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Effect of nutritional status on rat adipose tissue, muscle and post-heparin plasma clearing factor lipase activities: their relationship to triglyceride fatty acid uptake by fat-cells and to plasma insulin concentrations

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Summary

1. In rats in a variety of nutritional states, the adipose tissue clearing factor lipase activity is strongly, positively correlated with fat-cell triglyceride fatty acid uptake.

2. In the same animals, muscle clearing factor lipase activity is inversely correlated with the activity of the enzyme in adipose tissue and with the plasma insulin concentration.

3. In starved animals that are given glucose, adipose tissue clearing factor lipase activity is positively correlated with the plasma insulin concentration.

4. The effect of changes in nutritional status on the activity of clearing factor lipase in rat post-heparin plasma depends on the heparin dosage used. The administration of glucose, but not of fructose or sucrose, to starved rats alters the response to heparin injection towards that found in rats in the fed state.

Key words: adipose tissue, clearing factor lipase, fat-cell, insulin, lipoprotein lipase, muscle, post-heparin plasma, triglyceride fatty acid.

Introduction

There is now considerable circumstantial evidence that the plasma triglycerides are hydrolysed by the enzyme, clearing factor lipase or lipoprotein lipase, during the course of their removal from the blood-stream. Furthermore, it appears that most of this hydrolysis takes place in the extrahepatic tissues, particularly adipose tissue and muscle, and occurs at the luminal surfaces of the capillary endothelial cells of these tissues (Robinson, 1970).

In adipose tissue, the activity of clearing factor lipase changes markedly with alterations in nutritional status and in several studies such changes have been correlated with alterations in the uptake of triglyceride fatty acids by the tissue (Bezman, Felts & Havel, 1962; Garfinkel, Baker & Schotz, 1967; Austin & Nestel, 1968). In all such investigations to date, the extent of the uptake has been determined by analyses carried out on the intact tissue. In such a situation, however, it is difficult to distinguish material that is taken up by the adipose tissue cells from that which is associated non-specifically with the tissue, either by adsorption or by entrapment in the blood capillaries (see Bezman *et al.*, 1962). In order to overcome this problem, we have made use of the method introduced by Rodbell (1964) for the preparation of fat-cells from adipose tissue and have studied the uptake of chylomicron triglyceride fatty acids by these cells in rats in which we have also measured adipose tissue clearing factor lipase activities. Animals in a variety of nutritional states have been employed in these studies in order to cover a wide range of lipase activities and in the same animals we have also measured the plasma insulin concentrations, in view of the substantial body of evidence which suggests that this hormone plays an important role in controlling adipose tissue clearing factor lipase activity (Robinson, 1970). In addition, the clearing factor lipase activities of various muscular masses and of post-heparin plasma have been deter-

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mined. This paper reports the results of these investigations.

A brief account of some of this work has been presented elsewhere (Cryer, Riley, Williams & Robinson, 1974).

Materials and methods

Animals and tissue preparations

Male rats of the Wistar strain were used throughout. They were maintained on Oxoid pasteurized diet 41 B (H. Styles Ltd, Bewdley, Worcs.) and weighed 175–195 g in the fed state when the experiments were begun. The rats were divided into groups and treated by one of the following procedures: (a) continued provision of the maintenance diet *ad libitum*; (b) starvation for 24 h; (c) starvation for 24 h, followed by the administration of glucose; (d) starvation for 24 h, followed by the administration of fructose; (e) starvation for 24 h, followed by the administration of sucrose.

The sugars administered to the rats in groups (c) to (e) were of AnalaR grade and were given by stomach tube between 09.00 and 09.30 hours and again between 10.00 and 10.30 hours, while the animals were under light ether anaesthesia. The dosage of glucose was 3 ml of a 100% (w/v) solution in water at each time and the other sugars were given in equicaloric amounts. All the rats were allowed to recover from the anaesthesia after the sugars had been administered.

Group (b) includes animals that were starved for 24 h and then given 3 ml of water by stomach tube. They were originally intended to act as controls for the animals in groups (c) to (e). However, the results obtained did not differ from those obtained in 24 h starved animals which were not given water and they have therefore been grouped together.

