow in LPL-rich tissues in the rat. Stress was produced by body immobilization (Immo): the rat's limbs were taped to metal mounts, and its head was placed in a plastic tube. Chronic stress (2 h daily of Immo) decreased total LPL activity in mesenteric and epididymal white adipose tissue (WAT) and was accompanied by a weight reduction of these tissues. In limb muscle, heart, and adrenals, total LPL activity and mRNA levels increased, and, in plasma, LPL activity and mass also increased. Acute stress (30-min Immo) caused a decrease in total LPL activity only in retroperitoneal WAT and an increase in preheparin plasma active LPL, but the overall weight of this tissue did not vary significantly. We propose an early release of the enzyme from this tissue into the bloodstream by some unknown extracellular pathways or other local mechanisms. These changes in this key energy-regulating enzyme are probably induced by catecholamines. They modify the flow of energy substrates between tissues, switching the WAT from importer to exporter of free fatty acids and favoring the uptake by muscle of circulating triacylglycerides for energy supply. Moreover, we found that acute stress almost doubled blood flow in all WAT studied, favoring the export of free fatty acids.

immobilization stress; lipoprotein lipase messenger ribonucleic acid; muscle; white adipose tissue; blood flow

LIPOPROTEIN LIPASE (LPL) (EC 3.1.1.34) is a dimeric enzyme responsible for the hydrolysis of the circulating triacylglycerides (TAG) that form part of the chylomicra and very-low-density lipoproteins, thus enabling the underlying tissues to capture the fatty acids produced by this hydrolysis. Low-density lipoprotein is synthesized and expressed in all tissues of the organism except the liver (7). Due to the relative weight of the contribution of each tissue to the organism as a whole and due to the different levels of LPL activity in each of these tissues, almost 99% of total LPL activity takes place in adipose tissue and in muscle (41, 42).

The physiologically active form of LPL is located at the surface of the vascular endothelium of tissues from which it is synthesized and to which it is anchored by a membrane-bound glycosaminoglycan chain (heparan and dermatan sulfate) (36). However, the synthesis of the enzyme occurs in parenchymal cells, from which it is secreted and then transported to the lumen surface of endothelial cells. After a short half-life, the enzyme is released from its anchorage in a monomeric and inactive form and travels through the blood bound to low- and high-density lipoprotein (58). The released enzyme is readily captured by the liver (56, 57), where it degrades. Only very few LPL molecules are released from tissue in dimeric form (active). This gives rise to a very low level of LPL activity in plasma and, temporarily, in the liver (8).

The extracellular location of LPL determines and limits the mechanisms by which it is regulated. As this regulation is tissue dependent and subject to energy requirements, LPL is key in TAG distribution between tissues. LPL is regulated in the medium term, according to the degree of its synthesis (transcription, translation, and posttranslation processing) (2, 13), but a tissue's enzyme activity can also be regulated by the release of LPL from the endothelium to plasma.

Catecholamines, a powerful factor involved in LPL regulation, can have opposite effects on LPL-rich tissues. We reported that in vivo administration to rats of catecholamines decreases extracellular active LPL in white adipose tissue (WAT) but increases it in plasma (3). Previous studies give heterogeneous results, although the effects we found in WAT have been previously reported (2, 12). It has been described that catecholamines increase LPL in muscle (12). These changes in LPL activity might involve changes in circulating lipid metabolism.

In a previous paper (46), our laboratory described the alterations to metabolism, especially lipid metabolism, induced by immobilization (Immo) stress, an in vivo experimental model in which catecholamines increase greatly (60).

The phenomenon of stress, which occurs widely, both in nature and in human society, can be defined as a phylogenetically developed response of the organism to those agents that pose a threat to its overall well-being (55). This individual response is activated when the effect of a stimulus is greater than the capacity of homeostatic mechanisms to deal with changes in the internal medium. Stress involves activation of both the sympato-medullo-adrenal axis (with the secretion of catecholamines) and the hypothalamus-hypophysis-adrenal axis (which leads to glucocorticoid release). Catecholamines and glucocorticoids act on their target tissues, provoking a metabolic response that prepares the organism for the reaction known as "fight or flight." This metabolic response is characterized by an increase in available energy substrates. Cardiac rate and blood pressure also rise (19).

Our laboratory's previous paper (46) described how, in rats fed a standard diet, acute and/or chronic stress produced by body Immo induced a notable response in both lipid (increase in plasma nonesterified fatty acid and glycerol) and lipoprotein (decrease in plasma TAG and increase in total cholesterol)

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metabolism. These changes suggest that catecholamines and glucocorticoids, which are segregated and synthesized under such conditions, may alter LPL activity directly or indirectly in various tissues.

Here we examine the variations in LPL activity and LPL mRNA expression in LPL-rich tissues and their importance in the shift of energy substrates from storage sites to oxidative sites in response to acute and/or chronic stress. Variations in local blood flow in these tissues are also analyzed to study its relation to energy substrate distribution.

There are significant areas of investigation in the fields of LPL regulation and energy balance during stress involving alterations of lipid metabolism, such as atherosclerosis, obesity, diabetes, and X syndrome. This paper focuses for the first time on the importance of tissue LPL changes under stress adaptation and energy substrate distribution.

MATERIALS AND METHODS

Model of Stress by Body Immo

The Immo procedure was based on one described by Kvetnansky and Mikulaj (29). Male Wistar rats (Harlan Interfauna Ibèrica, Barcelona, Spain), weighing 190–240 g, were attached to wooden boards in the prone position by the taping of their forelimbs and rear hindlimbs to metal mounts. The head was placed in a plastic tube fixed over the neck to restrict its movement.

