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Lipoprotein Lipase Activity in the Tissues of Guinea Pigs Exposed to Different Dietary Fats from Conception to Three Months of Age

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Long-term exposure of guinea pigs to a diet rich in maize oil caused an increase in adipose-tissue lipoprotein lipase activity. A similar diet rich in beef tallow had no such effect, and neither diet affected the enzyme activity in heart, lung, diaphragm or skeletal muscle.

Lipoprotein lipase (EC 3.1.1.3) is the extrahepatic enzyme responsible for the hydrolysis of plasma lipoprotein triacylglycerol, and its activity in tissues is the factor regulating plasma triacylglycerol removal (Robinson, 1970; Brunzell *et al.*, 1973; Harris & Felts, 1973; Kompiang *et al.*, 1976). The total activity of lipoprotein lipase in adipose tissue and a variety of muscle types changes markedly with alterations in nutritional status (Cryer *et al.*, 1976). In adipose tissue (Cryer *et al.*, 1976), cardiac muscle (Rogers & Robinson, 1974) and skeletal muscle (Linder *et al.*, 1976) such total-activity changes have been correlated with changes in the uptake of triacylglycerol fatty acids by the tissues. The total enzyme activity present in tissues also responds to changes in the composition of the diet (Harris & Felts, 1973). However, only a few studies have investigated the effect of high-fat diets or the fatty acid composition of the diet on lipoprotein lipase activity, and these have been exclusively short-term and confined to the rat (Pokrojak & Lassow, 1967; Weisenburg-Delorme & Harris, 1975; Pawar & Tidwell, 1968; Jansen *et al.*, 1975) or man (Bagdade *et al.*, 1970; Chait *et al.*, 1974).

For man, reports are conflicting in that post-heparin lipolytic activity has been reported to be increased (Bagdade *et al.*, 1970) or unchanged (Chait *et al.*, 1974) when diets rich in polyunsaturated fats have been compared with saturated-fat diets under similar conditions. For rats, it has been shown that gastric intubation of corn oil to previously starved animals leads to a transient increase in the total enzyme activity present in adipose tissue (Pokrojak & Lassow, 1967). This activity was also elevated after feeding rats on a high-butterfat diet for 2 weeks (Weisenburg-Delorme & Harris, 1975). However, in the latter set of experiments the only control group used consisted of animals starved for 24 h. When rats fed on diets rich in polyunsaturated fatty acids were

compared directly with others receiving a predominantly saturated-fat diet for 6 weeks, a 50% increase in adipose-tissue lipoprotein lipase activity was observed (Pawar & Tidwell, 1968). There is little information, however, on the comparative effects of such diets on the total activity of the enzyme in other tissues, although their contribution to the overall removal of triacylglycerol from the plasma is probably considerable.

Gurr and co-workers (Kirtland *et al.*, 1976; Pavey *et al.*, 1976; Gurr *et al.*, 1976) have documented the changes in body, plasma and tissue composition occurring at birth and at 3 months of age when guinea pigs were fed on diets containing different amounts and types of fats from mid-gestation onwards. Because of the involvement of unsaturated fatty acids in regulating plasma lipid concentrations (Grundy, 1975), and because the guinea pigs described above provided a well-characterized model, the current studies were undertaken to measure plasma lipids and the total activity of lipoprotein lipase in tissues. Two white-adipose-tissue sites and lung, cardiac, skeletal and diaphragm muscle in guinea pigs exposed to diets rich in maize oil or beef tallow from foetal to adult life were studied.

Materials and Methods

Female guinea pigs (Colworth House strain derived from the Duncan–Hartley strain) were allocated at the start of pregnancy (when they were 12, 14 or 16 weeks of age) to one of the three diets described by Kirtland *et al.* (1976). The diets were a low-fat ‘control’ diet, and high-fat diets containing either beef tallow (‘beef’ diet) or maize oil (‘maize’ diet). The control diet contained 3% by weight of fat and the high-fat diets 20% by weight of fat. The male progeny of the three groups of females were weaned on to the same diet as the mothers when they reached 180 g

body weight (about 2 weeks of age), and continued on the diet until studied at the termination of the experiment at 14–15 weeks of age.

Animals had free access to food and water up to the time of death. At 09:30h they were anaesthetized with cyclopropane, bled by cardiac puncture and then killed with CO₂. Blood was allowed to clot for 1 h at 20°C in glass tubes. Clotted blood was centrifuged at 1500g_{av.} for 7 min. Serum was removed and used immediately to determine triacylglycerol and cholesterol concentrations. Samples of white adipose tissue (epididymal and perirenal), the heart, the lungs, the diaphragm and samples of quadriceps femoris skeletal muscle were removed within 30 min of death, rinsed in 150mM-NaCl, blotted and weighed.

