

Lipoprotein Lipase Activity in the Adipose Tissue of Rats Adapted to Controlled Feeding Schedules

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1. The relationships between nutritional state, lipoprotein lipase activity in epididymal fat-pads, and the concentrations of glucose, insulin and unesterified fatty acids in the plasma were studied in rats that had been adapted for 3 weeks to one of two controlled feeding schedules. In one of these, rats had access to food for 14h during each 24h period, and in the other, they had access to food for 14h during each 48h period. Groups of animals were killed at different times during the 14h when they had access to food and during the following period when they were deprived of food. 2. Low lipoprotein lipase activity, low concentrations of plasma glucose and insulin and high concentrations of plasma unesterified fatty acids were found in rats deprived of food for 34h. Feeding resulted in increases in lipoprotein lipase activity and in the concentrations of glucose and insulin in the plasma. Enzyme activity continued to increase during the first 6–9h of the feeding period. 3. After adapted rats had been deprived of food for 12–16h there was a marked and unexpected increase in lipoprotein lipase activity; this occurred even when the rats were kept in an isolated environment. 4. The findings suggest that factors other than the absolute concentrations of insulin and glucose in the blood can exert a considerable influence on lipoprotein lipase activity in the epididymal fat-pad of a rat.

Lipoprotein lipase is an enzyme that hydrolyses lipoprotein-bound triglycerides (Korn, 1955). It is present in various tissues and is involved in the transport of fatty acids from circulating triglycerides into these tissues (Hollenberg, 1959; Robinson, 1960). The activity of the lipoprotein lipase in adipose tissue is proportional to the rate of uptake of triglycerides; therefore the enzyme may have a significant role in controlling fat deposition in this tissue (Bezman *et al.*, 1962; Garfinkel *et al.*, 1967). In the adipose tissue of fed animals lipoprotein lipase activity is greater than in the adipose tissue of starved animals (Cherkes & Gordon, 1959; Hollenberg, 1959; Robinson, 1960; Pav & Wenkeova, 1960). Feeding restores enzyme activity in the adipose tissue of starved animals, carbohydrate being the most efficient nutrient to do so (Wenkeova *et al.*, 1962). Moreover, the activity of lipoprotein lipase in adipose tissue is decreased in alloxan-diabetes (Pav & Wenkeova, 1960; Schnatz & Williams, 1963; Kessler, 1963). Thus the metabolism of glucose and the secretion of insulin would appear to be involved in the transition from low enzyme activity in starved animals to high enzyme activity in fed animals. The experiments reported below were conducted to investigate lipoprotein lipase activity of adipose tissue

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in different nutritional states and to study the relationships between enzyme activity and the concentrations of glucose, insulin and unesterified fatty acids in the blood of rats that had been adapted to controlled feeding schedules.

A brief account of this work has been presented (Reichl, 1970).

Experimental

Animals, feeding schedules and experimental procedure

Male albino rats of the Wistar strain were given a stock laboratory diet (no. 8, supplied by Dixon and Sons Ltd., Ware, Herts., U.K.) and were adapted to one of two feeding schedules. In Expt. 1, a group of 40 rats had access to food for 14h during each 24h period; food was available from 19.00h every evening until 09.00h the next morning. In Expt. 2, a group of 70 rats had access to food for 14h during each 48h period; food was available from 19.00h on alternate evenings until 09.00h the next morning. The rats in Expts. 1 and 2 were housed in the same animal room together with other experimental rats. In Expt. 3, the feeding schedule of a group of 40 rats was exactly the same as that of the rats in Expt. 2, but the animals were housed in a separate room so that they could be completely isolated from all rats on other feeding treatments. In all three experiments the lights

in the animal houses were turned off at 19.00h and were turned on at 07.00h every day; water was available *ad lib.* at all times and food was delivered *ad lib.* from an automatic machine during the specified feeding periods. In each experiment, the rats were adapted to the particular feeding schedule for 3 weeks. According to Fabry (1967), a period of 3 weeks is sufficient for rats to adapt to different feeding schedules of this type. The mean weights (\pm s.d.) of the rats at the beginning and at the end of the adaptation periods for all three experiments were 202.2 ± 38.4 and 262.2 ± 58.4 g respectively.

At the end of the period of adaptation, groups of rats were killed by decapitation at different times during the 14h when the animals had access to food and during the following period when food was not available (see Figs. 1–5). In Expts. 1 and 2, the first groups of rats were killed just before 19.00h, i.e. just before the beginning of the feeding period. Thus in Expts. 1 and 2 these first groups of rats had been without food for 10 and 34h respectively. In Expt. 3, the first group of rats was killed at 19.15h and therefore had access to food for 15min after starvation for 34h. The period of observation extended over 2 days in Expt. 1 and 3 days in Expts. 2 and 3 (see Figs. 1–5).

