Lipid content and lipoprotein lipase activity in skeletal muscle of lactating and weaned rats

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Abstract The aim of the investigation was to study the changes in lipid content and lipoprotein lipase (LPL) activity of skeletal muscle of lactating and weaned rats, in order to gain insight on the role of skeletal muscle in the metabolism of triacylglycerols not used by the mammary gland after weaning. Sprague-Dawley rats fed ad libitum were killed 0, 8, 12, and 24 h after litter separation on the 14th day of lactation. Electron microscopy, as well as determinations of the total fat and phospholipid content were performed on muscles of the right hind limb. Lipoprotein lipase activity was determined in extracts of acetone powder of skeletal muscle, mammary gland, and adipose tissue using dibutyryl fluorescine as substrate. Fat droplets were identified in the muscle interfiber spaces of weaning rats. Muscle total fat and phospholipids were higher in weaned than in lactating rats. After litter separation, lipoprotein lipase activity increased significantly in muscle and in adipose tissue (fourfold), while activity in the mammary gland decreased. The increased muscle lipoprotein lipase activity in the weaned rats seems to be associated with the higher content of fat and with the presence of fat droplets.—**Del Prado, M., R. Ramos, H. Hernández-Montes, and S. Villalpando.** Lipid content and lipoprotein lipase activity in skeletal muscle of lactating and weaned rats. *J. Lipid Res.* 1993. **34:** 1115–1120.

Supplementary key words muscle • lipoprotein lipase • weaning • lactation

During lactation, the mammary gland takes up large amounts of circulating glucose, triacylglycerols, and amino acids for milk synthesis (1, 2). The uptake of plasma amino acids is related to gamma-glutamyl transpeptidase activity, whereas the uptake of triacylglycerols by most tissues is dependent on lipoprotein lipase (3–5). Lipoprotein lipase (LPL) hydrolyzes plasma triglycerides at the surface of capillary endothelial cells, and the released fatty acids are taken up by the cells to be stored or oxidized (6).

During lactation, lipoprotein lipase activity is greatly increased in the mammary gland. It is markedly decreased in adipose tissue just before parturition, remaining low throughout lactation and increasing again on removal of pups (7–9). As a result, during lactation, a large proportion of the dietary fat is diverted to the mammary gland for milk synthesis and, to a lesser extent, to adipose tissue for storage. After weaning, substrates are shifted to other tissues for storage or oxidation. Skeletal muscle represents an important site of utilization of fatty acids from plasma chylomicrons and VLDL triglyceride (10, 11). Muscle LPL activity has been extensively studied in male rats, in which activity varies with circadian rhythm, as well as with diet, exercise, and hormones (12–16). Changes occurring in lactating mothers are poorly documented (17); to our knowledge no data in weaned dams are available.

This paper describes the changes in lipid content and LPL activity in skeletal muscles of lactating and weaned dams.

METHODS

Animals

Adult female Sprague-Dawley rats were used. Their body weights ranged from 220 to 260 g and they were maintained at an ambient temperature of 22 ± 2°C, under light-dark cycles of 12 h (light 7:00–19:00 h). Animals had free access to water and commercial nonpurified diet containing 55% carbohydrates, 2.8% fat, and 26% protein (Nutricubos, Purina Guadalajara, México). The NIH and Secretaria de Salubridad of Mexico guidelines for experimental animals were complied with.

After normal pregnancy and delivery, litters were adjusted to eight pups per dam the first day of lactation. Food intake was determined the day before the experiment; 400 g of commercial food was provided to each rat at 9:00 AM, the residual food was registered at the same hour the next day, and the food ingested was calculated by difference.

Abbreviations: LPL, lipoprotein lipase; VLDL, very low density lipoprotein; DBF, dibutyryl fluorescine.

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Electron microscopy

In a first experiment, groups of six dams in their 12–14th day of lactation were killed by cervical dislocation, immediately or 24 h after separation from their pups. All the experiments started between 9:00 and 10:00 AM. Five pieces of 1 mm^3 from the deep portion of quadriceps were excised and submerged 2 h in a fixative solution of 0.1 M phosphate buffer, pH 7.2, containing 2.5% glutaraldehyde, and washed with 0.1 M phosphate buffer, pH 7.2, for 24 h. The fragments were postfixed in a 1% solution of osmium tetroxide in 0.1 M phosphate buffer, pH 7.2, and washed with the buffer alone for 24 h. Later, they were dehydrated with increasing concentrations of ethanol (60–96%). The tissue was then embedded in propylene oxide-epoxy resins by a standard procedure (18). The blocks were sectioned on a Sorvall-Porter-Blum MT2-B ultramicrotome and thin sections (90–150 nm) were stained with 4% uranyl acetate followed by 0.4% lead citrate, and then examined in a Zeiss 9.S.2 electron microscope.