Blood and tissue samples were taken from the rats in groups (a) and (b) between 09.00 and 09.30 hours and from those in groups (c) to (e) between 12.00 and 12.30 hours—that is, 3 h after the first administration of the sugars. The 3 h period was chosen on the basis of previous work (Cryer *et al.*, 1974), which showed that, in rats given glucose, the adipose tissue clearing factor lipase activity is significantly increased at this time above that of starved animals although it is not as high as in animals fed *ad libitum*.

The rats were lightly anaesthetized with ether and killed by exsanguination from the abdominal aorta. The blood was collected into tubes containing heparin (Cryer *et al.*, 1974) and, while the collection was proceeding, the epididymal fat-bodies, the heart, the diaphragm and a sample of the thigh sartorius muscle were removed. All the tissues were rinsed in NaCl solution (150 mmol/l), blotted dry and weighed. Then the muscles and one of the fat-bodies were used for the assay of clearing factor lipase, and the other fat-body was used for the isolation of fat-cells (see below).

Rats killed by decapitation without ether anaesthesia were used in preliminary studies on adipose tissue clearing factor lipase (A. Cryer, S. E. Riley, E. R. Williams & D. S. Robinson, unpublished observations). The enzyme activities were not significantly different from those in tissue taken from animals killed under ether anaesthesia. Nor were the plasma glucose and free fatty acid concentrations (see below) altered by the ether anaesthesia procedure. Previous studies (Wing, Salaman & Robinson, 1966) have also shown that there is no significant difference between the clearing factor lipase activities of left and right fat-bodies. Nevertheless, in the present work alternate left and right fat-bodies were taken from consecutive animals for clearing factor lipase measurement, the other fat-body of each animal being used for the fat-cell isolation.

In many of the above experiments, the rats were first injected with chylomicrons in which the triglyceride fatty acids were labelled with ^{14}C. The chylomicrons were collected from the cannulated thoracic ducts of rats that had been fed with 200 μCi of glyceryl tri[1-^{14}C]oleate (The Radiochemical Centre, Amersham, Bucks.; 63 mCi/mmol), in 1 ml of olive oil (Bezman-Tarcher, Otway & Robinson, 1965). Before its administration, the chyle was washed and its triglyceride and radioactivity content was measured as previously described (Bezman-Tarcher *et al.*, 1965). Immediately before use, it was diluted with NaCl solution (150 mmol/l) so that approximately 10^6 c.p.m. and between 5 and 10 mg of triglyceride were injected in a volume of 0·4 ml. The chyle was injected into the femoral vein of the rats while they were maintained under light ether anaesthesia and, after 30 min, the animals were exsanguinated from the aorta and both fat-bodies were removed.

Measurement of fat-cell radioactive lipid

Fat-cells were isolated by the modification of

the method of Rodbell (1964) described previously (Cunningham & Robinson, 1969). The washed cells derived from each fat-body were finally suspended in 0·5 ml of Krebs–Ringer bicarbonate solution at pH 7·4 containing 4% (w/v) of bovine serum albumin and 3 ml of methanol was added to this suspension. After mixing, 6 ml of chloroform was added and the resulting mixture was left for 24 h at room temperature before filtration through defatted filter paper. The precipitate was washed on the filter paper with chloroform/methanol (2:1, v/v) and the combined filtrate was reduced in volume under a stream of air and transferred quantitatively to a weighed aluminium planchet. After evaporation of the solvent at room temperature, the planchets were reweighed and the lipid radioactivity was counted to an error of less than 5% in a Nuclear-Chicago gas-flow counter. All the radioactivity count rates were corrected to zero mass. The recovery of fat-cell lipid, as a proportion of the original wet weight of the fat-body, was 65·6 ± 10·1% (mean ± SD for seventy observations).

Measurement of tissue and plasma clearing factor lipase

Adipose tissue and muscle clearing factor lipase activities were measured in homogenates of acetone/ether-dried preparations of the tissues as described by Cunningham & Robinson (1969). However, the chylomicron preparation employed as a triglyceride substrate in their assay was replaced by the artificial triglyceride emulsion, Intralipid (Vitrum, Stockholm, Sweden). Recent work has shown that the rates of free fatty acid release are the same with chylomicron and Intralipid triglycerides as substrates (Riley & Robinson, 1974). The acetone/ether-dried preparations were made as previously described (Borensztajn, Otway & Robinson, 1970), except that, before being dispersed in acetone, the fresh tissue was homogenized in 1·5 ml of a solution of 5% (w/v) casein (soluble, light white, British Drug Houses Ltd, Poole, Dorset) that had been dialysed against NaCl solution (150 mmol/l) (Cryer, Davies, Williams & Robinson, 1975). The preparations were stored at −20°C and assayed within 24 h.

