Stimulation of lipoprotein lipase synthesis by refeeding, insulin and dexamethasone

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Lipoprotein lipase synthesis in adipose tissue was greater in rats fed *ad libitum* or refed than in fasted rats. Insulin alone and together with dexamethasone increased lipoprotein lipase synthesis in adipose tissue incubated *in vitro*. The changes in relative lipoprotein lipase synthesis (immunoprecipitable ^{35}S -labelled lipoprotein lipase as a fraction of general ^{35}S protein after pulse-

labelling with ^{35}S methionine) indicate that insulin and dexamethasone exert a selective effect on lipoprotein lipase synthesis. There was no evidence for an inverse relationship between lipoprotein lipase synthesis and activity for any of the conditions studied.

INTRODUCTION

Lipoprotein lipase (EC 3.1.1.34) is a glycoprotein that hydrolyses the triacylglycerol components of very-low-density lipoproteins and chylomicrons, thus releasing non-esterified fatty acids (NEFA) which are taken up by the surrounding tissue. In the fed state, lipoprotein lipase activity is increased in adipose tissue and decreased in muscle, whereas in the fasted state lipoprotein lipase activity is increased in muscle and correspondingly decreased in adipose tissue [1,2]. This response of lipoprotein lipase activity to changes in nutritional state is likely to be brought about by the interaction of several hormones [1,3], and early work suggested that insulin and glucocorticoids might play an important role [4]. A number of workers showed that insulin *in vitro* increased lipoprotein lipase activity in rat adipose tissue and adipocytes [5,6] and adipocyte cell lines [7,8]. Evidence was produced for insulin action on transcription [7], synthesis [5–7] and secretion [8] of lipoprotein lipase. Dexamethasone was found to potentiate the effect of insulin on both the activity and synthesis of lipoprotein lipase in adipose tissue [4,9] and on cell-surface content of the enzyme in isolated adipocytes [10].

The availability of antiserum to lipoprotein lipase and of cDNA clones has enabled the molecular mechanisms of the nutritional and hormonal changes in lipoprotein lipase activity to be investigated directly. Decreases in lipoprotein lipase mRNA levels have been reported in response to fasting in adipose tissue of chicken [11] and guinea pig [12], in which there was a close parallelism between the abundance of lipoprotein lipase mRNA and relative lipoprotein lipase synthesis (immunoprecipitable ^{35}S -labelled lipoprotein lipase as a fraction of general ^{35}S protein after pulse-labelling with ^{35}S methionine). However, whereas in old guinea pigs the decreases in lipase activity and synthesis were comparable, in younger animals the change in lipase activity was greater than the change in lipase synthesis [12]. In rat adipocytes insulin stimulated the production of lipoprotein lipase mRNA [13] and of lipoprotein lipase synthesis with only a slight increase in general protein synthesis [13,14]. Taken together, these results suggested that in a number of situations lipoprotein lipase activity was controlled at the level of transcription and was synthesis-driven, whereas in others additional regulation was exerted after translation.

Evidence for post-translational control of lipoprotein lipase through an increase in specific activity was reported after feeding in humans [15]. Olivecrona et al. [16] had previously observed inactive enzyme in 3T3-L1 cells, but were unable to determine whether this was synthesized *de novo* or arose as the result of inactivation. Support for post-translational control came from studies by Semenkovich et al. [17], who showed that in 3T3-L1 adipocytes insulin increased lipoprotein lipase activity while producing a decrease in lipoprotein lipase synthesis and very little change in lipoprotein lipase mRNA levels. An inverse relation between synthesis and activity was also found in studies on the response of lipoprotein lipase in rat adipose tissue to fasting and refeeding [18]. The 50% decrease in catalytic activity on fasting was accompanied by a nearly 2-fold increase in lipase mRNA levels and relative rate of synthesis, although the enzyme mass remained unchanged. The explanation for this was a change in the apparent specific activity due to a decrease in the complex form of lipoprotein lipase found in the Golgi and at the functional site, and an increase in the high-mannose form of lipoprotein lipase located in the endoplasmic reticulum [19].