Experimental Groups

The pattern of experimental groups was the same as described previously by our group (46). Briefly, animals were housed in a specially controlled, noise-free environment (lights on from 8 AM to 8 PM, temperature $23 \pm 2°C$, humidity 45–55%) and fed ad libitum on a standard laboratory diet (Panlab) and water. The animals were randomly assigned to experimental groups: *1*) Co: control, intact animals (nonstressed); *2*) Co+Ac: acute stress, animals only immobilized for 30 min immediately before being killed; *3*) Ch: chronic stress, animals immobilized for 2 h daily during two periods (of 5 and 4 days), separated by 2 days of rest to avoid stress habituation (46); and *4*) Ch+Ac: chronic + acute stress, animals receiving the same Immo schedule as *group 3* plus 30 min immediately before being killed.

All procedures involving animals were approved by the Committee on Animal Bioethics and Care of the University of Barcelona and the Generalitat (Autonomous Regional Government) of Catalonia.

The animals were killed by decapitation between 2:00 and 3:00 PM. Immediately, a muscular pack from the upper hindlimb (containing gluteus superficialis, biceps femoris, semitendinosus, caudo femoralis, gracilis, rectus femoris, semimembranosus, and vastus lateralis) was removed and frozen in liquid nitrogen. White and red fibers in this skeletal muscle pack were more or less balanced. Other tissues were also removed and frozen in liquid nitrogen in the following order: complete pads of WAT (epididymal, retroperitoneal, and mesenteric), heart, and adrenal glands. A blood sample was collected in EDTA vessels.

LPL Activity Assays

Tissues were homogenized (150–200 mg/ml) in HEPES-dithio-threitol-EDTA-saccharose buffer (pH 7.5), containing heparin (5 mU/ml), and LPL activity was determined according to Ref. 22. As hepatic lipase in plasma cross reacts with LPL lipolytic assay, samples were preincubated (1:1) with an antihepatic lipase serum for 90 min at 4°C.

RNA Analyses and DNA Hybridization Probes

Total RNA was extracted from frozen rat tissues by using a commercial reagent (TriPure Isolation Reagent, Roche Diagnostics, Barcelona, Spain) based on the single-step RNA isolation method developed by Chomczynski and Sacchi (10). RNA dot blots were prepared, as described previously (37), filtered through a Minifold apparatus, and immobilized on positively charged nylon membranes (Roche Diagnostics). For Northern blots, a 1.36-kb cDNA fragment encoding rat LPL was used as a probe for hybridizations, and an oligo(dT) probe (Tibmolbiol, Berlin, Germany) for total mRNA was used as the loading control (results not shown). Digoxigenin-11-UTP (Roche Diagnostics) was used in accordance with the manufacturer's instructions for random-primed probe labeling. Prehybridization, hybridization, and detection were performed, as described in Roche Diagnostics' digoxigenin system user's guide. Each film was scanned and image-quantified by Phoretix software.

Plasma LPL Mass by Western Blots

Plasma samples were loaded in a 9% gel for subsequent polyacryl-amide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane (Bio-Rad, Segrate MI, Italy), followed by blotting in Tris-buffered saline containing 5% (wt/vol) nonfat dry milk. Incubation with chicken IgG against bovine LPL (kindly provided by Dr. T. Olivecrona, University of Umeå, Umeå, Sweden), 1:2,000, was performed overnight in the same buffer at 4°C. A rabbit horseradish peroxidase labeled against chicken IgG (Chemicon International, Hofheim, Germany), 1:10,000, was used for immunoblotting detection, with the SuperSignal West Pico Chemiluminescent Substrate system (Pierce, Rockford, IL). Each film was scanned and image-quantified by Phoretix software.

Studies of Regional Blood Flow in Conscious Rats

These studies are based on the intra-arterial infusion in the conscious animal of a known number of fluorescent microspheres of polystyrene through a catheter inserted in the ascending aorta (16). The microspheres are distributed in a few seconds throughout the body by cardiac output. The fluorescence obtained is directly related to the blood flow of the tissue. We used fluorescent microspheres of two colors in the same animal, as proposed in other studies (1), to test the blood flow variations in each selected tissue before and after acute stress. Below, we describe various improvements made to the method described and its adaptation to studies of stress.

Experimental groups and surgical procedures. To study the in vivo variations of tissue blood flow in the same animal (before and after stress by Immo), another group of male Wistar rats, chronically catheterized, was used. Two catheters or cannulas were inserted in all animals into the left carotid artery (Critchley Electrical, 0.80/0.50 mm, inner diameter/outer diameter) and the left femoral artery (Clay-Adams, 0.20/0.61 mm, inner diameter/outer diameter), to measure the concentration of microspheres in a reference blood sample. Catheters were inserted beneath the skin and tunneled subcutaneously as far as the back of the neck. They were then exteriorized, heat-sealed, and fastened with special plaster (armorlike) until the day of the experiment.

Standard rat chow and water were provided ad libitum throughout the study. The environmental conditions and the experimental Immo procedure were the same as described above. There were only two groups of animals, weighing around 250 g, randomized into the Co group and the Ch group. The acute effect of Immo was tested in the same animal (Co or Ch), with its baseline situation taken as (−) (one color of fluorescent microsphere) and its postacute stress situation as (+) (another microsphere color).

The animals were catheterized after the first 5 days of Immo (see MATERIALS AND METHODS). They were then housed in individual cages, to avoid their being bitten by another rat, and underwent the same

procedure of stress by Immo over the next 4 days (see MATERIALS AND METHODS). Chronic Immo sessions after catheterization were only begun if the growth rate was recovered after surgery. Microsphere infusion and blood sampling on the day of the experiment were done in the cage itself, thus avoiding additional stress to the animal and allowing its free movement. Blood flow was always measured without anesthesia, which let animals in the cage act spontaneously.

