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Effects of Glucose and Insulin on the Activation of Lipoprotein Lipase and on Protein Synthesis in Rat Adipose Tissue

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Glucose, and certain sugars that can readily be converted to glucose 6-phosphate, bring about an activation of adipose-tissue lipoprotein lipase when epididymal fat-bodies from starved rats are incubated in the presence of cycloheximide. Other substrates do not support the activation. If the tissue is preincubated in the presence of cycloheximide for longer than 2h, the ability of added glucose to activate the enzyme is lost. On the other hand, the addition of glucose still brings about an increase in lipoprotein lipase activity after preincubation in the absence of cycloheximide for as long as 4h. The magnitude of the increase in enzyme activity brought about by the addition of glucose is increased when protein synthesis is stimulated during the preincubation period by insulin. The results are interpreted in terms of the existence in adipose tissue of a proenzyme pool of lipoprotein lipase that is normally maintained by protein synthesis and that is converted to complete enzyme of higher specific activity by a process that specifically requires glucose.

Lipoprotein lipase (EC 3.1.1.34) is the enzyme that regulates the uptake of the triacylglycerol fatty acids of chylomicrons and very-low-density lipoproteins from the bloodstream into the extrahepatic tissues. In adipose tissue, it is synthesized and secreted from the adipocyte and transported to the luminal surfaces of the capillary endothelial cells where its activity is expressed (Robinson, 1970; Scow *et al.*, 1976).

Variations in physiological and nutritional states can bring about dramatic changes in the activity of lipoprotein lipase in adipose tissue, and it is now recognized that many of these changes are hormonally regulated (Robinson & Wing, 1971). There is good evidence, for example, that insulin and glucocorticoids promote the synthesis of the enzyme in the adipocyte, whereas catecholamines bring about its rapid inactivation (Patten, 1970; Desai & Hollenberg, 1975; Vydelingum *et al.*, 1978; Ashby *et al.*, 1978*b*, 1980).

Part of the evidence for hormonal control of the activity of the enzyme derives from experiments in which epididymal fat-bodies from 24h-starved rats, which have low lipoprotein lipase activity, are incubated *in vitro* in the presence of glucose and various hormone combinations (Robinson & Wing, 1971). Using such systems we have recently studied the protein-synthesis-independent activation of the enzyme and found it to be independent of hormonal control (Ashby *et al.*, 1978*b*). We have, therefore suggested (Ashby *et al.*, 1978*b*, 1980) an overall scheme for the synthesis and release of the enzyme from the adipocyte that involves: (*a*) control of the synthesis of a proenzyme form by insulin and glucocorticoids; (*b*) post-translational modification of the proenzyme by a process that is independent of hormonal control and associated with an increase in specific activity; (*c*) further hormonal control through a catecholamine-stimulated inactivation of the enzyme that can occur before its secretion from the adipocyte and transport to the endothelial cell surface.

We report in this paper further studies on the enzyme in epididymal fat-body incubation systems which suggest that the activation of the proenzyme requires the presence of glucose or a closely related sugar, but that the effect of insulin on the synthesis of the proenzyme is independent of glucose.

Materials and Methods

Materials

Bovine serum albumin (fraction V), glucose, sodium pyruvate and cycloheximide were obtained from the Sigma Chemical Co., Kingston-upon-Thames, Surrey, U.K. The high-specific-radioactivity ^{3}H-labelled amino acid mixture, which contained L-[3,4-^{3}H]leucine, L-[4,5-^{3}H]lysine, L-[2,4,6-^{3}H]phenylalanine, L-[2,3,4,5-^{3}H]proline and L-[2,3,5,6-^{3}H]tyrosine, each at 60–130Ci/mmol, was

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supplied by The Radiochemical Centre, Amersham, Bucks., U.K. Horse serum was obtained from Oxoid, Basingstoke, Hants., U.K., and insulin was supplied by Duncan, Flockhart and Co. London E2 6LA, U.K. Intralipid (20%) was purchased from Vitrum, Stockholm, Sweden. Amino acids and other chemicals were obtained from BDH Chemicals, Poole, Dorset, U.K.

