Two different mechanisms are involved in nutritional regulation of lipoprotein lipase in guinea-pig adipose tissue

Henrik SEMB* and Thomas OLIVECRONA

Department of Physiological Chemistry, University of Umeå, S-90187 Umeå, Sweden

Lipoprotein lipase activity in adipose tissue responds rapidly to changes in the physiological state. To study what mechanisms are involved in the regulation, guinea pigs were fasted and the decrease in adipose-tissue lipoprotein lipase activity was compared with the decreases in mRNA and lipase synthesis. The mRNA pattern (three species) did not change. There was a close parallelism between the abundance of lipase mRNA and relative lipase synthesis (immunoprecipitable ^{35}S -labelled lipoprotein lipase as fraction of total [^{35}S]protein after pulse-labelling with [^{35}S]methionine). Total protein synthesis decreased on fasting, compounding the decrease in relative lipase synthesis. Lipoprotein lipase mRNA changed similarly in fat-pads and in isolated adipocytes, whereas lipase activity changed more in the pads, indicating disproportionally large changes in extracellularly located lipase. In old guinea pigs the decreases in lipoprotein lipase activity and lipase synthesis were comparable, but in young animals the change in lipase activity was substantially larger than the change in lipase synthesis. Refeeding of fasted young guinea pigs with glucose resulted in a rapid increase in lipoprotein lipase activity, but there was only a small change in lipase mRNA. Old animals responded slowly to refeeding. The results indicate that in older animals the major mechanism for regulation of adipose lipoprotein lipase activity is a relatively slow change in lipase mRNA, whereas in younger animals an additional, more rapid, regulation is exerted on the transport and turnover of the enzyme.

INTRODUCTION

In adipose tissue lipoprotein lipase is synthesized in adipocytes, but is then transferred, by a yet unknown mechanism, to the vascular endothelium (reviewed in refs. [1–3]). Here the enzyme hydrolyses triacylglycerols in lipoproteins (chylomicrons and very-low-density lipoproteins). The released fatty acids are taken up in adipocytes, where they are re-esterified into triacylglycerols or used for other metabolic processes. Thus local changes of lipoprotein lipase activity regulate the amount of lipoprotein fatty acids taken up by the tissues.

Lipoprotein lipase activity in adipose tissue responds rapidly to changes in the physiological state, e.g. feeding/fasting [4], cold adaptation [5], physical exercise [6,7], lactation [8] and infections/tumours [9]. Hormones involved in the regulation include insulin, catecholamines, glucocorticoids and tumour necrosis factor (reviewed in refs. [4] and [9]). It is not clear, however, what the molecular mechanisms of the regulation are. To study this we have used guinea pigs, since nutritional regulation of adipose-tissue lipoprotein lipase activity has a larger amplitude in this species than in the more studied rat or mouse models [10]. Results indicating that inactive forms of the enzyme could be recruited into active enzyme without synthesis of new protein had been reported by other groups [11–17]. Studies on the synthesis, processing and transport of the enzyme in adipocytes showed, however, that the enzyme becomes active soon after synthesis and travels a direct path out of the cell [18,19]. These experiments give no evidence for major delay in a storage pool. There are several reports that the rate of

lipoprotein lipase synthesis varies with nutritional and hormonal factors [20–22], but this regulation does not appear to account fully for the changes in lipoprotein lipase activity [23]. Pulse-chase experiments have shown that only a fraction, often less than half, of newly synthesized lipoprotein lipase is released from the adipocytes [18,24]. The rest is rapidly degraded in the cells. This provides an opportunity for regulation, by modulation of the fraction released versus that degraded. Such a possibility is supported by the finding that the amount of enzyme located extracellularly in the tissue undergoes more marked changes with nutritional state than does the intracellular lipoprotein lipase activity [1–4,10].

In the present study we have compared changes of lipoprotein lipase activity in guinea-pig adipose tissue on fasting with the changes in its mRNA and in the rate at which the enzyme is synthesized. The results indicate that two mechanisms operate, control of enzyme synthesis and control of enzyme turnover.