Sampling for blood flow measurements in vivo. As other studies in rats showed low blood flow in WAT (17, 28), we decided to improve the method by using microspheres with very intense fluorescence, such as yellow-green and orange ones, and with a 15-μm diameter (FluorSpheres Blood Flow Determination, Molecular Probes, Leiden, The Netherlands). The solutions used contained ~1,000,000 microspheres in a saline solution. In the same animal, the yellow-green microspheres (maximum emission to 505 nm) were used to study the baseline situation (−), whereas the orange microspheres (maximum emission to 554 nm) were infused after acute Immo (+). The dose used in each animal (700 microspheres/g body wt) was higher than in other studies (100–400), because we found that this dose showed minimal variability in the results from assayed WAT and blood.

On the day of the experiment, the microspheres (sonicated previously) were infused for 15 s with a Hamilton syringe through the cannula inserted into the left carotid artery. At the same time and using a peristaltic pump, a saline solution (37°C) was infused (0.4 ml/min) for 2 min and 30 s. Simultaneously, an extraction of reference blood (also 0.4 ml/min) was started through the other cannula (inserted into the left femoral artery). The reference blood was sampled for 60 s from the beginning of the microsphere infusion.

After this baseline situation (−), the cannulas were disconnected from the pump and sealed. The conscious animal was immediately immobilized for 30 min [acute stress (+)], and, at the end of this time, the chronic catheters were again connected to the peristaltic pump so as to repeat the infusion and extraction protocol: this time with the orange microspheres. The rats were then decapitated, immediately after acute stress, between 2:00 and 3:00 PM (as in the case of the main experiment), and tissues were dissected and weighed.

Treatment of the samples and quantification of fluorescence. Whole tissues and reference blood (minimum 1 ml) were digested in polypropylene tubes with 2 N KOH (in ethanol and Tween 20 to 0.05% vol/vol) in a relation of 1:10 (g/ml). The closed tubes were then left for 24–36 h at 60°C. They were occasionally shaken and always protected from light. The digests of whole tissues and blood were passed through glass fiber filter paper disks, which retained the microspheres (Whatman GF/D of 2.7-μm pore, Springfield Mill, Maidstone, UK) by means of a filtration collector system (Millipore, Bedford, MA). The filters were washed successively in distilled water, 2% Tween 20 (vol/vol) (in distilled water), and increased concentrations of ethanol solutions [from 50% (vol/vol) up to absolute ethanol]. The filters were then dried at 50°C (30 min) and placed in tubes containing 2.5 ml of 2-ethoxyethylacetate (cellosolve) (Merck, Darmstadt, Germany) for 90 min at room temperature and protected from light. The fluorochrome retained in the microspheres was dissolved in the organic solvent. After centrifugation at 200 *g* (5 min), the clear solution was transferred to quartz cuvettes, and its fluorescence was measured with an RF5001PC spectrofluorimeter (Shimadzu, Kyoto, Japan) to maximum fluorescence emissions of 505 nm (yellow-green) and 554 nm (orange). A standard of a known number of microspheres (yellow and orange together) was first processed in the same way as the samples (see paragraph above). We prepared five ascending solutions, each with a different total number of yellow-green microspheres (0, 100, 500, 5,000, and 10,000), along with five descending solutions of orange microspheres (10,000, 5,000, 500, 100, and 0) in the same kind of tube. Then, two standards were prepared, one for tissues and one for blood assays; either 1.5 g of a mixture of different rat tissues (representative of the body) or 1 ml of rat blood was added to each standard tube. The total number of microspheres retained (yellow-green and orange) in each tissue, or the microspheres diluted in the reference blood, was calculated by means of the corresponding standard.

The organ blood flows (F) were calculated from *1*) the reference blood flow (0.4 ml/min) (Fs); *2*) the total number of microspheres retained in the whole organ (No); and *3*) the number of microspheres from the reference blood sample collected in 1 min (Ns), following the equation F = (Fs × No)/Ns.

The aortic blood flow (A) was calculated from *1*) the reference blood flow (0.4 ml/min) (Fs); *2*) the number of microspheres infused (dose) (D); and *3*) the number of microspheres from the reference blood sample collected in 1 min (Ns), following the equation: A = (Fs × D)/Ns.

Unfortunately, the use of a microsphere injection protocol does not allow heart flow to be estimated, because the microspheres enter through the left carotid artery and go directly to the aortic arch, where they are rapidly diluted in the blood. They leave the left ventricle and are then distributed throughout the circulatory system, with no possibility of their moving back into the heart.

Statistics

Results were given as the means ± SE of 7–12 animals/group. Significance was assessed by ANOVA by using the Graph Pad Prisma program (Graph Pad Software). Individual comparisons were made using the Bonferroni *t*-test as follows: acute stress vs. control (to find acute effects), chronic stress vs. control (to find chronic effects), and chronic + acute stress vs. chronic stress (to find additional effects of acute over chronic stress). Statistical comparisons were significant when $P < 0.05$.

RESULTS

Figure 1 shows the variations in total LPL activity of each tissue. Acute stress in control animals had no effect on LPL activity in either skeletal (taken from the upper part of the hind leg) or cardiac muscle, although, in adrenal glands, there was a significant increase (*left*). However, in the adipose tissues (*right*), LPL activity decreased in retroperitoneal tissue (100 mU) and tended to decrease slightly with acute stress in mesenteric tissue, although these variations were not significant. Chronic stress caused increases (over the control group) in total LPL activity in both muscle (skeletal and cardiac) and adrenal glands (over 30 times more than in the control situation). In contrast, this LPL activity decreased in mesenteric and epididymal adipose tissue and remained unchanged in retroperitoneal tissue. In these latter tissues, chronic stress caused a total decrease of over 275 mU. In none of the tissues studied did acute stress add significantly to chronic stress.

The body weight of the chronically stressed animals was ~80% of the controls on the day they were killed (Table 1). As expected, acute stress did not cause any variations in this figure. The total weight of the tissues studied did not vary significantly under acute stress (Table 1). However, after chronic stress, the total weight of certain tissues changed noticeably (Table 1). The adrenal glands almost doubled in weight, while the heart's weight was somewhat lower and the weight of the adipose tissues decreased significantly more than in the nonstressed group.

