Short Communications

Adipose-Tissue Lipoprotein Lipase Activity and Cellularity in the Genetically Obese Zucker Rat (*fa/fa*)

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The lipoprotein lipase activity per adipocyte was increased in the genetically obese rat (*fa/fa*). However, there was no difference between obese and lean animals when the enzyme activities were related to adipocyte surface area. The possible implications of the findings are discussed.

Uptake of lipoprotein triglyceride fatty acids into adipose tissue of normal animals is believed to depend on the activity of the enzyme lipoprotein lipase (clearing-factor lipase) (Bragdon & Gordon, 1958; Bezman *et al.*, 1962; Pokrajac & Lossow, 1967; Garfinkel *et al.*, 1967; Austin & Nestel, 1968). Since obesity is characterized by an increase of the fat stores, one might expect that lipoprotein lipase activity in adipose tissue would be higher in obese than in lean animals. In fact the activity of the enzyme has been found to be increased in the adipose tissue of the obese hyperglycaemic (*ob/ob*) mouse (de Gasquet & Péquignot, 1972; Enser, 1972).

Enlargement of the fat depots is the consequence of an increase in adipocyte size or number or both. Moreover the extent of the increase varies with the age and sex of animals, adipose-tissue localization and diet (Hirsch & Han, 1969; Knittle & Hirsch, 1968; Johnson *et al.*, 1971; Johnson & Hirsch, 1972; Lemonnier, 1971*a*, 1972). Therefore variations of enzyme activity in a particular fat depot might only reflect variations in the cellularity. Accordingly, the activity of lipoprotein lipase and cellularity were determined simultaneously in two adipose-tissue depots of male and female 1-year-old genetically obese Zucker rats (*fa/fa*). Lean litter mates were used as controls.

Experimental

Male and female genetically obese Zucker rats (*fa/fa*) and their lean litter mates (*Fa/-*) were bred in our laboratory from heterozygous (*Fa/fa*) parents kindly supplied by Dr. L. M. Zucker, Harriet G. Bird

Memorial Laboratory, Stow, Mass., U.S.A. They were fed *ad libitum* on a low-fat diet consisting of (w/w): casein, 12.2%; DL-methionine, 0.4%; lard, 1.1%; wheat flour, 77%; bran, 3%; salt mixture, 4%; vitamin mixture, 2.2%. When 12 months old the animals were killed by decapitation, between 9 a.m. and 11 a.m. Portions of the perigenital and perirenal fat-fads were homogenized in 0.25M-sucrose, pH 8.1, containing heparin (2 i.u./ml). The activity of lipoprotein lipase was measured in the fresh-tissue homogenates as described by de Gasquet & Péquignot (1972). Homogenates were incubated at 37°C for 1 h with an equal volume (0.4 ml) of an emulsion consisting of 1.2 vol. of 10% Intralipide (Laboratoire Vitrum, 94100 Vitry, France) and 4.8 vol. of normal rat serum previously incubated together for 30 min at 37°C, 10.5 vol. of 18% (w/v) bovine serum albumin, pH 8.1 (fraction V; Miles Laboratories Inc., Kankakee, Ill., U.S.A.), and 3.5 vol. of 1.6M-Tris-HCl buffer, pH 8.1. The amount of free fatty acids released in the incubation medium was determined by titration (Dole, 1956). Control experiments performed either in the presence of 0.5M-NaCl or by omitting the added serum were conducted with fresh-tissue homogenates of pooled perigenital and perirenal fat-pads from three lean and three obese rats of either sex. The percentage inhibition in the presence of 0.5M-NaCl was 95–100% except for the parametrial adipose tissue from lean animals (81%). In the absence of serum the percentage inhibition was 92–100% except for the parametrial adipose tissue from lean animals (86%). Most of the lipolytic activity of the adipose-tissue homogenates was therefore attributed to the lipoprotein lipase enzyme.

Adipocyte volume and number were determined in fragments of fat-pads by a histological technique (Lemonnier, 1970, 1972). Dissection of the perirenal fat-pads was as described by Lemonnier (1972).