The clearing factor lipase activity of post-heparin plasma was measured in some experiments. Rats maintained under light ether anaesthesia were injected by the femoral vein with either 5 or 200 i.u. of heparin (Pularin; Evans Medical Ltd, Speke, Liverpool)/kg body weight and bled from the abdominal aorta respectively 2·5 or 5 min after the injection. The blood was collected as previously described (Robinson, Harris & Ricketts, 1959) and the clearing factor lipase activity of a suitable volume of the plasma was measured with Intralipid as the triglyceride substrate as described by Riley & Robinson (1974).

One unit of clearing factor lipase is defined as that which released 1 μmol of free fatty acid from its triglyceride substrate/h at 37°C.

Analytical methods and statistical analysis

Plasma immunoreactive insulin and triglyceride concentrations were measured in duplicate as described previously (Cryer *et al.*, 1974). The statistical difference between groups of measurements were assessed by Behren's modification of Student's *t*-test (Fisher & Yates, 1957).

Results

Adipose tissue clearing factor lipase activity and triglyceride fatty acid uptake

Fig. 1 shows, for animals in a variety of different nutritional states, the relationship between the clearing factor lipase activity of rat epididymal adipose tissue and the ability of the tissue to incorporate injected ^{14}C-labelled chylomicron triglyceride fatty acids into its fat-cell lipids. The wide range of adipose tissue clearing factor lipase activities in such animals has already been described (Cryer *et al.*, 1974). It is evident that, over this range, the activity of the enzyme is strongly correlated with the capacity of the tissue to take up triglyceride fatty acids, the correlation coefficient (r) in the fifty-seven animals studied here being 0·88 ($P < 0·001$; $y = 0·058 + 0·06x$). This correlation is not altered when the triglyceride fatty acid uptake is expressed per g wet weight of tissue or per g of recovered fat-cell lipid. Thus the variations in fat-cell lipid recovery noted in the Materials and methods section do not significantly affect the relationship.

Included in Fig. 1 are values for twenty-four rats that were fed their maintenance diet *ad libitum* and it is noteworthy that in this group of animals the adipose tissue clearing factor lipase activity

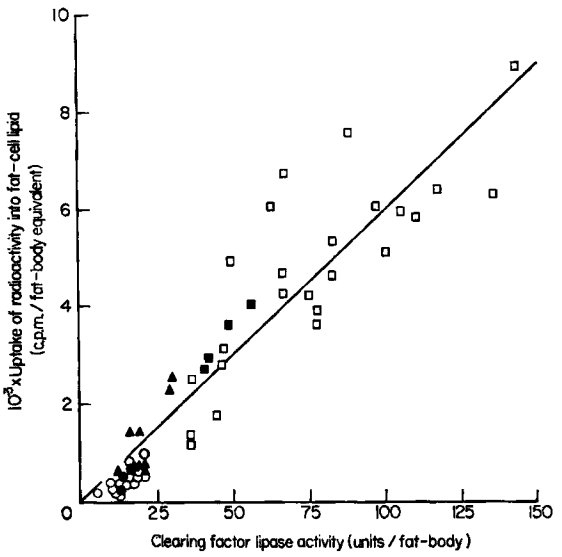

FIG. 1. Adipose tissue clearing factor lipase activity and fat-cell triglyceride fatty acid uptake. Rats in different nutritional states were injected intravenously with chylomicron triglycerides labelled in their fatty acid moiety with ^{14}C. After 30 min, clearing factor lipase was measured in one epididymal fat-body and fat-cell lipid radioactivity was estimated in the other (see the Materials and methods section). The fat-cell radioactivity was corrected to an injected dose of 10^6 c.p.m., the actual dose being within 10% of this value in all cases. Values were determined for rats (figures in parentheses are the numbers of animals in each group): □, given their maintenance diet *ad libitum* (24); ○, starved for 24 h (13); ■, starved for 24 h and then given glucose (4); ●, starved for 24 h and then given fructose (5); ▲, starved for 24 h and then given sucrose (11).