Chronic stress decreased food intake (data not shown), especially during the first days of Immo. The amount of food the animals ate varied from 55% of intake compared with controls on the first day, up to 70% on the day the animals were killed (see previous study, Ref. 46).

To determine whether these differences in LPL activity in different tissues were due to variations in enzyme expression,
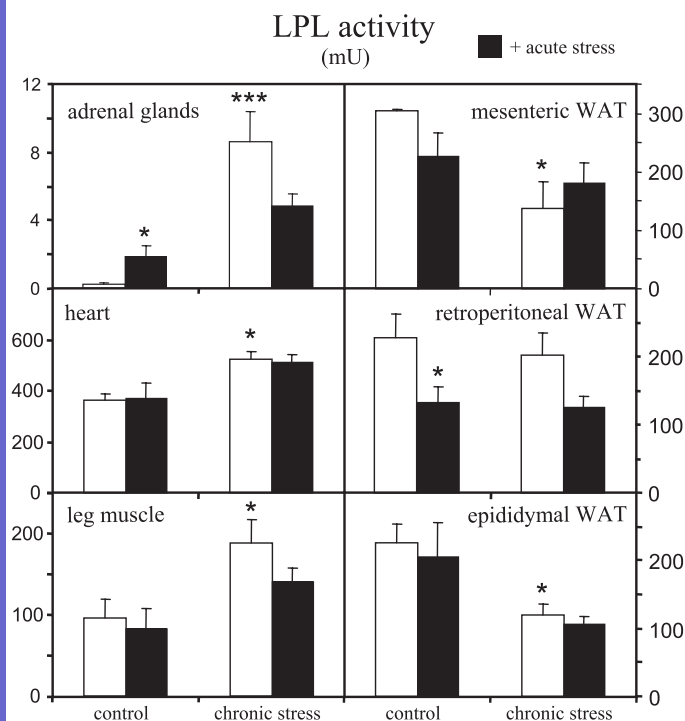
Fig. 1. Total lipoprotein lipase (LPL) activity in tissues from controls (Co) and rats chronically (Ch) and/or acutely stressed (Ac) by immobilization (Immo). At the end of the chronic Immo schedule (see MATERIALS AND METHODS), one-half of the animals from each group (Co and Ch) were exposed to 30 min of Immo, starting at 2 PM (Ac, solid bars) and then immediately killed. The remaining animals, nonacutely stressed (open bars), were killed at the same time. Values are expressed as means ± SE of $n = 7$ for Co groups or 12 for Ch groups. WAT, white adipose tissue. Statistical comparisons by ANOVA and Bonferroni t-test are as follows: both Ac and Ch vs. Co group (*$P < 0.05$, ***$P < 0.001$) and Ch + Ac vs. Ch group [not significant (NS)]. F values for adrenals = 7.710 ($P < 0.001$), heart = 5.399 ($P < 0.01$), leg muscle = 3.753 ($P < 0.05$), mesenteric WAT = 3.650 ($P < 0.05$), retroperitoneal WAT = 3.107 ($P < 0.05$), and epididymal WAT = 4.779 ($P < 0.05$).

Fig. 2. LPL mRNA by Northern blot and dot-blot assay in tissues from Co and Ch rats chronically stressed by Immo. *A*: single band (around 3.7 kb) in different Northern blot assays of tissues from nonstressed (Co) or Ch animals. Pool samples from each tissue were performed in duplicate. *B*: dot-blot assay of same tissues as in *A* plus liver, in nonstressed animals (Co). Different quantities tested for each tissue are indicated. eWAT, rWAT, mWAT: epididymal, retroperitoneal, and mesenteric WAT, respectively.

we analyzed LPL mRNA levels in the various tissues by means of dot blot (Figs. 2 and 3). The specificity of the signal produced by rat tissue RNA was assessed by Northern blot hybridization (Fig. 2). The LPL cDNA probe reacted with a single mRNA band in all tissues from the experimental groups studied (Fig. 2*A*). On use of this probe in dot-blot assays, the intensity of the band obtained was related linearly to the amount of RNA loaded, at least between 1 and 10 μg for heart and WAT, and between 5 and 45 μg for muscle and adrenal
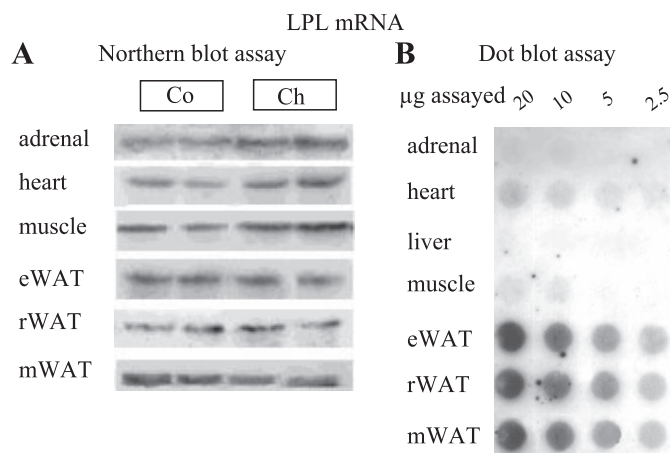
glands (data not shown). LPL mRNA was mainly expressed in WAT, with there being no differences between the different depots (Fig. 2*B*).

Figure 3 gives the results of the quantification by dot blot of LPL mRNA in the various tissues. As expected, 30-min acute stress caused no change in this parameter in any of the tissues. However, chronic stress significantly increased LPL-mRNA levels in the heart and skeletal muscle, but the increase was not significant in the adrenal glands. These increases can also be observed in the Northern blots shown in Fig. 2*A* and are due to the increased expression of the same single mRNA band. Furthermore, they coincide with the increases in total LPL activity detected under the same conditions in heart and skeletal muscle (see Fig. 1). However, the abovementioned decreases in total LPL activity detected in adipose tissues under acute and chronic stress (see Fig. 1) were not caused by a decrease in the levels of the enzyme's mRNA (Fig. 3).

