Retroperitoneal white adipose tissue lipoprotein lipase activity is rapidly down-regulated in response to acute stress

Albert Casanovas, Núria Parramon, Ferran de la Cruz, Olga Andrés, José Terencio, M. Dolores López-Tejero, and Miquel Llobera[1]

Departament de Bioquímica i Biologia Molecular, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain

Abstract **Tissue-specific regulation of LPL has been widely studied in rats. Previous studies reported that in vivo administration of adrenaline and acute stress cause an increase in plasma LPL activity coinciding with a decrease in white adipose tissue (WAT) LPL activity. We studied the speed of LPL activity changes during 30 min of stress by immobilization (IMMO) in rats. A first experimental approach in permanently cannulated rats permitted sequential blood sampling in the same animal during IMMO and the obtaining of hemodynamic parameters. In a second experimental approach, animals were euthanized at different times after the start of IMMO to determine LPL activity in tissues. Stress was characterized by rises in blood pressure, heart rate, plasma corticosterone, and available circulating energy substrates. Five min after the start of IMMO, LPL activity fell in retroperitoneal WAT and increased in plasma. These data show the quickest LPL activity change ever described in response to a physiological situation. The speed and simultaneity of these changes suggest that the release from endothelium to the bloodstream may constitute a fast nonexplored mechanism of tissue LPL activity regulation, involved in the lipid energy-substrate redistribution between tissues needed to prepare the "fight-or-flight" response.—** Casanovas, A., N. Parramon, F. de la Cruz, O. Andrés, J. Terencio, M. D. López-Tejero, and M. Llobera. **Retroperitoneal white adipose tissue lipoprotein lipase activity is rapidly down-regulated in response to acute stress. *J. Lipid Res.* 2007.** 48: **863–868.**

Supplementary key words plasma • fight-or-flight • rat • immobilization • blood pressure • heart rate

LPL belongs to the triacylglyceride (TAG) lipase family that includes pancreatic lipase, hepatic lipase, and endothelial lipase (1). The enzyme is synthesized and secreted by parenchymal cells from a variety of tissues, notably in adipose and muscle. After its secretion, LPL migrates to the luminal surface of endothelial cells, where it is anchored to heparan sulfate proteoglycans. After a short half-life (2), normal pathway for degradation of LPL at the capillary is believed to be the endothelium-plasma-liver pathway. LPL is released from vessels to plasma, where it is present mainly in an inactive form (3), and readily captured by the liver, in which degradation takes place (4). At the vascular surface, LPL acts as a homodimer and its primary function is the hydrolysis of circulating TAG from chylomicra and VLDLs (generating fatty acids), which can be stored as TAG in adipocytes, used as a source of energy in muscle, or reesterified for TAG synthesis in mammary glands.

LPL is under tissue-specific modulation and has a key role in whole-body energy balance. It has been widely reported that white adipose tissue (WAT) and muscle LPL are inversely regulated in several situations, such as exercise (5), detraining of runners (6), feeding and fasting (7), insulin-glucose infusion (8), rexinoid administration (9), and/or stress (10).

LPL expression and activity in tissues are sensitive to a wide array of hormones. Catecholamines are one of the most powerful factors involved in LPL regulation, with opposite effects on LPL-rich tissues. In previous studies in the rat (11), we first reported that in vivo administration of catecholamines decreases LPL activity in WAT and increases it in plasma. Later, we studied the response to immobilization stress (IMMO) in the rat (10), as an in vivo model of endogenous secretion of adrenaline. Chronic IMMO (2 h daily for 10 days) decreased total LPL activity in mesenteric and epididymal WAT and increased LPL activity in limb muscle, heart, and adrenal glands. In contrast, acute stress (30 min IMMO) caused a decrease in LPL activity only in retroperitoneal WAT (rWAT). Both chronic and acute stress induced a significant increase in plasma LPL activity.