Animals

Specific-pathogen-free male rats of the Wistar strain and of body weight 170–190g were used (A. Tuck and Son, Rayleigh, Essex, U.K.). They were maintained as described by Ashby *et al.* (1978*a*) and were starved for 24h from 09:00h before the start of the experiment.

Incubation of epididymal fat-bodies

The procedure for the collection and incubation of fat-bodies was that described by Ashby *et al.* (1978*b*).

The basic incubation medium was Krebs–Henseleit bicarbonate buffer solution, pH 7.3–7.5 (Krebs & Henseleit, 1932) gassed with O_2/CO_2 (19:1) and supplemented with bovine serum albumin that had been dialysed against water (final concentration, 2%, w/v), Intralipid (final concentration, 5%, v/v) and, unless otherwise stated, the amino acid mixture used for HeLa-cell culture (Eagle, 1955). In addition a ^{3}H-labelled amino acid mixture (0.2μCi/ml) was normally present in the medium. Other additions to the medium were as specified in the text.

Assay of lipoprotein lipase

Acetone/ether-dried preparations of the fat-bodies and the incubation medium combined were made as described by Ashby *et al.* (1978*a*). Subsequent homogenization of the dried preparations and assay of the homogenate was as described by Ashby *et al.* (1978*b*), except that the assay volume was reduced to 1ml and horse serum, rather than rat serum, was used as the source of apoprotein CII. The measured activities were similar to those obtained with rat serum and the assay was linear with respect to time and enzyme concentration over the same range. One unit of enzyme activity is defined as the amount of enzyme that releases 1μmol of fatty acid/h in the assay.

Incorporation of radioactive amino acid into fat-body protein

The procedure for determining the incorporation of ^{3}H-labelled amino acids into the fat-body protein has been described by Ashby *et al.* (1978*b*). Under the conditions of the experiments, rates of amino acid incorporation in particular media were linear for up to 8h.

The results are expressed in c.p.m. because of the difficulty of obtaining meaningful estimates of the counting efficiency for radioactive samples precipitated on to filter discs. The constancy of the sampling procedures and of the sample geometry ensures identical counting efficiency within each experiment (Mans & Novelli, 1961).

Statistical analysis

Appropriate results have been analysed for significance by the paired sample *t* test, which eliminates the effect of variations in fat-body lipoprotein lipase activity between consignments of rats (Campbell, 1974). This method of analysis has been discussed by Ashby *et al.* (1978*b*). Because of the variations in absolute lipoprotein lipase activities in experiments using different batches of rats, the standard deviations of the activities are not given in the Figures. However, the number of experiments of which each Figure is typical is indicated in the legends.

Results

Hexose requirement for lipoprotein lipase activation at 25°C

We have shown previously that the lipoprotein lipase activity of epididymal fat-bodies from 24h-starved rats increases by an energy-dependent activation process when they are incubated in media containing glucose and cycloheximide at 25°C (Cryer *et al.*, 1973; Ashby *et al.*, 1978*b*). The data in Table 1 now show that the requirement for glucose in the medium is relatively specific. There is some endogenous activation of lipoprotein lipase, however, the magnitude of the increase is significantly greater in the presence of glucose ($P<0.001$) or mannose ($P<0.025$) and fructose ($P<0.01$) which are readily converted to glucose 6-phosphate. On the other hand, with galactose, ribose, 2-deoxyglucose, malate or pyruvate in the medium, the rise is not significantly greater than in their absence.

Insulin was present in all the media used in the experiments described in Table 1. However, independent experiments have confirmed the findings of Ashby *et al.* (1978*b*) that it does not influence the activation process (K. Walker, unpublished work).