Details of these conditions are given in the Materials and methods section.

shows considerable variation from rat to rat. However, despite this, the correlation between the enzyme activity and triglyceride fatty acid uptake for this group alone remains high ($r = 0{\cdot}70$).

In all the experiments described in Fig. 1, over 90% of the injected triglyceride fatty acid has been removed from the plasma at 30 min after the injection when the measurements were made (A. Cryer, S. E. Riley, E. R. Williams & D. S. Robinson, unpublished work). This is in agreement with earlier studies showing that, at the dosage employed, the circulating half-life of chylomicron triglycerides in the rat is only a few minutes (Robinson, 1963). It is possible therefore to assess from the data the proportion of the injected triglyceride fatty acid that was present per g of adipose tissue at 30 min after the injection. The results of such calculations show a similar high positive correlation with the clearing factor lipase activity of the tissue. For example, in a single experiment with twenty-eight animals in a variety of nutritional states, the correlation coefficient was 0·92 and the values for percentage uptake and clearing factor lipase activity ranged from $0{\cdot}48 \pm 0{\cdot}13\%$ and 40 ± 5 units/g of epididymal adipose tissue in six rats starved for 24 h to $3{\cdot}2 \pm 0{\cdot}6\%$ and 233 ± 58 units/g of epididymal adipose tissue in eight rats fed *ad libitum*.

Adipose tissue clearing factor lipase activity and plasma insulin concentration

Plasma immunoreactive insulin concentrations were also determined in most of the experiments described in Fig. 1. These, together with values derived from other experiments, identical except that radioactive triglyceride fatty acids were not injected, are plotted in Fig. 2 against epididymal adipose tissue clearing factor lipase activities that were measured in the same animals. The correlation coefficient calculated from these values is 0·73 ($n = 134$, $P < 0{\cdot}0001$; $y = 12{\cdot}97 + 1{\cdot}57x$).

More detailed analysis of the data in Fig. 2 (Table 1) shows, however, that no significant positive correlation exists when the plasma insulin concentrations are either low or high: that is, neither in the 24 h starved animals and the 24 h starved animals given fructose and sucrose nor in the animals fed *ad libitum*. Only when both the plasma insulin concentrations and the adipose tissue enzyme activities are increasing—as in the starved animals given glucose—is the correlation between the two parameters significant ($P < 0{\cdot}001$).

The results in Table 1 also confirm previous findings (Cryer *et al.*, 1974). Thus the adipose tissue clearing factor lipase activity is significantly ($P < 0{\cdot}001$) raised above that in starved animals in the rats given glucose but not in those given fructose or sucrose. The activity in the rats given glucose is, however, still significantly lower ($P < 0{\cdot}001$) than in the rats fed *ad libitum*. Plasma insulin concentrations are also significantly ($P < 0{\cdot}001$) raised above those in starved animals in the rats given glucose, but not in those given sucrose and fructose.

Adipose tissue and muscle clearing factor lipase activity and plasma insulin concentration

