Lipoprotein lipase and hormone-sensitive lipase activity and mRNA in rat adipose tissue during pregnancy

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Martin-Hidalgo, A., C. Holm, P. Belfrage, M. C. Schotz, and E. Herrera. Lipoprotein lipase and hormone-sensitive lipase activity and mRNA in rat adipose tissue during pregnancy. *Am. J. Physiol.* 266 (*Endocrinol. Metab.* 29): E930-E935, 1994.—To investigate the factors controlling maternal depot fat accumulation during early pregnancy and net decrease during late pregnancy, the activity and mRNA expression of adipose tissue lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL) were related to several other lipid metabolic parameters. Virgin control rats, pregnant rats (at *days 12, 15, 19,* and *21*), and lactating rats (at *days 5* and *10* postpartum) were studied. In adipose lumbar tissue of late pregnant rats, LPL activity decreased to about one-third that of the virgin control animals, with < 10% of initial LPL mRNA expressed as determined by Northern blots. HSL activity increased maximally 1.5-fold with a fourfold increase of HSL expression at *days 12–15* of pregnancy and decreased to control levels after parturition. The HSL-to-LPL mRNA and activity ratios were enhanced from *days 15* and *19* of pregnancy, respectively, and remained so even during lactation, mainly because of the marked lowering of the LPL values. This enhancement coincided with increments in plasma free fatty acids and glycerol levels indicating an increased depot fat breakdown. These results give no indication of an involvement of LPL and HSL gene expression changes in the accumulation of maternal depot during early pregnancy. In contrast, such changes could be responsible for the net breakdown of this fat depot during late gestation. Thus, during this physiological state, long-term (e.g., transcriptional) regulation of LPL and HSL gene expression could be an important mechanism for the control of adipose tissue mass breakdown during late gestation.

rat pregnancy; lumbar adipose tissue; lipolysis

LIPOPROTEIN LIPASE (LPL) and hormone-sensitive lipase (HSL) in adipose tissue are involved in lipid deposition and mobilization, respectively (1). LPL is synthesized in the parenchymal cells of most tissues in the body and transported to its site of action on the capillary endothelium, where it hydrolyzes triglycerides in circulating chylomicrons and very-low-density lipoproteins. LPL activity is especially high in adipose tissue, where it has a key role in the regulation of triglyceride storage (4). In contrast, HSL catalyzes the rate-limiting step in adipose tissue lipolysis (1), thereby controlling the breakdown of stored triglycerides. This enzyme is regulated by an adenosine 3′,5′-cyclic monophosphate-dependent phosphorylation mechanism. Both LPL and HSL cDNA sequences have been recently determined in the mouse (14), the rat (11, 12), and humans (17, 30). The HSL sequence has no homology with the lipase gene family sequences, including LPL, hepatic lipase, and pancreatic lipase, or with any other known eukaryotic protein (12, 17).

Although changes in the mRNA expression of HSL and LPL have been studied under many different conditions, simultaneous comparison has been made in only a very few cases. It has recently been reported that, in hibernating mammals such as marmots, body fat deposition during summer and fat depot release during winter are modulated by the reciprocal expression of adipose tissue LPL and HSL genes. This regulation may be responsible for the large changes in adipose tissue mass at different seasons of the year (29).

Pregnancy is another physiological state characterized by changes in maternal adipose tissue mass. Depot fat accumulates during early stages of pregnancy and decreases during late phases. These changes presumably result from the net catabolic state responding to the rapid fetal accretion during this latter period (13, 19). During late gestation in the rat, the activity of LPL in adipose tissue is greatly reduced (9, 24, 26) whereas the lipolytic activity is highly stimulated (15). In addition to the known increased lipogenic activity (25), changes in LPL and HSL activities most likely contribute to the sequential maternal adipose tissue anabolic-to-catabolic transition that occurs in the course of gestation. To test this hypothesis, we examined the activities and the mRNA expression of adipose tissue LPL and HSL at different days of pregnancy and lactation in the rat.