Total lipid content of muscle

Total content of lipids and phospholipids of all the right hind limb muscles of the rats included in the electron microscopy study were determined gravimetrically in triplicate samples after extraction with chloroform–methanol 2:1 as described by Folch, Lees, and Sloane Stanley (19). Phospholipid content was determined by quantitation of the amount of inorganic phosphate in a percholoric acid digest of the lipid extract using the method described by Fiske-Subbarow (20).

Lipoprotein lipase activity in tissues

In a second experiment, groups of six rats in their 10–14th day of lactation were weighed and then killed by cervical dislocation, immediately, or 8 and 12 h after separation from their pups. In order to avoid circadian and eating pattern effects on the enzyme activity, all the animals were killed at 9:00 AM.

Blood was collected for plasma triglyceride determination. The pectoral and abdominal mammary glands, parametrial adipose tissue, and all the muscles of the right hind limb were dissected and weighed.

Lipoprotein lipase activity was determined in acetone powders of the tissues, obtained as follows. Muscle and mammary gland were minced in ice and rinsed in cold 0.85% NaCl; this step was repeated until the supernatant was clear and free from blood and milk. The tissues were then homogenized in cold 0.85% NaCl and added drop by drop to −20°C cold acetone. Adipose tissue was immediately homogenized in cold acetone.

The tissue constituents insoluble in acetone were collected by filtering through Whatman paper on a Buchner funnel and washed with acetone and diethyl ether at room temperature. These insoluble fractions were scraped off the filter papers and maintained at −20°C until use.

The enzyme was extracted by mixing variable amounts of acetone powder with 1 ml of 25 mM NH_4Cl buffer, pH 8.1, containing 1 U/ml of heparin. The mixture was kept on ice for 60 min and the supernatant obtained after centrifugation at 5,000 *g* for 10 min at 4°C was used as enzyme source. Lipoprotein lipase activity in the extract was stable for at least 2 h at 4°C. When acetone-ether powders of muscle were extracted three times with ammonia buffer containing heparin as described, 76.12 ± 1.5% of the total activity was recovered in the first extract.

Lipoprotein lipase assay

Lipoprotein lipase was assayed by a fluorometric method using dibutyryl fluorescine (DBF) as substrate. Synthesis of DBF was described by Kramer and Guilbault (21). Dibutyryl fluorescine solution was prepared just before assay with 1 mg of DBF in 5.0 ml of ethylene glycol monomethylether to obtain a final concentration of 400 nmol/ml. Substrate emulsion was obtained by dilution of concentrated substrate in 0.1 M phosphate buffer, pH 8.0, (1:20 v/v). Validation of the assay was published elsewhere (22). This method has a high correlation with the method using tritiated triolein as substrate (r=0.84).

Incubations were carried out at 36°C in a total volume of 1.0 ml containing 10 nmol DBF, 5% ethylene glycol monomethyl ether, 0.1 M phosphate buffer, pH 8.0, and 100 µl of the enzyme extract. The fluoresceine produced was measured 120 min after addition of the enzyme in an Aminco Bowman fluorometer at 530 nm for emission and 490 nm for excitation. The release of fluoresceine was linear in response to increasing amounts of the enzyme source at saturating levels of substrate. Lipoprotein lipase activity was calculated by subtracting the fluoresceine liberated when 1 M NaCl was added to the system from the fluoresceine liberated in a system free of NaCl.

TABLE 1. Body weight, weight, lipid and phospholipid content of muscle of dams during lactation and 24 h after weaning

Variable	Lactating	Weaned, 24 h
Number of animals	8	12
Body weight (g)	274.3 ± 39.6	303.7 ± 37.4
Muscle		
Wet weight (g)	9.6 ± 1.7	11.4 ± 1.4[a]
Lipid content		
mg/g tissue	13.5 ± 4.7	14.4 ± 4.6
mg/total tissue	119.9 ± 47.7	175.0 ± 60.3[a]
Phospholipid content		
µg/g tissue	1183.9 ± 433.3	1303.1 ± 344.6
mg/total tissue	11.0 ± 5.6	14.2 ± 4.4

Values are given as means ± SD. Comparison between means was made by t test.
[a] $P < 0.05$.