MATERIALS AND METHODS

Materials

The antiserum used was the same as in our previous studies [10,18,25,26]. It was raised in a rabbit, by using lipoprotein lipase purified from guinea-pig milk as antigen [27]. Bovine serum albumin, bovine insulin and Trasylol were from Sigma, St. Louis, MO, U.S.A. Heparin was from Löwens, Malmö, Sweden. Cell-culture media were from Flow Laboratories. [α - ^{32}P]dCTP was from Amersham Sweden AB, Solna, Sweden.

* To whom correspondence should be addressed.

Leupeptin and pepstatin were from The Peptide Institute, Osaka, Japan. Collagenase was from Boehringer, Mannheim, Germany. Hepes and phenylmethanesulphonyl fluoride were from Serva, Heidelberg, Germany.

Animals

Male guinea pigs (Dunkin Hartley) were used. The animals that we designated 'young' and 'old' were of a mean age of 40 and 105 days and weighed 400–500 g and 750–850 g respectively. They were housed with a 12 h daylight cycle. Fed animals had free access to food pellets and drinking water. The composition of the diet (by wt.) was: 19.6% total protein, 17% digestible protein, 3.4% fat and 46% carbohydrate. Fasting, which started in the morning, was for 24 h, 48 h and 72 h with access to water only. For each experiment animals from a single consignment were used. Some of the fasted animals were tube-fed with glucose [6.8 ml of a 75% (w/v) glucose solution in water/kg body wt.] and returned to their cages with access to water only. Corresponding controls were given the same volume of water. All experiments started between 08:00 and 10:00 h, when the animals were either killed or tube-fed with glucose. Animals were killed by a blow on the neck. Tissue studied was epididymal adipose tissue.

Assay of lipoprotein lipase activity and of DNA

Adipocytes were prepared by collagenase digestion of epididymal adipose tissue and washed in Krebs–Ringer bicarbonate buffer as detailed in ref. [10]. Tissue and adipocytes were homogenized in a buffer containing detergents as described [10]. The homogenate was centrifuged, and the clear solution between a floating fat layer and a sediment was used for assay of lipoprotein lipase activity and DNA [10]. Lipoprotein lipase activity was determined by the method of Peterson *et al.* [28] as detailed by Semb *et al.* [25]. DNA was measured by determining the fluorescence of Hoechst 33258 bound to DNA [29]. One unit of enzyme represents 1 nmol of fatty acid released/min at 25 °C.

[³⁵S]Methionine incorporation

To study synthesis of lipoprotein lipase, pieces of, or intact, epididymal fat-pads from guinea pigs were rinsed at room temperature in medium A [Eagle's minimal essential medium (modified) with Earle's salts and 2 g of NaHCO₃/l, without glutamine and methionine, supplemented with 5% (w/v) bovine serum albumin/1 mM-glutamine/0.1 μM-insulin (bovine; Sigma)/1.25 mM-Hepes, pH 7.4]. The fat-pads or pieces were then incubated in a shaking water bath for 20 min at 37 °C, in 4 ml of gassed (O₂/CO₂, 19:1) medium A supplemented with [³⁵S]methionine (DuPont–New England Nuclear; 1100 μCi/mmol, 45 μCi/ml). To study protein synthesis in adipocytes, the cells were labelled for 10 min under the conditions described above for fat-pads. Cells and media were separated by centrifugation at room temperature (30 s at 700 g). Adipose tissue and cells were homogenized in a detergent-containing buffer, as described above, and lipoprotein lipase was immunoprecipitated as detailed in ref. [25]. For quantification of ³⁵S-labelled lipoprotein lipase, the immunoprecipitates were electrophoresed on SDS/polyacrylamide gels and the lipoprotein lipase zone was cut out and counted for radioactivity in a LKB β-scintillation counter. Total incorporation of [³⁵S]methionine was determined by

precipitation of proteins with trichloroacetic acid as detailed in ref. [26].

Isolation of RNA

Total RNA was prepared by the method of Chirgwin *et al.* [30]. Briefly, the tissue was homogenized in 4 M-guanidinium thiocyanate. The homogenate was layered on a 5.7 M-CsCl cushion and centrifuged at 25 °C (18 h at 94000 g). The RNA pellet was collected. RNA concentration was calculated from the A₂₆₀.

