A comparison of lipoprotein lipase activity and adipocyte differentiation in growing male rats

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Abstract During adipose tissue development changes in lipoprotein lipase activity per adipocyte precede significant changes in fat cell size. Lipoprotein lipase activity per adipocyte increases fourfold from the second to seventh postnatal week. Furthermore, when isolated adipocytes and stromal–vascular cells are prepared by collagenase digestion of adipose tissue, there is a progressive shift in enzyme activity during development from the stromal–vascular compartment to the adipocyte fraction. The data support the concept that during normal development a "bed" of preadipocytes is synthesized during the suckling period. The data further suggest a regulatory role for lipoprotein lipase in the control of "lipid-filling" during early postnatal development.

Supplementary key words adipose tissue · isolated fat cells · isolated stromal–vascular cells · adipose tissue cellularity

Numerous studies have documented the normal development of adipose depot cellularity in rodents (1–4), man (5, 6), and other mammals (7). In all of these studies it has been pointed out that the number of fat cells reaches a maximum early in life in the normal animal. The exact time at which precursor cellular proliferation ceases may vary from species to species and is difficult to determine because of methodological limitations. However, using radioactive thymidine incorporation into precursor fat cells, it was shown that precursor fat cell proliferation ceases by the fourth postnatal week in the epididymal fat pad of the normal rat (8). Furthermore, the data suggested that numerous preadipocytes were synthesized during the postnatal proliferative period, and that they did not fill with lipid until as long as 30 days later.

Although early postnatal regulation of enzymes associated with cellular proliferation is likely to play a decisive role in determining the size of the ultimate adult adipose tissue mass (9), it seems equally credible that regulation of the rate of "lipid-filling" during development must also contribute to the pattern of expansion of adipose depots. If a preadipocyte does exist, it may differ from the mature adipocyte with respect to its complement of active lipogenic enzymes, and the process of differentiation of preadipocyte into adipocyte could be marked by the induction and/or activation of those enzymes which are necessary for lipid-filling to occur at appreciable rates. Since little is known about the development of the enzymes controlling the rate of lipid-filling in adipose tissue during normal development, we have conducted a series of experiments to characterize the developmental pattern of adipose tissue lipoprotein lipase (LPL).

In the experiments reported here, total LPL activity has been measured in adipose tissue during postnatal development. Its levels were also determined in isolated adipocytes and stromal–vascular cells to determine if there is a relationship between the timing of adipocyte differentiation (lipid-filling) and the localization of the LPL activity between adipocyte and stromal–vascular compartments during growth. The data from these experiments help to substantiate and evaluate the existence of a preadipocyte pool in the stromal–vascular compartment and to assess the manner in which preadipocytes are recruited during the early postnatal growth of this tissue.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Holtzman Company) were used in all experiments. The rats were maintained in a temperature controlled room (24°C) with a 12-hr light-dark cycle. Rats were fed Purina lab chow ad libitum, except in fasting experiments. When rats were fasted they were allowed ad libitum access to tap water. The enzyme methodology studies

Abbreviations: LPL, lipoprotein lipase; FFA, free fatty acids.

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were conducted on tissue from adult, 3–4 month old rats. In the developmental study a total of 188 animals ranging in age from 10 to 235 days were used.

Reagents

Disodium EDTA, fatty acid-free bovine serum albumin from fraction V, Triton X-100, Trizma base, and L-α-phosphatidylcholine (egg yolk, Type I, 98% pure) were purchased from Sigma Chemical Company (St. Louis, MO). Bacterial collagenase type III (141 U/mg) was purchased from Worthington Biochemical, Inc. (Freehold, NJ). The radioactive tri[1-^{14}C]oleoyl glycerol, sp act 75 mCi/mmol, from Applied Sciences Laboratories (State College, PA), and sp act 55 mCi/mmol from Amersham/Searle Corp. (Arlington Heights, IL) was purified by thin-layer chromatography. Hexane–diethyl ether–acetic acid 85:15:1 (v/v/v) was used as the developing solvent and the labeled triolein was eluted from silica gel first by chloroform, and then by chloroform–methanol 2:1 (v/v) to yield a material of approximately 99% radiopurity. Unlabeled triolein was purchased from Applied Sciences Laboratories. Insta-Gel (Packard Instrument Company, Downers Grove, IL) was used as the scintillation liquid. Sodium heparin (5000 I.U. per ml) was purchased from Organon, Inc., W. Orange, NJ.

Tissue and cell preparation

Rats were weighed and decapitated between 9 and 10 AM. The epididymal fat pads were removed and placed in ice-cold 0.25 M sucrose–1 mM EDTA buffer, pH 7.4, and kept on ice. After weighing both fat pads, a 20% (w/v) tissue homogenate in 0.25 M sucrose–1 mM EDTA buffer, pH 7.4, was prepared using a Potter-Elvehjem type glass homogenizer with a Teflon pestle. Samples from 10-day-old rats were prepared as a 10% homogenate due to the small amount of tissue available. The homogenates were centrifuged at 12,000 *g* for 15 min in a Sorval RC2-B refrigerated centrifuge at 4°C and the fat-free postmitochondrial supernatant was aspirated from below the fat cake layer. For some of the experiments in which methodology was compared, ammonium extracts (0.05 M NH_4OH/Cl buffer, pH 8.1) of acetone–ether powders from adipose tissue samples were extracted with 0.05 M NH_4OH–NH_4Cl buffer, pH 8.1, as described by Nilsson-Ehle (10).