Following on from the observations above, the initial aim of this work was to determine whether insulin *in vitro* exerted translational and post-translational control of lipoprotein lipase in rat adipose tissue from fed and fasted rats. However, our preliminary results on lipoprotein lipase synthesis were in disagreement with those of Doolittle et al. [18], and so we had to reinvestigate the response of lipoprotein lipase synthesis to refeeding and fasting.

EXPERIMENTAL

Materials

Dexamethasone phosphate was a gift from Merck, Sharp and Dohme. Bovine insulin, BSA (Fraction V for lipoprotein lipase assays, NEFA-free and devoid of insulin by radioimmunoassay for incubations), rabbit anti-goat IgG and non-immune goat serum were from Sigma. Endoglycosidase H was from Boehringer Mannheim and Pansorbin Cells were from Calbiochem. Tran ^{35}S -label was obtained from ICN Biomedicals. The product contained 70% L- ^{35}S methionine and 15% L- ^{35}S cysteine, and the specific radioactivity was approx. 1000 Ci/mmol with respect to

[^{35}S]methionine. The antiserum to purified rat adipose-tissue lipoprotein lipase prepared in goat [20] was the same as used in our previous study [21] and was a gift from J. Etienne, Paris.

Animals

Male Porton-Wistar rats were fed *ad libitum* on standard chow pellets consisting of (by weight) 51.6% carbohydrate, 22.7% protein and 4.3% fat (the residue was non-digestible material; Special Diet Services, Witham, Essex, U.K.) and had free access to drinking water. A 14 h-light/10 h-dark cycle began at 07:00 h. Fasted rats were without food for 24 h before removal of adipose tissue. Refed rats were without food for 36 h and then were provided, the night before slaughter, with the food described above with a supplement of 15% glucose in the drinking water. Epididymal adipose tissue was removed at 09:00 h.

Incubations

Epididymal fat-pads were cut into five or six pieces of approx. 100 mg. They were incubated in a shaking water bath at 37 °C in gassed (O_2/CO_2 , 19:1) Eagle's minimal essential medium (modified) with Earle's salts without methionine, pH 7.4 (MEM), supplemented with 1 mM glutamine [12], 5% (w/v) fatty-acid-free BSA and 10 units/ml streptomycin and penicillin, herein-after referred to as Medium A. The ratio of tissue to medium was approx. 0.1 g to 0.5 ml of medium. [^{35}S]Methionine (from Tran ^{35}S -label) was included at 50 or 100 $\mu\text{Ci/ml}$ as indicated. In some experiments 0.2 μM L-methionine was also added as indicated.

Measurement of general protein synthesis and lipoprotein lipase synthesis

At the end of the incubations, the adipose-tissue pieces were removed and washed once in MEM supplemented with 20 mM L-methionine and three times in Krebs-Ringer bicarbonate buffer, pH 7.4 [18], supplemented with 20 mM Hepes and 20 mM L-methionine, before homogenization in lysis buffer [0.01 M sodium phosphate, 0.1 M NaCl, 5 mM EDTA, 0.5% (w/v) sodium deoxycholate, 1% (v/v) Triton X-100, 0.1% (w/v) SDS, 0.1 mM phenylmethanesulphonyl fluoride and (per ml) 20 μg of leupeptin, 0.7 μg of pepstatin and 3.5 units of heparin, pH 7.5]. The homogenates were centrifuged for 20 min at 10000 g at 4 °C, and samples of the clear infranatant were taken for protein estimation.

Total incorporation of ^{35}S was determined by precipitation of proteins in 50 μl samples of infranatant in 5 ml of ice-cold 10% trichloroacetic acid and 20 mM L-methionine, trapping on GF-C filters under vacuum and washing with 4 $\times$ 5 ml of 10% trichloroacetic acid. The filters were dried overnight and counted for radioactivity in 5 ml of Ecoscint A.