The fall in rat adipose tissue clearing factor

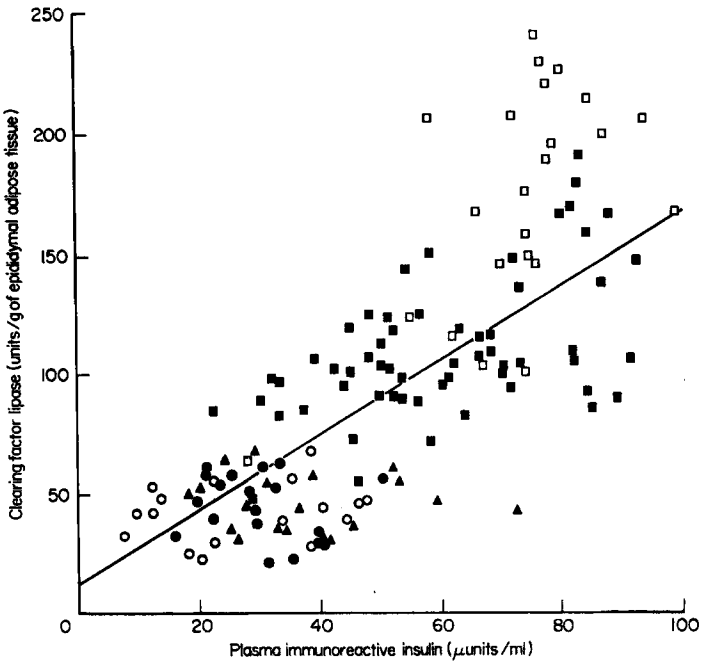

FIG. 2. Adipose tissue clearing factor lipase activities and plasma insulin concentrations in rats in different nutritional states. Epididymal adipose tissue clearing factor lipase activities and the plasma immunoreactive insulin concentrations are shown for rats in the following nutritional states (figures in parentheses are the numbers of animals in each group): □, given their maintenance diet *ad libitum* (22); ○, starved for 24 h (17); ■, starved for 24 h and then given glucose (59); ●, starved for 24 h and then given fructose (18); ▲, starved for 24 h and then given sucrose (18). Details of these conditions are given in the Materials and methods section.

lipase activity that occurs on starvation is accompanied over the first 24 h by a rise in the activity of the enzyme in muscle tissue (see Robinson, 1970). The extent of this inverse correlation has not previously been documented, however. In the present study we therefore also measured the activity of the enzyme in the heart, the thigh sartorius and the diaphragm muscle in rats in the various nutritional states described in the legend to Fig. 1. These are plotted against the adipose tissue enzyme activities measured in the same animals in Fig. 3. In every case, there is a highly significant negative correlation ($P < 0.001$).

The particular clearing factor lipase activities of these three muscular tissues in the different nutritional states studied are shown in Table 2. As

TABLE 1. *Effect of nutritional state on adipose tissue clearing factor activity and plasma insulin concentration*

The plasma insulin concentrations and adipose tissue clearing factor lipase activities are derived from Fig. 2. For each nutritional state, mean values (±SD) are given and the number of observations is shown in parentheses.

Nutritional state of rats	Plasma insulin concn. (μunits/ml) (*a*)	Adipose tissue clearing factor lipase activity (units/g of tissue) (*b*)	Correlation coefficient (*a* v. *b*)
Fed *ad libitum*	69±14 (22)	175±56 (22)	+0·26
Starved 24 h	26±14 (17)	40±10 (17)	+0·10
Starved 24 h, then given glucose	60±12 (59)	110±29 (59)	+0·51
Starved 24 h, then given fructose	29±9 (18)	44±14 (18)	−0·28
Starved 24 h, then given sucrose	36±12 (18)	51±14 (18)	−0·20

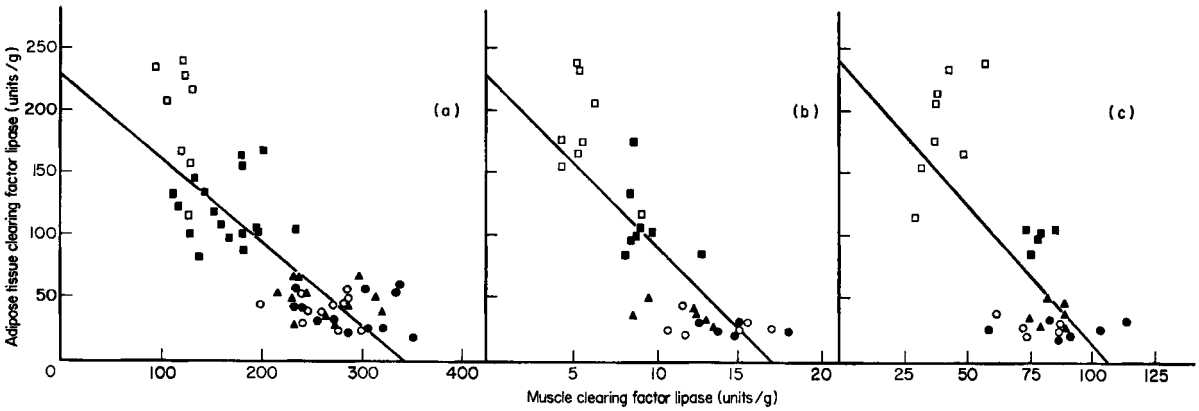

FIG. 3. Relationship between the clearing factor lipase activities of epididymal adipose tissue and (a) heart, (b) thigh and (c) diaphragm muscle in rats in the nutritional states described in the legend to Fig. 1. The correlation coefficients (r), the number of animals in each group (n) and the equations of the regression lines for the different muscle types are: heart: $r = -0{\cdot}79$, $n = 62$, $y = 226 - 0{\cdot}66x$; thigh: $r = -0{\cdot}76$, $n = 32$, $y = 229 - 13{\cdot}9x$; diaphragm: $r = -0{\cdot}75$, $n = 30$, $y = 242 - 2{\cdot}28x$.