In some experiments, isolated adipocytes and stromal cells were prepared by the collagenase digestion technique as described by Rodbel (11) and modified by Greenwood and Hirsch (8). Minced pieces of the fat pad in 3 ml of Krebs-Ringer bicarbonate buffer with 10 mM glucose, containing 1 mg/ml collagenase, were incubated in a shaking water bath at 37°C for 1 hr. The digested pieces were filtered through a nylon mesh with 25 ml of Krebs-Ringer bicarbonate buffer and centrifuged gently (200–400 *g*) in a table centrifuge for 30 sec. The fat cells floated to the surface; the bottom part of the buffer containing stromal–vascular cells was aspirated. The procedure was repeated three times and the first two washings were combined and used to isolate stromal cells by centrifugation in a Sorval RC2-B at 800 *g* for 15 min at 4°C. The stromal cells were washed twice and the final pellet containing stromal–vascular cells was suspended in 5 ml of Krebs-Ringer bicarbonate buffer with 10 mM glucose. The isolated adipocytes were suspended in 5–10 ml of the same buffer. In one series of experiments, both isolated intact adipocyte and stromal–vascular cells were homogenized manually with a glass-on-glass homogenizer and centrifuged at 12,000 *g* for 15 min at 4°C and the soluble, postmitochondrial fraction was used for enzyme assays.

It was of concern that the use of intact cells (both adipocytes and stromal–vascular cells) rather than postmitochondrial supernatant solutions from homogenates of the two cell fractions might result in artifactually lower enzyme activity. It is preferable to use intact cells, especially in young rats where preparation of appropriate postmitochondrial supernatants would have required an excessive number of rats in order to obtain sufficient tissue. We considered it a possibility that the plasmalemma might limit the accessibility of LPL to the triolein substrate. Therefore, in one series of experiments, LPL activity in intact adipocytes and stromal–vascular cells from adult rats was compared with the LPL activity present in postmitochondrial supernatants prepared from isolated adipocytes and stromal–vascular cells. **Table 1** illustrates that there were no significant differences between activity in the intact cells or the respective postmitochondrial supernatant fraction on a tissue weight basis. Although there was a modest enrichment of enzyme activity in the adipocyte supernatant preparation when the data were expressed on a per mg of protein basis, it was considered that the measurement of the LPL activity in the intact isolated cell fractions was satisfactory.

Lipoprotein lipase assay

Lipoprotein lipase activity (glycerol ester hydrolase, E.C.3.1.1.3) was determined using purified [^{14}C]-triolein as a substrate as described by Schotz et al. (12). The substrate emulsion was prepared by pipetting 1.8 μCi of labeled triolein and 36 mg of cold triolein in benzene into a plastic scintillation vial

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TABLE 1. LPL activity in isolated adipocytes and stromal–vascular cells from epididymal fat pads and in respective postmitochondrial supernatant fractions of adult rats

	Adipocyte Fraction		Stromal–Vascular Fraction	
	Intact Cells	Supernatant	Intact Cells	Supernatant
μmol FFA $hr^{-1}g^{-1}$ (wet wt)	0.60 ± 0.15	0.45 ± 0.22	0.39 ± 0.31	0.15 ± 0.05
μmol FFA $hr^{-1}g^{-1}$ (protein)	0.20 ± 0.04	0.52 ± 0.14[a]	0.22 ± 0.18	0.24 ± 0.05
n	10	8	5	8

[a] $P < 0.05$, all other comparisons were not significant.

Means and standard errors of the means are shown. Student's t test was used to determine significance of differences of LPL activity between intact and supernatant fractions of adipocyte and stromal–vascular compartments respectively.

together with 1.5 mg of lysolecithin in 3 ml of heptane. The organic solvents were evaporated in a water bath under a nitrogen flow. 0.2 M Tris-HCl buffer (2.55 ml), pH 8.0, 0.45 ml of 1% bovine serum albumin, and 3.0 ml of thawed human serum taken originally after a 12 hr fast were added to the vial and the mixture was sonicated in an ice bath at 60 W for 4 min at 30-sec intervals by a Branson sonicator (Danbury, CT) equipped with a microtip. In order to control for nonspecific lipolysis the [^{14}C]triolein substrate was prepared as described above, but in the absence of serum. Only the lipolysis resulting from serum activation was specified as lipoprotein lipase activity.

The LPL activity was assayed in duplicate plastic test tubes containing 0.1 ml of substrate emulsion (0.7 μmol of triolein, if not stated otherwise). All reaction mixture tubes were preincubated at 37°C for 30 min (12, 13). The reaction was started by the addition of 0.1 ml of adipose tissue postmitochondrial supernatant solution (or acetone–ether extracts); the incubation period was 30 min. The reaction was stopped by adding 3.25 ml of the fatty acid extraction mixture, chloroform–methanol–heptane 2.3:2.5:1.8, as described by Belfrage and Vaughan (14). Later, 1.05 ml of 0.1 M bicarbonate buffer, pH 10.5, was added to facilitate separation of the two phases (14, 15). The contents of the tubes were mixed on a Vortex mixer and centrifuged at 800 g for 20 min at room temperature. One-ml aliquots of the upper phase containing released free fatty acids were pipetted into plastic scintillation vials and 8 ml of Insta-Gel was added. The recovered radioactivity was counted in a refrigerated Packard TriCarb liquid scintillation spectrometer, and the results were converted to μmol of FFA released per hr. The counting efficiency was 85%.