Blood was collected from the decapitated animals into tubes containing dry sodium fluoride and sodium citrate; plasma was obtained by centrifugation. The concentrations of glucose and unesterified fatty acids were determined in portions of each plasma sample on the same day that the rats were killed. The remainder of each plasma sample was frozen and stored at -20°C ; it was used subsequently for the determination of the concentrations of immunoreactive insulin. As rapidly as possible after death the stomach and both epididymal fat-pads were excised from each animal. The fat-pads were rinsed with 0.9% NaCl, blotted on filter paper, weighed and then used for the determination of lipoprotein lipase activity. The mean weights (\pm s.d.) of single epididymal fat-pads in Expts. 1, 2 and 3 were 0.65 ± 0.1 , 0.81 ± 0.3 and 1.05 ± 0.2 g respectively. For each animal the weight of the stomach together with its contents was recorded. No attempt was made to separate the stomach from its contents in all rats, as it was found that the weight of the empty stomach varied only between 1.7 and 1.9g. Therefore Figs. 1(a), 2(a) and 4(a) reflect changes in the weight of stomach contents.

Determination of lipoprotein lipase activity

The epididymal fat-pads were homogenized with acetone in a MSE top-drive homogenizer: approx. 30ml of acetone, cooled to -20°C , was used per g of fresh tissue. The homogenate was filtered under reduced pressure and the residue was washed with

cold acetone and then cold peroxide-free diethyl ether. About 30ml of each solvent was used per g of fresh tissue. The washed material was air-dried and stored at -20°C ; enzyme activities were determined in this material within 3 days of its preparation.

The acetone–ether-dried preparation from both fat-pads of each rat was incubated for 30min at 4°C with aq. 0.025M-NH₃ soln. (2ml/g of original fresh tissue). The incubation mixture was centrifuged for 20min at 9000 g and 4°C . The supernatant layer was carefully removed and portions of it were used for the determination of protein content and lipoprotein lipase activity. The sediment in the centrifuge tube was resuspended in the appropriate volume of fresh aq. 0.025M-NH₃ soln. and was treated in the same way as the original solvent-dried material. These two extractions were sufficient to remove virtually all of the protein that was soluble in aq. 0.025M-NH₃ soln. and the sum of the amounts of protein in the two extracts was considered to be the total protein extractable with aq. 0.025M-NH₃ soln. The yield of protein from the second extraction was about 22% of the yield from the first extraction.

For the determination of lipoprotein lipase activity in the aq. 0.025M-NH₃ extracts, the substrate was prepared as described by Korn (1955), but Prosparol (an emulsion of arachis oil supplied by BDH Pharmaceutical Ltd., Poole, Dorset, U.K.) was used instead of Ediol as the artificial fat emulsion. The composition (% w/v) of the Prosparol emulsion was: arachis oil, 50; glycerol monostearate, 2.9; polyoxyethylene sorbitol monostearate, 1.0; sodium benzoate, 0.1; butylated hydroxyanisole, 0.01. Each reaction vessel contained 0.02ml of Prosparol, 0.2ml of pooled human blood serum and 40mg of bovine serum albumin (fraction V; Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex, U.K.); aq. 0.05M-NH₃–NH₄Cl buffer was added to bring the total volume to 1.0ml. The pH was adjusted to 8.5 with aq. NH₃ soln. and the mixture was incubated for 30min at 30°C to activate the substrate. A measured volume of the ammoniacal extract of the solvent-dried tissue was then added to the substrate and the reaction mixture was incubated at 30°C . For the determination of the release of unesterified fatty acids, one sample of the reaction mixture was taken at zero time and duplicate samples were taken after incubation for 60min. A further portion of the ammoniacal extract of each solvent-dried tissue was incubated with the substrate in the presence of NaCl at a final concentration of 0.5M in the reaction mixture. With all tissue extracts it was found that when NaCl was present at this concentration in the incubation mixture, lipolytic activity was less than 10% of that determined in the absence of 0.5M-NaCl. In agreement with Garfinkel *et al.* (1967) it was observed that the lipoprotein lipase activity was stable in dilute ammoniacal solutions for more than

1h at 4°C. The two extractions with aq. 0.025M-NH₃ soln. were sufficient to remove all of the extractable lipoprotein lipase from the solvent-dried fat-pads. The enzyme activity in the second extract amounted to about 21% of that in the first extract; no activity was found in a third extract. The sum of the activities in the first and second extracts was considered to represent the total extractable lipoprotein lipase. Results are expressed as lipoprotein lipase activity (μ equiv. of unesterified fatty acids released/h) per fat-pad or per mg of extractable protein.