Analysis of RNA

Dot-blot dilution series were performed with glyoxylated total RNA preparations, on nitrocellulose filters saturated with 15 × SSC [31], with a Bio-Rad dot-blot apparatus. Six dilutions were analysed for each RNA preparation. The blots were probed with a subclone of the lipoprotein lipase recombinant LPLA [32], labelled with [α-³²P]dCTP by the 'oligo-labelling' technique [33]. This probe corresponds to nucleotides 690–1240 in Fig. 1 of a previous publication by Enerbäck *et al.* [32]. This segment is located within the coding sequence of the mRNA and encompasses the C-terminal half of the mature protein. Pre-hybridization and hybridization were carried out under stringent conditions with 50% formamide at 42 °C [34]. Stringent post-hybridization washes were performed (0.1% SDS and 0.1 × SSC at 50 °C). After exposure to X-ray film, the filters were cut and each dot was counted for radioactivity in a LKB β-scintillation counter. The radioactivity data were analysed by multiple regression analysis, and the best fit to a regression line was determined. The slopes of the regression lines were compared, as a measure of relative lipoprotein lipase mRNA abundance. For Northern-blot analysis, electrophoresis in a 1% agarose gel in 10 mM-phosphate buffer, pH 7.0, was carried out after denaturation with glyoxal and dimethyl sulphoxide [35]. Glyoxylated RNA was then transferred on to nitrocellulose filters [36]. The blots were probed and hybridized as described for the dot-blots. To exclude loss and/or degradation of total RNA, all RNA preparations were tested for intact 28 S rRNA by Acridine Orange staining and analysed by Northern-blot analysis probing for lipoprotein lipase mRNA.

RESULTS

The first step in this research was to study the effect of changes in nutritional conditions on lipoprotein lipase activity and on lipoprotein lipase synthesis in adipose tissue in young and older guinea pigs (Table 1). Fasting suppressed the enzyme activity to less than 20% within 24 h in both groups. Synthesis of lipoprotein lipase also decreased on fasting, but this was more marked in the older guinea pigs. Lipoprotein lipase is known to turn over rapidly, so that active lipoprotein lipase represents enzyme molecules that have been synthesized within the last few hours [16,18,24]. If the rate of lipoprotein lipase synthesis was the sole determinant of tissue lipoprotein lipase activity, one would expect the values for the two parameters roughly to parallel each other at times such as 24 and 48 h, i.e. a decrease in lipoprotein lipase synthesis by about 50% would cause a decrease in lipoprotein lipase activity also to about 50% of its value in fed controls. In Fig. 1 we have plotted individual values for lipoprotein lipase activity and lipoprotein

Table 1. Changes in general protein synthesis, lipoprotein lipase synthesis, lipoprotein lipase relative synthesis and lipoprotein lipase activity in adipose tissue during fasting in young and old guinea pigs

Epididymal adipose tissue from fed (controls) guinea pigs and animals fasted for up to 72 h were dissected out, washed and incubated in [³⁵S]methionine-containing medium as detailed in the Materials and methods section. Incorporation of radioactivity into immunoprecipitable lipoprotein lipase (LPL) and total proteins was determined. For young guinea pigs there were three animals in each group. For old guinea pigs there were two animals in each group, except for the fed animals, for which there were three. For each animal lipoprotein lipase activity was determined in one fat-pad and incorporation of radioactivity into proteins and lipoprotein lipase was determined in the contralateral fat-pad. Both analyses were done in duplicate. The mean values for lipoprotein lipase activity in fed controls were 3.3 and 1.3 units/mg of DNA for young and old guinea pigs respectively.

		General protein synthesis*	LPL synthesis†	LPL relative synthesis‡	LPL activity§
Young					
Fed	1	1203	7478	0.62	3606
	2	1192	7432	0.62	3066
	3	1118	5476	0.49	3291
24 h	1	282	2036	0.72	508
	2	381	1555	0.41	375
	3	368	2524	0.69	597
48 h	1	499	3092	0.62	387
	2	624	2518	0.40	733
	3	439	2292	0.52	409
Old					
Fed	1	3085	12 500	0.41	793
	2	3469	11 102	0.32	2512
	3	3821	10 751	0.28	595
24 h	1	1663	2636	0.16	208
	2	2262	3021	0.13	266
48 h	1	2542	1942	0.08	64
	2	1242	1053	0.08	77
72 h	1	1856	1654	0.09	47
	2	1241	1010	0.08	52

* $10^{-3} \times$ c.p.m. in trichloroacetic acid-precipitable proteins.