For immunoprecipitation of lipoprotein lipase [22], the IgG-enriched fraction of antiserum to purified rat adipose-tissue lipoprotein lipase was obtained by $(\text{NH}_4)_2\text{SO}_4$ precipitation and added to the infranatants at a ratio of 120 $\mu\text{l/mg}$ of protein. Similarly treated non-immune goat serum was added to identical samples of infranatant to act as control immunoprecipitations. All procedures were at 4 °C. After an overnight incubation, rabbit anti-goat IgG was added to the samples, which were left for a further 2 h. Pansorbin Cells were added to the samples, and after 30 min the immunocomplexes were collected by centrifugation at 10000 g for 5 min. The pellets were washed twice with lysis buffer, twice with 50 mM Tris/HCl (pH 7.5)/0.5 M LiCl, twice with lysis buffer and finally once with 0.01 M sodium

phosphate (pH 7.5)/0.1 M NaCl/5 mM EDTA [22]. All wash solutions contained 20 mM L-methionine, and the pellets were sonicated for 4 min in a Dawe Sonicleaner after every second wash. For quantification of lipoprotein lipase synthesis, the immunoprecipitates were dried, resuspended in Laemmli sample buffer, sonicated as above for 4 min, boiled for 4 min and then electrophoresed on SDS/PAGE 10% gels. The gels were fixed in 10% acetic acid and subjected to sodium salicylate fluorography [23]. After autoradiography, lipoprotein lipase was revealed as a band of M_r around 56000 which was not present in the control (non-immune goat serum) immunoprecipitates (as shown in Figure 2). The intensity of the lipoprotein lipase band was quantified on a Joyce-Loebl scanning densitometer within the linear range of the system. The area of the lipoprotein lipase band was cut out of the dried gel, rehydrated, and the protein was extracted with NCS tissue solubilizer at 50 °C for 2 h, followed by neutralization with acetic acid and scintillation counting in Ecoscint A. The data from gel-slicing agreed with data from densitometry. Equal amounts of tissue protein were applied to the gel, but when gels were loaded with equal amounts of trichloroacetic acid-precipitable label similar results were obtained.

Lipoprotein lipase activity was assayed in infranatants of homogenates of adipose tissue as previously described [24]. Activity is expressed as μmol of NEFA released/h per g wet wt. of tissue.

Endoglycosidase H digestion of lipoprotein lipase immunoprecipitates

After 80 min incubation, radiolabelled lipoprotein lipase was immunoprecipitated as described above. The washed immunoprecipitates were sonicated for 5 min and boiled for 5 min in 50 mM sodium phosphate (pH 6.0)/0.5% SDS and then centrifuged at 10000 g for 2 min. The supernatants were incubated at 37 °C for 16 h in the presence or absence of Endoglycosidase H (2 m-units/250 μg of general protein taken for immunoprecipitation). An equal volume of 2 $\times$ Laemmli buffer was added, followed by boiling for 3 min, separation by SDS/PAGE and quantification of the lipoprotein lipase bands by fluorography as above.

Results were analysed by Student's *t* test and are expressed as means $\pm$ S.D.

RESULTS

Effect of nutritional state on lipoprotein lipase synthesis

As our initial aim was to determine whether insulin *in vitro* altered lipoprotein lipase synthesis or the proportion of lipoprotein lipase in the complex and high-mannose forms, we investigated in preliminary experiments the effect of insulin on adipose tissue from both fed and fasted rats. Insulin increased lipoprotein lipase synthesis in adipose tissue from fed rats (Table 1), but its effect on protein synthesis was smaller. In two separate experiments insulin did not alter the activity of lipoprotein lipase released into the medium. Combining the results from the two experiments, the lipoprotein lipase activity released during 1 h incubation was 1.8 ± 0.7 μmol of NEFA/h per g wet wt. of tissue ($n = 5$) in the presence of insulin and 2.0 ± 0.9 μmol of NEFA/h per g wet wt. ($n = 5$) in control incubations. Enzyme activity in the tissue was 72 ± 7 and 56 ± 15 μmol of NEFA/h per g wet wt. ($n = 5$) in the presence and absence of insulin respectively.