MATERIALS AND METHODS

Animals and tissue collection. Female Wistar rats from our colony, weighing 180–200 g, were maintained at 22 ± 1°C under standard conditions of illumination (from 0800 to 2000 h) and feeding (Purina Chow diet; Panlab, Barcelona, Spain).

The experimental groups were virgin control rats, pregnant rats (*days 12, 15, 19,* and *21*), and lactating rats (*days 5* and *10*). The rats were mated with normal males, and positive pregnancy was determined by the appearance of spermatozoids in vaginal smears. Litter sizes were adjusted to 9–11 pups at birth. The animals were fed ad libitum and had constant access to tap water. They were killed between 1000 and 1100 h by decapitation after normal night access to food at the days listed above, and trunk blood was collected in ice-chilled heparinized tubes for immediate separation of plasma at 4°C.

The two uterine horns were immediately dissected and weighed with their content to obtain the whole conceptus weight. This value was subtracted from the body weight before death to obtain the net maternal body weight. Lumbar fat pads

were rapidly excised, placed in liquid nitrogen, and stored at −80°C until processing (see *RNA preparation, LPL assay*, and *HSL assay* below). Plasma aliquots were kept at −20°C until analyzed for glycerol (7) in supernatants after protein precipitation (27) and for free fatty acids (22).

RNA preparation. Total cellular RNA was isolated from frozen rat adipose tissue by use of a single-step acid guanidinium thiocyanate-phenol-chloroform extraction method (2). All glassware and plastic ware were autoclaved before use. Briefly, tissues were homogenized with a polytron in the presence of 4 M guanidinium thiocyanate, 0.5% sodium *N*-lauroylsarcosine, 25 mM sodium citrate (pH 7.0), and 0.1 M 2-mercaptoethanol (added just before use). RNA was purified via a series of ethanol precipitations. RNA pellets were dissolved in diethyl pyrocarbonate-treated sterile water, and samples of whole cell RNA were quantified by optical density at 260 nm. Northern blot analysis was used to determine LPL and HSL mRNA levels from each individual animal.

Northern analysis. Equal amounts (5 μg) of total RNA were fractionated on 1% agarose gels containing 2.2 M formaldehyde. Electrophoresis was carried out for 18 h at 50 V in 3-(*N*-morpholino)propanesulfonic acid, pH 7.0, running buffer. RNA was transferred to nylon membrane (Hybond-N; Amersham) for 1 h in 3 M NaCl, 0.3 M sodium citrate, pH 7.0, by a vacu-aid vacuum blotting system with a 80-cmH_20 valve (Hybaid) and immobilized by cross-linking with ultraviolet light (3) by using an ultraviolet Stratalinker 1800 (Stratagene).

The nylon membranes were prehybridized for 1 h at 60°C in 0.5 M sodium phosphate, pH 7.0, 1 mM EDTA, 7% (wt/vol) sodium dodecyl sulfate (SDS), and 1% (wt/vol) bovine serum albumin. Northern hybridization was performed with denatured ^{32}P-labeled cDNA probes (1×10^6 cpm/ml) for 17–18 h at 60°C in the same buffer system as above. cDNA sequences used as probes were the mouse LPL clone ML 2 (14), the full-length rat HSL cDNA (11, 12), and the human β-actin cDNA (8). Cloned cDNAs were radiolabeled as described by Feinberg and Vogelstein (6) by use of an oligolabeling kit (LKB biotechnology, Pharmacia). DNA (25–50 ng) was labeled to a specific activity of $1–2 \times 10^9$ disintegrations·min^{-1}·μg^{-1} using [^{32}P]deoxycytidine triphosphate (3,000 Ci/mmol, Amersham). Northern filters were washed twice (20 min each wash) with 0.3 M NaCl, 30 mM sodium citrate, pH 7.0, 0.1% SDS at either 60°C (HSL and β-actin cDNAs) or at room temperature (LPL cDNA) and twice (20 min each wash) with 15 mM NaCl, 1.5 mM sodium citrate, pH 7, 0.1% SDS at 60°C. The blots were dried at room temperature and exposed to Kodak XAR-5 film. Autoradiography was performed with a single intensifying screen at −80°C. Densitometry of bands was performed with a videodensitometric system developed by Lars Kopp (Makab, Göteborg, Sweden). The linear relationship between RNA loaded and the LPL mRNA signal was checked by measuring different amounts of total RNA from control rat lumbar adipose tissue (Fig. 1).