Figure 4 shows that both acute and chronic stress induced a significant twofold increase in plasma LPL activity levels. However, no effect of acute stress over chronic stress was observed. To determine whether this increase in plasma LPL activity was reflected in an increase in the levels of this protein,

Table 1. *Body and tissue weights in control and chronically and/or acutely stressed rats due to immobilization*

	Control			Chronic Stress			
		Co+Ac		Ch		Ch+Ac	
Statistics vs. Co or Ch	Co		vs. Co		vs. Co		vs. Ch
Body weight, g	315±2	310±2	NS	259±5	↓ ↓ ↓	253±5	NS
Adrenals, mg	35±1	37±3	NS	59±5	↑ ↑	46±3	↓
Heart, g	0.97±0.03	1.00±0.03	NS	0.80±0.03	↓ ↓ ↓	0.79±0.03	NS
Mesenteric WAT, g	2.6±0.3	1.8±0.1	NS	1.1±0.2	NS	1.2±0.2	NS
Retroperitoneal WAT, g	3.09±0.32	2.90±0.23	NS	1.95±0.16	↓ ↓	1.75±0.12	NS
Epididymal WAT, g	1.87±0.12	2.13±0.12	NS	1.40±0.08	↓ ↓	1.29±0.06	NS

Values are means ± SE of $n = 7$ for control groups or 12 for chronic stress groups. Co, control; Co+Ac, acutely stressed; Ch, chronically stressed; Ch+Ac, chronically and acutely stressed; WAT, white adipose tissue; NS, not significant. Statistical comparisons by ANOVA and Bonferroni t-test vs. Co or Ch groups are shown by ↑ (higher than) and ↓ (lower than). One arrow, $P < 0.05$; two arrows, $P < 0.01$; three arrows, $P < 0.001$.
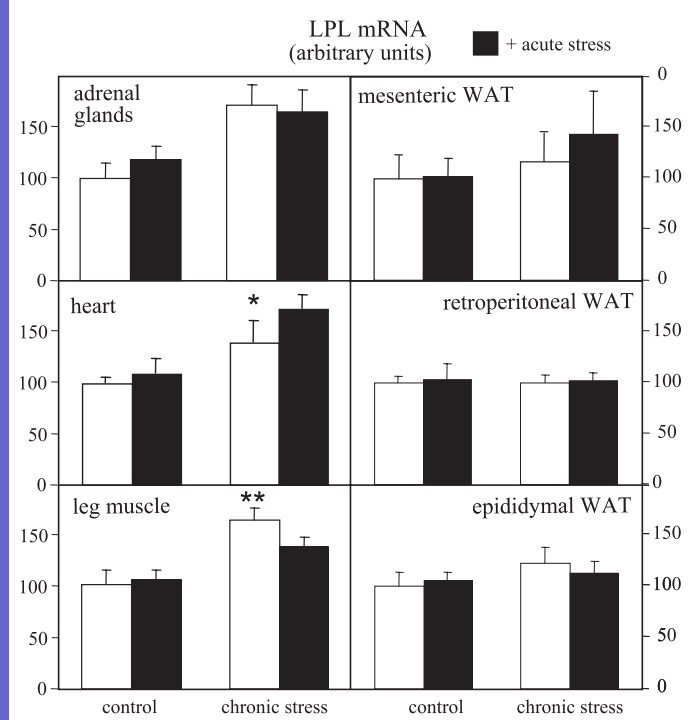
Fig. 3. Quantification of LPL mRNA by dot-blot assay in tissues from Co and Ch and/or Ac rats stressed by Immo. One dot-blot assay was performed for each tissue. Quantification is expressed as percentage of corresponding Co group. Values are means ± SE. Statistical comparisons by ANOVA and Bonferroni t-test are as follows: both Ac and Ch vs. Co group (*$P < 0.05$, **$P < 0.01$) and Ch + Ac vs. Ch group (NS). F values for adrenal glands = 2.839 (NS), heart = 3.981 ($P < 0.05$), leg muscle = 6.077 ($P < 0.01$), mesenteric WAT = 0.359 (NS), retroperitoneal WAT = 0.036 (NS), and epididymal WAT = 0.466 (NS).

we measured the mass of plasma LPL (monomeric form) by means of Western blot (Fig. 5). It can be seen (Fig. 5A), in each experimental group, that the antibody recognizes a single band with an estimated mass of 60 kDa, corresponding to the molecular mass of the LPL monomer. Chronic stress increases the levels of the enzyme present in plasma. Quantification by densitometry (Fig. 5B) shows that chronic stress increases plasma LPL levels by 40%. This increase, though considerable, is much less than that in enzyme plasma activity (70%) (Fig. 4).
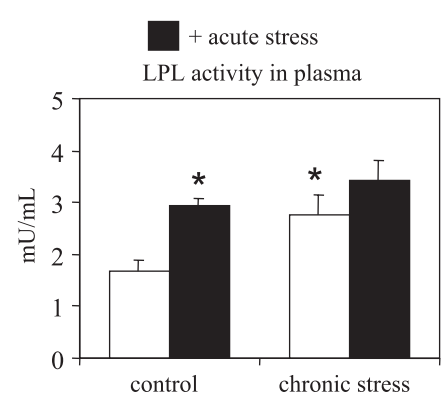