Lipoprotein lipase activation at 37°C

Fig. 1 shows that a protein-synthesis-independent rise in lipoprotein lipase activity, similar to that observed at 25°C (Ashby *et al.*, 1978*b*), also occurs during incubations of epididymal fat-bodies from 24h-starved rats in the presence of glucose at 37°C. However, whereas a plateau of lipoprotein lipase activity is maintained for several hours at 25°C, the activity during incubations at 37°C falls rapidly from the peak value that is achieved. The rapid fall may explain why the initial rise was not detected

Table 1. *Effects of various energy substrates on adipose-tissue lipoprotein lipase activity*

Groups of four fat-bodies were incubated at 25°C in the basal incubation medium described in the Materials and Methods section from which the amino acid mixture had been omitted. The medium also contained cycloheximide (36 μM), insulin (0.2 m-i.u./ml) and the energy substrates indicated at a final concentration of 10 mM. After 2.5 h, lipoprotein lipase was assayed in the fat-bodies and medium combined. The mean initial lipoprotein lipase activity of 10 groups of fat-bodies was 5.7 units/fat-body. Incorporation of ^{3}H-labelled amino acids into the fat-body protein was inhibited by 94–97%. The number of experiments (n) signifies the number of direct comparisons made with independent preparations of tissue, and the significance of the differences between the means (P) was calculated by the paired sample t test (see the Materials and Methods section).

Addition to medium	Mean lipoprotein lipase activity (units/fat-body) No additions	+ Substrate	Mean difference (±S.D.)	n	Significance of increase with added substrate ($P<$)
Glucose	6.8	10.1	3.3 ± 1.6	9	0.001
Mannose	6.6	8.8	2.3 ± 1.7	6	0.025
Fructose	6.6	8.8	2.2 ± 1.9	9	0.01
Galactose	6.5	5.5	−1.0 ± 1.4	4	NS
Ribose	6.0	6.4	0.4 ± 0.8	5	NS
2-Deoxy-glucose	6.0	5.3	−0.7 ± 0.6	7	NS
Malate	6.3	7.2	0.9 ± 0.6	4	NS
Pyruvate	6.2	5.8	−0.4 ± 0.9	6	NS

during earlier similar studies at 37°C (Wing *et al.*, 1966; Wing & Robinson, 1968), and probably reflects a greater instability of the activated enzyme at the higher temperature (Robinson & Wing, 1971).

Effects of preincubation of fat-bodies on activation at 37°C

Fig. 2 shows the results of experiments in which epididymal fat-bodies were first incubated at 37°C in the presence of pyruvate, insulin and cycloheximide, and glucose was then added at various times after the start of the incubations. The ability of the added glucose to bring about activation of lipoprotein lipase is seen to be lost when the period of the preliminary incubation is 2 h or longer.

On the other hand, when similar experiments were carried out with cycloheximide as well as glucose absent from the medium used for the preliminary incubations, glucose addition produced a rapid rise in lipoprotein lipase activity after preliminary incubations of as long as 4 h (Fig. 3).

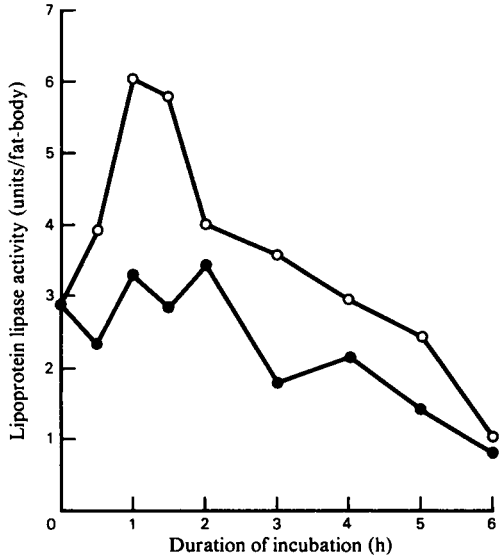

Fig. 1. *Effect of glucose on fat-body lipoprotein lipase activity during incubations in the presence of cycloheximide at 37°C*