expected, the activity of the enzyme in all three muscles is higher in the 24 h starved rats than in the fed rats. In heart muscle, the activity 3 h after the administration of glucose to the 24 h starved animals has declined towards that characteristic of the fed state. There is also some evidence for a decline at this time in the enzyme activity of thigh muscle, though that of diaphragm muscle is unchanged. The clearing factor lipase activity of none of the muscles examined is altered at 3 h after the administration of either sucrose or fructose to 24 h starved rats.

Table 2 also shows the plasma insulin concentrations in the same group of rats. In view of the strong positive correlation between adipose tissue clearing factor lipase activity and plasma insulin concentration (Fig. 2) and the negative correlation between muscle and adipose tissue enzyme activities (Fig. 3), it is not surprising that there is an overall negative correlation between the muscle clearing factor lipase activity and the plasma insulin concentration. The correlation coefficient (r) for heart muscle calculated from the data in Table 2 is 0·67, the equation of the regression line being $y = 308{\cdot}7 - 1{\cdot}86x$; for thigh muscle and diaphragm muscle respectively the values of r are $-0{\cdot}62$ and $-0{\cdot}54$ and the regression line equations are $y = 15{\cdot}53 - 0{\cdot}09x$ and $y = 98{\cdot}7 - 0{\cdot}51x$ (P in all cases is $<0{\cdot}001$).

TABLE 2. *Effect of nutritional state on muscle clearing factor lipase activity and plasma insulin concentration*

The activities of clearing factor lipase in heart, thigh and diaphragm muscle in rats in the nutritional states described in the legend to Fig. 1 are compared with plasma insulin concentrations in the same animals; for each muscle type and nutritional state mean values (± SD) are given and the numbers of observations are shown in parentheses.

Nutritional state of rats	Muscle clearing factor lipase activity (units/g of tissue)			Plasma insulin concn. (μunits/ml)
	Heart	Diaphragm	Thigh	
Fed *ad libitum*	119±13 (8)	41±9 (8)	6±2 (8)	75±12 (8)
Starved 24 h	258±29 (12)	76±11 (6)	13±4 (4)	32±12 (12)
Starved 24 h, then given glucose	156±31 (12)	79±5 (6)	10±2 (6)	69±19 (12)
Starved 24 h, then given fructose	280±39 (12)	89±19 (6)	14±2 (6)	31±10 (12)
Starved 24 h, then given sucrose	261±37 (12)	84±6 (6)	12±2 (6)	39±12 (12)

TABLE 3. *Effect of heparin dosage on plasma post-heparin clearing factor lipase activity in rats in different nutritional states*

Groups of six rats in the nutritional states described in the legend to Fig. 1 were each injected by the femoral vein with either 5 or 200 i.u. of heparin/kg body weight. After 2·5 or 5 min respectively the rats were bled from the abdominal aorta and clearing factor lipase was assayed in the plasma as described in the Materials and methods section.

Nutritional state of rats	Plasma clearing factor lipase activity (units/ml)	
	After 5 units of heparin/kg body wt.	After 200 units of heparin/kg body wt.
Fed *ad libitum*	3·61±0·86	70·4±10·1
Starved 24 h	6·79±1·22	47·0±3·3
Starved 24 h, then given glucose	2·87±0·72	55·3±4·5
Starved 24 h, then given fructose	7·48±1·48	42·9±6·7
Starved 24 h, then given sucrose	5·69±0·86	40·1±6·0

Post-heparin plasma clearing factor lipase activity

We have previously reported that, whereas the amount of clearing factor lipase that appears in the plasma after a high dose of heparin is lower in starved rats than in rats in the fed state, the response to a low heparin dose is higher in the starved animals (Salaman & Robinson, 1961), and these findings are confirmed in Table 3.