Comparison of emulsification agents in ammonium extracts of acetone–ether powders and in postmitochondrial supernatants

Two different methods for obtaining the LPL activity were compared. A postmitochondrial supernatant prepared as described above and ammonium extracts of acetone–ether powders as described by Nilsson-Ehle et al. (15) were prepared. The emulsification of the triolein is an important step in the preparation of substrate, and surfactants facilitate this procedure. Therefore, a comparison was made between two commonly used surfactants, Triton X-100 and lysolecithin, (**Figs. 1A** and **1B**) in concentrations reported as optimal by others (12, 15). In ammonium extracts of acetone–ether powders, no marked differences were found in the LPL activity, regardless of the emulsifier, but in the postmitochondrial supernatant fraction, emulsification in the

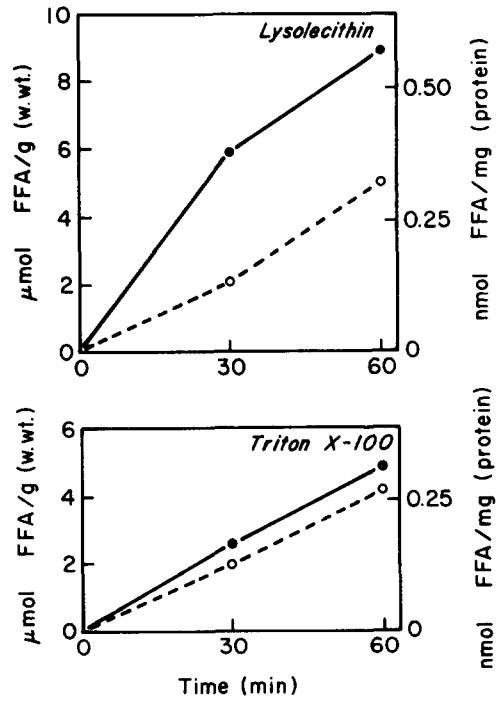

Fig. 1. The effects of emulsifying agent lysolecithin (10 μg/assay) or Triton X-100 (8 μg/assay) on LPL activity in modified postmitochondrial supernatant (solid line) and acetone–ether powders prepared from postmitochondrial supernatants (dotted line). Otherwise, incubation conditions are as described in Methods and Materials. Each point represents mean of duplicate assays from three rats.

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presence of lysolecithin resulted in higher activity. Thus, lysolecithin was used in the substrate preparation and the postmitochondrial supernatant was used as the enzyme source for all characterization studies and for the developmental study.

Protein, lipid, and cellularity determinations

Protein was determined according to Lowry et al. (16). Total lipids were extracted according to Folch, Lees and Sloane Stanley (17). Total lipid was determined by oxidization of the extracted lipid with potassium dichromate in 36 N H_2SO_4 and by measuring the disappearance of yellow-brown color in a spectrophotometer at 350 nm (18).

Adipose tissue cellularity was determined using the method of electronic counting of osmium-fixed cells as described by Hirsch and Gallian (19). The animals used to determine cellularity were of the same strain, age, and body weight as the rats used for LPL assay, but cellularity was determined at less frequent time intervals than LPL activity.

RESULTS

Enzyme characterization study

Substrate saturation occurred at a level of 2.0 mM triolein and a further increase in triolein concentration did not enhance the LPL activity to any great extent. (**Fig. 2A**). The K_m derived from a Lineweaver–Burk plot was 0.62 mM. The reaction at 37°C was linear up to 45 min of incubation; thereafter, the reaction continued at a slower rate (Fig. 2B). The LPL activity increased in a linear fashion up to 0.27 mg of supernatant protein in the incubation mixture (Fig. 2C). The pH optimum showed a broad spectrum with peak activity at pH 7.5–8.0 (Fig. 2D). In vitro addition of heparin caused only a slight (ca. 10%) activation of the supernatant LPL activity and a higher concentration inhibited the activity (Fig. 2E). Since LPL activity is characterized by serum activation and NaCl inhibition, the effects of these factors were also studied. The peak activation of LPL activity was achieved at a final serum concentration of 25% (Fig. 2F); a 0.5 M final NaCl concentration caused over 90% inhibition (Fig. 2G). Fasting rapidly decreased the LPL activity. After a 6 hr fast, only about 40% of the LPL activity remained and further fasting resulted in only a slight additional decrease in enzyme activity (**Table 2**).