Groups of four fat-bodies were incubated at 37°C in the basal incubation medium described in the Materials and Methods section to which cycloheximide (36 μM), insulin (12 m-i.u./ml) and pyruvate (26.6 mM) were added. Glucose (13.3 mM) was either absent (●) or present (○). The lipoprotein lipase activity of the fat-bodies and medium combined was determined at the times shown. The incorporation of ^{3}H-labelled amino acids into the fat-body protein was inhibited by 97–98%. The results shown are typical of three paired experiments. The mean (± S.D.) differences between the lipoprotein lipase activities observed in the presence (○) or absence (●) of glucose after 1 h, 2 h and 3 h were 2.3 ± 0.87 ($P<0.05$), 0.47 ± 0.15 ($P<0.05$) and 0.5 ± 0.80 (not significant) units/fat-body respectively.

These results are consistent with the existence of an activatable pool of lipoprotein lipase proenzyme molecules within the fat-bodies at the time of their removal from the rats (Ashby *et al.*, 1978*b*). However, they suggest that the half-life of the proenzyme is relatively short at 37°C and that the glucose-dependent rise in enzyme activity, therefore, can only be observed during incubations at this temperature if the proenzyme is renewed through continuing protein synthesis.

The rapid increase in lipoprotein lipase activity that followed the addition of glucose in the experiments of Fig. 3 was not accompanied by any stimulation of the rate of protein synthesis, beyond that observed during the preliminary incubations in the presence of insulin and pyruvate. This has been

Table 2. *Effects of glucose, pyruvate and insulin on incorporation of ^{3}H-labelled amino acids into fat-body protein*
Groups of four fat-bodies were incubated at 37°C in the basal incubation medium described in the Materials and Methods section with the additions shown. The final concentrations of the substances added were: insulin, 12 m-i.u./ml; pyruvate, 26.6 mM; glucose, 13.3 mM. The incorporation of ^{3}H-labelled amino acid into the fat-body protein was determined after 5 h. The number of experiments was six, and this signifies the number of direct comparisons; the significance of the increases in incorporation due to insulin was calculated by the paired sample *t* test (see the Materials and Methods section).

Additions to the incubation medium	10^{-3} × Mean incorporation of ^{3}H into fat-body protein (c.p.m./fat-body)	Relative increase in incorporation due to insulin (fold)	Significance of increase in incorporation due to insulin ($P<$)
Glucose	9.2		
Glucose + insulin	12.7	1.39	0.005
Pyruvate	8.1		
Pyruvate + insulin	10.6	1.31	0.005

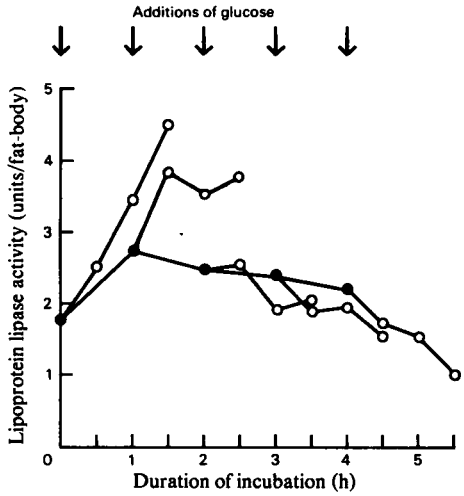

Fig. 2. *Effect of preincubation of fat-body in the presence of cycloheximide at 37°C on glucose-induced activation of lipoprotein lipase*
The experiment was carried out as described in the legend to Fig. 1. Glucose (13.3 mM) was either absent (●) or was added (○) at 0 h, 1 h, 2 h, 3 h or 4 h. The results shown are typical of three paired experiments. The mean (± S.D.) differences in lipoprotein lipase activity 1.5 h after glucose additions at 0 h, 1 h, 2 h and 3 h were 1.78 ± 0.66 ($P<0.05$), 2.15 ± 0.69 ($P<0.05$), 0.68 ± 1.13 (not significant) and 0.15 ± 0.5 (not significant) units/fat-body respectively.

