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Lipoprotein Lipase Activities in Adipose Tissues, Heart and Diaphragm of the Genetically Obese Mouse (*ob/ob*)

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Obesity occurs in genetically obese (*ob/ob*) mice as a consequence of spontaneous overeating. An intense hepatic lipogenesis (Jansen *et al.*, 1967) allows the excess of caloric intake to be converted into fatty acids, which are conveyed to the fat depots as triglycerides. Fat storage in this tissue is believed to depend on the activity of the enzyme lipoprotein lipase (clearing-factor lipase) (Bragdon & Gordon, 1958; Bezman *et al.*, 1962; Garfinkel *et al.*, 1967; Austin & Nestel, 1968). In non-obese animals the activity of this enzyme in adipose tissue is nutritionally regulated, being high in the fed state and low in starvation (Cherkes & Gordon, 1959; Hollenberg, 1959). Administration of glucose *in vivo* (Garfinkel *et al.*, 1967) or the addition of glucose plus insulin to incubated tissue preparations *in vitro* (Hollenberg, 1959; Salaman & Robinson, 1966; Austin & Nestel, 1968; Patten, 1970) is necessary to induce or to maintain a high lipoprotein lipase activity in adipose tissue. By contrast, heart and diaphragm lipoprotein lipase activities are low in the fed state and high in starvation (Hollenberg, 1960; Borensztajn *et al.*, 1970), and the administration of glucose *in vivo* to starved animals decreases the heart enzyme activity (Borensztajn *et al.*, 1970).

Since glucose and insulin appear to play a major role in regulating the lipoprotein lipase activity of extrahepatic tissues, it seemed worthwhile to determine the lipoprotein lipase activities in the tissues of genetically obese hyperglycaemic (Bleish *et al.*, 1952) and hyperinsulinaemic (Mayer *et al.*, 1953) mice in the fed state.

Experimental

Male and female obese mice (*ob/ob*) and their lean littermates, obtained from La Source (45–Orléans, France), were fed on laboratory biscuits *ad libitum* and killed when 3–5 months old, between 9a.m. and 11a.m. Perigenital and perirenal fat tissue, heart and diaphragm were excised, rapidly washed in 0.9% NaCl, blotted on filter paper, weighed and kept at −20°C. There was no loss of lipoprotein lipase activity after 8 days of storage at this temperature. Portions (200–500mg) of adipose tissue were homogenized (Ultra-Turrax) in 8ml of 0.25M-sucrose, pH8.1. Whole heart and whole diaphragm were homogenized in 8ml of 25mM-NH_3–NH_4Cl buffer, pH8.1. Both homogenizing media contained heparin (2i.u./ml).

An emulsion was prepared as follows: to a mixture of 1.2vol. of 10% Intra-lipid (Laboratoire Vitrum, 94-Vitry, France) and 4.8vol. of rat serum, previously incubated together for 30min at 37°C, were added 10.5vol. of aq. 18% (w/v) bovine serum albumin, pH8.1, and 3.5vol. of 1.6M-tris–HCl buffer, pH8.1. Four assay tubes per tissue preparation were prepared by mixing 0.4ml of homogenate and 0.4ml of the above emulsion and they were then placed in a metabolic shaker at 37°C. The two pairs of tubes were removed 5min and 65min later, and the amount of free fatty acid in the whole content of each tube was determined (Dole, 1956). The difference, corrected for blank values (without homogenate), was taken as a measure of lipolysis. The reaction proceeded linearly during the incubation period and there was proportionality between lipolysis and the amount of added homogenate.

Most of the lipolytic activity thus measured was assessed to be lipoprotein lipase activity since it was inhibited by 94–100% by 0.5M-NaCl and by 91–100% when serum was omitted; it was 1.5–3-fold higher when heparin (1i.u./ml) was present. There was only a slight inhibition (3–20%) with 10mM-NaF. These values were obtained in a series of paired assays performed with acetone-dried-powder homogenates and the corresponding fresh-tissue homogenates. Quite similar results were found, in a given tissue, with both tissue preparations and when results for homologous tissues from obese mice and from lean mice were compared. Thus in a typical experiment the percentages of inhibition found with fresh-tissue homogenate and defatted-tissue homogenate of pooled epididymal fat-pads from four lean mice and with fresh-tissue homogenate and defatted-tissue homogenate of pooled epididymal fat-pads from two obese mice were respectively 98.1, 95.5, 97.5 and 99.0% when 0.5M-NaCl was present, and 99.8, 100, 91.2 and 93.7% when serum was omitted.

