Lipoprotein Lipase Activity at Onset of Development of White Adipose Tissue in Newborn Rats

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The low triacylglycerol concentration in inguinal tissue of newborn rats did not change during the first 6 h after birth, despite the relatively high lipoprotein lipase activity in the tissue. Subsequently triacylglycerol concentration and enzyme activity rose in parallel. The results show that lipoprotein lipase activity was present in the tissue before fat accumulation.

Morphological studies on human and on animal foetuses and newborns (see reviews by Wassermann, 1965; Napolitano, 1965; Desnoyers, 1973) have shown that the primitive cells, which eventually differentiate to form adipose tissue, contain small fat-vacuoles. These cells are arranged in lobes and are irrigated by capillary networks. Wassermann (1926) termed this structure the 'primitive fat organ'. Differentiation of the primitive cells occurs concomitantly with lipid deposition. The resulting mature adipocytes, which are characterized by a single large lipid vacuole, first appear at times that vary with the tissue location and species. The onset of adipose-tissue development in rats and mice takes place after birth.

In weaned rats, the lipids that are stored in adipocytes are essentially triacylglycerols. Their fatty acid moieties are supplied mainly either by synthesis *de novo* in the fat-cell, or by transfer of plasma triacylglycerol fatty acids made available to the adipocytes after hydrolysis by lipoprotein lipase (clearing-factor lipase) (Robinson, 1970). Maternal milk has a high fat content, and high-fat diets are known to inhibit lipogenesis in adipose tissue (Hausberger & Milstein, 1955). It is most likely therefore that fatty acid synthesis *de novo* is minimal in the adipose tissue of newborns (Hahn & Novak, 1975). However, as first suggested by Wassermann & MacDonald (1963), it is possible that the newly deposited lipid in newborn mice is of chylomicron origin, which in turn would be indicative of lipoprotein lipase activity in the primitive fat-organ.

The present study was undertaken to see if a lipoprotein lipase activity could be detected in the inguinal region of newborn rats during the early development of adipose tissue at this site.

Experimental

Wistar rats (E.V.I.C.-C.E.B.A., 33290 Blanquefort, France) were bred in an animal room at 23 ±

1°C with lighting from 07:30 to 19:30 h. The females were caged with males until mating occurred. Each morning vaginal smears were taken and examined. The day spermatozoa were found in a smear was considered to be the first day of pregnancy. On day 23, when birth usually occurred, the pregnant rats were kept under constant observation until delivery. The time of birth was noted and two or three pups were taken from the new litter either at birth (zero time) or at 6, 12, 18 or 24 h thereafter; they were weighed and then killed by decapitation. The body weights (g) at 0, 6, 12, 18 and 24 h were respectively (means ± S.E.M., with numbers of pups in parentheses): 5.49 ± 0.09 (23), 5.56 ± 0.08 (23), 6.05 ± 0.12 (19), 6.08 ± 0.07 (18) and 6.25 ± 0.13 (18). Pups at zero time were taken away from their mothers before suckling commenced. All other pups were nursed normally and their stomachs always contained milk.

The identification and dissection of the developing inguinal pad were carried out as follows. In preliminary experiments, it had been noticed that the inguinal adipose pad did not become macroscopically visible before 12 h after birth. Before this, a gelatinous material irrigated by numerous small vessels, which were plainly visible under a magnifying glass, was observed in the subcutaneous inguinal region. At 12 h the inguinal pad appeared as a thin brown line approx. 1 mm wide and 6 mm long, lying in the gluteal fold. At 18 and 24 h, a well-defined white adipose pad was present. When visible, i.e. from 12 to 24 h, the inguinal pad was carefully dissected out under a magnifying glass. The gelatinous material of the inguinal region was also sampled at 0 and 6 h. The left-hand pads from two or three pups were pooled for assay of lipoprotein lipase, and the corresponding right-hand pads were used for triacylglycerol determinations. The individual wet weights (mg) of samples taken for enzyme assay at 0, 6, 12, 18 and 24 h were respectively (means ± S.E.M., with numbers of tissues in parentheses):

15.6 ± 2.1 (23), 16.0 ± 1.0 (23), 23.9 ± 1.9 (18), 25.8 ± 1.6 (18) and 26.3 ± 1.9 (18).

Tissue triacylglycerols were extracted in chloroform/methanol (2:1, v/v) by the method of Folch *et al.* (1957). After evaporation of the solvent phase and saponification of the residue, glyceride glycerol was measured by an enzymic assay (Eggstein and Kreutz, 1966) with reagents supplied by Boehringer, Mannheim, Germany. The triacylglycerol content of the tissues was expressed as mg of triolein/g wet wt.

Lipoprotein lipase activity was determined by using defatted preparations of the tissues by the method of Wing & Robinson (1968). The acetone/diethyl ether-dried powders were homogenized in 2.5 ml of 50 mM-NH₄Cl, adjusted to pH 8.1 with dilute NH₃, containing heparin (4 i.u./ml). Portions (0.4 ml) of the homogenate were mixed with an equal volume of an emulsion containing triacylglycerol substrate (Intralipid; Vitrium, 94100 Vitry, France), activated by rat serum, as previously described (de Gasquet & Péquignot, 1972). The unesterified fatty acids that were released on incubation at 37°C for 60 min were extracted by the method of Dole (1956) and were measured by titration (Kaplan, 1970). The enzyme activity was expressed as μmol of fatty acid released/h per g wet wt. of tissue. The significance of the results was not altered when the parameters were expressed per whole tissue (one fat-pad).