LPL activity in the epididymal fat pads of growing rats

Rats used in these experiments had normal body and fat pad weight gains (**Figs. 3A** and **3B**). Fat pad

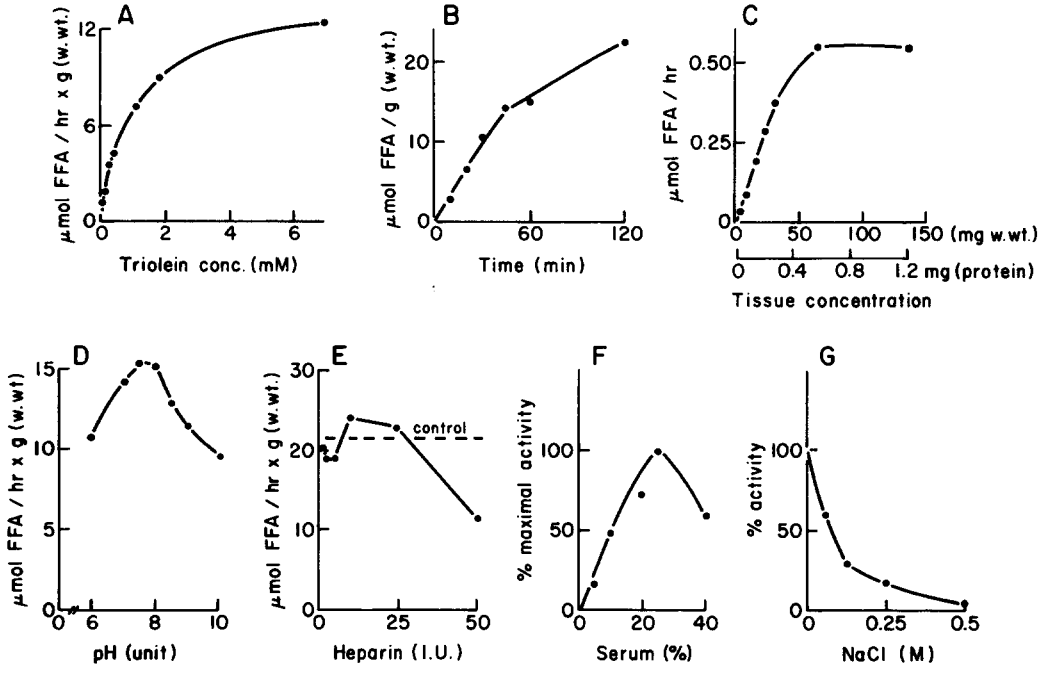

Fig. 2. Kinetics of LPL in postmitochondrial supernatant prepared from epididymal fat pads of adult (3–4 months of age) rats. *A*. Effect of substrate (triolein) concentration on LPL activity. *B*. Effect of time of incubation on LPL activity. *C*. Effect of tissue and/or protein concentration on LPL activity. *D*. Effect of pH on LPL activity. *E*. Effect of in vitro addition of heparin (International Units per assay) on LPL activity. *F*. Effect of serum concentration on LPL activity. *G*. Effect of NaCl concentration on LPL activity. Each data point represents the mean of quadruplicate assays.

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TABLE 2. Effect of fasting on LPL activity in rat epididymal fat pad

Fasting Period	n[a]	FFA Released			
		μmol/hr per g (wet wt.)	P	μmol/hr per mg protein	P
0	10	10.6 ± 1.2[b]		0.84 ± 0.08	
6	5	4.9 ± 0.6	0.01	0.42 ± 0.04	0.005
24	5	3.6 ± 0.6	0.005	0.40 ± 0.12	0.01
48	5	3.9 ± 1.5	0.01	0.29 ± 0.11	0.005

[a] Number of rats.
[b] Mean ± SEM.
The statistical significances between control group and fasted group were determined using Student's *t* test and *P* values are denoted.

weight began to increase markedly at 22 days of age (Fig. 3B); this is reflected in the increasing percentage of body weight contributed by the fat pad (Fig. 3C). These data show that from 22 to 99 days the weight of epididymal fat pads increased more rapidly than did total body weight. This pad growth occurs by early hyperplasia which is noted in Fig. 5 where the number of cells per gram remains constant as does fat cell size from 12 to 35 days of age. The increasing deposition of lipid after 35 days of age is reflected by an increased cell size (see Fig. 5) and a drop in the amount of protein extracted into the supernatant fraction per gram of epididymal adipose tissue, noted particularly between 50 and 100 days of age (Fig. 3D). Lipoprotein lipase activity expressed as μmol of FFA released per gram of tissue wet weight (**Fig. 4A**) increased sharply from 10 days, peaking at 42 days. From 42 to 57 days it decreased to levels that remained unchanged for the rest of the experimental period. When the enzyme activity was expressed on the basis of the amount of supernatant protein, (Fig. 4B), a sharp increase in LPL activity was apparent between 10 and 42 days of age, and then it stabilized at the elevated levels. When expressed on a cellular basis (Fig. 4C), LPL activity was unchanged from 10 to 17 days, increased fourfold between 17 and 42 days, and increased more gradually an additional twofold by 235 days of age. As might have been expected, this pattern was also reflected in total epididymal pad LPL activity during normal tissue growth, shown in panel D.