There was no effect of insulin on lipoprotein lipase activity in adipose tissue from fasted rats. In a further two experiments, lipoprotein lipase activity was measured in adipose tissue at the

Table 1 Effect of insulin on lipoprotein lipase and general protein synthesis and lipoprotein lipase activity in adipose tissue from fed and fasted rats

Adipose tissue was removed from two fasted and two fed rats and the pieces from the two rats were pooled and incubated for 60 min in Medium A containing 25 $\mu\text{Ci/ml}$ [^{35}S]methionine. Insulin (0.04 μM) was added as indicated. Lipoprotein lipase synthesis and general protein synthesis were measured in the pooled samples as described in the Experimental section. Separate incubations in the absence of [^{35}S]methionine were carried out for the measurement of lipoprotein lipase activity. Either values are expressed as a percentage of the value in the absence of insulin, or the fed values are expressed as a percentage of the fasted.

Measurement	Comparison ...		Fed + insulin Fed - insulin	Fed Fasted
	Fasted + insulin	Fasted - insulin		
Lipoprotein lipase synthesis	127		193	218
General protein synthesis	91		127	148
Relative lipoprotein lipase synthesis	141		152	148
Lipoprotein lipase activity	106		126	200

end of 60 min incubation. For fed tissue the values in the presence of insulin, $76 \pm 10 \mu\text{mol}$ of NEFA/h per g, were significantly higher ($P < 0.05$, $n = 6$) than in its absence, $48 \pm 10 \mu\text{mol}$ of NEFA/h per g. The values for fasted tissue were $38 \pm 4 \mu\text{mol}$ of NEFA/h per g and $29 \pm 7 \mu\text{mol}$ of NEFA/h per g respectively in the presence and absence of insulin. These results are in agreement with our earlier experience (J. D. Oliver and M. P. Rogers, unpublished work) that stimulation of lipoprotein lipase activity by insulin in adipose tissue from fasted rats is not reproducible and is not as high as reported by others.

When comparing fed and fasted animals, the results in Table 1 show that lipoprotein lipase and general protein synthesis in tissue from fed animals was higher than in fasted animals and relative lipoprotein lipase synthesis was also increased. Similar results were observed in another experiment when tissue from fed and fasted animals were compared under conditions identical with those in Table 1, except that the incubation with [^{35}S]methionine was for 20 min. Lipoprotein lipase synthesis, general protein synthesis and relative lipoprotein lipase synthesis (in pooled samples) was 178%, 119% and 149%, respectively, of the values in fasted animals.

When adipose-tissue pieces from fed and fasted rats were incubated under the conditions given in Table 1, general protein synthesis was linear over the 60 min, and at each time point protein synthesis was less in adipose tissue from fasted animals than from fed animals. At 60 min the value for tissue from fasted animals was $63.1 \pm 10.2\%$ ($P < 0.05$, $n = 3$) of the value for fed animals.

Doolittle et al. [18] compared lipoprotein lipase synthesis in adipose tissue from fasted rats and from fasted rats refed for 12 h, and found a significant decrease in relative lipoprotein lipase synthesis in the refed rats. An explanation for the lack of a finding of decreased lipoprotein lipase synthesis in our study could be that we used rats fed *ad libitum*, as opposed to refed rats. On the other hand, the omission of amino acids from the incubations in the study by Doolittle et al. [18] could have affected protein synthesis.

To test the latter point, protein synthesis was measured in adipose tissue from fasted and refed rats incubated in either Medium A or Krebs-Ringer bicarbonate buffer, pH 7.4 [18], containing 20 mM Hepes, 1% BSA and 10 units/ml streptomycin and penicillin (in the absence of amino acids). The amount of [^{35}S]methionine was increased to 100 $\mu\text{Ci/ml}$, and

Figure 1 Changes in lipoprotein lipase synthesis, general protein synthesis, relative lipoprotein lipase synthesis and lipoprotein lipase activity in adipose tissue during refeeding

In two separate experiments adipose tissue was removed from fasted and refed rats, and five pieces from each fat-pad were pooled. In the first experiment the pieces were incubated for 20 min in Krebs-Ringer buffer containing 20 mM Hepes, 1% BSA and 10 units/ml streptomycin and penicillin and 100 $\mu\text{Ci/ml}$ [^{35}S]methionine ($n = 4$). In the second experiment pieces were incubated for 80 min in Medium A containing 100 $\mu\text{Ci/ml}$ [^{35}S]methionine and 0.2 μM methionine ($n = 5$). Lipoprotein lipase synthesis ($\square$), general protein synthesis ($\boxtimes$) and relative lipoprotein lipase synthesis ($\boxminus$) were determined as described in the Experimental section. Lipoprotein lipase activity ($\square$) was determined in separate incubations in the absence of [^{35}S]methionine. Values are means $\pm$ S.D. and are for refed rats expressed as a percentage of fasted: * $P < 0.05$, ** $P < 0.001$.