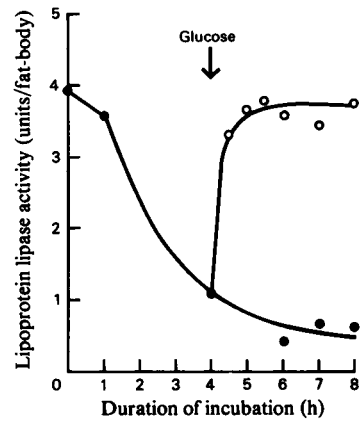

Fig. 3. *Effect of glucose on lipoprotein lipase activity after preincubation of fat-bodies in the absence of cycloheximide at 37°C*
The experiment was carried out as described in the legend to Fig. 1, except that cycloheximide was omitted from the medium. Glucose (13.3 mM) was either absent (●) or added at 4 h (○). At intervals, lipoprotein lipase activity was determined in the fat-bodies and medium combined. The results shown are typical of 10 experiments. In these experiments the mean (± S.D.) differences in the lipoprotein lipase activity (units/fat-body) at 0.5, 1 and 1.5 h after glucose additions were as follows: 2.54 ± 1.17 ($P<0.001$); 3.07 ± 1.01 ($P<0.001$); 4.14 ± 1.47 ($P<0.001$).

confirmed in six further experiments in which the mean (± S.D.) incorporation of ^{3}H-labelled amino acid measured at 6 h, after the addition of glucose at 4 h, was 103 ± 8% of that in paired incubations to which no glucose was added. These results are consistent with previous work (Krahl, 1964) and with the results in Table 2 showing that insulin's effect on adipose-tissue protein synthesis is of the same magnitude in the presence of pyruvate as in that of glucose.

During incubations of fat-bodies in the absence of insulin (Table 2), the rate of protein synthesis was decreased. When the preliminary incubations were carried out under such conditions, the extent of the increase in lipoprotein lipase activity after the addition of glucose was also diminished (Fig. 4*b* versus 4*a*), suggesting that the rate of protein

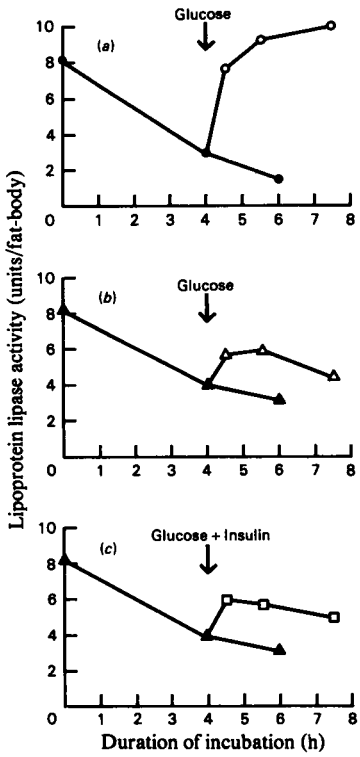

Fig. 4. *Effect of preincubation of fat-body in the presence and absence of insulin at 37°C on glucose-induced increase in lipoprotein lipase activity*

Groups of four fat-bodies were incubated at 37°C in the basal incubation medium described in the Materials and Methods section to which pyruvate (26.6mM) was added. Insulin (12m-i.u./ml) was either present (●) or absent (▲). After 4h, glucose (13.3mM) was added to some of the incubations (○ and △) (*a* and *b* respectively) and glucose (13.3mM) and insulin (12m-i.u./ml) were added to others (□) (*c*). Lipoprotein lipase activity was determined in the fat-bodies and medium combined, at the intervals shown. The results are typical of three paired experiments. In six experiments, the mean (± S.D.) difference in lipoprotein lipase activity between *a* and *b*, 1.5h after the addition of glucose was 2.94 ± 1.74 ($P < 0.01$) units/fat-body. The mean (± S.D.) difference in lipoprotein lipase activity between *a* and *c* at 1.5h after the addition was 2.52 ± 0.83 ($P < 0.05$) units/fat-body (three experiments).

synthesis during the preincubation period determines the supply of proenzyme available for activation. The possibility that the smaller rise in enzyme activity in Fig. 4(*b*) was a result of diminished glucose transport in the absence of insulin is excluded by the results in Fig. 4(*c*), where the addition of glucose and insulin together did not increase the extent of the rise in enzyme activity beyond that following the addition of glucose alone.