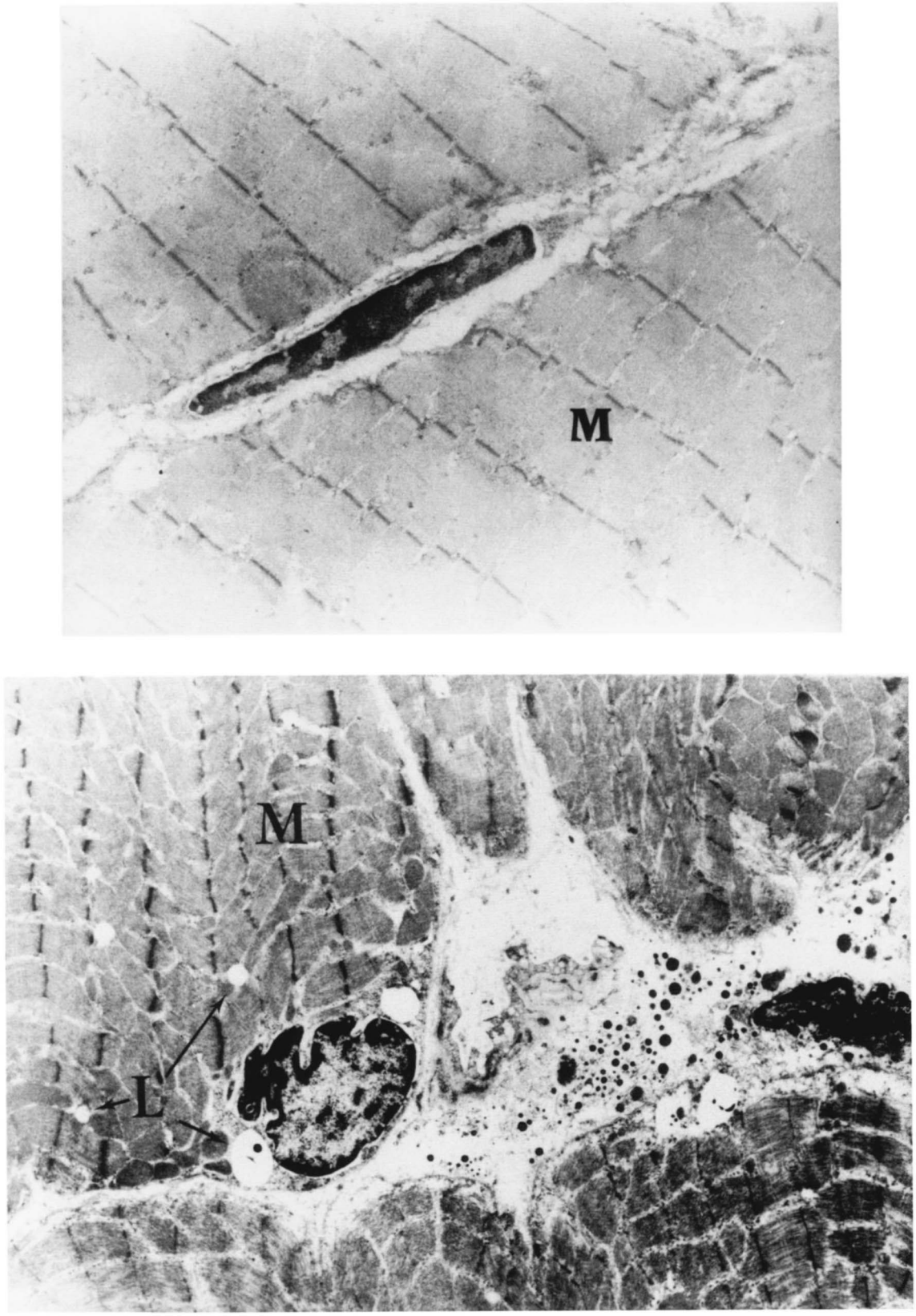

Fig. 1. Electron microscopy of a representative section of skeletal muscle of lactating (upper panel) and 24-h weaned dams (lower panel); M, for muscle fibers. Lipid droplets (L) are evident in the inter-fiber spaces of the muscle of weaned dams, while they are not identified in the muscle of lactating dams.