Table 2 Effect of fasting and refeeding on the distribution of lipoprotein lipase between the complex, intermediate and high-mannose forms of the enzyme

Five pieces of adipose tissue from three fasted and three refed rats were incubated in Medium A containing 100 $\mu\text{Ci/ml}$ [^{35}S]methionine and 0.2 μM methionine for 80 min. For each tissue piece the high-mannose, intermediate and complex forms of lipoprotein lipase were separated and quantified as described in the Experimental section. Values are means $\pm$ S.D. and are expressed as a percentage of total lipoprotein lipase. Fasted values significantly different from refed: * $P < 0.02$, ** $P < 0.001$.

	Distribution (%)		
	Complex	Intermediate	High-mannose
Refed	39.2 ± 4.2	21.5 ± 1.7	38.3 ± 3.8
Fasted	$25.3 \pm 1.3^{**}$	$18.3 \pm 2.1^*$	$56.4 \pm 2.1^{**}$

0.2 μM unlabelled methionine was added. The incorporation of [^{35}S]methionine into protein was linear throughout the 100 min. There was no significant difference in general protein synthesis in the two media at any time point.

Next, lipoprotein lipase synthesis was compared in adipose tissue from rats that had been fasted or refed. The results in Figure 1, after a 20 min pulse, show that refeeding led to an increase in lipoprotein lipase synthesis and a significant increase in lipoprotein lipase activity and general protein synthesis. Also

Figure 2 Endoglycosidase H digestion of lipoprotein lipase

Adipose-tissue lipoprotein lipase was immunoprecipitated and solubilized as described in the Experimental section. The supernatants were incubated in the absence (lane 2) and presence (lane 3) of Endoglycosidase H and then subjected to SDS/PAGE. Lane 1 is a control immunoprecipitation with non-immune goat serum. The positions of the three forms of lipoprotein lipase (C, complex; I, intermediate; H, high-mannose) are shown along with M_r marker proteins.

shown in Figure 1 are the results, after an 80 min pulse in Medium A, obtained from the experiment to determine the glycosylation state of lipoprotein lipase (Table 2). A similar pattern to that with the 20 min pulse was found, although in this experiment both lipoprotein lipase synthesis and protein synthesis were significantly increased after refeeding (Figure 1). However, if more of the newly synthesized lipoprotein lipase is degraded in adipose tissue from fasted animals (see [12]), the synthesis in fed animals will be over-estimated after this length of pulse. In both experiments, the relative lipoprotein lipase synthesis was lower in refed rats, but not significantly so.

Post-translational control of glycosylation state of lipoprotein lipase

Endoglycosidase H sensitivity distinguishes between the high-mannose form of lipoprotein lipase in the endoplasmic reticulum and the intermediate and complex forms in the Golgi and secretory pathway [18,19]. After Endoglycosidase H treatment and SDS/PAGE, undigested lipoprotein lipase, in the complex form, migrated as a band of M_r approx. 56000, whereas the intermediate and high-mannose forms migrated as two distinct bands of lower M_r (Figure 2). The distribution of the three forms in adipose tissue from fasted and refed rats was measured after an 80 min pulse [18] and expressed as a percentage of total lipoprotein lipase (Table 2). As the complex and intermediate forms are both found to be secreted and active, they can be considered together [18]. In the refed state the distribution was 60.7 ± 3.8% complex + intermediate: 39.3 ± 3.8% high-mannose, whereas in the fasted state this ratio was 43.6 ± 2.1% complex + intermediate: 56.4 ± 2.1% high-mannose. Refeeding caused a significant ($P < 0.001$) increase in the amount of lipoprotein lipase in the complex + intermediate form.

Our initial intention was to determine whether insulin altered the proportion of lipoprotein lipase in the complex form.