Effect of glucose concentration

Fig. 5 shows the results of two experiments in which, after the incubation of fat-bodies for 4h at 37°C in the presence of pyruvate and insulin, at concentrations of either 0.2 or 12m-i.u./ml, the amount of glucose added to the incubation medium was varied. The extent of the rise in enzyme activity measured at 90min after the glucose addition increased up to a glucose concentration in the medium of about 3mM, and was greater when the insulin concentration during the preceding period of incubation was 12m-i.u./ml than when it was 0.2m-i.u./ml. Since the rate of protein synthesis was also higher at the higher insulin concentration, this can again be interpreted in terms of the dependence of the extent of activation on the rate of prior synthesis of proenzyme in the tissue.

Discussion

Role of glucose in the activation of lipoprotein lipase

The results of the present study show that the protein-synthesis-independent activation of lipoprotein lipase requires glucose, or certain sugars that are readily converted to glucose 6-phosphate. This is consistent with the possibility that glycosylation may be an essential step in the formation of the complete enzyme from a proenzyme form. In common with other secreted enzymes (Czichi & Lennarz, 1977; Lingappa *et al.*, 1978), lipoprotein lipase is known to be glycosylated (Bensadoun *et al.*, 1974; Iverius & Östlund-Lindqvist, 1976). It has been reported that glucose or mannose is specifically required for protein glycosylation in mouse myeloma cells, which, like adipose tissue, cannot carry out gluconeogenesis (Stark & Heath, 1979).

We have confirmed the findings of Ashby *et al.* (1978*b*) that the effect of glucose on the activation process is not promoted by insulin under the conditions of our experiments. However, in three paired experiments, carried out as described in the legend to Fig. 4, the increases in activity 1.5h after the addition of glucose were reduced by 25 ± 9% and 42 ± 10% respectively when phlorrhizin (5mM) or phloretin (1mM) were added immediately prior to the glucose. These effects of inhibitors of glucose transport clearly leave open the possibility that insulin may play a role in the activation process *in vivo* if the plasma concentration of glucose is such that its transport into the cell is rate-limiting.