Northern analyses of whole adipose tissue RNA were performed for each animal. RNA blots were first probed with mouse LPL cDNA, and the same blot was then reprobed with rat HSL and human β-actin (internal standard). Bands of 3.6, 3.3, and 2.2 kb were expressed in adipose tissue from all the groups and corresponded to LPL, HSL, and β-actin mRNA. A representative Northern blot analysis corresponding to the tissue from a virgin rat is shown in Fig. 2. There were no significant differences in loading as verified by ethidium bromide staining of the gels. Additionally, to check for possible differences in loading, transference to the membrane, and hybridization, the same sample of total RNA isolated from control adipose tissue was also introduced into each blot as a second internal standard. The densitometric values of the LPL mRNA for this sample in four different gels were 1,177, 1,234, 1,110, and 1,423. There was thus no significant difference between different gels.

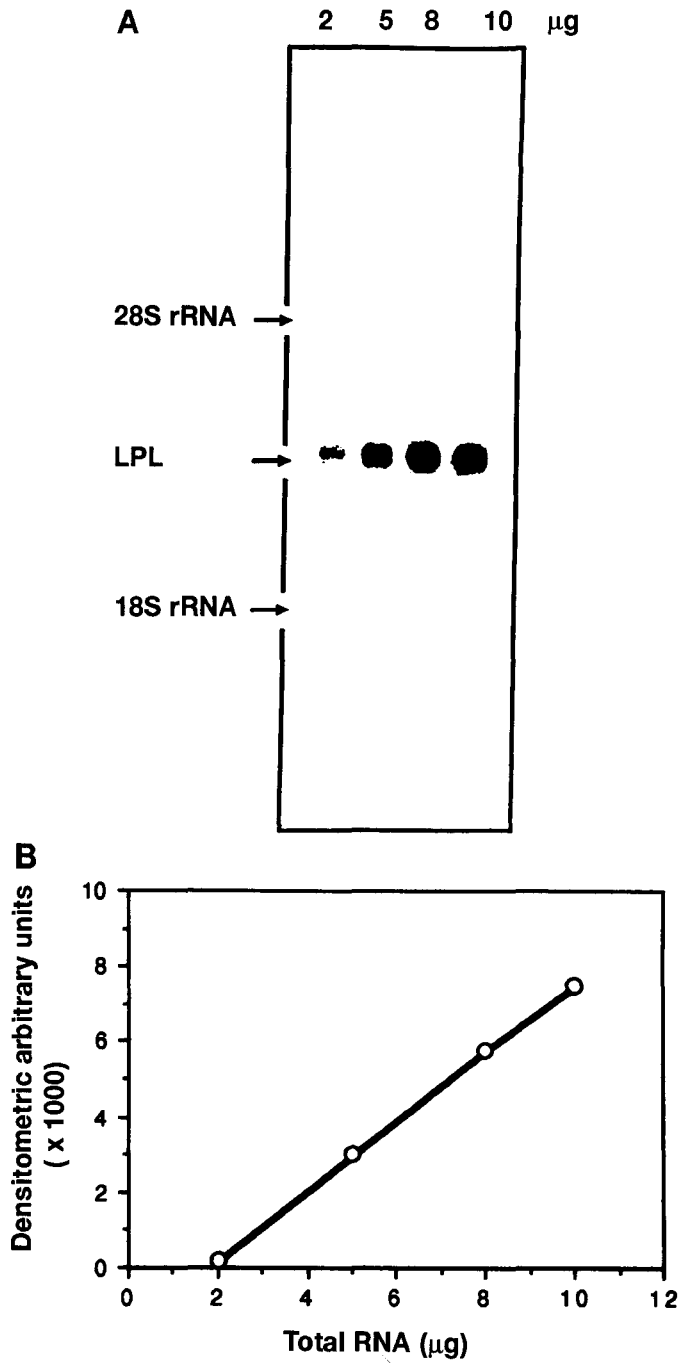

Fig. 1. Northern blot analysis of lipoprotein lipase (LPL) mRNA from virgin control rat lumbar adipose tissue. *A*: total RNA (2, 5, 8, and 10 μg) from rat lumbar adipose tissue was electrophoresed in denaturing (formaldehyde) agarose gels, transferred to nylon membranes, and then hybridized with LPL probe. Autoradiography performed for 3 days. *B*: relationship between RNA loaded and densitometric values of LPL mRNA bands.