TABLE 2. Food intake, plasma triglycerides and lipoprotein lipase activity in several tissues of lactating and weaned dams

Variable	12-14th Day of Lactation	Hours after Weaning 8	Hours after Weaning 12
Food intake (g/24 h)	41.9 ± 5.0	47.0 ± 4.8	40.6 ± 6.2
Plasma triglyceride (mg/dl)	48.4 ± 21.3	68.9 ± 27.9[a]	88.9 ± 10.7[a]
Adipose tissue			
Wet weight (g)	4.5 ± 0.08	4.6 ± 1.6	4.3 ± 2.3
Acetone powder yield (mg)	95.0 ± 56.0	87.0 ± 26.0	100.0 ± 38.0
Lipoprotein lipase activity (nmol/120 min/mg protein)	984 ± 203	4059 ± 1449[a]	1199 ± 519
Mammary gland			
Wet weight (g)	11.3 ± 3.2	18.1 ± 4.8[a]	21.2 ± 3.6[a]
Acetone powder yield (g)	1.6 ± 0.03	2.4 ± 0.33[a]	2.2 ± 0.46
Lipoprotein lipase activity			
nmol/120 min/mg protein	850 ± 158	728 ± 278	756 ± 197
nmol/120 min/g tissue	9691 ± 2769	7360 ± 3091	5013 ± 1043[a]
Muscle			
Wet weight (g)	8.8 ± 0.9	9.0 ± 0.63	9.0 ± 0.6
Lipoprotein lipase (nmol/120 min/mg protein)	23.7 ± 9.0	30.3 ± 11.9[a]	29.7 ± 12.9

Values are given as means ± SD. Comparisons between means of groups were done by ANOVA test.
[a] $P < 0.05$.

Enzyme activity was expressed as nmoles of fluoresceine per 120 min per mg protein. The protein concentration of each extract was measured by the method of Itzhaki and Gill (23). Plasma triglyceride was determined using a commercial kit (Sigma Co.).

Statistical analysis

Differences between treatment groups were analyzed by one-way ANOVA, followed by a Tukey test (24).

RESULTS

Body weight, muscle wet weight, and muscle lipid content of animals included in experiment 1 are presented in **Table 1.** There were no differences in body weight among the groups. The wet weight and total lipid content of all the right hind limb muscles were significantly higher in 24-h weaned than in lactating dams ($P < 0.05$).

Fat droplets were identified by electron microscopy in the inter-fiber spaces of the muscle of 24-h weaned dams, but they were not present in the muscles of the lactating rats (**Fig. 1**).

In the second experiment no differences were observed in food intake among lactating and 8-h or 12-h weaned rats. Plasma triglyceride concentration was significantly higher in 8-h and 12-h weaned dams than in lactating dams ($P < 0.05$), being higher at 12 than at 8 h ($P < 0.05$) (**Table 2**).

Adipose tissue

There were no differences among groups in wet weight and in fat-free dry weight (Table 2). However, lipoprotein lipase activity was threefold higher in 8-h weaned dams than in lactating dams. There was no difference in lipoprotein lipase activity between 8-h and 12-h weaned dams.

Mammary gland

Wet weight was significantly higher in 8-h and 12-h weaned than in lactating dams (Table 2). Mean lipoprotein lipase activity was not different in 8-h weaned than in lactating dams. After 12 h of litter separation, mammary gland lipoprotein lipase activity was significantly lower in weaned than in lactating dams.

Muscle

Lipoprotein lipase was significantly higher in 8-h weaned than in lactating dams (Table 2).

Table 3 shows the kinetic properties of LPL in different tissues. LPL had similar characteristics in mammary gland and adipose tissue while muscle LPL showed the lowest specific activity and K_m.