Methods of analysis

The concentration of glucose in the plasma was determined by the glucose oxidase method of Keston (1956) with the reagents supplied by Boehringer Corp. (London) Ltd., London W.5, U.K. The concentrations of unesterified fatty acids in the plasma and in the enzyme-assay media were determined by the method of Novak (1965). The protein contents of the tissue extracts were determined by the method of Lowry *et al.* (1951) with crystalline bovine serum albumin (Sigma Chemical Co., St Louis, Mo., U.S.A.) as standard. Plasma immunoreactive insulin concentrations were determined by the double-antibody radioimmunoassay technique of Samols & Bilkus (1964) with rat insulin (supplied by Novo Ltd., Copenhagen, Denmark) as a standard.

Results

Expt. 1: rats adapted to feed for 14h during every 24h

As is indicated by the changes in weights of the stomachs plus contents (Fig. 1a), the rats began to eat as soon as food was presented to them at 19.00h on day 1 and they continued to do so during the whole of the 14h period that food was available. After a period of 7h without food, i.e. at 16.00h on day 2, the stomachs of the rats were virtually empty.

There was a progressive increase in the lipoprotein lipase activity of the epididymal fat-pads during the first 6h of the period that food was available to the rats (Figs. 1b and 1c). After food was removed from the cages at 09.00h on day 2, there was a decrease in the lipoprotein lipase activity of the fat-pads (Figs. 1b and 1c). It should be noted that the activity of the enzyme at 19.00h on day 1, after the rats had been without food for 10h, was greater than that at 16.00h on day 2, after the rats had been without food for only 7h.

Expt. 2: rats adapted to feed for 14h during every 48h

The pattern of food intake was different from that observed in Expt. 1. In Expt. 2, the rate of food consumption was particularly rapid during the first

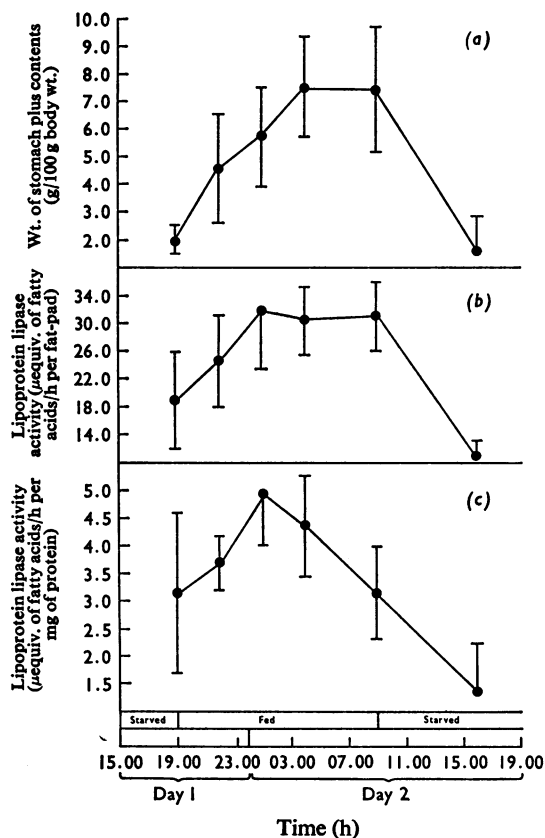


Fig. 1. Expt. 1: rats adapted to feed for 14h during every 24h

Changes in the weight of the stomach plus contents, and in the lipoprotein lipase activity of the epididymal fat-pads, during the periods when food was available and when food was withheld are shown. The weights of the stomach plus contents are expressed as g per 100g body wt. (a), and the lipoprotein lipase activities are expressed per fat-pad (b) or per mg of protein in the enzyme extract of the solvent-dried tissue (c). Each value is the mean derived from the results of six or seven rats per group; the vertical bars represent \pm S.D.

6h of the feeding period, and at 01.00h on day 2 the weight of stomachs plus contents (Fig. 2a) accounted for almost 20% of the total body weight and was about twice the maximum weight of stomach plus contents observed for the rats in Expt. 1 (Fig. 1a). Although food was still available, the weights of the stomachs plus contents began to decrease after 01.00h on day 2 and continued to do so during the remainder of the observation period (Fig. 2a).