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Abbreviations: IMMO, immobilization; rWAT, retroperitoneal white adipose tissue; TAG, triacylglyceride; WAT, white adipose tissue.

[1] To whom correspondence should be addressed.
e-mail: millobera@ub.edu

These data suggest that acute stress and/or catecholamine administration could release LPL from adipose tissue to plasma. This might be interpreted as a rapid mechanism that enables this tissue to change from TAG importer to TAG exporter. If this regulator mechanism is a part of the metabolic response known as "fight-or-flight", which is characterized by increases in available energy substrates (12, 13), cardiac rate, and blood pressure (14), it has to be extremely fast.

The aim of the present study was to determine the speed of LPL activity changes in plasma and tissues during 30 min of IMMO in the rat. In one experimental approach (Experiment 1), permanent cannulation of rats was performed to study hemodynamic parameters and to allow sequential blood sampling in the same animal during IMMO. In a second experiment (Experiment 2), animals were euthanized at different times after the start of IMMO to determine LPL activity variations in tissue during acute stress.

EXPERIMENTAL PROCEDURES

Animals

Male Wistar rats (Harlan Interfauna Ibèrica, Barcelona, Spain) weighing 220–250 g upon arrival were housed in a controlled noise-free environment (lights on from 8:00 AM to 8:00 PM, temperature at 23 ± 2°C, humidity at 45–55%) and fed ad libitum with a standard rat chow diet and water.

Stress

Rats were acutely stressed by IMMO. The procedure was similar to that described by Kvetnansky and Mikulaj (15) and used previously by our group (10, 12). Briefly, the rats were attached to wooden boards in prone position by taping their forelimbs and hind limbs to metal mounts. Head motion was restricted by introducing it into a transparent plastic tube fixed over the neck area. Experiments were always done between 2:00 and 4:00 PM to minimize the effects of circadian rhythms on the LPL activity described elsewhere (16). 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, Spain.

Experiment 1

To make individual monitoring of blood parameters possible during 30 min of IMMO, rats were cannulated as described below. Animals were only submitted to stress if their growth rate recovered after surgery.

Cannulation procedure. A cannula was implanted into the carotid artery to record blood pressure in conscious animals. Briefly, rats were anesthetized by intraperitoneal injection of ketamine (90 mg/kg body weight) and xylacine (10 mg/kg body weight). A polyethylene cannula (PE50), especially shaped in our laboratory and filled with 1% (w/v) citrate isotonic solution, was passed subcutaneously until coming out at the back of the neck. The cannula was introduced into the common carotid artery until it reached the aortic arch and then tied. After cannulation was completed, the cannula was filled with a 3% (w/v) citrate solution (citrate lock) to avoid thrombus formation and sealed with a metallic tip. Rats were left in their original individual cages to avoid their being bitten by another rat; and the citrate lock was renewed every two days. Heparin solution was not used in this experiment to avoid its well-known ability to release LPL from its endothelial anchorage (17).

Hemodynamic measurements and blood sampling. On the day of the experiment, fluency of the arterial cannula was checked. A first blood sample was withdrawn directly from the cannula into a heparinized glass capillary (Clinitube, Radiometer, Copenhagen, Denmark), sealed, and left on ice. The animal was placed in a cage and the cannula was connected to the assembly for recording hemodynamic parameters. Briefly, a swivel, placed in a holder above the cage, was connected to a pressure transducer (SensoNor 840, SensoNor, Horten, Norway) through a polyethylene tube. A two-way stopcock with a syringe connected to the transducer was used to fill the circuit with 1% (w/v) citrate isotonic solution. Arterial blood pressure and heart rate were recorded continuously from the start of the experiment by means of software (DATAWIN, Panlab, Barcelona, Spain) switched on to the pressure transducer through an amplifier. After the rat cannula was connected, it was filled with fresh 1% (w/v) citrate solution and the rat was left undisturbed until a second blood sample was withdrawn 23 min later, i.e., 2 min before starting IMMO (time −2). For this second sample, the circuit was opened and blood was left to flow passively until reaching the stopcock. The stopcock was then closed and the sample was withdrawn at the level of the swivel where blood was undiluted. After this sample, the animal was immobilized at time 0 for 30 min, as described above. During this IMMO period, three samples were withdrawn at the level of the animal cannula (5, 15, and 25 min after starting IMMO). After 30 min of IMMO, the animal was released and put back into its cage, and 2 min later another sample was withdrawn at the level of the swivel, as described above. During the entire experiment, the fluency of the circuit was maintained by filling with fresh citrate when necessary, with the aim of obtaining a good pressure signal. Thus, from each animal we recorded hemodynamic parameters continuously throughout the experiment and obtained blood samples (≈ 80 μl) at 25 and 2 min before starting IMMO (times −25 and −2; **Figs. 1 and 2**), at 5, 15, and 25 min after starting IMMO (times 5, 15, and 25; Figs. 1 and 2) and 2 min after the end of the 30 min of IMMO (time 32; Figs. 1 and 2).