LPL assay. Total LPL activity was measured as previously described (17, 23). Briefly, tissue samples were homogenized in 0.2 M tris(hydroxymethyl)*aminomethane* (Tris)-HCl, pH 8.2, at 4°C and delipidated with acetone-diethyl ether. LPL activity

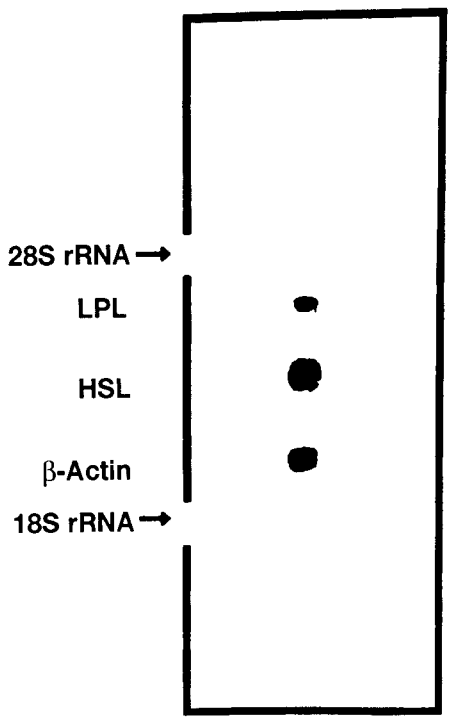

Fig. 2. Northern blot analysis of virgin control rat lumbar adipose tissue for LPL, hormone-sensitive lipase (HSL), and β-actin mRNA. Total RNA (5 μg) from rat lumbar adipose tissue was electrophoresed in denaturing (formaldehyde) agarose gels, transferred to nylon membranes, and then hybridized with LPL, HSL, and β-actin cDNA probes.

was assayed in triplicate using an egg lecithin-stabilized emulsion of ^{14}C-fatty acid-labeled triolein as substrate (final concentration 2.5 mM triolein, 2.4% bovine serum albumin, 0.2 M Tris, pH 8.5, 0.1 M NaCl, and 8% heated rat serum in 0.25 ml) in the absence and presence of 1 M NaCl (high salt). LPL activity was determined by subtracting the non-LPL-dependent activity (high salt) from the total lipolytic activity. Enzyme activity is expressed in nanomoles of fatty acids released per milligram of protein.

HSL assay. Adipose tissue samples were homogenized at 4°C with a knife homogenizer in 3 vol of 0.25 M sucrose containing 1 mM EDTA, 1 mM dithioerythreitol, 10 μg/ml leupeptin, and 10 μg/ml antipain, at pH 7.4. Infranatants were obtained by centrifugation at 110,000 *g* in a Beckman centrifuge (model TL-100) for 45 min at 4°C. A phospholipid-stabilized emulsion of a dioleoylglycerol ether analogue, 1(3)-mono[^{3}H]oleyl-2–O-oleoylglycerol, was used to assay the hormone-sensitive diacylglycerol lipase activity (28). Protein was determined according to the method of Lowry et al. (21) using bovine serum albumin as a standard.

Statistical analysis. Values are means ± SE. The significance of difference between means of two groups was obtained with Student's *t* test. All comparisons were judged to be significant at $P < 0.05$.

RESULTS

Corporal parameters in rats at different days of gestation and lactation (postparturition) are shown in Table 1. The maternal body weight, conceptus-free, is already significantly higher at *day 12* of gestation than in virgin animals. This difference is sustained throughout pregnancy and during lactation. The conceptus mass is very small at *day 12* of gestation but increases exponentially from the 15th day to term.