† c.p.m. in immunoprecipitable lipoprotein lipase.

‡ (c.p.m. in immunoprecipitable lipoprotein lipase/c.p.m. in trichloroacetic acid-precipitable proteins) $\times 100$.

§ munits/mg of DNA.

lipase synthesis, as percentages of the values in corresponding fed controls. In old guinea pigs, decreases in lipoprotein lipase synthesis and activity were comparable. For the young animals, however, the changes in synthesis were clearly less than for activity. There are data points in Fig. 1 where synthesis remained at 35–45 %, but activity had decreases to less than 20 %. Hence some additional factor must operate to regulate lipoprotein lipase activity in the young guinea pigs.

Lipoprotein lipase synthesis depends on two factors, the rate of general protein synthesis and the fraction devoted to lipoprotein lipase (relative synthesis). Incorporation of [³⁵S]methionine in the fat-pads, presumably reflecting general protein synthesis, decreased on fasting in both young and old guinea pigs (Table 1). This was more marked in the young animals, where the

Fig. 1. Relationship between the changes in lipoprotein lipase activity and lipoprotein lipase synthesis in adipose tissue during fasting in young and old guinea pigs

Individual values for lipoprotein lipase (LPL) activity and lipoprotein lipase synthesis from fasted young (■) and old (○) guinea pigs from Table 1 and additional experiments were plotted relative to corresponding fed controls (100 %). The mean value in fed guinea pigs was taken as 100 %, and individual values in the fasted animals were calculated relative to this. The broken line shows where data points would lie if lipoprotein lipase synthesis and activity changed in parallel.

incorporation was only about 30 % of that in fed controls, whereas in the older animals methionine incorporation remained around 60 % of control (Table 1, 24 h). In contrast, relative synthesis of lipoprotein lipase decreased more in fat-pads from old than from younger guinea pigs (Table 1). In the fed state mean relative synthesis of lipoprotein lipase in fat-pads was 0.58 and 0.34 % respectively in young and old guinea pigs. In the old guinea pigs the value had decreased to 0.14 % after 24 h, and to 0.08 % after 48 h of fasting. In the young guinea pigs the value remained at 0.61 % after 24 h and at 0.51 % after 48 h of fasting.

In the next experiment we compared lipoprotein lipase mRNA, lipoprotein lipase synthesis and lipoprotein lipase activity in fat-pads and in adipocytes isolated from the same pads after digestion with collagenase (Fig. 2). This experiment was with older guinea pigs fasted for 48 h and corresponding fed controls. Lipoprotein lipase mRNA and relative lipoprotein lipase synthesis decreased both in fat-pads and in adipocytes. The decrease was, if anything, more marked in the adipocytes than in the pads. In contrast, the lipoprotein lipase activity decreased more in the pads than in the adipocytes (Fig. 2). In a separate experiment fat-pads were pulse-labelled with [³⁵S]methionine. Then part of the pads were digested with collagenase, and the adipocytes were isolated. About 17 % (results not shown) of the methionine incorporated by the fat-pads was recovered with the adipocytes. Thus most of the methionine was incorporated by other cells in the tissue. From the values for relative synthesis of lipoprotein lipase in fat-pads (0.37 %) and in adipocytes (0.54 %) from the experiment in Fig. 2 and from additional

Fig. 2. Changes in lipoprotein lipase mRNA, relative lipoprotein lipase synthesis, general protein synthesis and lipoprotein lipase activity in adipose tissue and in adipocytes during fasting in old guinea pigs

Old guinea pigs were fasted for 48 h. Groups of five animals were compared. Epididymal fat-pads from the five animals were pooled and lipoprotein lipase mRNA abundance (dot-dot), relative lipoprotein lipase synthesis (diagonal lines), general protein synthesis (cross-hatch) and lipoprotein lipase activity (white) were determined, as described in the Materials and methods section. Each assay was in duplicate, except for mRNA analysis (see the Materials and methods section). Values in fed animals were taken as 1.0, and values from fasted guinea pigs were calculated relative to this. Data are means $\pm$ S.E.M. from three identical experiments.

data, it can be calculated that about 25% of the radioactive lipoprotein lipase synthesized by the fat-pads was in the adipocytes as isolated.