Heparinized glass capillaries containing blood samples were centrifuged (2,000 × *g*, 15 min, 4°C) and hematocrit was determined. Using a fine knife, the glass capillary was cut at the level of the fibrin clot, and plasma was discarded into a tube. Plasma was then aliquoted for biochemical determinations and stored at −80°C until assayed.

Experiment 2

Intact animals, not manipulated by surgery, were randomly assigned to one of five experimental groups: control or nonstress (0 min of IMMO, time 0), 5, 15, or 25 min of IMMO (times 5, 15, and 25), or 30 min of IMMO followed by 2 min of rest (time 32) (**Figs. 3–5**).

Blood and tissue sampling. Rats were decapitated immediately after IMMO. The 2 rats inhabiting each cage were immobilized and euthanized simultaneously. Blood was collected from the neck, placed into a recipient with EDTA as anticoagulant and kept on ice until centrifugation (2,000 × *g*, 15 min, 4°C). Hematocrit was determined and the separated plasma was stored at −80°C until biochemical determinations. After blood collection, complete pads of WAT (epididymal, retroperitoneal, and mesenteric), interscapular brown adipose tissue, a muscular pack from the upper hind limb, and the heart were harvested, weighed, immediately frozen in liquid nitrogen, and stored at −80°C.

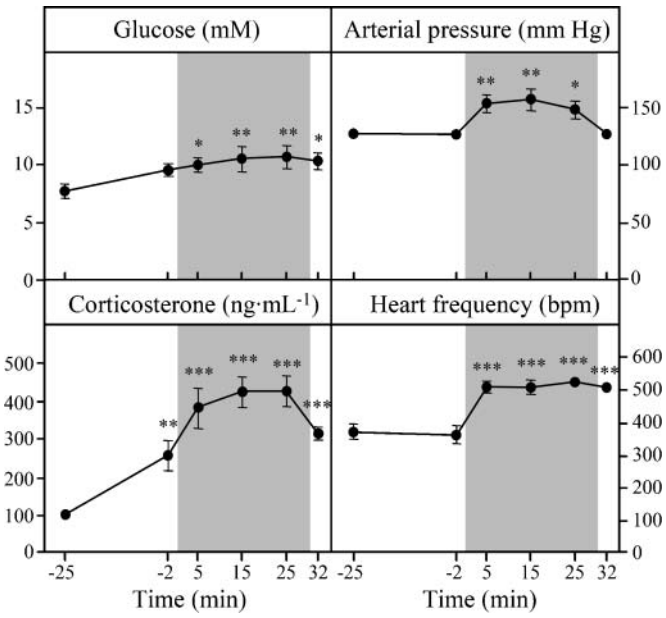

Fig. 1. Plasma and hemodynamic parameters during acute stress by IMMO in cannulated rats (Experiment 1). Heart rate frequency in beats per minute (bpm). Shaded areas correspond to immobilization period. Results are expressed as means ± SEM of 6 animals. Each animal contributes with one value to each time studied. Statistical comparisons by repeated-measures ANOVA and post hoc Tukey tests. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, compared with basal situation (−25 min).