Figure 3 illustrates changes in the plasma lipid levels in rats at different days of pregnancy and lactation. Plasma free fatty acid levels appear lower in 12-day-pregnant rats than in virgins, but later they increase progressively to attain, at *day 19* of pregnancy, values significantly higher than in the virgin control group. This high level is still found on *day 10* of lactation (Fig. 3*A*). Plasma triglycerides in the 12-day-pregnant rat are at the same level as in virgins, but they increase progressively between *days 15* and *19* of gestation and then decrease at *day 21* and during lactation to values not different from those in nonpregnant rats (Fig. 3*B*). On the other hand, plasma glycerol levels remain unchanged up to the 19th day of gestation but increase significantly at *day 21* and remain augmented on *day 10* of lactation (Fig. 3*C*).

The enzyme activities of both HSL and LPL in lumbar adipose tissue at different days of gestation and lactation are shown in Fig. 4, *A* and *B*, respectively. HSL activity is significantly increased at *day 12* of gestation and remains so until term before returning to control values at *days 5* and *10* after parturition. Change in LPL activity takes a different direction from that of HSL. LPL activity remains unchanged up to the 15th day of gestation, decreases slightly but not significantly at *day 19*, and appears significantly decreased at *day 21* of gestation, remaining low after parturition compared with nonpregnant control rats. Because the HSL-to-LPL activity ratio may indicate the relative triglyceride breakdown activity compared with storage, this parameter was estimated as shown in Fig. 4*C*. The HSL-to-LPL activity ratio remains unchanged until the 15th day of gestation, whereas it increases significantly at the 19th day and remains increased after parturition.

The mRNA levels for both LPL and HSL in RNA extracts from lumbar adipose tissue are illustrated in Fig. 5, *A* and *B*, respectively. HSL mRNA increased significantly on *day 12* of gestation compared with values in nonpregnant rats, and values remained increased on *day 19* of pregnancy but returned to control levels by *day 21* of pregnancy. As seen in Fig. 5*A*, the level of HSL mRNA is two to four times higher in the

Table 1. *Effect of pregnancy and lactation on maternal and conceptus weights in the rat*

Condition	Day	Maternal Body Weight, g	Conceptus Weight, g
Virginity		195 ± 4	
Pregnancy	*12*	240 ± 7.1*	5.20 ± 0.4
	15	257 ± 5.8*	14.9 ± 0.9
	19	244 ± 5.6*	49.8 ± 1.8
	21	254 ± 5.8*	87.2 ± 2.5
Lactation	*5*	272 ± 8.3*	
	10	265 ± 8.9*	

Values are means ± SE of 5–6 animals for each group. Maternal body weight is free-of-conceptus weight. *Significantly different from weight of virgin rats ($P < 0.001$).