There was a close parallelism between the abundance of lipoprotein lipase mRNA and relative lipoprotein lipase synthesis in both adipocytes and fat-pads (Fig. 2). There are several species of lipoprotein lipase mRNA in guinea-pig tissues. Northern-blot analysis of RNA from fat-pads and adipocytes of fed and fasted young and old guinea pigs showed that fasting did not cause any marked changes in the lipoprotein lipase mRNA patterns (Fig. 3).

In the next set of experiments we studied the effect of refeeding fasted guinea pigs with glucose. Figs. 4(a) and 4(b) show examples of such experiments carried out with young guinea pigs. In both parts the fast resulted in a decrease of lipoprotein lipase activity, in Fig. 4(a) to about 30% and in Fig. 4(b) to about 10% of the value in corresponding fed controls. In these experiments lipoprotein lipase mRNA abundance had decreased to 40–50%. In both experiments glucose refeeding resulted in a rapid increase in lipoprotein lipase activity, which after 5 h exceeded that in fed controls. In Fig. 4(a) the increase was about 4-fold, and in Fig. 4(b) it was about 14-fold. In contrast, there were only small changes in lipoprotein lipase mRNA. These experiments demonstrate that in the young guinea pigs changes in lipoprotein lipase activity in adipose tissue are larger than the changes in lipoprotein lipase mRNA abundance.

Figs. 4(c) and 4(d) show examples of corresponding experiments carried out in old guinea pigs. Fasting

Fig. 3. Northern-blot analysis of lipoprotein lipase mRNA from adipose tissue and adipocytes from fed and fasted young and old guinea pigs

Total RNA was prepared from adipose tissue and adipocytes (see the Materials and methods section) from young and old animals fasted for 24 h and 48 h respectively. There were two animals in each group, except for five animals when RNA was prepared from adipocytes. Total RNA was analysed for steady-state levels of lipoprotein lipase mRNA by Northern-blot analysis, as detailed in the Materials and methods section. Lanes 1–4: adipose tissue (10 μ g of RNA). Lane 1: young fed animals. Lane 2: young animals fasted for 24 h. Lane 3: old fed animals. Lane 4: old animals fasted for 48 h. Lanes 5 and 6: adipocytes (4 μ g of RNA). Lane 5: old fed animals. Lane 6: old animals fasted for 48 h.

decreased lipoprotein lipase activity to 10–20% and lipoprotein lipase mRNA to 30–40% of the values in fed controls. Glucose refeeding resulted in rather small changes in lipoprotein lipase activity, in contrast with what was seen with the younger animals (compare Figs. 4c and 4d with Figs. 4a and 4b). After 5 h the activity was still only half or less of that in fed controls. The increases were less than 4-fold in both cases. There were only small (not significant) changes in lipoprotein lipase mRNA.

The above experiments indicated that in young guinea pigs changes in lipoprotein lipase synthesis were not sufficient to explain the rather large changes in lipoprotein lipase activity in the fat-pads. There was, however,

Fig. 4. Time courses for the changes in lipoprotein lipase mRNA and lipoprotein lipase activity in adipose tissue during refeeding of fasted young and old guinea pigs

Guinea pigs were fasted for 24 h and then tube-fed with a glucose solution, as described in the Materials and methods section. Data are means of duplicate assays (except for RNA; see the Materials and methods section) on samples from two animals and are expressed relative to the mean value of two corresponding controls. (a) and (b), experiments with young guinea pigs; (c) and (d), experiments with old animals; ●, lipoprotein lipase activity; □, lipoprotein lipase mRNA.

Table 2. Changes of lipoprotein lipase mRNA and lipoprotein lipase activity in adipose tissue during fasting and refeeding in young guinea pigs

Three identical experiments were carried out on three successive days. In each experiment groups of two guinea pigs were fed, fasted for 24 h, or refed with glucose for 5 h after a 24 h fast. Adipose-tissue lipoprotein lipase (LPL) activity (assayed in duplicate) and lipoprotein lipase mRNA (dot-blot) were analysed from each animal, as described in the Materials and methods section. Values are means $\pm$ S.E.M. ($n = 6$). The P values for fed versus fasted and glucose-refed versus fasted were all less than 0.05 (Student's t test).