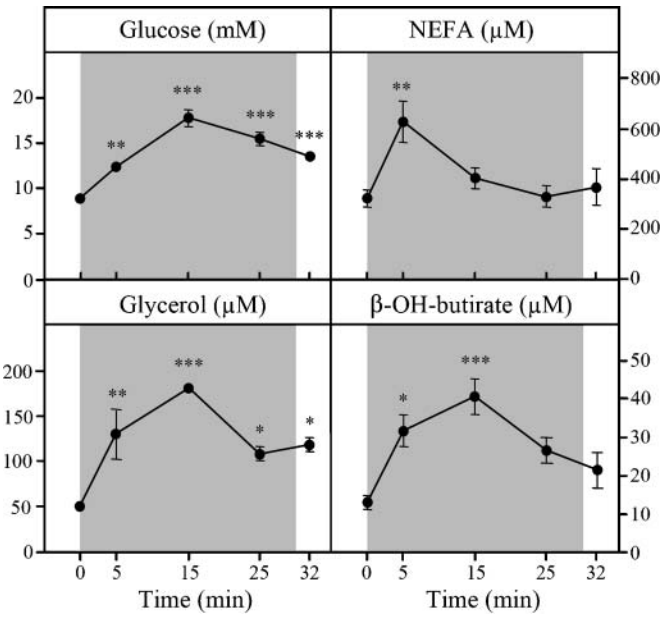

Fig. 3. Plasma metabolites during acute stress by IMMO in rats (Experiment 2). Shaded areas correspond to immobilization period. Results are expressed as means ± SEM of 6 animals/group. Statistical comparisons by one-way ANOVA (time of stress) and post hoc Tukey tests. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, compared with basal situation (0 min). β−OH-butyrate, β-hydroxybutyrate; NEFA, nonesterified fatty acids.

Plasma determinations

Plasma samples were tested for the following parameters: corticosterone was determined by radioimmunoassay, as described elsewhere (18); glucose, by a commercially available kit from HoribaABX (Montpellier, France); and NEFA, by a commercially available kit from Wako Chemicals (Neuss, Germany). For glycerol (19) and β-hydroxybutyrate (20) analysis, plasma was previously deproteinized (70:3 v:v) in perchloric acid 60% (w/v).

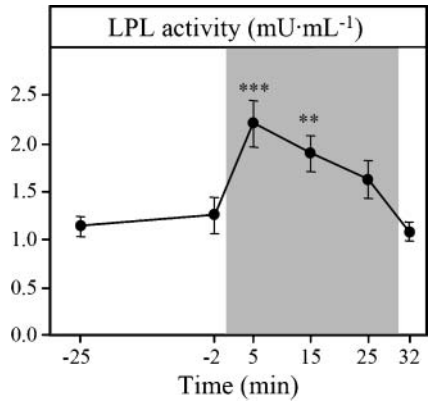

Fig. 2. LPL activity in plasma during acute stress by IMMO in cannulated rats (Experiment 1). Shaded area corresponds to immobilization period. Results are expressed as means ± SEM of 6 animals. Each animal contributes with one value to each time studied. Statistical comparisons by repeated-measures ANOVA and post hoc Tukey tests. ** $P < 0.01$; *** $P < 0.001$, compared with basal situation (−25 min).

LPL activity assay

Tissues were homogenized (≈ 200 mg) immediately before LPL activity assay in 1 ml of buffer (10 mM HEPES, 1 mM EDTA, 1 mM DTT, 5 U/ml heparin, pH 7.5). LPL activity was determined as described elsewhere (21). As hepatic lipase in plasma cross-reacts with LPL lipolytic assay, samples were previously incubated (1:1 v:v) with antihepatic-lipase serum for 90 min at 4°C.