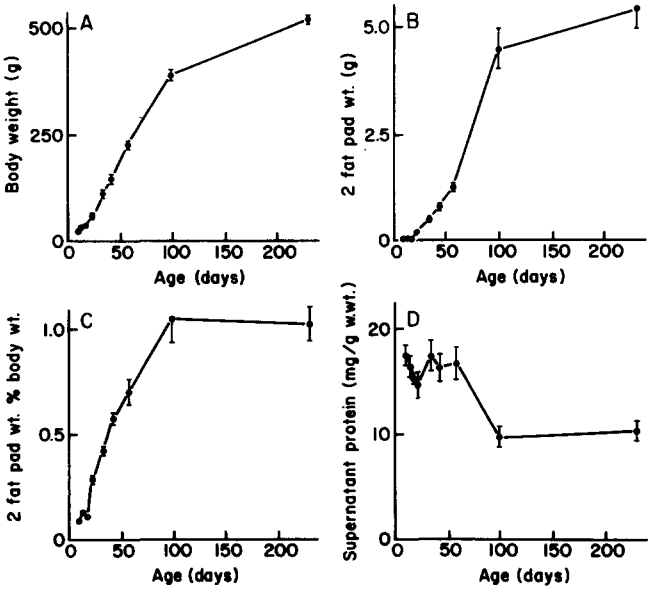

Fig. 3. Growth characteristics of rats used for the LPL development. The means and standard errors of the means are shown. The number of experiments and (rats per pool) in each age group were as follows: 10 days, 4 (4–5); 13 days, 14 (4); 17 days, 4 (4); 22 days, 16 (3); 33 days, 12 (2); 42 days, 8 (1); 57 days, 6(1); 99 days, 6 (1); 235 days, 6 (1). Both epididymal pads from each rat were used. *A*. Body weight curve. *B*. Epididymal fat pad growth. *C*. Percent of body weight contributed by epididymal fat pads. *D*. supernatant protein extracted per gram of epididymal fat pad at various ages.

Isolated adipocytes and stromal–vascular cells

We examined the tissue compartmentalization of LPL in order to provide further information that might aid in understanding adipose tissue development. In an effort to prepare two separate isolated cell fractions free from contamination, one from the other, repeated washings of the two fractions were carried out, which resulted in considerable cell loss from both fractions. As the rats aged, both the amount of tissue used for collagenase digestion and the amount of lipid in the tissue increased. As noted in **Table 3**, stromal–vascular fractions were free of lipid and, therefore, presumably free of fat cells. Repeated washings of the fat cells produced an isolated adipocyte fraction free from stromal–vascular cell contamination at the light microscopic level. When the isolated cell fractions are prepared in this fashion, there are several technical problems to be considered. The first is that the isolation procedure itself results in selective cell loss from one or another of the isolated cell fractions. Another consideration is the possibility that the LPL itself is differentially responsive to collagenase digestion during development. Since there are no really adequate methods of con-

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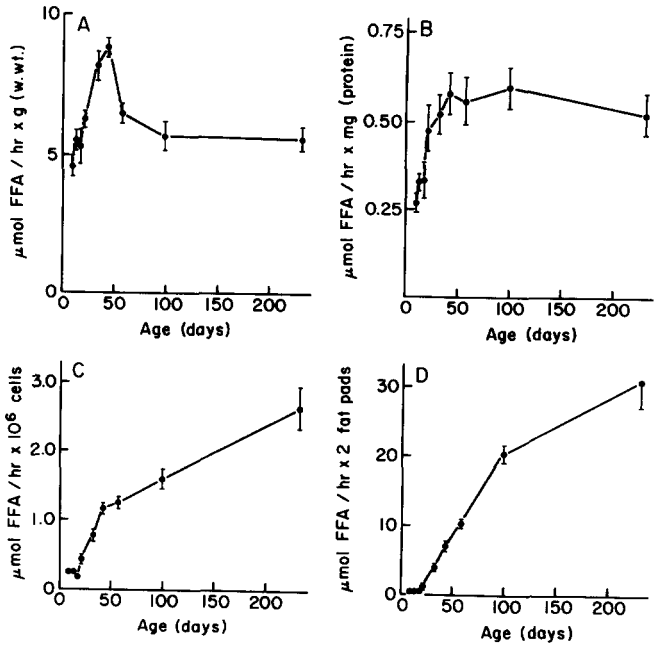

Fig. 4. LPL activity in epididymal fat pads during development. *A*. LPL activity per gram of tissue wet weight. *B*. LPL activity per mg of supernatant protein. *C*. LPL activity on a cellular basis. *D*. Total LPL activity per two epididymal fat pads. Data are reported as mean values ± SEM for 4–16 separate groups of rats at each age studied (see Fig. 3).

trol for these contingencies, caution must be observed when interpreting these data.

The protein yield in these isolated cell preparations, as one would expect, was a function of the amount of tissue used in the collagenase digestion procedure, the increased with both tissue weight and age of the rats used (Table 3). The protein concentration of the isolated adipocyte fraction (mg/g of tissue) remained constant except at 42 days when an apparent, but not significant, decrease occurred. The protein concentration of the isolated stromal–vascular cell fraction decreased as rat age increased. When the total amount of protein recovered in both isolated adipocytes and stromal–vascular cells is divided by the amount of tissue used for collagenase digestion, the ratio, although variable, remains relatively constant, decreasing only 20–30% from 12 to 90–105 days. The proportion of adipocyte to stromal–vascular protein recovered also remained comparable except at 90–105 days when there was more of the recovered protein in the adipocyte fraction. In both isolated fractions, the amount of protein used to assay LPL was well within the linear range of the reaction.