	LPL activity*	LPL mRNA†
Fed	3238 $\pm$ 483	75.1 $\pm$ 7.2
Fasted	451 $\pm$ 113	29.8 $\pm$ 1.7
Glucose-refed	1638 $\pm$ 240	43.8 $\pm$ 3.9

* munits/mg of DNA.

† arbitrary units (the slope for the regression lines for the RNA dilutions).

considerable variation between individual experiments and between batches of guinea pigs in the magnitude of the changes. To obtain data sufficient for statistical analysis, we carried out a series of experiments each comprising groups of six young guinea pigs which were

fed, fasted for 24 h, or refed glucose for 5 h after a 24 h fast (Table 2). Fasting decreased lipoprotein lipase activity by 86% but decreased lipoprotein lipase mRNA by only 60%. Glucose refeeding increased lipoprotein lipase activity by 260%, but increased lipoprotein lipase mRNA by only 47%. These results demonstrate again that changes in lipoprotein lipase activity in fat-pads of young guinea pigs are substantially greater than the changes in lipoprotein lipase mRNA.

DISCUSSION

This study indicates that there are at least two mechanisms which regulate lipoprotein lipase activity in guinea-pig adipose tissue. The rate at which the lipase is synthesized is modulated by changes in the abundance of its mRNA and by changes in the rate of general protein synthesis, but these changes cannot fully account for the changes in lipoprotein lipase activity in the tissue.

The rate of protein synthesis in adipocytes [26] and adipose tissue [37] is dependent on the nutritional state, perhaps mediated by insulin [37]. A marked decrease on fasting was observed both with fat-pads and with isolated adipocytes. The difference seemed to be somewhat larger in the younger animals, but this was not studied in detail. In all experiments relative synthesis of lipoprotein lipase (as measured by incorporation of [35 S]methionine into immunoprecipitable lipoprotein lipase) correlated closely with changes in lipoprotein lipase mRNA abundance. Thus the changes in relative synthesis of lipoprotein

lipase were most likely caused by the changes in lipoprotein lipase mRNA. For total synthesis of lipoprotein lipase, however, the changes in mRNA were compounded by changes in general protein synthesis.

Fasting consistently decreased lipoprotein lipase mRNA markedly in the older guinea pigs. In the young guinea pigs, on the other hand, the response varied and in some experiments the decrease was small. These results suggest that with age adipocytes acquire an ability to down-regulate lipoprotein lipase mRNA in response to fasting, whereas in young animals this parameter is less responsive to nutritional variations.

In the young animals glucose refeeding caused a rapid increase in lipoprotein lipase activity, despite little change in lipoprotein lipase mRNA. The difference in adipose lipoprotein lipase activity between fed and fasted animals concerns primarily extracellularly localized lipoprotein-lipase [2,10], and there are reports that insulin can increase release of lipoprotein lipase from adipocytes [17,38–40]. Recent studies indicate that the lipoprotein lipase which is not secreted is rapidly degraded within the adipocytes [18,24,41]. Enhanced secretion would lead to increased total lipoprotein lipase in the tissue, by rescuing some of the newly synthesized enzyme from degradation. Results supporting such a mechanism have been obtained in experiments with rat fat-pads. Olivecrona *et al.* [42] found that incorporation of [³⁵S]methionine into immunoprecipitable lipoprotein lipase was initially similar in fat-pads from fed and fasted rats, but with time substantially more radioactivity was recovered from the pads from fed animals, indicating that less of the newly synthesized lipoprotein lipase had been degraded. Ball *et al.* [43] found that adrenaline increased degradation of newly synthesized lipoprotein lipase in rat fat-pads, explaining, at least in part, the lower lipoprotein lipase activity found in these pads compared with controls. It is plausible that such changes in the partitioning of newly synthesized lipoprotein lipase between release and degradation is the mechanism behind the more pronounced changes in lipoprotein lipase activity compared with lipoprotein lipase synthesis demonstrated here in the young animals. Whatever the mechanism is, our data indicate that it is more important in young animals; in older guinea pigs adipose lipoprotein lipase activity was less responsive to refeeding.