Statistics

Results are given as mean ± SEM of six animals/group. Data obtained in Experiment 1 were statistically analyzed by repeated-measures ANOVA; and data obtained in Experiment 2, by one-way ANOVA. In both cases, a post hoc Tukey test was used to compare the effect of different times of stress with the basal situation (−25 min in Experiment 1 and 0 min in Experiment 2). Group differences were significant when $P < 0.05$.

RESULTS

Experiment 1

In this experiment, arterial cannulation of rats made the individual monitoring of plasma and hemodynamic parameters during acute stress possible. Figure 1 shows the evolution of several parameters relating to stress from 25 min before starting IMMO (basal situation) to 2 min of rest after ending stress. Glucose and corticosterone have been defined as acute stress markers (22, 23). Both increased significantly during IMMO and tended to decrease when the stressor stopped. In a similar way, arterial pressure and heart rate frequency increased quickly and

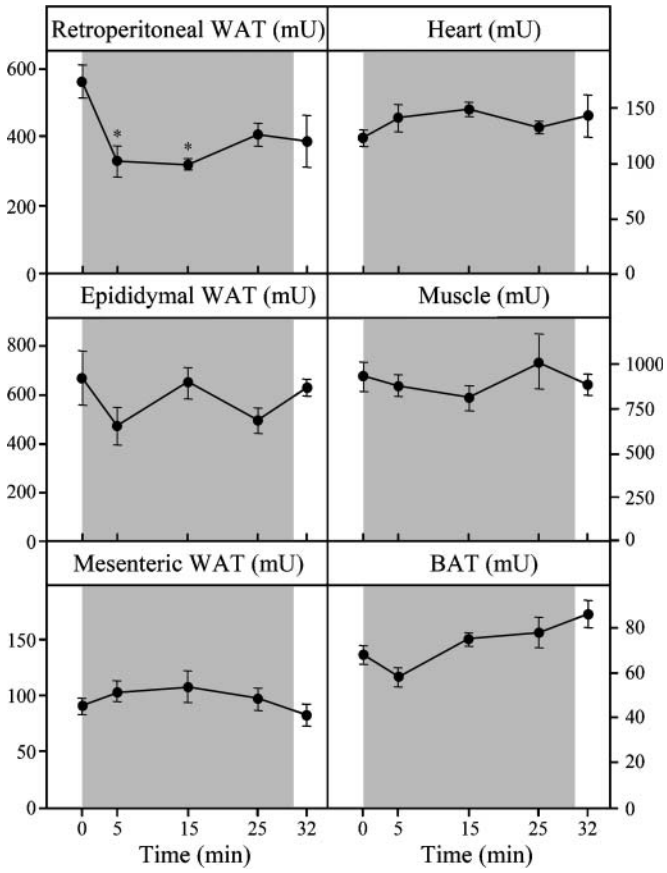

Fig. 4. Total LPL activity in several tissues during acute stress by IMMO in rats (Experiment 2). Shaded area corresponds to immobilization period. Results are expressed as means ± SEM of 6 animals/group. Statistical comparisons by one-way ANOVA (time of stress) and post hoc Tukey tests. * $P < 0.05$, compared with basal situation (0 min). BAT, brown adipose tissue; WAT, white adipose tissue.

very significantly when IMMO started. When stress finished, heart rate frequency remained high, whereas blood pressure returned to initial levels.

LPL activity in plasma) (Fig. 2) also increased rapidly on stress starting (increase of 94% in 5 min) and diminished gradually during the period of IMMO.

Experiment 2

In this experiment, rats did not undergo surgical procedures. On the contrary, different animals were submitted to various periods of IMMO and then euthanized immediately. This protocol made it possible to obtain tissues at different times after the beginning of stress.