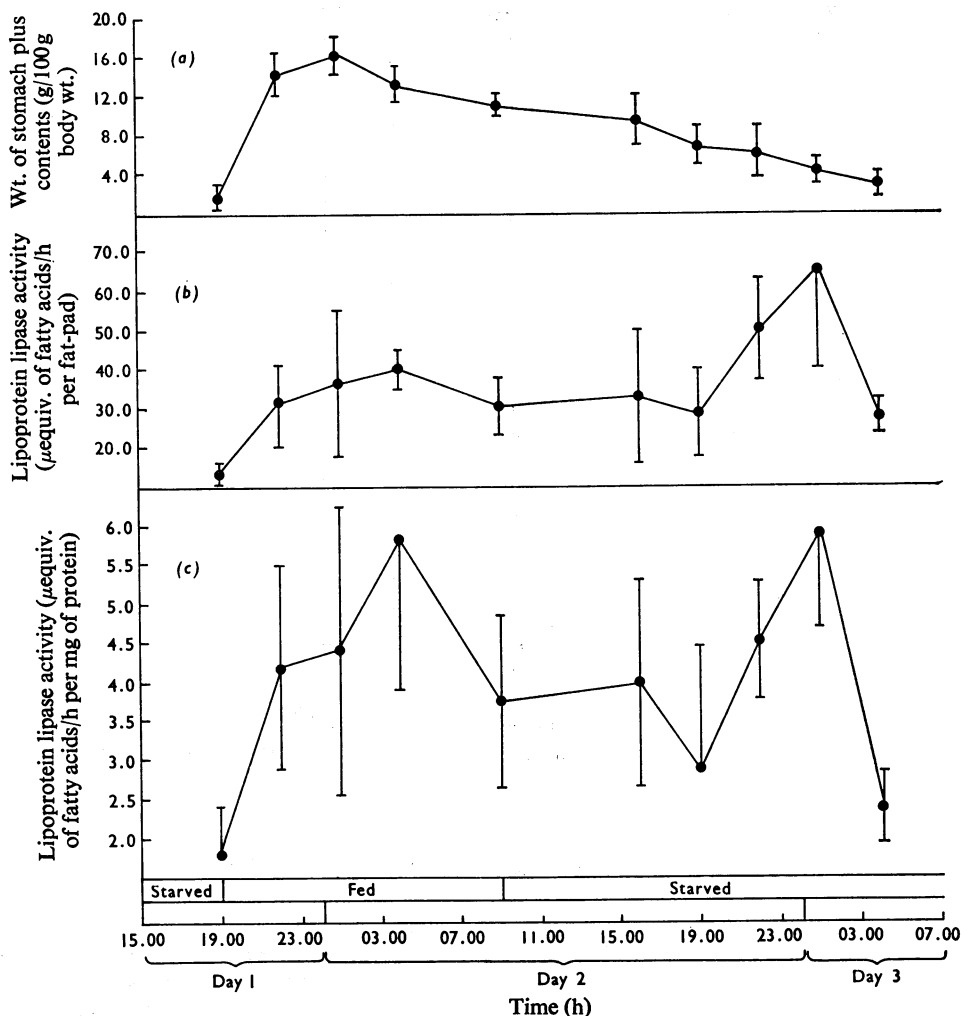


Fig. 2. Expt. 2: rats adapted to feed for 14h during every 48h

Changes in the weight of the stomach plus contents, and in the lipoprotein lipase activity of the epididymal fat-pads, during the periods when food was available and when food was withheld are shown. The weights of the stomach plus contents are expressed as g per 100g body wt. (a), and the lipoprotein lipase activities are expressed per fat-pad (b) or per mg of protein in the enzyme extract of the solvent-dried tissue (c). Each value is the mean derived from the results of four to eight rats per group; the vertical bars represent \pm S.D.

On this dietary treatment, the lipoprotein lipase activity of the fat-pads increased progressively during the first 9h of the period that food was available to the rats (Figs. 2b and 2c). After food was withheld at 09.00h on day 2 there were no consistent changes in lipoprotein lipase activity until 22.00h, when there was a pronounced increase in the enzyme activity of the fat-pads; this increase continued until 01.00h on day 3, after which there was a marked decrease in enzyme activity (Figs. 2b and 2c).