Table 3 also shows that the administration of fructose or sucrose to 24 h starved rats neither reduces significantly the response to a low dose of heparin nor raises significantly the response to a high dose of heparin. On the other hand, within 3 h of the administration of glucose to 24 h starved rats the response to a low heparin dose is lowered significantly ($P<0·001$) to a value which is not significantly different from that found in animals in the fed state. The response to a high heparin dose in starved rats given glucose is significantly increased ($P<0·01$), though it does not reach that found in fed animals.

The clearing factor lipase activities in Table 3 have also been related to the plasma insulin concentrations that were measured in the same animals. There is a highly significant positive correlation between these two parameters in the experiments with high doses of heparin ($r = 0·77$, $P<0·001$) and a negative correlation of equal significance in the experiments with low doses of heparin ($r = -0·69$, $P<0·001$).

Discussion

A major purpose of the present work was to study the correlation between the activity of clearing factor lipase in adipose tissue and the uptake of chylomicron triglyceride fatty acids from the plasma by the fat-cell component of the tissue. The results establish the degree of this correlation both for rats in different nutritional states displaying a wide range of adipose tissue clearing factor lipase activities and for rats in a single nutritional state (animals fed *ad libitum*) in which there is nevertheless considerable variation in the tissue enzyme activity. They confirm and extend the findings in previous studies in which only triglyceride fatty acid uptake by the intact tissue was measured (see the Introduction section) and are entirely consistent with the view that the activity of the tissue enzyme is a major factor controlling the uptake of triglyceride fatty acids from the bloodstream.

The present work also provides further evidence that the plasma insulin concentration is an important determinant of the activity of clearing factor lipase in adipose tissue (Fig. 2). However, it is only when both the plasma insulin concentration and the tissue enzyme activity are increasing, as in starved rats given glucose, that the correlation between the two parameters is highly significant (Table 1). This is of particular interest in the light of earlier investigations which have shown that it is the increase in clearing factor lipase activity that occurs when adipose tissue from starved rats is incubated in appropriate media *in vitro* which depends on the presence of insulin in those media (see Robinson & Wing, 1970). Furthermore, the results support the view that insulin is not the only hormone that affects

the activity of the enzyme in adipose tissue *in vivo*. There is now good evidence, for example, that glucagon, glucocorticoids and the catecholamines may each affect the activity of the enzyme in adipose tissue (Robinson & Wing, 1970; Reichl, 1972; De Gasquet & Pequignot, 1973; De Gasquet, Pequignot-Planche, Tonnu & Diaby, 1975). Differences in the plasma concentrations of these hormones could evidently occur in the animals investigated in the present study—either because of the differences in nutritional status or because of variations in the degree of exposure to stressful conditions arising from the use of ether anaesthesia or the sugar administration procedures. These could therefore be responsible for the lack of any significant correlation between the plasma insulin concentration and the adipose tissue enzyme activity when these parameters were either low or high (Table 1).

Changes in plasma glucagon, glucocorticoid and catecholamine concentrations could also have affected the activity of clearing factor lipase in muscle tissue in the present work. Thus there is direct evidence that glucagon and glucocorticoids are each important determinants of the activity of the enzyme in this tissue (Borensztajn, Keig & Rubinstein, 1973; De Gasquet *et al.*, 1975) and indirect evidence that the catecholamines may also be involved in its control (Rogers & Robinson, 1974). Therefore, although the finding that the muscle enzyme activity is inversely correlated with the plasma insulin concentration (Table 2) is consistent with other work (Borensztajn, Samols & Rubinstein, 1972) suggesting that insulin plays a role in the control of the enzyme's activity in this tissue, it also seems likely that, as in adipose tissue, the enzyme activity displayed by the muscles in particular situations depends on the combined effects of a variety of hormonal influences.

The present findings on post-heparin plasma clearing factor lipase activities in rats in different nutritional states cannot be fully assessed at the present time. They do, however, stress the complexity of the factors that are involved in determining the activity of the plasma enzyme after heparin injection. It is evident, in fact, that extreme caution needs to be exercised in attempts to relate such activities, determined at particular times after particular heparin dosages, to the activity of the enzyme in triglyceride fatty acid removal as this is exercised at the endothelial cell surface.

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