Results and discussion

In both male and female mice the lipoprotein lipase activities of adipose tissue increased with the weight of the fat tissue (Table 1) and, as a consequence, the activities per g (not shown in Table 1) were quite

Table 1. *Body weights, tissue weights and lipoprotein lipase activities in homogenates of tissues of lean and genetically obese mice*

The enzyme activities are expressed as units (μmol of free fatty acid released/h at 37°C) per whole tissue or per g fresh wt., and are means ± S.D. of the numbers of observations given in parentheses. Values that are statistically significantly different from control (lean mice) values are indicated by: *$P<0.01$; †$P<0.05$.

	Males						Females					
	Lean			Obese			Lean			Obese		
Body wt. (g)	36.7	±1.3	(12)	67.4	± 2.6	(12)*	30.5	± 1.0	(13)	73.8	± 2.2	(12)*
Perigenital adipose tissue												
Tissue wt. (g)	1.2	±0.13	(12)	5.46	± 0.28	(12)*	1.04	± 0.14	(12)	9.32	± 0.77	(12)*
Lipoprotein lipase (units/tissue)	43.3	±6.7	(12)	190.5	±17.1	(12)*	47.7	± 8.0	(12)	448.1	±51.6	(12)*
Perirenal adipose tissue												
Tissue wt. (g)	0.409	±0.040	(12)	3.63	± 0.18	(12)*	0.304	± 0.055	(13)	3.37	± 0.27	(12)*
Lipoprotein lipase (units/tissue)	17.9	±1.7	(12)	146.8	±11.3	(12)*	13.8	± 2.6	(13)	149.8	±16.8	(12)*
Heart												
Tissue wt. (mg)	155.4	±3.6	(12)	170.4	± 3.2	(12)	130.8	± 4.1	(13)	152.5	± 4.4	(12)
Lipoprotein lipase (units/g)	260.4	±7.6	(12)	304.4	±12.3	(12)†	306.3	±11.0	(13)	366.2	±15.1	(12)*
Lipoprotein lipase (units/tissue)	42.1	±1.6	(12)	52.0	± 2.7	(12)*	39.7	± 1.2	(13)	55.8	± 2.6	(12)*
Diaphragm												
Tissue wt. (mg)	97.3	±3.1	(11)	104.2	± 3.5	(12)	82.7	± 3.7	(13)	96.7	± 2.7	(12)*
Lipoprotein lipase (units/g)	163.6	±6.0	(11)	195.8	± 7.8	(12)†	159.5	± 8.3	(13)	227.5	±11.9	(12)*
Lipoprotein lipase (units/tissue)	15.9	±0.7	(11)	20.4	± 1.0	(12)*	13.1	± 0.8	(13)	21.8	± 1.0	(12)*

similar regardless of the extent of the obesity or the localization of the adipose tissue. Clearly the significance of the findings depends on the extent of hyperplasia in the fat tissue of the obese mice. In the epididymal fat-pad of genetically obese mice no increase of the number of adipocytes has been found (Hellman *et al.*, 1962; Lemonnier *et al.*, 1971). Thus the 5-fold increase in lipoprotein lipase activity of this tissue reported in Table 1 corresponds to a 5-fold increase of activity per cell. A definite hyperplasia, along with cell hypertrophy, occurs in the parametrial fat tissue of the genetically obese rat (*fa/fa*) (Lemonnier, 1971) and in that of the Swiss mouse made obese with a high-fat diet (Lemonnier, 1970). In these two instances the fat tissue from the obese animals contained respectively 2.3 and 2.5 times as many adipocytes as did the tissue from lean animals. Recently P. R. Johnson & J. Hirsch (unpublished work) have shown hyperplasia of the same magnitude in perigenital adipose tissue of the female and in perirenal adipose tissue of both sexes, in 6-month-old genetically obese hyperglycaemic mice. Such reported hyperplasia cannot entirely account for the 10-fold increase of lipoprotein lipase activity shown for these tissues in Table 1, however. Hence the lipoprotein lipase activity per adipocyte is likely to be several-fold higher in obese mice than in lean controls.

The heart and diaphragm enzyme activities (Table 1) were also higher in obese mice, ranging from 123% (in the heart of the males) to 166% (in the diaphragm of the females) of the lean mice values (units/tissue). However, although statistically significant, these increases were far below those observed in adipose tissue.

The enhanced activity of the enzyme lipoprotein lipase in the tissues of genetically obese hyperglycaemic mice established in this study awaits further investigation before being given an interpretation. At least three questions may be raised. First, to what extent do other genetically or nutritionally obese animals show a high lipoprotein lipase activity per adipocyte? Secondly, are the enzyme activities lowered to normal values when the obese mice are pair-fed with their lean littermates? Thirdly, does starvation enhance further the enzyme activity in the heart of the obese mice and depress the enzyme activity to the same low value in adipose tissue of both obese and lean mice?

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