TABLE 3. Kinetic properties of LPL from adipose tissue, mammary gland, and muscle

Tissue	Specific Activity	K_m
	nmol/min/mg protein	*nM*
Adipose tissue	1.83	0.862
Mammary gland	1.256	0.65
Skeletal muscle	0.1468	0.481

DISCUSSION

The results herein presented demonstrate an increased lipid content of skeletal muscle in weaned rat dams, along with microscopic evidence of its interstitial accumulation. This is associated with an increased LPL activity in muscle and augmented plasma levels of triglycerides. The novel contribution of this study is the evidence of the important role played by skeletal muscle in the metabolic adaptations post-weaning, i.e., increased LPL activity and total lipid content. Others have found increments in muscle LPL activity due to starvation and exercise, but no changes had been found in pregnant and lactating animals (17). To our best knowledge no information is available about the changes in weaned rats.

The increased muscle LPL activity in the presence of elevated circulating triacylglycerol could explain the fat deposition in the interstitial space we observed in the 8-h weaned dams. The interstitial lipid deposits are the largest portion of the intramuscular lipid compartment and are considered to be an integral part of the intramuscular lipid stores available for metabolism during contractions (25). If so, the increased intramuscular lipid stores found in this study in the first hours after weaning could be used later on by the tissue to obtain energy. In fed rats, the skeletal muscle uses considerable amounts of lipids as fuel, accounting for a significant portion of total lipid uptake (11, 26). Plasma free fatty acids are oxidized immediately, and used to replace fatty acids hydrolyzed from the triglyceride pool, or to maintain the free fatty acid pool size in skeletal muscle (27–29). Direct utilization of fatty acids from plasma triacylglycerols by this tissue depends upon the activity of LPL in the capillary beds (10).

In weaned rats lipoprotein lipase activity in skeletal muscle was lower than in adipose tissue. The K_m of muscle LPL in humans (30) as well as in rats (present study) is lower than in adipose tissue, suggesting that skeletal muscle hydrolyzes triglycerides at a given serum triglyceride concentration more actively than adipose tissue. As a consequence, the uptake of lipids by the muscle might be saturated before that in adipose tissue.

The progressive decline of LPL in mammary gland after weaning is consistent with the decrease of total accumulation of lipid occurring in the gland (31). However, we did not observe the marked decrease in the mammary gland LPL activity after 8 h of weaning reported by Hamosh et al. (9), perhaps because we studied rats in middle lactation (12 days) while Hamosh et al. studied dams in their early stages, 5th or 6th day postpartum. The fact that the wet weight of the mammary gland of 8-h and 12-h weaned rats was higher than that of lactating rats might be misleading; this was due mainly to milk accumulation.

In summary, the weaning process increases the availability of plasma triglycerides; under these circumstances muscle seems to play a relevant role in their handling. Evidence is presented herein that part of these triglycerides is stored within the muscle. It will be of interest to study the final fate and the importance of such deposits in the metabolism of muscle in the weaned rat.

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REFERENCES

1. Williamson, D. H. 1980. Integration of metabolism in tissues of the lactating rat. *FEBS Lett.* **117 (Suppl.):** K93–K105.
2. Williamson, D. H., M. R. Munday, and R. G. Jones. 1984. Biochemical basis of dietary influences on the synthesis of the macronutrients of rat milk. *Fed. Proc.* **43:** 2443–2447.
3. Pocious, P. A., C. R. Baumrucker, J. P. McNamara, and D. E. Bauman. 1980. Gamma glutamyl transpeptidase in rat mammary tissue. Activity during lactogenesis and regulation by prolactin. *Biochem. J.* **188:** 565–568.
4. Eckel, R. H. 1989. Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. *N. Engl. J. Med.* **320:** 1060–1068.
5. Nilsson-Ehle, P. 1980. Lipolytic enzymes and plasma lipoprotein metabolism. *Annu. Rev. Biochem.* **49:** 667–693.
6. Robinson, D. S. 1963. The clearing factor lipase and its action in the transport of fatty acids between the blood and the tissues. *Adv. Lipid Res.* **1:** 133–182.
7. Scow, R. O., and S. S. Chernick. 1987. Role of lipoprotein lipase during lactation. *In* Lipoprotein Lipase. J. Borensztajn, editor. Evener Publishers, Inc. Chicago. 149–186.
8. Scow, R. O., S. S. Chernick, and T. R. Fleck. 1977. Lipoprotein lipase and uptake of triacylglycerol cholesterol and phosphatidylcholine from chylomicrons by mammary and adipose tissue of lactating rats in vivo. *Biochim. Biophys. Acta.* **487:** 297–306.
9. Hamosh, M., T. R. Clary, S. S. Chernick, and R. O. Scow. 1970. Lipoprotein lipase activity of adipose and mammary tissue and plasma triglyceride in pregnant and lactating rats. *Biochim. Biophys. Acta.* **210:** 473–482.
10. Tan, M. H., T. Sata, and R. J. Havel. 1977. The significance of lipoprotein lipase in rat skeletal muscles. *J. Lipid Res.* **18:** 363–370.
11. Linder, C., S. S. Chernick, T. R. Fleck, and R. O. Scow. 1976. Lipoprotein lipase and uptake of chylomicron triglyceride by skeletal muscle of rats. *Am. J. Physiol.* **231:** 860–864.
12. Borensztajn, J., M. S. Rone, S. P. Bavirak, J. A. McGarr, and L. B. Oscai. 1975. Effect of exercise on lipoprotein lipase activity in rat heart and skeletal muscle. *Am. J. Physiol.* **229:** 394–397.
13. Kotlar, T. J., and J. Borensztajn. 1977. Oscillatory changes in muscle lipoprotein lipase activity of fed and starved rats. *Am. J. Physiol.* **233:** E316–E319.
14. Weisenburg, C. L., and K. L. Harris. 1975. Effects of diet on lipoprotein lipase activity in the rat. *J. Nutr.* **105:** 447–451.
15. Gorski, J., and B. Stankievicz-Choroszucha. 1982. The effect of hormones on LPL activity in skeletal muscles of the rat. *Horm. Metab. Res.* **14:** 189–191.