In culture some fibroblast-like cells can be committed into a pre-adipocyte state [44] and express early adipocyte markers in a reversible manner [45–47]. Lipoprotein lipase is such an early marker, and its expression precedes accumulation of lipid in the cells [48]. On continued development the cells divide once, which appears to lock in the adipocyte state, and allow appearance of a number of late markers [49]. Presumably corresponding populations of cells are present in adipose tissue. In fact isolation of different cell types from adipose tissue has shown that lipoprotein lipase is produced both in fat-filled adipocytes [4,10,11,17], and in relatively fat-poor pre-adipocytes [50–51]. It is tempting to speculate that the earlier stages may produce lipoprotein lipase at a high rate independent of the nutritional state and that the main regulation on feeding–fasting in these cells occurs by modulation of the transport and turnover of the enzyme. This would endow the cells with a mechanism to provide rapidly more lipoprotein lipase to nearby endothelial sites after a meal. Later stages of adipocyte maturation may acquire an ability to down-regulate

lipoprotein lipase mRNA, but at the same time have a decreased ability to change rapidly the turnover of the enzyme protein.

We thank Dr. Sven Enerbäck for the kind supply of lipoprotein lipase probe, and gratefully acknowledge the technical assistance of Ms. Agneta Brännström. This work was supported by a grant from the Swedish Medical Research Council (B13X-727).

REFERENCES

- Olivecrona, T. & Bengtsson-Olivecrona, G. (1989) in Heparin (Lane, D. & Lindahl, U., eds.), pp. 335–361, Edward Arnold, London
- Garfinkel, A. S. & Schotz, M. C. (1987) in Plasma Lipoproteins (Gotto, A. M., ed.), pp. 335–357, Elsevier Science Publishers, Amsterdam
- Robinson, D. S. (1987) in Lipoprotein Lipase (Borensztajn, J., ed.), pp. 1–13, Evener Publishers, Chicago
- Eckel, R. H. (1987) in Lipoprotein Lipase (Borensztajn, J., ed.), pp. 79–132, Evener Publishers, Chicago
- Carneheim, C., Nedergaard, J. & Cannon, B. (1983) *Am. J. Physiol.* **246**, E327–E333
- Borensztajn, J., Rone, M. S., Babirak, S. P., McGarr, J. A. & Oscai, L. B. (1975) *Am. J. Physiol.* **29**, 394–397
- Lithell, H., Orlander, J., Schele, R., Sjödin, B. & Karsson, J. (1979) *Acta Physiol. Scand.* **107**, 257–261
- Scow, R. O. & Chernick, S. S. (1987) in Lipoprotein Lipase (Borensztajn, J., ed.), pp. 149–185, Evener Publishers, Chicago
- Beutler, B. & Cerami, A. (1986) *Nature (London)* **320**, 584–588
- Semb, H. & Olivecrona, T. (1986) *Biochim. Biophys. Acta* **876**, 249–255
- Stewart, J. E. & Schotz, M. C. (1974) *J. Biol. Chem.* **249**, 904–907
- Spencer, I. M., Hutchinson, A. & Robinson, D. S. (1978) *Biochim. Biophys. Acta* **530**, 375–384
- Cryer, A., Davies, P., Williams, E. R. & Robinson, D. S. (1975) *Biochem. J.* **146**, 481–488
- Friedman, G., Chajek-Shaul, T., Stein, O., Noe, L., Etienne, J. & Stein, Y. (1986) *Biochim. Biophys. Acta* **87**, 112–120
- Friedman, G., Chajek-Shaul, T., Etienne, J., Stein, O. & Stein, Y. (1987) *Biochim. Biophys. Acta* **919**, 1–12
- Vannier, C., Amri, E. Z., Etienne, J., Negrel, R. & Ailhaud, G. (1985) *J. Biol. Chem.* **260**, 4424–4431
- Pradines-Figueres, A., Vannier, C. & Ailhaud, G. (1988) *Biochem. Biophys. Res. Commun.* **154**, 982–990
- Semb, H. & Olivecrona, T. (1987) *Biochim. Biophys. Acta* **921**, 104–115
- Semb, H. & Olivecrona, T. (1989) *J. Biol. Chem.* **264**, 4195–4200
- Speake, B. K., Parkinson, C. & Robinson, D. S. (1985) *Horm. Metab. Res.* **17**, 637–640
- Strieleman, P. J. & Bensadoun, A. (1987) *Biochim. Biophys. Acta* **908**, 268–274
- Ong, J. M., Kirchgessner, T. G., Schotz, M. C. & Kern, P. A. (1988) *J. Biol. Chem.* **263**, 12933–12938
- Enerbäck, S., Semb, H., Tavernier, J., Bjursell, G. & Olivecrona, T. (1988) *Gene* **64**, 97–106
- Cupp, M., Bensadoun, A. & Melford, K. (1987) *J. Biol. Chem.* **262**, 6383–6388
- Semb, H., Peterson, J., Tavernier, J. & Olivecrona, T. (1987) *J. Biol. Chem.* **262**, 8390–8394
- Semb, H. & Olivecrona, T. (1986) *Biochim. Biophys. Acta* **878**, 330–337