Fig. 4. LPL activity in plasma from Co and Ch and/or Ac rats stressed by Immo. Values are means ± SE. Statistical comparisons by ANOVA (F value = 5.865, $P < 0.001$) and Bonferroni t-test are as follows: both Ac and Ch vs. Co group (*$P < 0.05$) and Ch + Ac vs. Ch group (NS).
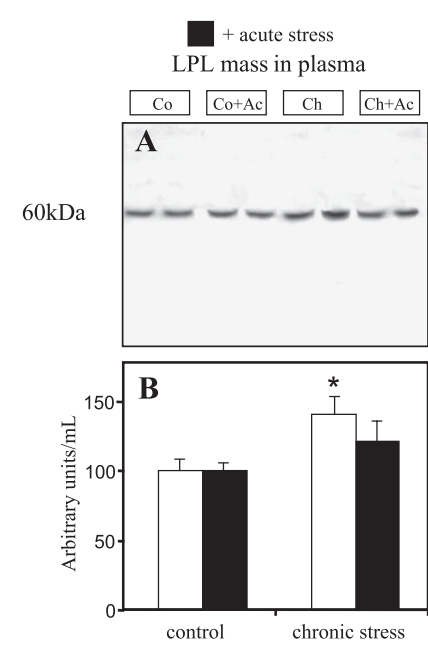

Fig. 5. LPL mass by Western blot assay in plasma from Co and Ch and/or Ac rats stressed by Immo. Co+Ac, acutely stressed; Ch+Ac, chronically plus acutely stressed animals. A: Western blot of pool samples from each group performed in duplicate. B: quantification of LPL mass of 5–8 individual Western blots, with each group expressed as percentage of the Co group present in the same membrane. Values are means ± SE. Statistical comparisons by ANOVA (F value = 2.867, $P < 0.05$) and Bonferroni t-test are as follows: both Ac and Ch vs. Co group (*$P < 0.05$) and Ch + Ac vs. Ch group (NS).

In a parallel study, we developed a method of determining tissue blood flow in the same animal before the baseline situation (−) and after Immo [acute stress (+)], by using two types of fluorescent microspheres (see MATERIALS AND METHODS). Table 2 shows the changes in blood flow per unit of weight in the studied tissues as a result of Immo, so that these can be compared with the variations in total LPL activity in the same tissues (see Fig. 1). They are shown in descending order in the control situation. The greatest flow was found in the aorta, and the least in WATs. Both Co+Ac and Ch significantly increased blood flow in the adrenal glands. Aortic flow also tended to increase with stress, but the differences were not significant.

In general, blood flow in WATs was 10 times less than in leg muscle, spleen, and diaphragm, and 100 times less than in the adrenal glands, kidney, and lung. For WAT, acute stress (+) significantly increased blood flow in both the Co and Ch groups. Although in absolute terms the increase was small, it was double that found in the corresponding situation without acute stress. We also studied the flow in other tissues, to see whether the variations found in adipose tissues were a generalized response to Immo. Acute stress was found to increase flow considerably in kidney and the adrenal glands, regardless of whether the animals had also been chronically stressed. In liver, however, this increase was only observed when animals had not been chronically stressed. The remaining tissues studied showed no important changes in flow in response to stress by Immo.

DISCUSSION

Changes in LPL Activity in Tissues

Our model of stress caused LPL to behave differently in the various tissues. Total LPL activity in muscle and heart in-

Table 2. *Tissue blood flow in controls and rats chronically and/or acutely stressed by immobilization*

Acute stress Statistics vs. Co or Ch	Control			Chronic Stress			
	−	+	vs. Co	−	vs. Co	+	vs. Ch
Aortic, ml/min	94±4	129±21	NS	126±16	NS	121±12	NS
Adrenal glands	4.6±1.3	9.4±1.3	↑	10.2±1.5	↑	11.1±2.2	NS
Kidney	2.9±0.4	6.0±0.5	↑ ↑ ↑	3.1±0.4	NS	5.6±0.7	↑
Lung	1.0±0.2	1.1±0.3	NS	1.2±0.2	NS	1.5±0.3	NS
Diaphragm	0.76±0.08	0.63±0.08	NS	0.83±0.8	NS	0.82±0.13	NS
Spleen	0.72±0.18	0.49±0.07	NS	0.89±0.19	NS	0.63±0.14	NS
Leg muscle	0.41±0.06	0.40±0.06	NS	0.40±0.06	NS	0.37±0.05	NS
Liver	0.09±0.02	0.22±0.03	↑ ↑	0.08±0.02	NS	0.09±0.02	NS
Retroperitoneal WAT	0.08±0.03	0.16±0.04	↑	0.04±0.01	NS	0.15±0.04	↑
Epididymal WAT	0.02±0.01	0.05±0.02	↑	0.03±0.01	NS	0.07±0.01	↑

Values are expressed as means $\pm$ SE in $ml \cdot min^{-1} \cdot g\ tissue^{-1}$ (except for aortic); $n = 6$ for control groups or 5 for chronically stressed groups. Each animal was studied both nonacutely and acutely stressed: Co and Ch animals were studied before (−) and after (+) 30 min of acute stress immobilization. Statistical comparisons by ANOVA and Bonferroni *t*-test vs. Co or Ch groups are shown by ↑ (higher than) and ↓ (lower than). One arrow, $P < 0.05$; two arrows, $P < 0.01$; three arrows, $P < 0.001$. F values for aortic flow = 0.912 (NS), adrenals = 4.354 *(P < 0.05)*, kidney = 10.56 *(P < 0.001)*, lung = 0.688 (NS), diaphragm = 0.063 (NS), spleen = 1.233 (NS), leg muscle = 0.084 (NS), liver = 8.503 *(P < 0.01)*, retroperitoneal WAT = 6.020 *(P < 0.01)*, and epididymal WAT = 7.430 *(P < 0.01)*.

creases with chronic stress. These increases are not related to the changes observed in tissue weight: muscle weight did not vary, and heart weight decreased slightly after chronic stress. In these tissues, LPL activity increases in parallel to LPL mRNA levels, showing that tissue activity rises together with protein expression. Our results also suggest that, in chronic stress, adrenal glands increase LPL expression.