16. Planche, E., A. Boulange, P. de Gasquet, and N. T. Tonnu. 1980. Importance of muscle lipoprotein lipase in rats during suckling. *Am. J. Physiol.* **238:** E511–E517.
17. Champigny, O., and Y. Hittier. 1987. Lipoprotein lipase activity in skeletal muscle and brown adipose tissue of pregnant and lactating rats. *J. Nutr.* **117:** 349–354.
18. Pease, D. C. 1964. Histological Techniques for Electron Microscopy. Academic Press, New York. 354–356.
19. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.
20. Lindberg, O., and L. Ernster. 1956. Determination of organic phosphorus compounds by phosphate analysis. *Methods Biochem. Anal.* **3:** 1–22.
21. Kramer, D. N., and G. G. Guilbault. 1963. A substrate for the fluorometric determination of lipase activity. *Anal. Chem.* **5:** 588–591.
22. Del Prado, M., H. Hernández-Montes, and S. Villalpando. 1993. Characterization of a fluorometric method for assay of lipoprotein lipase. *Arch. Biochem.* Submitted for publication.
23. Itzhaki, R. F., and D. M. Gill. 1964. A micro-biuret method for estimating proteins. *Anal. Biochem.* **9:** 401–410.
24. Keppel, G. 1973. Design and Analysis. A Researcher's Handbook. Prentice Hall, Englewood Cliffs, N.J. 329–344.
25. Barclay, J. K., and W. N. Stainsby. 1972. Intramuscular lipid store utilization by contracting dog skeletal muscle in situ. *Am. J. Physiol.* **223:** 115–119.
26. McNamara, J. P., M. Azain, T. R. Kasser, and R. J. Martin. 1982. Lipoprotein lipase and lipid metabolism in muscle and adipose tissues of Zucker rats. *Am. J. Physiol.* **243:** R258–R264.
27. Davidson, M. B. 1979. Studies on the oxidation of muscle triglycerides. *J. Endocrinol. Invest.* **2:** 367–374.
28. Pande, S. V., and M. C. Blanchaer. 1971. Carbohydrate and fat in energy metabolism of red and white muscle. *Am. J. Physiol.* **220:** 549–553.
29. Dagenais, G. R., R. G. Tancredi, and K. L. Zierler. 1976. Free fatty acid oxidation by forearm muscle at rest, and evidence for an intramuscular lipid pool in the human forearm. *J. Clin. Invest.* **58:** 421–431.
30. Lithell, H., and J. Boberg. 1978. Determination of lipoprotein lipase activity in human skeletal muscle tissue. *Biochim. Biophys. Acta.* **528:** 58–68.
31. Oller Do Nascimento, C. M., and D. H. Williamson. 1988. Tissue-specific effects of starvation and refeeding on the disposal of oral [1-^{14}C]triolein in the rat during lactation and on removal of litter. *Biochem. J.* **254:** 539–546.