As might be expected, low concentrations of glucose and high concentrations of unesterified fatty acids were observed in the plasma of the rats killed just before 19.00h on day 1 (Figs. 3c and 3b); this group of rats had been without food for 34h. The intensive food consumption that occurred during the first 3h of the feeding period (Fig. 2a) was accompanied by a marked increase in the concentration of glucose and a marked decrease in the concentration of unesterified fatty acids in the plasma (Figs. 3c and

3b). During the remainder of the feeding period there was a progressive decrease in the concentration of plasma glucose, but after food was removed from the cages at 09.00h on day 2, there appeared to be an increase in plasma glucose concentration. Between 01.00h and 04.00h on day 3, when it may be assumed that the absorption of food was virtually complete (Fig. 2a), there was an increase in the concentration of unesterified fatty acids and a decrease in the concentration of glucose in the plasma. The concentration of immunoreactive insulin in the plasma increased during the first 6h of the period that food was

available to the rats, but from 01.00h on day 2 until the end of the period of observation, the insulin concentration in the plasma decreased progressively (Fig. 3a).

Expt. 3: rats adapted to feed for 14h during every 48h in an isolated environment

In Expt. 2 it was noteworthy that the second peak of lipoprotein lipase activity in the fat-pads occurred on day 3 at a time similar to that at which the first peak of activity had occurred on day 2. Since the rats

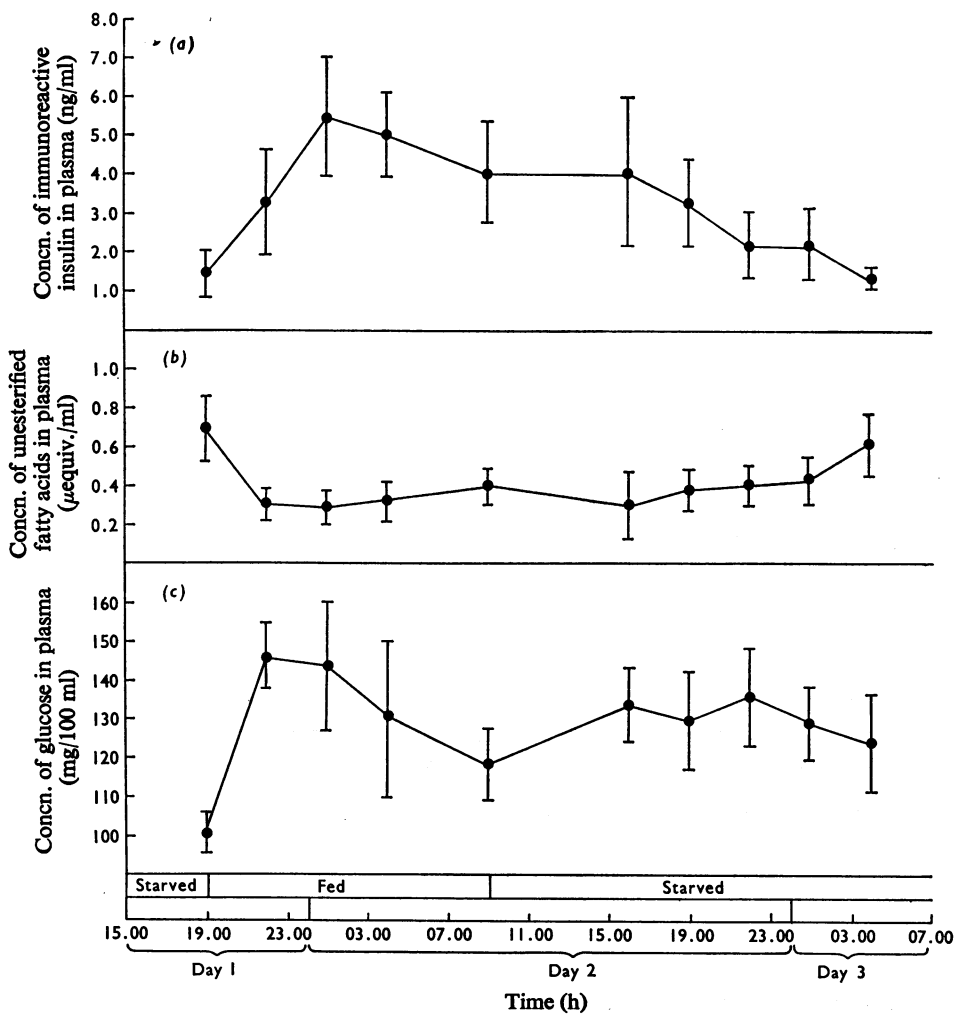


Fig. 3. *Expt. 2: rats adapted to feed for 14h during every 48h*

Changes in the concentrations of immunoreactive insulin (a), unesterified fatty acids (b) and glucose in the plasma (c) during the periods when food was available and when food was withheld are shown. The numbers of animals per group and the significance of the vertical bars are as given in Fig. 2.