We found that increased mRNA corresponds to a single band of ~3.7 kb observed in all tissues studied except liver, in which LPL is expressed only in special situations (37). Other authors also described a single mRNA band of 3.6 kb in the same rat tissues (24, 25).

The increased LPL activity in these tissues from chronically stressed rats could be related to high catecholamine levels in plasma. It is well known that catecholamines are highly released under stress (66). On the other hand, it has been reported (12) that 4-day infusion to rats of the β-agonist isoproterenol increased muscle LPL activity. Heart heparin-releasable LPL is also increased by isoproterenol perfusion in rat (52). Other authors using muscle inactivity (hindlimb Immo) have described how LPL activity in muscle is regulated by contractile activity through local signals (5), with LPL increasing during physical exercise and decreasing during physical inactivity (18). Two studies of Immo (Ref. 5 and the present study) found opposing effects of muscle LPL activity. In the earlier study, restraint and stress were avoided, and large decreases in muscle LPL activity during acute and chronic leg inactivity were observed; whereas, in the present study, stressful restraint, causing a fight-or-flight response, was induced, which had a very different effect, increased activity, on muscle LPL.

In fact, the control of LPL activity is tissue specific: in heart and muscle it is associated with transcriptional changes, but this is not the case in other types of tissue, such as WAT (25).

Overall, unlike in muscle, stress significantly decreases total LPL activity in WAT by ~100 mU under acute stress and 275 mU under chronic stress. However, as our results show, there are differences in the response profile of WAT types to acute and chronic stress, probably due to their different sensitivities to the same stimulus. Similarly, other studies (45) described the variable response of LPL activity and the regional specificity of fat accumulation at different hormone concentrations.

The food intake of chronically stressed animals was 30% less than that of control animals, which suggests that insulin could be involved in modifications of LPL activity. However, in a previous study, our laboratory demonstrated that, under our experimental conditions of chronic stress, the plasma levels of insulin did not change vs. controls (46). So, both in chronic and in acute stress, circulating catecholamines are probably the main factor responsible for changes induced by Immo in LPL activity in WAT. Our laboratory found (3) that epinephrine administration in vivo decreased WAT LPL activity. Other studies reported that 4-day infusion to rats of the β-agonist isoproterenol (12) decreased WAT LPL activity. This effect could be due to increased cAMP levels, since WAT LPL activity decreased in rats treated with cholera toxin (20, 26).

The different sensitivities observed in different fat depots due to Immo are probably related to differences in the pool of adrenergic receptors (30) and the physiological role of each WAT in the body. For instance, subcutaneous WAT is less sensitive to circulating catecholamines than other WAT depots (47).

Surprisingly, the decrease in LPL activity observed in WAT from chronically stressed rats was not accompanied by a decrease in LPL mRNA levels. Similarly, although mRNA expression increases in fasting, LPL activity in WAT drops, probably as a result of enzyme redistribution to the degradation pathway (13). Many studies have demonstrated that the intracellular regulation of LPL is complex and may occur at the level of transcription, translation, and/or posttranslational processing. Some have shown that LPL changes in response to the addition of epinephrine. In fact, the degradation of newly synthesized LPL is known to increase in adipocytes exposed to epinephrine (2). Also, when adipocytes in culture were treated with epinephrine, LPL translation was inhibited (43), through the production of an RNA binding protein (30 kDa) that binds to a region on the proximal 3′ untranslated region of the LPL mRNA by a PKA-mediated mechanism (44). This factor or protein is only present in cytoplasmic extracts of adipocyte cell lines previously treated with epinephrine. However, in epinephrine- or isoproterenol-treated rats (3), there is an increased release of active LPL from WAT to plasma and/or degradation of active LPL. In this study, WAT extracellular active LPL

(heparin-releasable) was decreased by epinephrine, whereas intracellular active LPL was not affected by it, which suggested an increased release to plasma of active extracellular LPL from this tissue (3).

Thus the rapid (with only 30 min of Immo) decrease observed in retroperitoneal WAT in acutely stressed rats might be due to an increased release of LPL as a result of high catecholamine levels. The LPL decrease in mesenteric and epididymal WAT under chronic stress might be attributed to long-term epinephrine action, reducing the secretory pathway from adipocyte to endothelium due to inhibition of its intracellular processing, as well as to sustained LPL release into the bloodstream.

LPL Activity in Plasma

Under stress, LPL activity increases in plasma. This increase is rapid, occurring in only 30 min of Immo.

It is well known that intravenous injection of heparin increases LPL activity in plasma dramatically (35) and that, in preheparin plasma, LPL mass is high, but active LPL is very low (the pathological significance of preheparin LPL mass and activity in plasma was recently reviewed; Ref. 27). Nevertheless, it has been reported that LPL activity in plasma does increase in various situations, such as surgery (49), fasting (9), and exercise (18), and that LPL has a circadian rhythm (4). There are also several substances that cause the release of the enzyme to the plasma, such as intralipid (38), epinephrine (3), cholera toxin (20, 26), or tumor necrosis factor-α (51). It has been shown that increased plasma LPL activity does not directly correlate with LPL mass increase (53), as we observed in acute Immo.

We suggest that the increase in LPL activity observed in plasma with stress could be due to an increased release of the enzyme from some WAT depots, as in the case of retroperitoneal WAT in acutely stressed rats. Thus increased release of LPL as a result of high catecholamine levels explains the rapid decrease of LPL activity in WAT under stress.