Role of insulin in the regulation of lipoprotein lipase activity

We have suggested that insulin increases the rate

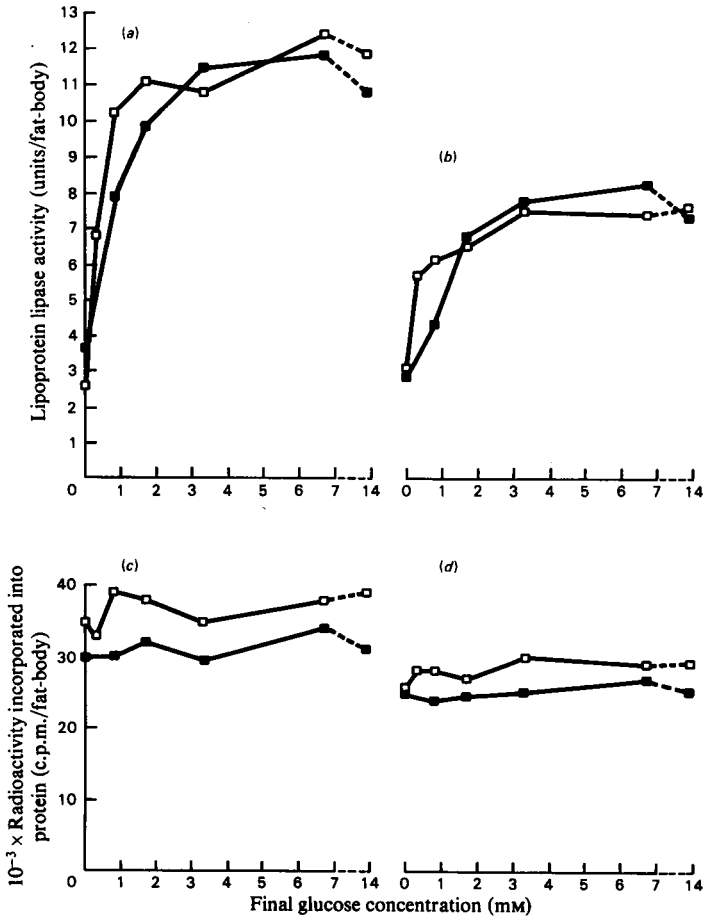

Fig. 5. *Effect of glucose concentration on the increase in activity of fat-body lipoprotein lipase*
Groups of four fat-bodies were incubated at 37°C in the basal incubation medium described in the Materials and Methods section to which insulin [12 m-i.u./ml (*a* and *c*) or 0.2 m-i.u./ml (*b* and *d*)] and pyruvate (26.6 mM) were added. Glucose (0–13.3 mM) was added to the medium after 4 h. Lipoprotein lipase activity in the fat-bodies and medium combined (*a* and *b*), and the incorporation of ^{3}H-labelled amino acid into the fat-body protein (*c* and *d*), were determined after 5.5 h. The results shown (■ and □) are from two paired experiments in each of which paired fat-bodies were incubated in media containing either 12 or 0.2 m-i.u. of insulin/ml.

of synthesis of a proenzyme form of lipoprotein lipase in adipose tissue (Ashby *et al.*, 1980). Consistent with this are the experiments presented here in which the rates of protein synthesis observed during preincubations of fat-bodies in the presence of pyruvate, and in either the absence of insulin or in its presence at 0.2 or 12 m-i.u./ml, were directly related to the extent of the increase in lipoprotein lipase activity that followed the addition of glucose to the incubation medium.

Whether such an effect of insulin reflects its general stimulation of adipocyte protein synthesis (Muller & Beigelman, 1967; Minemura *et al.*, 1970; Jarrett *et al.*, 1972; Jacobs & Krahl, 1973; Sakai *et al.*, 1973), or involves a specific action on the synthesis of lipoprotein lipase, will require further work. However, the present studies show that it does not require the presence of glucose. Since insulin does not influence the influx of pyruvate into the cell (Winegrad & Renold, 1958; Kneer & Ball, 1968), it can be concluded that its action is independent of any effects on the transport of energy substrates across the adipocyte cell membrane.

In previous studies it was reported that effects of insulin on the activity of lipoprotein lipase in rat adipose tissue were dependent on the presence of glucose. For example, Wing *et al.* (1966) claimed that the increases in enzyme activity that occurred

under their conditions of fat-body incubation at 37°C required both insulin and glucose, whereas Patten (1970) found that the activity of the enzyme in isolated adipocytes fell more dramatically in the presence of insulin alone than in the presence of insulin and glucose. Both these observations appear capable of explanation, however, in terms of our present concepts, whereby a proenzyme form of lipoprotein lipase is synthesized in the presence of pyruvate and insulin, but the expression of its activity requires conversion to the fully active enzyme in the presence of glucose.

Use of epididymal fat-body preincubation systems

We believe that the magnitude of the initial rise in lipoprotein lipase activity that occurs when fat-bodies are incubated in the presence of cycloheximide provides an estimate of the size of the pre-existing proenzyme pool that is present in the tissue *in vivo*. On the other hand, we think we have been able to separate the process of synthesis of the proenzyme form from the process of its activation, through the procedure of preincubation of the fat-bodies in media that are adequate for synthesis but that lack the glucose required for activation. If we are correct, the extent of the rise in enzyme activity that occurs on the addition of glucose provides an indirect measure of the rate of proenzyme synthesis during the preceding incubation period. This could provide a relatively simple means to study the effect of other hormones on the synthesis of the proenzyme in different nutritional conditions.

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