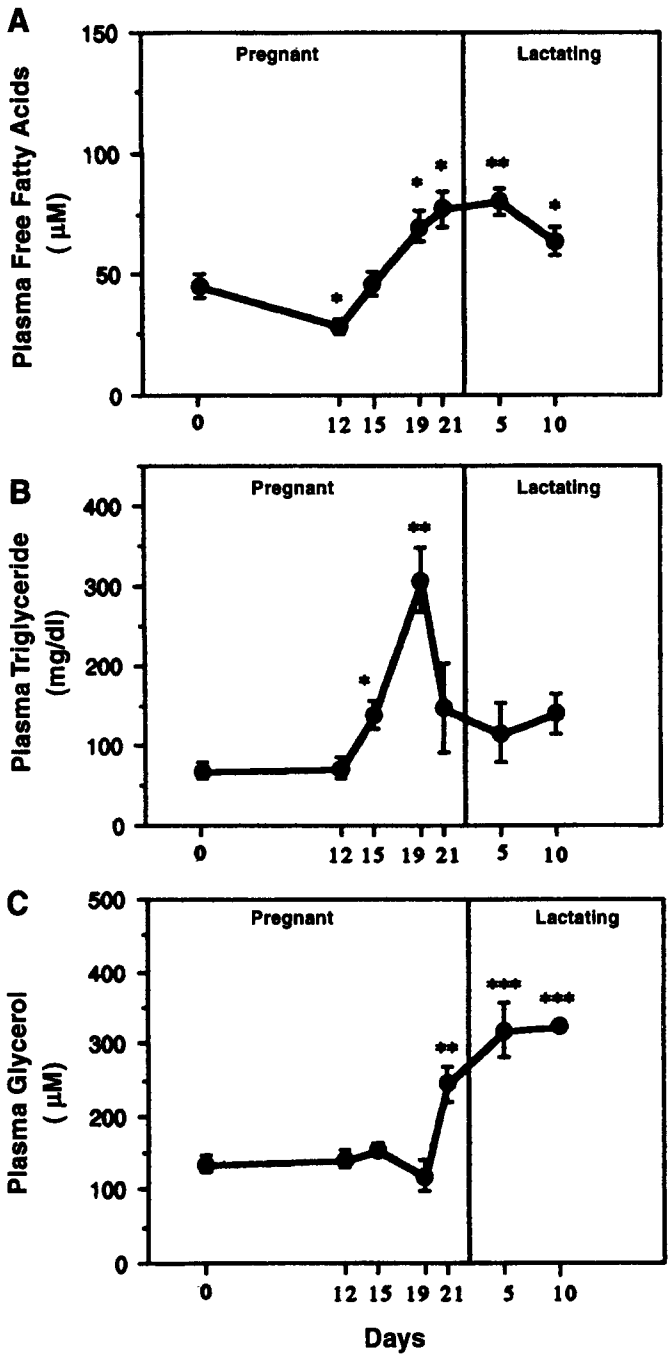

Fig. 3. Changes in plasma free fatty acids (*A*), triglycerides (*B*), and glycerol (*C*) during pregnancy and lactation in the rat. Values are means ± SE of 5–6 animals for each time point. Significantly different from virgin values: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

12-, 15-, and 19-day-pregnant than in the virgin rats. In contrast, the amount of LPL mRNA showed a statistically significant reduction on the 19th day of gestation and remained so through the 10th day of lactation (Fig. 5*B*). As shown in Fig. 5*C*, the HSL-to-LPL mRNA ratio showed a significant and progressive increase from the 15th until the 21st day of gestation, whereas the ratio decreased sharply after parturition. The values at *days 5* and *10* of lactation are still increased with respect to the virgin animals (Fig. 5*C*).

DISCUSSION

The present study demonstrates that gestation in the rat causes different responses of both LPL and HSL