Because of the liver's high LPL uptake rate (57), one would expect plasma LPL to decrease rapidly after its release from tissues due to a stress stimulus. However, our chronic stress results show that, at time of death, 3 h after the last Immo, LPL activity remains high in plasma. This could be due to sustained LPL release or desensitization of liver uptake during the Immo period for acute and chronic stress groups, as well as during the first few hours after a stress session for chronically stressed animals. This effect is probably due to catecholamines: the effects of epinephrine on metabolism and heart rate persist long after their return to baseline levels (15). Recently, we found that, 24 h after the last Immo in chronic stress, plasma LPL activity was similar to that in control animals (D. Ricart-Jané and M. Llobera, unpublished observations). Thus LPL activity levels return to baseline when stress is removed, but this process takes more than 3 h. This means that the increased LPL activity in plasma is extended in time after acute stress, but does not become chronic.

We conclude that this phenomenon occurs only during and after acute Immo stress and under β-adrenergic stimulation. Recently, we found that a β-adrenergic blocking agent (propranolol, 30 mg/kg ip injected 90 min before the 30 min of Immo) prevents the acute stress-induced changes in plasma LPL activity (D. Ricart-Jané and M. Llobera, unpublished observations). Also, after surgery in rats (which increases catecholamine levels), LPL activity increases in plasma (49).

Because LPL did not decrease in muscle with acute stress, it seems that WAT is the main source for LPL increase in plasma. However, in chronic stress, we cannot rule out that the muscle might also contribute to increased plasma LPL activity due to increased expression of the enzyme in this tissue. Another experimental approach will be needed to tackle this question.

Circulating Candidates Involved in LPL Release From Tissues

Several candidates involved in LPL release and stabilization in plasma have been described: TAG-rich lipoproteins (56) or TAG emulsions (21), heparan sulfate oligosaccharides (35), or free fatty acids (FFA) (50). Some reports have suggested that fatty acids exported from tissues, which also increased in our stressed animals (46), could release active LPL from tissues in vivo (39), although no correlation has been found between LPL release and local WAT FFA concentrations (23). Furthermore, FFA failed to release LPL from perfused rat hearts (48). New experiments will be needed to tackle this question.

In addition, the in vivo administration of TNF-α (51, 54) and lipopolysaccharide (40) to rats decreases LPL activity in WAT. In both cases, these effects were related to inducible nitric oxide (NO) synthase expression and NO production. As NO rises with Immo stress (31) and is the main local blood flow modulator (34), it could have an additional function in the local LPL release mechanism from endothelial cells during Immo. Further studies are required in this field.

Local Blood Flow and Distribution of Energy Substrates Between Tissues

In the present study, the only tissue in which LPL decreased and flow increased under acute stress was WAT, in which blood flow doubled. The total weight of these tissues did not vary in 30 min of acute stress, as happens under epinephrine treatment in vivo (3) and in the main experiment of this study (Table 1), so the increases of blood flow expressed per unit of tissue weight were not affected by the weight of WAT. Other local and short-term mechanisms may be involved in the regulation of blood flow. Under chronic stress, no variations in blood flow were observed in WAT. However, the decreased weight of WAT during chronic stress must have increased the blood flow (per unit of weight) in these tissues. This means that other compensatory long-term mechanisms could affect total blood flow in WAT, decreasing in parallel with weight. However, flow probably increases during every Immo session in the chronic group, because it increased in rats subjected to additional acute stress.

Published studies give a baseline flow value for different types of WAT in different species of between 0.03 and 0.3 ml·min^{-1}·g tissue^{-1} (11), within which our values fall. WAT blood flow is largely regulated by the adrenoreceptors in blood vessels. Circulating catecholamines, such as those produced during stress, act via the β-receptors and have a vasodilating effect; whereas innervation induces vasoconstriction via α-receptors (11). There are several physiological situations in which WAT blood flow varies: exercise (6) and fasting (33, 14)

increase blood flow two- or threefold in different WAT depots in rats and humans, an effect that depends on the WAT type. However, during feeding in rats, WAT blood flow decreases (59), whereas LPL activity only increases in mesenteric and retroperitoneal WAT, with no changes occurring in epididymal and subcutaneous WAT. The reason blood flow through WAT decreases during feeding could be to favor TAG uptake.

In contrast to feeding, during stress by body Immo, a situation characterized by the mobilization of energy substrates, as our laboratory previously described (46), increased blood flow could help export FFA from WAT to other tissues, as in exercise and fasting. In this physiological context, LPL activity in WAT is not necessary and so decreases. However, TAG is required in muscle to prepare the animal for fight or flight and thus for exercise. Then LPL increases in muscle to uptake TAG (synthesized in the liver from WAT-derived FFA) and satisfy its energy demands, as has been described for training exercise (18).

Finally, increased flow in the adrenal glands with stress would *1*) increase the effect of pituitary hormones, such as ACTH; *2*) increase the capture of circulating substrates (TAG, as a result of the enormous increase in LPL activity) for hormone synthesis; and *3*) facilitate the export of synthesized hormones to the target tissues. As there was no increase in messenger levels and 30 min seems too short a time for synthesis to be activated, it may be that, during acute stress, adrenal glands uptake circulating LPL as a rapid source of enzyme to increase activity. Increased blood flow would facilitate this by increasing the amount of enzyme reaching the tissue. Other authors have suggested a similar process of LPL redistribution from muscle to WAT during feeding (23). Moreover, in vitro experiments have shown that the LPL dimer has a high affinity for heparin sulfates and that it rebinds to the endothelium after its release (32).

In conclusion, the main finding of this study is that stress modifies LPL activity and expression in LPL-rich tissues. The stress increases LPL activity in muscle, decreases it in WAT, and also increases it in plasma. Under acute stress, the rapid decrease of LPL activity in some WAT depots could be due to an increase in the release of the enzyme, a nonexplored mechanism of tissue LPL regulation. Under chronic stress, other posttranscriptional events must also be involved. Moreover, in all WAT depots studied, blood flow doubled. Overall, these changes allow the lipid energy-substrate redistribution between tissues that the organism needs to prepare for the fight-or-flight response.

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