The probability of the difference between means was tested by using Student's *t* test.

Results

The changes in triacylglycerol content of the inguinal pad during the first 24 h after birth are shown in Fig. 1(a). At birth, the triacylglycerol content at this site was 3.57 ± 0.57 mg/g (mean $\pm$ S.E.M. of nine determinations), significantly higher ($P < 0.05$), than that found in the thigh muscle (1.49 ± 0.20 mg/g, mean $\pm$ S.E.M. of three determinations) or in the serum (62.0 ± 1.3 mg/100 ml, mean $\pm$ S.E.M. of three determinations on pooled sera from three groups of three pups). The results indicate a small, but definite, accumulation of lipids at this site, suggesting that the primitive fat-organ was included in the tissue sample. The low triacylglycerol content of the inguinal pad remained unchanged during the first 6 h after birth. After this, it increased abruptly.

The lipoprotein lipase activities are shown in Fig. 1(b). At birth, the enzyme activity (mean $\pm$ S.E.M. of nine determinations) was 58.6 ± 11.5 μmol of fatty acid/h per g wet wt. of tissue. To characterize the lipolytic activity in the newborn, inhibition studies were performed, in a separate experiment, on three preparations of pooled tissues each from three pups. The activities were inhibited by 95–100% in the

Fig. 1. Changes in (a) triacylglycerol content and (b) lipoprotein lipase activity in the developing inguinal fat-pad of the newborn rat

The left-hand pads from two or three pups were pooled for assay of lipoprotein lipase, and the corresponding right-hand pads were used for triacylglycerol determinations. Values shown are means ($\pm$ S.E.M., indicated by the bars) of nine independent determinations. Statistically significant differences between successive groups are indicated by * ($P < 0.05$) and ** ($P < 0.01$).

presence of 0.5 M-NaCl, and by 91–100% when serum was omitted from the assay medium. This indicates that the enzyme activity was due to lipoprotein lipase. The activity at birth is relatively high, when compared with that measured either at birth, in brown interscapular adipose tissue (166.5 ± 20.6 μmol /h per g, mean $\pm$ S.E.M. of nine determinations) and the thigh muscle (23.9 ± 2.4 μmol /h per g, mean $\pm$ S.E.M. of 11 determinations), or at the age of 2 months in subcutaneous inguinal adipose tissue of fed rats (39.8 ± 3.5 μmol /h per g, mean $\pm$ S.E.M. of six determinations). Between 0 and 6 h lipoprotein lipase activity decreased significantly. Subsequently it increased in parallel with tissue triacylglycerol content.

Discussion

The lipoprotein lipase activity in adipose tissue of young adult rats is known to vary with nutritional

status, being low during deprivation of food, and rising on re-feeding (Robinson, 1970). This nutritional effect is insulin-mediated (Hollenberg, 1959; Wenkeova *et al.*, 1962; Salaman & Robinson, 1966; Borensztajn *et al.*, 1972). In the present study, lipoprotein lipase activity decreased during the first 6 h of life although the animals were in the fed state. This unexpected result seems to imply that either lipoprotein lipase in the newborn rat does not respond to the usual rise in insulinaemia observed in the adult, after feeding, or that insulinaemia does not rise after feeding in the newborn. Girard *et al.* (1973) recorded a sharp decrease in plasma insulin concentrations during the first hours following birth in the rat. However, this decrease was not unexpected since the pups were prevented from feeding. Low plasma insulin concentrations have also been observed in normally nursed pups from 0 to 6 h after birth (Blazquez *et al.*, 1974). These concentrations were well below those found in foetuses on day 22 of gestation or in 24 h-old pups. This suggested an impaired insulin response to the first meal. By using rats 1 h old, Blazquez *et al.* (1975) demonstrated both a lack of insulin secretion *in vivo* after a glucose load and a slow conversion rate of pro-insulin into insulin in pancreatic islets incubated *in vitro*. There is therefore a possibility that the decrease in adipose lipoprotein lipase activity during the first 6 h after birth resulted from a low rate of insulin release.

From 6 h after birth onwards, lipoprotein lipase activity increased. Again, these variations could be related to corresponding changes in plasma insulin concentrations. The extent of insulinemia in fed pups at 24 h is similar to that found in fed adults, approx. 2 times that in the 6 h pups (Blazquez *et al.*, 1974). Thus adipose lipoprotein lipase in the newborn rat apparently responds as in the adult to changes in insulinaemia (de Gasquet & Péquignot, 1973; Cryer *et al.*, 1974).

The rapid increase in adipose lipoprotein lipase activity paralleling that in triacylglycerol content between 6 and 24 h suggests that this enzyme may be involved in the filling of developing adipocytes with fat. Moreover the results indicate that fatty acid esterification was operating in these cells from 6 h onwards. Between 0 and 6 h, however, there was practically no accumulation of fat, and as shown here this was not due to a lack of lipoprotein lipase. The

possibility of a delay in the establishment of mechanisms for fatty acid esterification in the inguinal pad of the newborn cannot be excluded.

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