When LPL activity was assayed in each isolated cell fraction and the enzyme activity was expressed as μmol FFA released/hr per gram wet weight, the LPL activity in the adipocyte fraction (A) showed an eightfold increase between 22 and 42 days of age and then decreased somewhat by 90–105 days of age (**Table 4**). This pattern of LPL activity is also apparent in the isolated stromal–vascular fraction (S). When LPL activity was expressed per mg of protein, similarly, there was an increase in activity in both fractions (A and S) until 42 days and a subsequent decrease by 90–105 days (Table 4).

When the LPL activity was expressed per adipocyte, it increased with age and, consequently, with cell size. When the percent of total recovered LPL activity was calculated for each fraction, there was a continual increase in LPL activity with age in the adipocyte fraction while the stromal–vascular fraction LPL activity was highest in young rats and decreased with age (Table 4).

TABLE 3. Lipid and protein content of isolated adipocytes (A) and stromal–vascular cells (S) from epididymal fat pads of growing rats

Age (days)	n	Total Weight per Collagenase Digestion	Lipid Total Yield[a] A	Lipid A	Isolated Cell Fraction Supernatant Protein		Protein Recovered (A&S) per Gram Tissue[b]	Tissue Supernatant Protein		A Protein / S Protein
					A	S		A	S	
		g	*mg*	*mg/g*	*mg*		*mg*	*mg/g*		
10– 21	3	0.16 ± 0.01	3.7 ± 2.3	24.0 ± 15.9	0.43 ± 0.08	1.00 ± 0.14	8.99 ± 3.18	2.76 ± 0.60	6.34 ± 0.69	0.43
22	3	0.28 ± 0.07	3.0 ± 1.1	10.8 ± 8.5	0.76 ± 0.04	1.56 ± 0.13	9.19 ± 3.49	3.17 ± 0.82	6.37 ± 1.38	0.49
42	4	0.57 ± 0.05	30.7 ± 3.8	54.3 ± 5.9	0.87 ± 0.32	2.77 ± 0.58	6.51 ± 1.10	1.61 ± 0.68	4.97 ± 1.20	0.32
90–105	10	1.19 ± 0.10	75.7 ± 15.1	67.0 ± 13.4	4.0 ± 0.85	2.30 ± 0.10	6.33 ± 1.7	3.20 ± 0.40	2.20 ± 0.20	1.42

The means and standard errors of the means are given. The numbers of fat pads pooled to make each sample are from 1–9 rats, depending on the age. n = the number of pools.

[a] The amount of lipid in stromal fraction was below the sensitivity of the method.

[b] There are no statistically significant differences between age groups in total protein recovered per gram tissue used.

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TABLE 4. Lipoprotein lipase activity in isolated adipocytes (A) and in stromal–vascular cells (S) of epididymal fat pads from growing rats

Age (days)	n[a]	μmol FFA Released/hr						
		Per g (wet wt.)		Per mg Protein		Per 10^6 Adipocytes	Percent of Total Activity	
		A	S	A	S	A	A	S
10–12	3	0.0	0.32[b] ± 0.19	0.0	0.04 ± 0.02	0.0	0.0	100.0
22	3	0.10 ± 0.03	0.49 ± 0.05	0.40 ± 0.01	0.08 ± 0.01	0.75 ± 0.25	16.9 ± 3.0	83.1 ± 3.9
42	4	0.82 ± 0.26	1.77 ± 0.16	0.55 ± 0.05	0.43 ± 0.11	1.13 ± 0.33	30.3 ± 7.2	69.7 ± 7.2
90–105	13	0.54 ± 0.12	0.26 ± 0.14	0.20 ± 0.04	0.23 ± 0.07	1.70 ± 0.39	72.0 ± 4.8	28.0 ± 4.8

[a] Number of samples.
[b] Mean ± SEM.
The number of fat pads pooled to form each sample came from 1–9 rats, depending on age of the rat.

DISCUSSION

Lipoprotein lipase regulates the removal of triglycerides from serum and their entry into peripheral tissues. It has been studied in man, both in obese and in normal control subjects, in postheparin serum and in adipose tissue (20–24) and its activity has been shown to increase per cell as fat cell size enlarges in the adult (25). Since there are numerous methods available for measuring LPL activity (10, 15, 26–28) and we wished to measure it in very small amounts of adipose tissue, the optimal requirements for the enzyme assay were established. The enzyme kinetics of the present study generally agree with previous reports. The substrate saturation and Michaelis constant (0.62 mM) are in agreement with those described previously in perfused adipose tissue (0.70 mM) (29). The optimal incubation time, enzyme concentration, and pH, and the findings that LPL activity is not enhanced by heparin, is activated by serum, inhibited by NaCl (Fig. 2), and reduced by fasting (Table 2), are also in agreement with earlier reports (12, 13, 30–32). In the present experiments LPL activity was higher in the postmitochondrial supernatant fraction than in acetone–ether extracts, as also reported previously by others (33, 34). LPL activity was higher in the presence of lysolecithin than in the presence of Triton X-100 in the postmitochondrial supernatant fractions. Therefore, postmitochondrial supernatants prepared from adipose tissue homogenate, incubated in the presence of serum and [^{14}C]triolein substrate and prepared in the presence of lysolecithin, provided a reproducible assay system for use with very small amounts of tissue.