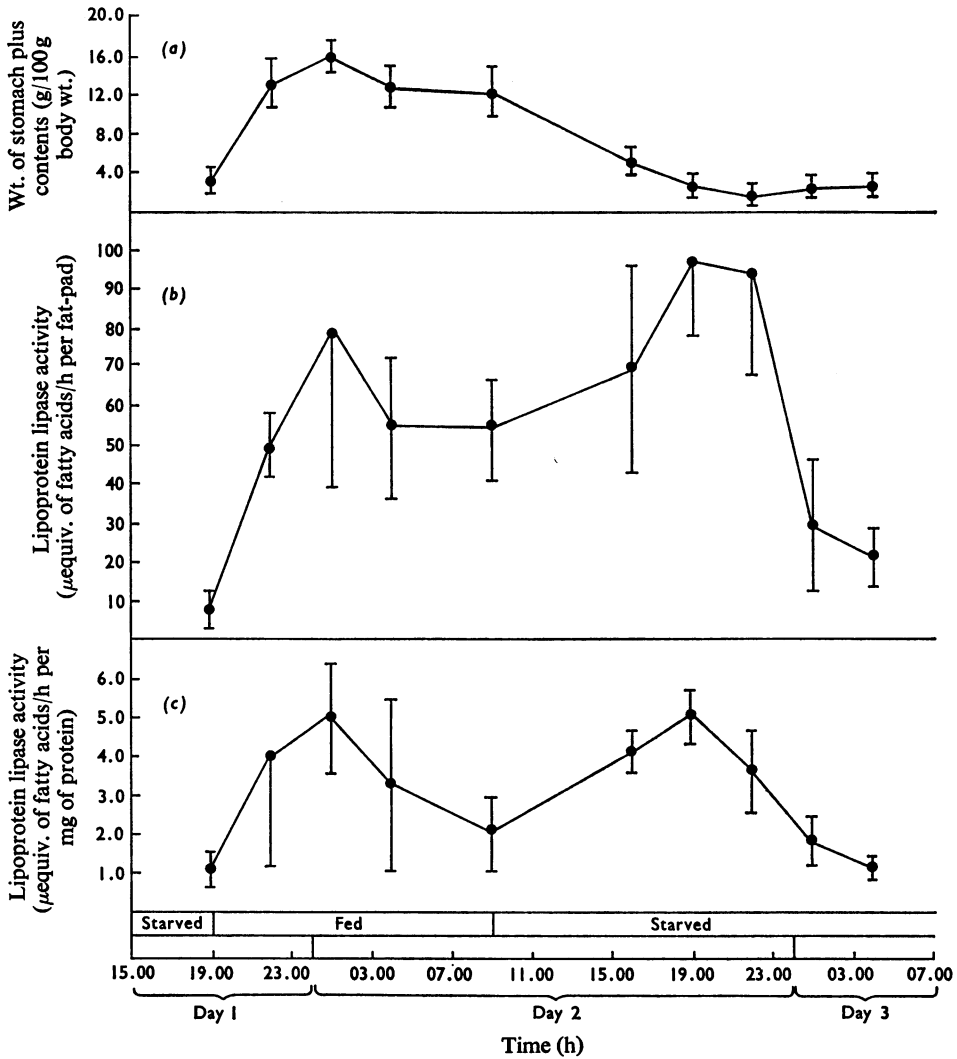


Fig. 4. Expt. 3: rats adapted to feed for 14h during every 48h in an isolated environment

Changes in the weight of the stomach plus contents, and in the lipoprotein lipase activity of the epididymal fat-pads, during the periods when food was available and when food was withheld are shown. The weights of the stomach plus contents are expressed as g per 100g body wt. (a), and the lipoprotein lipase activities are expressed per fat-pad (b) or per mg of protein in the enzyme extract of the solvent-dried tissue (c). With the exception of the value for the group killed at 09.00h on day 2, in which there were only three rats, each value is the mean derived from four rats per group; the vertical bars represent \pm S.D.

in Expt. 2 were housed in the same animal room as other rats that were given food at 19.00h every day (as in Expt. 1), it seemed possible that the lipoprotein lipase activity in the rats of Expt. 2 could have been influenced by the feeding activities of the other rats that were housed in the same room. Expt. 3 was

designed to investigate this possibility with rats that were kept in a completely isolated environment; the dietary treatment was exactly the same as that in Expt. 2.

In Expt. 3, the pattern of food intake (Fig. 4a) was similar to that observed in Expt. 2 (Fig. 2a), but, after

food was withheld, the rate of gastric emptying in the rats of Expt. 3 appeared to be somewhat greater than that in the rats of Expt. 2 (cf. Figs. 4a and 2a).