27. Wallinder, L., Bengtsson, G. & Olivecrona, T. (1982) *Biochim. Biophys. Acta* **711**, 107–113
28. Peterson, J., Olivecrona, T. & Bengtsson-Olivecrona, G. (1985) *Biochim. Biophys. Acta* **837**, 262–270
29. Labarca, C. & Paigen, K. (1980) *Anal. Biochem.* **102**, 344–352
30. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. (1979) *Biochemistry* **18**, 5294–5299
31. Thomas, P. S. (1983) *Methods Enzymol.* **100**, 255–266
32. Enerbäck, S., Semb, H., Bengtsson-Olivecrona, G., Carlsson, P., Hermansson, M.-L., Olivecrona, T. & Bjursell, G. (1987) *Gene* **58**, 1–12
33. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132**, 6–13
34. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
35. McMaster, G. K. & Carmichael, G. G. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 4835–4838
36. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5201–5205
37. Tischler, M. E., Ost, A. & Coffman, J. (1986) *Life Sci.* **39**, 1447–1452
38. Garfinkel, A. S., Nilsson-Ehle, P. & Schotz, M. C. (1976) *Biochim. Biophys. Acta* **424**, 264–273
39. Spooner, P. M., Chernick, S. S., Garrison, M. M. & Scow, R. O. (1979) *J. Biol. Chem.* **254**, 10021–10029
40. Chan, B. L., Lisanti, M. P., Rodriguez-Boulant, E. & Saltiel, A. R. (1988) *Science* **241**, 1670–1672
41. Vannier, C., Amri, E., Etienne, J., Negrel, R. & Ailhaud, G. (1985) *J. Biol. Chem.* **260**, 4424–4431
42. Olivecrona, T., Price, S. R., Pekala, P. H., Scow, R. O., Chernick, S. S., Semb, H., Vilaro, S. & Bengtsson-Olivecrona, G. (1987) in *Drugs Affecting Lipid Metabolism* (Paoletti, R., Kritchevsky, D. & Holmes, W. L., eds.), pp. 88–93, Springer Verlag, Heidelberg
43. Ball, K. L., Speake, B. K. & Robinson, D. S. (1986) *Biochim. Biophys. Acta* **877**, 399–405
44. Wise, L. S. & Green, H. (1978) *Cell* **13**, 237–242
45. Vannier, C., Gaillard, D., Grimaldi, P., Amri, E., Djian, P., Cermolacce, C., Forest, C., Etienne, J., Negrel, R. & Ailhaud, G. (1985) *Int. J. Obes.* **9**, Suppl. 1, 041–53
46. Amri, E., Grimaldi, P., Negrel, R. & Ailhaud, G. (1984) *Exp. Cell Res.* **152**, 368–377
47. Doglio, A., Dani, C., Grimaldi, P. & Ailhaud, G. (1986) *Biochem. J.* **238**, 123–129
48. Amri, E., Dani, C., Doglio, A., Etienne, J., Grimaldi, P. & Ailhaud, G. (1986) *Biochem. J.* **238**, 115–122
49. Amri, E., Dani, C., Doglio, A., Grimaldi, P. & Ailhaud, G. (1986) *Biochem. Biophys. Res. Commun.* **137**, 903–910
50. Glick, J. M. & Rothblat, G. (1980) *Biochim. Biophys. Acta* **618**, 163–172
51. Björntorp, P., Karlsson, M., Pettersson, P. & Synpiewska, G. (1980) *J. Lipid Res.* **21**, 714–723

Received 27 January 1989/24 April 1989; accepted 2 May 1989