Table 3 Effects of insulin and dexamethasone on lipoprotein lipase and general protein synthesis and lipoprotein lipase activity in adipose tissue

Adipose tissue was removed from three refed rats and two tissue pieces from each fat-pad were incubated for 60 min in Medium A containing 100 μ Ci/ml [35 S]methionine and 0.2 μ M methionine with either no further additions (controls) ($n = 4$) or 0.04 μ M insulin ($n = 4$) or 0.04 μ M insulin and 0.75 μ M dexamethasone phosphate ($n = 6$). Lipoprotein lipase synthesis and general protein synthesis were determined as described in the Experimental section. Lipoprotein lipase activity was measured in a separate experiment with incubations in the absence of [35 S]methionine ($n = 5$). Results are means ± S.D. and are expressed as a percentage of the control. Significantly different from controls: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Measurement	Treatment ... Insulin	Insulin + dexamethasone
Lipoprotein lipase synthesis	223 ± 91*	314 ± 79***
General protein synthesis	163 ± 36*	195 ± 25***
Relative lipoprotein lipase synthesis	141 ± 68	163 ± 49
Lipoprotein lipase activity	126 ± 13	171 ± 35**

However, although in other experiments we found the proportion of the complex form to be near 60% in adipose tissue from fed animals, that in tissue from fasted rats was more variable, and higher than the 35% reported in Table 2. Therefore we decided that a conclusive answer as to whether insulin stimulated formation of the complex form was unlikely to be obtained.

Effect of insulin and dexamethasone on lipoprotein lipase synthesis

The nutritional changes in lipoprotein lipase activity may be brought about in part by insulin and glucocorticoids. We had previously shown that both insulin and dexamethasone are required to maintain lipoprotein lipase activity in whole adipose-tissue pads incubated for up to 4 h [24]. Therefore, we next studied the effect on lipoprotein lipase synthesis of insulin alone and together with dexamethasone. Because of the poor response to insulin of adipose tissue from fasted rats, these experiments were performed with tissue from refed rats. The results in Table 3 show that insulin on its own significantly increased lipoprotein lipase and general protein synthesis. However, the addition of insulin and dexamethasone together produced a large, significant, increase in both parameters above, as well as in lipoprotein lipase activity. The observation that lipoprotein lipase synthesis was increased more than activity is consistent with the short half-life of lipoprotein lipase and the need for some enzyme to be replaced by synthesis in the control incubations. Relative lipoprotein lipase synthesis, although raised, was not significantly higher than controls.

In separate experiments under similar conditions insulin produced a significant increase in general protein synthesis of 155 ± 25% ($P < 0.05$, $n = 3$) and insulin with dexamethasone a significant increase of 175 ± 30% ($P < 0.05$, $n = 3$). With tissue from fasted rats, insulin and dexamethasone together produced a significant increase of 149 ± 21% ($P < 0.05$, $n = 3$) in general protein synthesis whereas the value for insulin alone was 113 ± 2% ($n = 3$).

DISCUSSION

There are two major questions as regards control of the synthesis of lipoprotein lipase which this work addressed. The first is whether there is an inverse relation between lipoprotein lipase synthesis and activity in fed and fasted rats, and secondly

whether changes in lipoprotein lipase synthesis can be accounted for by changes in general protein synthesis.

In this study, lipoprotein lipase synthesis was always greater, although not always significantly, in adipose tissue from fed rats than from fasted rats. This was so whether the rats were fed *ad libitum* or fasted and refed. When the change in lipoprotein lipase synthesis was related to the change in general protein synthesis, it was observed that relative lipoprotein lipase synthesis was lower in the refed state compared with fasting, but not in the fed state compared with fasting.

We have also shown, for the first time by direct measurement of lipoprotein lipase synthesis by immunoprecipitation, that insulin and dexamethasone together significantly increased lipoprotein lipase synthesis in rat adipose tissue. These results are in agreement with the early indirect measurements by Speake et al. [9]. General protein synthesis was also significantly increased under these conditions, so that relative lipoprotein lipase synthesis, although higher than in the absence of insulin and dexamethasone, was not significantly increased. Insulin alone also significantly increased both lipoprotein lipase and general protein synthesis in adipose tissue from fed rats, although the increase was not as great as when dexamethasone was also present.