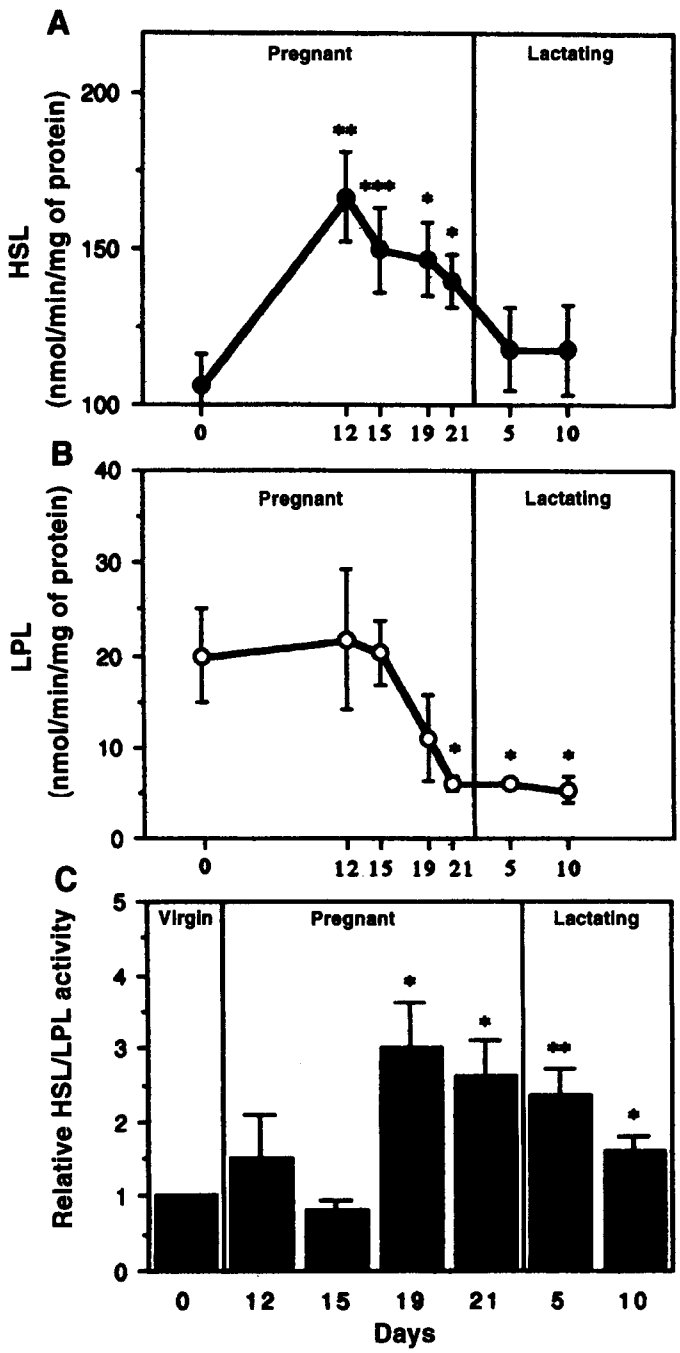

Fig. 4. Changes in HSL (*A*) and LPL enzyme activity (*B*) and HSL-to-LPL activity ratio (*C*) in rat lumbar adipose tissue during pregnancy and lactation. Values are means ± SE of 5–6 animals for each time point. Relative HSL-to-LPL activity is expressed relative to values of virgin control rats, which were arbitrarily set as unity (1.0). Significantly different from virgin values: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