Results and discussion

In obese animals of both sexes the perigenital adipose tissue was 2.4 times the mass of that in lean animals (Table 1). Adipocyte hypertrophy alone accounted for this increase, as the numbers of adipocytes were similar in the four groups of rats under study. In younger obese Zucker rats the number of adipocytes in the epididymal adipose tissue was lower (Johnson *et al.*, 1971; Lemonnier, 1971a), whereas it was higher in the parametrial adipose tissue, when compared with their respective controls (Lemonnier, 1971a). However, when the animals grew older, the differences between obese and lean animals became much smaller. In epididymal adipose tissue from

6-month-old obese and lean animals, Johnson *et al.* (1971) found between 5×10^6 and 7×10^6 adipocytes (per one fat-pad), values that are close to the values (per two fat-pads) reported in Table 1 for the 1-year-old rats.

The enlargement of the perirenal adipose tissue in obese animals was the result of cell hypertrophy of the same magnitude as in the perigenital adipose tissue, but in addition there was hyperplasia (Table 1). Hyperplasia was also found by Johnson *et al.* (1971) in the retroperitoneal adipose tissue from 6-month-old obese male rats.

The lipoprotein lipase activity per whole adipose tissue was approx. 2–8-fold higher in obese than in lean animals, depending on the adipose-tissue site and the sex of the animals. When expressed in terms of adipocyte numbers, the lipoprotein lipase activity in both types of adipose tissue was 1.5–2-fold higher in obese rats. The 'basal' value of the enzyme activity in the lean rats (30.3–38.1 enzyme units/ 10^6 adipo-

Table 1. *Perigenital and perirenal adipose-tissue lipoprotein lipase activities and cellularity in genetically obese Zucker rats and their lean litter mates*

Lipoprotein lipase activity was measured in fresh-tissue homogenates and is expressed as units (μmol of free fatty acid released/h at 37°C). Each value represents the mean $\pm$ S.E.M. Values that are statistically significantly different from control (lean rats) values are indicated by: * $P < 0.01$; † $P < 0.05$.

	Males		Females	
	Lean	Obese	Lean	Obese
No. of animals ...	7	6	8	7
Body wt. (g) ...	534 $\pm$ 13	671 $\pm$ 36*	288 $\pm$ 11	596 $\pm$ 17*
Perigenital adipose-tissue wt. and cellularity				
Tissue wt. of two fat-pads (g)	7.62 $\pm$ 0.35	18.5 $\pm$ 2.0*	7.50 $\pm$ 0.56	18.1 $\pm$ 1.5*
Adipocyte number (millions)	11.9 $\pm$ 0.8	12.9 $\pm$ 2.2	10.0 $\pm$ 0.8	11.2 $\pm$ 1.3
Volume of 10^6 adipocytes (mm^3)	722 $\pm$ 76	1674 $\pm$ 140*	853 $\pm$ 101	1805 $\pm$ 102*
Surface area of 10^3 adipocytes (mm^2)	38.7 $\pm$ 2.7	67.7 $\pm$ 3.8*	42.6 $\pm$ 3.5	71.8 $\pm$ 2.7*
Perigenital adipose-tissue lipoprotein lipase activity:				
units/g	50.7 $\pm$ 4.5	39.6 $\pm$ 4.8	47.0 $\pm$ 6.4	33.5 $\pm$ 1.9†
units/two fat-pads	378 $\pm$ 31	690 $\pm$ 36*	354 $\pm$ 56	595 $\pm$ 29*
units/ 10^6 adipocytes	33.2 $\pm$ 4.2	62.6 $\pm$ 11.2*	38.1 $\pm$ 7.2	55.9 $\pm$ 4.9
munits/ cm^2 of adipocyte surface area	85.2 $\pm$ 7.8	90.1 $\pm$ 12.6	85.9 $\pm$ 12.7	77.3 $\pm$ 5.0
Perirenal adipose-tissue wt. and cellularity:				
Tissue wt. of two fat-pads (g)	7.87 $\pm$ 0.73	49.3 $\pm$ 4.4*	4.32 $\pm$ 0.40	46.7 $\pm$ 4.5*
Adipocyte number (millions)	12.4 $\pm$ 1.5	33.6 $\pm$ 4.3*	5.43 $\pm$ 0.50	25.1 $\pm$ 2.6*
Volume of 10^6 adipocytes (mm^3)	708 $\pm$ 52	1631 $\pm$ 97*	871 $\pm$ 103	2044 $\pm$ 86*
Surface area of 10^3 adipocytes (mm^2)	38.1 $\pm$ 1.9	66.9 $\pm$ 2.7*	43.8 $\pm$ 3.3	77.6 $\pm$ 2.2*
Perirenal adipose-tissue lipoprotein lipase activity:				
units/g	45.8 $\pm$ 5.4	41.8 $\pm$ 5.6	42.6 $\pm$ 6.2	30.9 $\pm$ 3.5
units/two fat-pads	354 $\pm$ 47	1995 $\pm$ 205*	178 $\pm$ 24	1439 $\pm$ 210*
units/ 10^6 adipocytes	30.3 $\pm$ 4.7	60.8 $\pm$ 4.8*	33.4 $\pm$ 6.1	59.0 $\pm$ 7.7*
munits/ cm^2 of adipocyte surface area	77.8 $\pm$ 9.8	91.8 $\pm$ 9.0	75.0 $\pm$ 12.9	75.1 $\pm$ 8.9