Fresh samples of the tissues were homogenized in 5ml of 2.5% (w/v) casein solution in aq. 50mM-NH₃/HCl buffer, pH 8.1, in a motor-driven glass/Teflon Potter–Elvehjem homogenizer. The homogenizer had an overall clearance of 0.28mm and ten up-and-down strokes were used. The homogenates were dispersed in 150ml of ice-cold acetone and left for 15 min before the precipitated material was filtered off and washed with a further 100ml of acetone at room temperature (20°C), followed by 100ml of diethyl ether. The resultant powders were dried *in vacuo* and stored at –20°C until enzyme assay. No loss of enzyme activity occurred over the periods of storage used. Samples of the powders, homogenized in 50mM-NH₃/HCl buffer, pH 8.1 (100–200 mg of powder/2.5 ml of buffer), were used for the assay of enzyme activity (Riley & Robinson, 1974).

Total lipoprotein lipase activities were determined in tissues as described by Cryer *et al.* (1975), except that recalcified citrated plasma prepared from sheep blood was used to activate the triacylglycerol substrate (Intralipid; Vitrum, Stockholm, Sweden) instead of recalcified citrated plasma prepared from rat blood. Preliminary experiments showed that in the assay of the enzyme in guinea-pig tissue preparations the same activities were obtained with either rat or sheep

recalcified citrated plasma as the source of activator apoprotein for the artificial emulsion substrate. The assay incubations were carried out at 30°C for between 1 and 2 h and the enzyme activity was expressed as μmol of non-esterified fatty acid released/h per g fresh wt. of tissue.

Plasma total cholesterol and triacylglycerols were measured with test kits supplied by Boehringer Corp. (London), London W.5, U.K., and Calbiochem, Bishop's Stortford, Herts., U.K., respectively.

The lipoprotein lipase activity in the separate groups could not be shown to have a normal distribution about the mean, so comparisons between groups for each tissue used the non-parametric Mann–Whitney U-test, appropriate to such circumstances (Siegal, 1965). Unpaired Student's *t* test was used for the comparisons of plasma cholesterol and triacylglycerol concentrations, since the data complied with this test's requirements.

Results and Discussion

Table 1 shows the total activity of lipoprotein lipase in tissues taken from male guinea pigs fed on either the control (low-fat) diet or diets supplemented with either maize oil or beef tallow. The activities of the enzyme per g of tissue in the diaphragm, lung, cardiac muscle and skeletal muscle were not significantly different in any of the dietary groups. That is, the probabilities derived from the statistical test did not indicate that any of the dietary groups made up an independent population distinct from the total population studied. By contrast, the activity of the enzyme per g of tissue in both perirenal and epididymal adipose tissue of the animals fed on the maize-oil-supplemented diet was significantly higher than in the adipose tissue of the other two groups ($P < 0.01$ and $P < 0.001$ respectively). In the perirenal adipose tissue of animals fed on the 'maize' diet the activity of the enzyme was $505 \pm 308\%$ (13) higher ($P < 0.001$) than the controls and for the epididymal adipose tissue it was $245 \pm 95\%$ higher ($P < 0.01$) than controls.

Table 1. Activities of lipoprotein lipase in tissues of guinea pigs fed on a normal diet (control), a diet supplemented with beef tallow ('beef') or a diet supplemented with maize oil ('maize')

Lipoprotein lipase was measured in acetone/ether-dried powder preparations of tissues taken from fed animals in the three dietary groups, as described in the text. Activities (μmol of non-esterified fatty acid/h per g fresh wt. of tissue) are expressed as means $\pm$ S.D., with the numbers of independent observations in parentheses.