It is clear from Figs. 4(b) and 4(c) that the changes in lipoprotein lipase activity in Expt. 3 were broadly similar to those in Expt. 2. The first peak of enzyme activity occurred about 6h after the rats had begun to eat (i.e. at 01.00h on day 2), whereas the second peak of activity occurred some 10h after food had been removed from the cages (i.e. at 19.00h on day 2). In Expt. 3, the two peaks of lipoprotein lipase activity occurred somewhat earlier than did the corresponding peaks in Expt. 2 (cf. Figs. 2 and 4).

It is important to remember that, in Expt. 3, the first group of rats were killed at 19.15h on day 1,

i.e. 15min after the onset of feeding, whereas in Expt. 2 the first group of rats were killed just before 19.00h, when they had been without food for 34h. If the results for the first group of rats in Expt. 3 (Fig. 5) are compared with those for the first group of rats in Expt. 2 (Fig. 3) it would appear that food intake during this relatively short period of about 15min was sufficient to bring about pronounced increases in the concentrations of glucose and immunoreactive insulin in the plasma and a marked decrease in the concentration of unesterified fatty acids. The increase in the concentration of plasma unesterified fatty acids and the decrease in the concentration of plasma glucose observed towards the end of Expt. 3 (Figs. 5b and 5c) were more

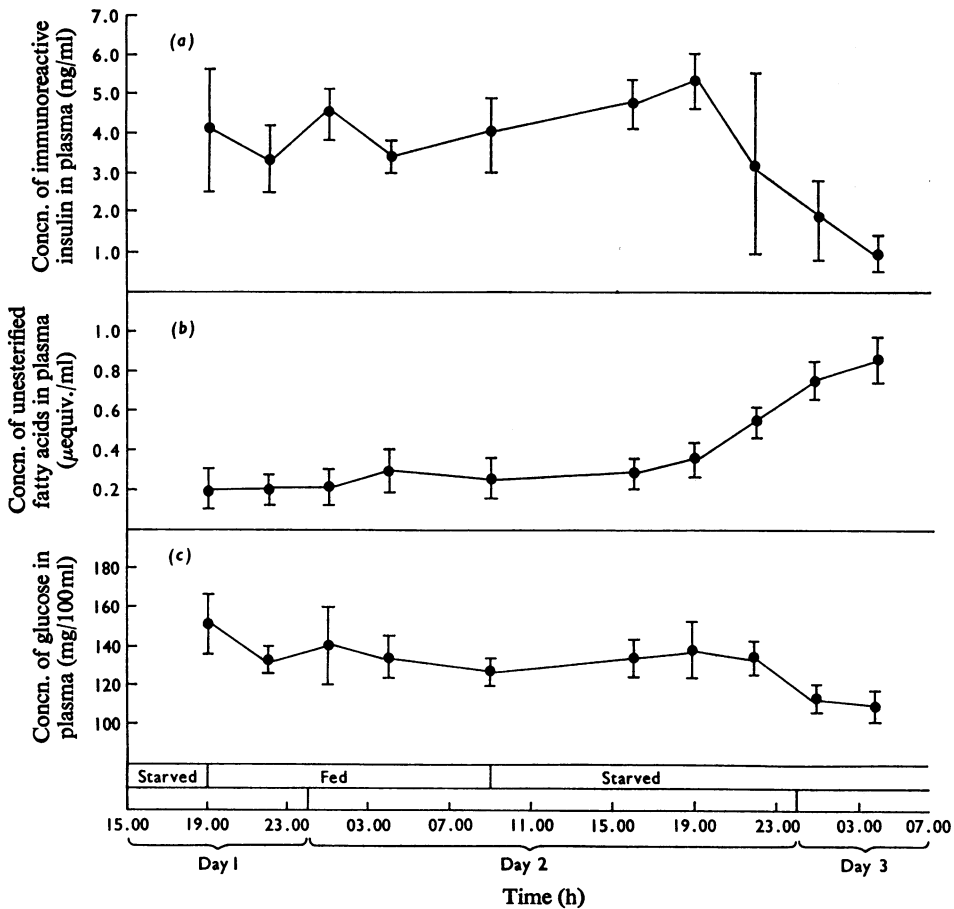


Fig. 5. Expt 3: rats adapted to feed for 14h during every 48h in an isolated environment

Changes in the concentrations of immunoreactive insulin (a), unesterified fatty acids (b) and glucose in the plasma (c) during the periods when food was available and when food was withheld are shown. The numbers of animals per group and the significance of the vertical bars are as given in Fig. 4.

pronounced than the corresponding changes observed at the end of Expt. 2 (Figs. 3b and 3c).