These results indicate that the elevated lipoprotein lipase activity observed in the fed state and in the presence of insulin and dexamethasone can be explained in part by an increase in lipoprotein lipase synthesis. We did not find any evidence for an inverse relation between lipoprotein lipase synthesis and activity.

Values for relative lipoprotein lipase synthesis suggest that, although the increased lipase synthesis in refed rats is accounted for by the increase in general protein synthesis, insulin and dexamethasone exhibit a selective effect on lipoprotein lipase synthesis. We have previously shown that lipoprotein lipase mRNA levels are higher in adipose tissue incubated in the presence of insulin and dexamethasone than in their absence [24].

Doolittle et al. [18] reported that the decreased lipoprotein lipase activity observed with fasting resulted in a nearly 2-fold increase in lipoprotein lipase mRNA levels and (relative) rates of synthesis. These results were of considerable importance, since they showed an inverse relation between mRNA levels and activity and between relative lipoprotein lipase synthesis (lipoprotein lipase synthesis itself was not reported) and activity and necessitated the involvement of post-translational controls. We have previously found no change in lipoprotein lipase mRNA levels in rats fasted for 24 h as compared with rats fed *ad libitum* [25]. Ladu et al. [26] reported a strong positive correlation between enzyme activity and lipoprotein lipase mRNA levels in adipose tissue of fed rats and rats fasted for 1 or 6 days. They suggested that explanations for the difference between their results and those of Doolittle et al. [18] could be the fasting/refeeding protocol, the length of fasting and animal body weight and age. It is more difficult to find explanations for the difference between our results and those of Doolittle et al. [18], since we measured lipoprotein lipase synthesis under conditions as similar as possible to those used by those workers.

While this work was in progress, a series of papers have been published on the regulation by insulin or dexamethasone of lipoprotein lipase in human and rat adipose tissue and adipocytes [27–32]. Fried et al. [27] found that in adipocytes of obese Zucker rats insulin stimulated lipoprotein lipase synthesis as a result of a general increase in protein synthesis. Appel and Fried [28] studying human adipose tissue cultured for 7 days, found that insulin stimulated lipoprotein lipase synthesis to a considerably greater extent than protein synthesis, and the simultaneous addition of dexamethasone led to additional increase in activity

with no change in general protein synthesis. They postulate that dexamethasone might inhibit degradation of newly synthesized lipoprotein lipase.

Ong et al. [30] observed yet another pattern in rat adipocytes, in that whereas the presence of insulin for 24 h increased lipoprotein lipase mRNA levels, synthesis and activity, the presence of both dexamethasone and insulin resulted in no change in any of these parameters. The authors suggest that in adipose tissue insulin and dexamethasone may induce differentiation of preadipocytes into adipocytes, resulting in more cells that are capable of producing lipoprotein lipase.

All these studies have one point in common, and that is that the change in direction of lipoprotein lipase synthesis is similar to the change in direction of lipoprotein lipase activity. Moreover, although the various studies at first sight seem to differ in detail, there is now considerable support for the view that regulation of lipoprotein lipase activity, at least in adipose tissue, may occur at many different points [12]. Control of lipoprotein lipase transcription and mRNA levels permits either changes in rates of lipoprotein lipase synthesis and activity to occur independently of changes in general protein synthesis or for lipoprotein lipase synthesis to keep pace with an increased capacity for general protein synthesis. There may also be control of mRNA stability [31]. Increased efficiency of either general protein synthesis or of a selected class of proteins, as may happen in the presence of insulin [33], could lead to an increase in lipoprotein lipase synthesis without a concomitant change in lipoprotein lipase mRNA levels. Post-translational control permits changes in lipoprotein lipase activity independent of changes in lipoprotein lipase synthesis. Alteration in rates of degradation and secretion of lipoprotein lipase and in the distribution of complex and mannose forms have been observed. Although all these controls are unlikely to operate simultaneously, it is no doubt this multiplicity of potential control points which gives rise to the variation in results with slightly different conditions. Moreover, a particular control may be more important in different species.

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