cytes), as well as the high enzyme activity in the obese rats (55.9–62.6 enzyme units/ 10^6 adipocytes), were each nearly constant, suggesting that the increase of the lipoprotein lipase activity per cell observed in the obese rats does not depend on the sex or the location of the adipose tissue. When expressed per cm^2 of adipocyte surface area, the lipoprotein lipase activity was similar in both sexes, in both types of tissue and in both lean and obese animals.

The findings are contrasted with those reported by Nestel *et al.* (1969), who found in normal growing non-obese rats a decrease of the lipoprotein lipase activity per cell in the more massive epididymal adipose tissue. However, their study differed from the present one with respect to at least two factors: genetic obesity and the age of the animals. Accordingly one cannot be certain whether the difference in lipoprotein lipase activity per cell between 1-year-old lean and obese rats (Table 1) would be seen in young animals under the critical age of about 15 weeks (Hirsch & Han, 1969; Johnson *et al.*, 1971; Lemonnier, 1972). Conversely one does not know either whether the lipoprotein lipase activity per cell decreases further in adult non-obese animals when the epididymal adipose tissue is growing only by cell enlargement.

The possibility of an increase in activity of lipoprotein lipase per adipocyte in genetically obese hyperglycaemic mice (*ob/ob*) was raised by de Gasquet & Péquignot (1972), as the enzyme activity per whole adipose tissue was increased in obese animals above the expected increase due to adipocyte numbers (Lemonnier *et al.*, 1971; Johnson & Hirsch, 1972). From the results presented in Table 1 the same conclusion can now be drawn with regard to the genetic obesity (*fa/fa*) of the Zucker rat.

The increase in the lipoprotein lipase activity per adipocyte in genetically obese mice and rats is probably not the direct expression of the genetic abnormalities conveyed by either the gene *ob* or *fa*, for the following reason: in normal Swiss mice made obese with a high-fat diet and compared with lean mice fed on a control low-fed diet, the activity of lipoprotein lipase per adipocyte in epididymal and parametrial adipose tissue is also increased (P. de Gasquet, E. Péquignot & D. Lemonnier, unpublished work). Therefore the increase in lipoprotein lipase activity per adipocyte appears to be a common feature of both genetic and nutritionally induced obesities.

In adipose tissue from normal fed rats a large part of the lipoprotein lipase appears to be located outside the adipocyte (Cunningham & Robinson, 1968). Consequently an increased lipoprotein lipase activity per adipocyte in obese animals would imply an increased capacity of the adipocyte to synthesize and transport the enzyme outside the cell, provided that

the half-life of the enzyme as well as its distribution within and outside the cell were similar in obese and lean animals. In the case of the genetically obese mice (*ob/ob*), Enser (1972) suggested that the latter condition was not fulfilled.

Insulin is believed to promote the synthesis of lipoprotein lipase in adipose tissue (Hollenberg, 1959; Wenkeová *et al.*, 1972; Salaman & Robinson, 1966). The 1-year-old obese animals used in the present study suffered from hyperinsulinism (G. Rosselin, unpublished work), as found by Lemonnier (1971b). In this context it is noteworthy that the activities of lipoprotein lipase per unit of adipocyte area were similar in obese and lean animals regardless of the size of the adipocyte (Table 1). Therefore the findings suggest that large adipocytes from obese animals respond to high concentrations of insulin in the circulation in a manner similar to adipocytes from control animals to normal concentrations of the hormone.

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