Figure 3 shows the evolution of several plasma metabolites during acute stress. As in Experiment 1, glucose increased significantly with the beginning of IMMO, reached a maximum value, and tended to decrease at the end of the period. The other parameters show the changes in lipid metabolism derived from acute stress. NEFAs (an index of lipolysis in WAT) rose significantly at 5 min, but rapidly recovered initial levels (at 15 min of stress). Glycerol (another lipolytic index in WAT) also increased rapidly and significantly (5 min), reached a maximum at

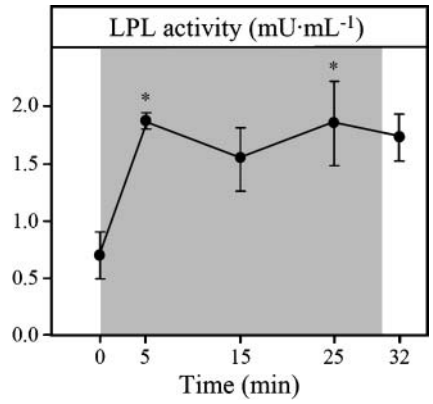

Fig. 5. LPL activity in plasma during acute stress by IMMO in rats (Experiment 2). Shaded area corresponds to immobilization period. Results are expressed as means ± SEM of 6 animals/group. Statistical comparisons by one-way ANOVA (time of stress) and post hoc Tukey tests. * $P < 0.05$, compared with basal situation (0 min).

15 min, and remained high until the end of the experiment. β-hydroxybutyrate (a hepatic transformation product of free fatty acids) had a similar profile, but at the end of the period returned to initial values and the differences with the basal situation disappeared.

Figure 4 shows total LPL activity in different tissues (adipose, heart, and muscle). In the rWAT, LPL activity decreased significantly at 5 and 15 min of IMMO, and recovered slightly at the end of stress. The other tissues studied had no significant differences. Plasma LPL activity, directly related with the fall in LPL activity in rWAT, increased) (Fig. 5). During the whole period of IMMO, LPL activity in plasma increased by more than 115% over the basal situation, with significant differences at 5 and 25 min.

All variations observed in plasma metabolites concentration and LPL activity are not caused by changes in the plasma volume of blood, as hematocrit did not change in any of the situations studied (A. Casanovas, F. de la Cruz, M. D. López-Tejero, and M. Llobera, unpublished observations).

DISCUSSION

LPL plays a central role in lipid metabolism, enabling tissues to uptake the fatty acids released from circulating TAG. The enzyme is under tissue-specific regulation related to the use of the imported fatty acids. Thus, when an individual is in a basal situation (rest and fed ad libitum), it shows increased levels of LPL activity in tissues that use fatty acids for storage (adipose tissues). However, when an organism faces a situation in which TAG-storage mobilization is required, as in physical exercise (5), fasting (7), or chronic stress (10), LPL activity increases in muscle or heart tissue and/or decreases in WAT.

Stress has been defined as a state of threatened homeostasis (24). During stress, adaptive compensatory specific mechanisms are activated to prepare the organism for a "fight-or-flight" response, which is characterized by an increase in available energy substrates (12, 13), and an in-

crease in cardiac rate and blood pressure (14). Variables that are modified by stress might be useful stress markers, i.e., glucose (22) and corticosterone (23) levels in plasma. In our model of IMMO, both parameters increased in a stress situation) (Figs. 1 and 3). The present results also show a fast and significant increase in heart rate frequency and arterial blood pressure during IMMO) (Fig. 1), which corroborates previous reports (25, 26).

In this study, an approach to lipid metabolism during acute IMMO stress showed variations in NEFA, glycerol, and β-hydroxybutyrate levels) (Fig. 3). These parameters proved the activation of lipolysis, through the action of adipose triglyceride lipase and hormone-sensitive lipase from WAT (27), increasing plasma glycerol and NEFA levels. Circulating NEFA were captured (at least partially) by the liver and transformed into ketone bodies (such as β-hydroxybutyrate). In fact, the plasma concentration of β-hydroxybutyrate rose significantly during stress in our model, and was probably used as an alternative energy substrate to glucose. This is the first time that the well-known response of mobilization of TAG from WAT and hepatic transformation of resulting NEFA into ketone bodies has been described as a very fast process. In our experimental model, all these metabolic processes took place less than 5 min after the start of IMMO.