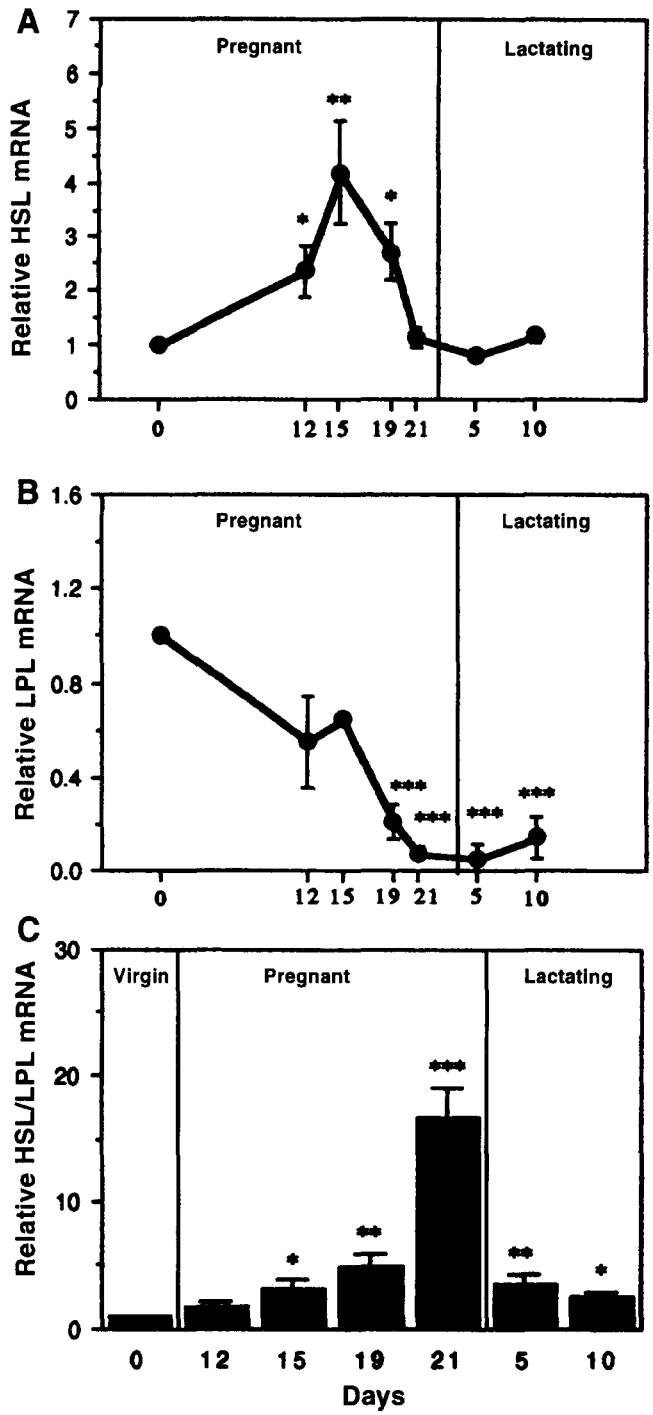

Fig. 5. Changes in HSL and LPL mRNA expression (*A* and *B*, respectively) and HSL-to-LPL mRNA ratio (*C*) in rat lumbar adipose tissue during pregnancy and lactation. Results are expressed relative to values of virgin control rats, which were arbitrarily set as unity (1.0). Relative HSL and LPL mRNA values are expressed in relation to β-actin and are means ± SE of 5–6 individual animals for each time point. Significantly different from virgin values: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

activity and their mRNA levels in adipose tissue. The parallel changes found between enzyme activity and mRNA level for an enzyme involved in lipid storage, LPL, and the lipolytic enzyme HSL during gestation and lactation are consistent with transcriptional control of these important genes involved in adipose tissue regulation. The lack of change in LPL activity in adipose tissue until *days 15–19* of gestation and the subsequent reduction suggest that LPL does not contribute to the progressive depot fat accumulation during gestation (10, 19, 20). Alternatively, the enhanced adipose tissue lipogenesis occurring at the beginning of gestation (5, 16, 25) may be responsible for net fat accumulation. Furthermore, the reduction in LPL activity seems unlikely to be the cause of maternal hypertriglyceridemia because the increase in plasma triglycerides occurs before the loss of LPL activity (24). The reduction in LPL activity seen in adipose tissue during late gestation, however, may have a role in decreasing the hydrolysis and uptake of circulating triglycerides by this tissue and thus in allowing circulating triglycerides to be directed to the mammary gland for milk synthesis. In fact, plasma triglycerides actually decreased on *day 21* of gestation, when the LPL activity in adipose tissue was at the lowest level. This decrease in enzyme activity coincided with the previously reported increase in mammary gland LPL activity, which most likely is a major cause of enhanced triglyceride clearance from the circulation at parturition (26).

An increase in HSL activity during gestation was seen in the fat pad. Although the increased HSL activity existed from *day 12* of gestation, plasma free fatty acids and glycerol levels did not rise until later. One possible explanation of this early stimulation of lipolytic activity may be that it is counteracted by enhanced lipogenesis and reesterification, which are already augmented by this gestational time (25). At late gestation, the HSL-to-LPL activity ratio was enhanced in the adipose tissue, indicating that net triglyceride breakdown was increased. This would also explain the decline in maternal body fat content known to occur on *day 21* (10, 19). In conclusion, the increase in maternal fat depots during the early stages of gestation is not explained by the changes in LPL and HSL gene expression. However, the enhanced fat depot breakdown taking place during late gestation is likely to account for the HSL-to-LPL activity and mRNA ratios.

The phosphorylation-dephosphorylation mechanism in the HSL is regulated rapidly by hormones and does not involve long-term control by gene expression. The recently reported seasonal cyclic change in the HSL mRNA level found in hibernating marmots (29) and our present findings showing a parallel increase in both HSL activity and mRNA in adipose tissue during gestation are the first reports of physiological conditions that suggest that HSL is transcriptionally regulated.

Elucidation of the factors controlling HSL and LPL mRNA expression and stability will undoubtedly enhance our understanding of depot fat regulation. The factors possibly responsible for this control remain to be established.

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