Very little, if anything, is known about the development of LPL activity during the differentiation of adipose tissue. The possible correlation between changes in tissue LPL activity and lipid-filling of adipocytes during normal growth is of interest in that it might form a biochemical basis for further evaluation of regulatory disturbances in lipid turnover during development and might suggest a mechanism whereby obesity could occur, particularly that form accompanied by large increases in adipocyte size. The hypothesis that precursor fat cell proliferation occurs primarily in postnatal development and that further increments in adipocyte number come about by lipid-filling of this "bed of precursor fat cells" made during early postnatal development was recently confirmed by measuring incorporation of radioactive thymidine into precursor adipocytes (8). The demonstration that predetermined, precursor, nonlipid-filled cells existed during the early postnatal, prepubertal period suggested that the induction of and regulation of the rate of lipid-filling of such cells might play a crucial role in the determination of adipose tissue mass in the postsuckling rat.

When one analyzes adipose tissue growth by examining changes in fat cell size, measured either as the amount of lipid per adipocyte or as the number of adipocytes per gram of tissue, an interesting pattern evolves (**Fig. 5**). During the early postnatal period, from 10 to 35 days of age, cell size (μg lipid/cell) did not change significantly ($P > 0.05$). Similarly, the number of adipocytes per gram of tissue did not change. Yet total pad weight (Fig. 3B) and total lipid per pad rose significantly during this same period. Therefore, significant numbers of new cells

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of the same average size must have been added to the pad during this interval. Beginning at approximately 35 days and continuing at least until 100 days of age, marked increases in fat cell size occurred. This is reflected both as a decrease in the number of fat cells per gram of tissue and as a sharp increase in the lipid per adipocyte.

The data from this developmental study support the concept that the changes in LPL activity per fat cell precede the changes seen in fat cell size. The early and marked postnatal increase in the lipoprotein lipase activity per cell occurred between 17 and 35 days of age which is before the increase in the amount of lipid per cell is noted (**Fig. 6**). Lipoprotein lipase activity per adipocyte showed a significant fourfold increase ($P < 0.01$) from the value at 17 days compared to that seen at 35 days of age while the average fat cell size did not significantly increase. From 10 to 42 days of age the specific activity of the enzyme, expressed per gram wet weight or per mg supernatant protein, also increased markedly (Fig. 4A and B). This suggests that these changes in activity that precede, and are probably causally related to, the active lipid-filling of preadipocytes, may have occurred as a result of enzyme activation and/or induction.

In a recent study of porcine LPL activity, enzyme activity expressed on a wet weight basis was highest at the age of 15–20 weeks, which is in accord with the beginning of differentiation of fat cells in this species (35). This agrees with the present data, suggesting that the increase in the LPL activity precedes the detectable lipid-filling of preadipocytes. In the study of Lee and Kauffman (35), as in the present study, an increase of the LPL activity per mg protein was noted, suggesting activation or induction of the LPL enzyme.

Further support for the concept that enhancement of LPL activity in "preadipocytes" occurs during early development is found in the tissue compartmentalization experiments; LPL activity was greatest at the age of 42 days, both in isolated adipocytes and in the stromal–vascular fraction (Table 4). The LPL activity increased about 3.5- to 5-fold from 10 to 42 days in the stromal–vascular fraction and this may well be due to initiation of differentiation in existing preadipocytes of the stromal–vascular fraction. However, since there were no adequate markers to identify preadipocytes, it is not possible to separate this pool from the rest of the stromal–vascular cells. Although the isolated adipocytes and stromal cells separately showed a pattern of development similar to that seen in supernatant fraction prepared from whole adipose tissue of growing rats, there were differences with respect to the distribution of

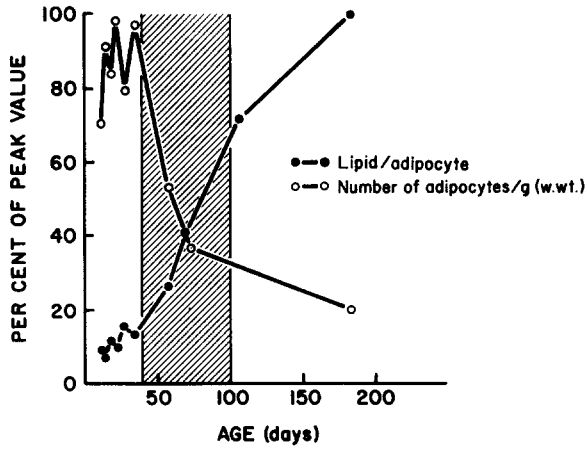

Fig. 5. Changes in lipid per adipocyte and number of adipocytes per gram of tissue in normally growing rats. For simplicity of representation the use of percent peak value was used. Each point represents the mean of 3–6 rats. The shaded area represents the period of apparent increase in fat cellularity due to lipid filling of preexistent cells.