Whole-body energy homeostasis depends on the precisely regulated balance of lipid storage and mobilization. Thus, the rapid metabolic change of WAT under stress from TAG importer to TAG exporter should make LPL activity in this tissue fall quickly (10).

Certainly, with a short period of stress (5 min) there is a significant decrease (over 40%) in rWAT LPL activity) (Fig. 4). This decrease, which is probably a response to the organism's need to quickly mobilize energy reserves to face a situation of stress, coincides in time with a rapid increase in plasma LPL activity, observed in both experiments) (Figs. 2 and 5).

The location of LPL, anchored to the endothelium, limits the mechanisms by which it is regulated. In our case, the simultaneity of the decrease in WAT LPL activity and the increase in plasma (also observed in previous studies of longer periods of stress) (10) may be explained in at least two ways: (i) through a release to the bloodstream of LPL anchored to the vascular surface of WAT or (ii) by means of a decreased uptake by liver/degradation of plasma LPL and a simultaneous fall in synthesis or activation of LPL in WAT. Several considerations, such as that the half-life of LPL is much longer (∼ 1 h) (2) than the time of response observed and the difficulty of activating two distinct mechanisms in only 5 min, suggest to us that release from vessels to the bloodstream is the most plausible option.

Although intravenous administration of heparin (17) or TAG emulsions (28) are known to cause a fast and massive release of active LPL to the bloodstream, this is the first time that such a fast increase in plasma LPL activity is reported in response to a physiological situation.

It has been described that catecholamines and isoproterenol (β-adrenergic agonist) selectively inhibit LPL activity in WAT, reducing LPL gene transcription (29), regulating LPL translation (30) and posttranscriptional maturation (31), or inducing LPL degradation (32). However, none of these mechanisms can explain by themselves the speed of response observed in this study. We suggest that the increase in LPL activity observed in plasma with 5 min of stress could be due to increased release of the enzyme from rWAT. This phenomenon probably occurs through an initial β-adrenergic stimulus, as treatment with propranolol (β-adrenergic blocking agent) prevents the acute IMMO stress-induced changes in plasma LPL activity (D. Ricart, M. D. López-Tejero and M. Llobera, unpublished observations).

Several candidates have been put forward as the main causes of the release of endothelium-associated LPL: TAG-rich lipoproteins (33), TAG emulsions (28), tumor necrosis factor-α (34) or NEFA (33, 35). Some reports have suggested that fatty acids exported from tissues, which are fast and significantly higher in our stressed animals, could release active LPL from tissues in vivo, although no correlation has been found between high local NEFA concentrations in WAT and LPL release (3). Furthermore, NEFA failed to release LPL from both perfused rat hearts (36) and perfused epididymal WAT (A. Moles and M. Llobera, unpublished observations). Nitric oxide has also been put forward as a mediator of LPL activity down-regulation observed in response to in vivo administration of lipopolysaccharide (37) or in cultured brown adipocytes exposed to tumor necrosis factor-α (38). It is known that nitric oxide rises in immobilization (39) and blood nitrates are also significantly increased during short acute IMMO stress (A. Casanovas, M. D. López-Tejero and M. Llobera, unpublished observations). However, further studies are required to determine which mediator or mediators cause the release of LPL from endothelium to the bloodstream in such a fast response to a physiological stimulus.

In conclusion, the main finding of our study is that acute stress causes an extremely fast decrease in rWAT LPL activity, coinciding with an increase in plasma LPL activity. This suggests that the release of the enzyme from endothelium to the bloodstream may constitute a fast unexplored mechanism of tissue LPL activity regulation, involved in the lipid energy-substrate redistribution between tissues that the organism needs to prepare for the "fight-or-flight" response.

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