Tissue	Lipoprotein lipase activity		
	Control	'Beef'	'Maize'
Perirenal adipose tissue	5.7 $\pm$ 3.2 (13)	8.8 $\pm$ 5.7 (12)	29.3 $\pm$ 30.9 (13)
Epididymal adipose tissue	6.5 $\pm$ 4.4 (13)	6.9 $\pm$ 6.8 (12)	16.9 $\pm$ 13.9 (13)
Diaphragm	21.1 $\pm$ 6.9 (4)	25.0 $\pm$ 4.8 (4)	32.7 $\pm$ 8.2 (4)
Lung	10.0 $\pm$ 6.2 (4)	5.9 $\pm$ 5.9 (4)	7.4 $\pm$ 4.1 (4)
Cardiac muscle	63.0 $\pm$ 15.0 (4)	71.9 $\pm$ 25.4 (4)	82.2 $\pm$ 27.1 (4)
Skeletal muscle	5.5 $\pm$ 4.5 (4)	3.5 $\pm$ 2.1 (4)	5.4 $\pm$ 2.6 (4)

Table 2. *Effect of diet on the plasma concentrations of triacylglycerol and cholesterol in fed guinea pigs*

The treatment of the dietary groups was as described in the text and the legend to Table 1. Results are means $\pm$ s.d. for *n* determinations.

Dietary group	Triacylglycerol (mM)	Cholesterol (mM)
Control (<i>n</i> =10)	1.26 $\pm$ 0.31	0.96 $\pm$ 0.18
'Beef' (<i>n</i> =9)	1.67 $\pm$ 0.56	1.78 $\pm$ 0.41
'Maize' (<i>n</i> =9)	1.20 $\pm$ 0.24	0.98 $\pm$ 0.34

The total activity of the enzyme in samples from both adipose-tissue sites of the animals fed on the 'beef' diet were not significantly different from the control values, but were significantly lower than those measured in tissue from the animals fed on the 'maize' diet (perirenal, $P < 0.001$; epididymal, $P < 0.01$).

Table 2 shows the effect of the long-term feeding of the experimental diets on plasma triacylglycerol and cholesterol concentrations. These were not significantly different in the control group and the groups on the 'maize' diet. In the group on the 'beef' diet, however, both triacylglycerol and cholesterol concentrations were elevated above those observed in the plasma of control animals ($P < 0.01$). The triacylglycerol concentrations in the group on the 'beef' diet were significantly higher than in either of the other two groups ($P < 0.01$).

The results of this study indicate that the long-term intake of a diet high in saturated fat by guinea pigs has no effect on the lipoprotein lipase activity of the tissues, but causes an increase in plasma cholesterol and triacylglycerol concentrations compared with those in the animals fed on the low-fat diet. By contrast, however, feeding a diet high in polyunsaturated fatty acids resulted in an elevation of the total enzyme activity in adipose tissue, with no change in the total activity in other tissues and no alteration in plasma lipid concentrations. This work extends the previous observations that exposure of rats to such diets increased the total activity of the enzyme in adipose tissue (Pawar & Tidwell, 1968). Whether the increased lipoprotein lipase present in the adipose tissue of the animals fed on polyunsaturated fat was a major factor contributing to the lower triacylglycerol and cholesterol concentrations in the plasma cannot be definitively ascertained from the experiments performed here. But, as discussed by Robinson (1970) and Cryer *et al.* (1976), an increased concentration of lipoprotein lipase present in a tissue usually correlates with an increased uptake of triacylglycerol fatty acids by that tissue. In the present experiments, however, this did not lead to an increase in the size of the adipose-tissue depots studied. For example, the epididymal and perirenal fat-body weights as percentages of total

body weight for the 'maize'-fed group were 0.53 ± 0.1 (9) and 0.51 ± 0.14 (9) respectively compared with the equivalent values of 0.62 ± 0.17 (9) and 0.52 ± 0.18 (9) calculated for the 'beef'-fed animals (the values are means $\pm$ s.d., with the numbers of observations in parentheses).

The increases in adipose-tissue lipoprotein lipase activity caused by the diet rich in polyunsaturated fatty acids are not easy to reconcile with the type of change in plasma insulin concentrations considered important in the regulation of the enzyme activity (Cryer *et al.*, 1976). Thus the effects of plasma lipids and particularly of triacylglycerols on, for example, insulin release or the insulin-sensitivity of fat-cells is under debate (Nilsson-Ehle *et al.*, 1975; Carroll & Nestel, 1972; Marks & Samols, 1970), and, although short-term feeding of saturated fat has been reported to decrease the sensitivity of adipose tissue to insulin and other hormones (Smith *et al.*, 1974) and to decrease the binding of insulin to adipocytes (Ip *et al.*, 1976), longer-term exposure (Cushman & Salans, 1975) resulted in no apparent alterations. Thus the effects on the enzyme's activity caused by feeding polyunsaturated fat cannot as yet be correlated with any change mediated by hormones or with changes in cell size, since this has previously been shown to be unchanged (Kirtland *et al.*, 1976).

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