recovered LPL activity between the two fractions. Care must be taken when interpreting the compartmentalization data since the protein recovery was not complete. The ratio of the amount of protein in both fractions recovered per gram of digested tissue was relatively constant from 12 to 42 days of age, as was the ratio of adipocyte:stromal protein. Although one cannot directly compare protein extracted in postmitrochondrial fraction (Fig. 3D) with total tissue protein, it is encouraging that postmitrochondrial protein/gram of tissue was constant until 42–50 days of age. Curtis-Prior (36) also reported that in younger rats the protein per gram of adipose tissue

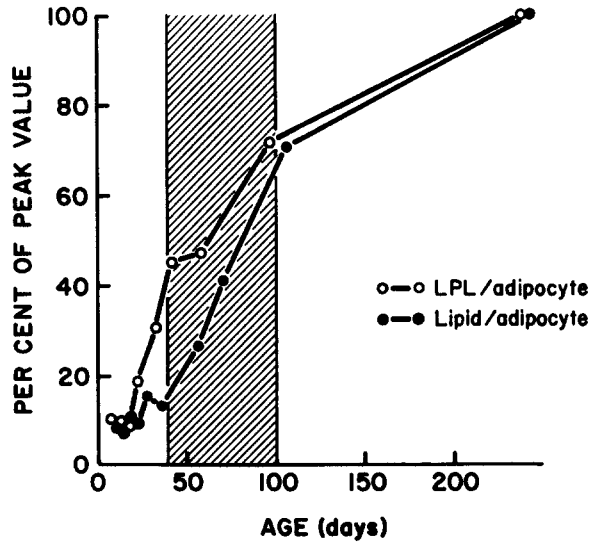

Fig. 6. Changes in LPL activity per adipocyte compared to the changes in fat cell size during development. For ease of comparison percent peak value is indicated on the ordinate.

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did not begin to change until body weight exceeded 200 g. Consequently the changes seen in the compartmentalization of LPL, at least until 42 days of age, can be most likely interpreted as genuine.

Total protein per gram of adipose tissue does decrease in older rats (36). Our total protein recovered per gram in the 90–105 day old rats was also somewhat lower and proportionately more of the recovered protein was associated with the adipocyte fraction. The recovery of adipocyte and stromal protein combined has been shown to be about 8.5 mg per gram of fat tissue in adult, fat-fed rats (37), yielding a 65% recovery of total tissue protein, which agrees well with this study in standard fed rats. However, the loss of LPL activity may be somewhat greater during the more extensive isolation procedures used in the present studies. Cunningham and Robinson (38) have reported only 20% recovery of LPL activity in the isolated fractions compared to respective total tissue activity, and Rodbell (11) has shown that about half of the activity remains in the tissue residue during the isolation. Part of the lost activity is due to a loss of adipocytes and stromal cells under repeated washings. In addition, some of the LPL activity may be released from adipocytes into washings. Nonetheless, with these considerations in mind, these changes in compartmentalization of LPL activity support the hypothesis that there are existing preadipocytes in the stromal fraction during early postnatal development that later differentiate and move to the adipocyte compartment.

In 10 to 12-day-old rats, all of the activity recovered is present in the stromal fraction. In fact, at this age, there are almost no fat-filled cells large enough to be isolated as an adipocyte fraction. As adipocyte lipid-filling procedes, increasing amounts of lipoprotein lipase activity are found in the adipocyte fraction (Table 4). At 22 days, 16% is found in adipocytes; at 42 days, 30% of the LPL activity is in the adipocytes; and in the mature rat of 90–105 days of age, 72% of the recovered lipoprotein lipase activity is detected in isolated adipocytes. Even considering the low yield of total LPL activity in the isolated adipocyte fraction and stromal–vascular fractions, the data support the interpretation that elevated LPL activity in the stromal–vascular fraction during early postnatal development is associated with the onset of lipid-filling of preexisting "preadipocytes".

The mechanism(s) that control LPL activity are unknown. In the more mature rat age and/or cell size may well play a role in the regulation of LPL activity, but the control of enzyme activity in the young rat, leading to differentiation of precursor cells into mature, lipid-filled adipocytes, is not understood. In adipose tissue, two forms of LPL activity have been described; one form is postulated to be a precursor undergoing a transformation into an active form (21, 39–41). The factors controlling the interconversion of these forms are presently unknown but it may be that the proportion of these forms varies in adipose tissue during critical periods of normal development.

In summary, LPL activity per adipocyte increases fourfold from the second to seventh postnatal week, a time during which adipocyte size shows no statistically significant change. The onset of the increased LPL activity per adipocyte and predominant localization of LPL activity in the stromal–vascular fraction of the tissues during early postnatal development supports the hypothesis that a "bed of preadipocytes", is formed during the postnatal hyperplastic growth phase of the tissue which then becomes lipid-filled as a result of the action of LPL to provide FFA for triglyceride synthesis. Furthermore, the early developmental changes in adipose tissue LPL activity may be among the factors that regulate the rate of triglyceride storage and, therefore, the size of adipose tissue mass in the long term. Since LPL activity is responsive to dietary and hormonal changes, factors that influence either the absolute level of its activity, its affinity for substrate, or the proportion of active to inactive enzyme form could play key roles in regulating adipocyte size during normal development and, if perturbed, might lead to the development of adipocyte hypertrophy and consequent obesity.

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