Discussion

In agreement with the results of others (Cherkes & Gordon, 1959; Hollenberg, 1959; Robinson, 1960; Pav & Wenkeova, 1960; Salaman & Robinson, 1966), low lipoprotein lipase activities were observed in the adipose tissues of those rats in Expts. 1 and 2 that had been deprived of food for 10 and 34 h respectively. In all three experiments, there was a marked increase in lipoprotein lipase activity during the first 6 h of the feeding period. The fact that the progressive increase in lipoprotein lipase activity in the adipose tissues coincided with an increase in the concentration of insulin in the plasma during the first 6 h of the feeding period in Expt. 2 (Figs. 2b, 2c, 3a) is consistent with the view that the increase in enzyme activity that occurs during the transition from the starved to the fed state is, at least in part, due to an increased secretion of insulin into the blood (Robinson & Wing, 1970). It has been well established that the ingestion of food elicits a rapid response in plasma insulin concentrations (see Taylor, 1967). With regard to lipoprotein lipase activity, the results of Expts. 2 and 3 are in keeping with those of Wenkeova *et al.* (1962), who showed that starved rats must be allowed access to food for about 3 h before any increase in enzyme activity is observed in the adipose tissue.

The appearance of a pronounced peak of lipoprotein lipase activity in the adipose tissue some 12–16 h after food had been withheld from the rats in Expts. 2 and 3 was a most unexpected finding. This peak did not depend on whether or not the rats were housed in an isolated environment. Moreover, in Expt. 2, it occurred at a time when there were decreases in the concentrations of glucose and insulin in the plasma; though it is perhaps noteworthy that the concentrations of plasma glucose and insulin at this time were greater than those observed when unadapted rats were starved for a similar period. Thus, contrary to the results of the investigation now reported, progressive decreases in the lipoprotein lipase activity of epididymal fat-pads were observed by Nikkila & Pykalisto (1968), who starved rats for up to 18 h, and by Otway *et al.* (1971), who starved rats for up to 48 h. However, in the studies of Nikkila & Pykalisto (1968) and Otway *et al.* (1971) the rats were not adapted to a period of starvation before the experiments were carried out. Experiments in this laboratory (D. Reichl, unpublished work) have confirmed that there is a progressive decrease in lipoprotein lipase activity in the epididymal fat-pads when unadapted rats are deprived of food. One major difference between the adapted and unadapted rat is to be found in the pattern of food consumption and

rate of gastric emptying. When food is withheld from an unadapted rat, the stomach is emptied fairly rapidly (D. Reichl, unpublished work); this is not so in an adapted rat (see, e.g., Fig. 2a). Such differences may be reflected in the insulin and glucose concentrations in the plasma of adapted and unadapted rats during the period of starvation. Contrary to the results for adapted rats (Figs. 3a and 5a), Nikkila & Pykalisto (1968) showed that when non-adapted rats were starved for 9 h there was a 50% decrease in the concentration of insulin in the plasma. It is thus possible that, in adapted rats, the moderately high concentrations of insulin and glucose in the plasma during the starvation period are necessary prerequisites for the appearance of a 'starvation' peak of enzyme activity in the adipose tissues. Small changes in these relatively high concentrations of plasma glucose and insulin might be critical in determining lipoprotein lipase activity. For instance, Nikkila (1967) observed a significant increase in lipoprotein lipase activity in the adipose tissues 10 min after an intravenous injection of glucose into rats that had been starved for only a short period. In such rats the concentrations of plasma glucose and insulin would have been moderately high even before the injection of glucose. Experiments *in vitro* have shown that insulin, at concentrations within the physiological range, induces increases in lipoprotein lipase activity in the adipose tissue of rats (Robinson & Wing, 1970), whereas adrenaline, noradrenaline, adrenocorticotrophin, glucagon and thyroid-stimulating hormone markedly inhibit such increases in enzyme activity (Wing *et al.*, 1966; Nestel & Austin, 1969). The activity of the enzyme *in vivo* will presumably depend, in part, on the ratio of the concentrations of these hormones in the blood that supplies the adipose tissues. It is noteworthy that with rats on a dietary regime similar to that in Expts. 2 and 3, Watanabe *et al.* (1968) observed a 'starvation' peak of tyrosine- α -oxoglutarate transaminase activity in the liver; this was tentatively attributed to daily oscillations in the concentration of plasma cortisone.

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