Changes in the Lipoprotein Lipase (Clearing-Factor Lipase) Activity of White Adipose Tissue during Development of the Rat

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The lipoprotein lipase (clearing-factor lipase) activity of the white adipose tissue from rats aged between 1 and 145 days was determined. Five adipose-tissue sites (epididymal, uterine, subcutaneous, perirenal and intramuscular) together with serum concentrations of triacylglycerol, cholesterol and glucose were studied. The pattern of enzyme-activity change was remarkably similar in all the sites studied, although the growth of the tissues proceeded non-uniformly. After a peak of activity early in suckling, lipoprotein lipase activity fell to low values by 20 days of age. At weaning (21 days) the activity increased sharply and within 5 days high values were regained. The serum triacylglycerol and cholesterol concentrations were low at birth and reached peaks of concentration coincidentally with the minima of white-adipose-tissue lipoprotein lipase activities, seen late in suckling. The changes in enzyme activity were related to other metabolic changes in adipose tissue and with the known changes in plasma insulin concentrations occurring during development.

Lipoprotein triacylglycerol fatty acids are removed from the plasma by the action of the lipoprotein lipase (clearing-factor lipase, EC 3.1.1.3) present in extrahepatic tissues (Robinson, 1970). Changes in this enzymic activity occur in response to a variety of situations and observed alterations correlate closely with changes in the triacylglycerol fatty acid uptake of tissues such as heart (Rogers & Robinson, 1974), adipose tissue (Cryer et al., 1976), mammary gland (Scow et al., 1976) and skeletal muscle (Linder et al., 1976). In the white adipose tissue of experimental animals, changes in the lipoprotein lipase have been shown to occur, for example, during alterations in nutritional status (Cryer et al., 1975; Garfinkel et al., 1967), diurnally (Reichl, 1972), during cold exposure (Rodomski & Orme, 1971), in genetic obesity (de Gasquet et al., 1973; Enser, 1972), during lactation (Otway & Robinson, 1968; Hamosh et al., 1970), in response to hormonal stimuli (Borensztajn et al., 1972) and in experimental diabetes (Pav & Wenkeova, 1960; Kessler, 1963). However, studies of such changes have been confined to the observation of adult animals and little information is available on the changes that may occur during development. Pequignot-Planche et al. (1977) have reported that the lipoprotein lipase activity of the immature inguinal white adipose tissue of male rats increased markedly over the first 24h of life. However, apart from the limited study by Hahn & Drahota (1966) and the prenatal studies by Jones (1976) and Harding (1971) in guinea pigs and rabbits respectively, little is known of what might occur at other times during development. The present study was therefore undertaken to investigate the changes in lipoprotein lipase activity present in the white adipose tissue of male and female rats throughout postnatal development.

Like other tissues, the size of the adipose organ is dependent on factors that affect both cell division and cell enlargement. Both these processes can be affected by nutritional factors (as can lipoprotein lipase activity), and the outcome depends, among other things, on the age of the animal (Hahn & Novak, 1975). Therefore, because of its role in the accretion of triacylglycerol fatty acids from the serum lipoproteins by adipose tissue, study of the developmental changes in lipoprotein lipase activity may be relevant not only to the ontogeny of the control of serum triacylglycerol concentrations but also to the control of the growth and development of white adipose tissue itself.

Materials and Methods

Materials

Casein (light white soluble), n-nonane and the clinical assay set for the determination of serum total cholesterol were obtained from BDH Chemicals, Poole, Dorset, U.K. Intralipid was obtained from Vitrum, Stockholm, Sweden, bovine serum albumin (fraction V) from Sigma (London) Chemical Co., London S.W.6, U.K., and heparin (Pularin) from Evans Medical, Speke, Liverpool, U.K.; 2,2'-azinodi-(3-ethylbenzthiazolin 6-sulphonic acid)diammonium,
glucose oxidase (grade II, of fungal origin) and glucose peroxidase (grade II, from horseradish) were supplied by Boehringer Corp. (London), London W.5, U.K.

**Animals and tissues**

Rats of the MRC hooded strain, from the colony of this Department, were used in all the experiments. Body-weight determinations indicated that foetal and suckling animals grew at acceptable rates (Lang, 1965). The upward shift from linearity of the graph relating logarithm of the body weight (g) versus the reciprocal of age (weeks) indicated that the animals after weaning grew well by accepted standards (Dunn et al., 1947). Litters were kept with their mothers until weaning at 21 days. From about 14 days of age until weaning and after weaning animals consumed a mixed diet of solid food (modified diet 4IB; Pilsbury and Co., Birmingham B5 7UG, U.K.) and milk. Animal ages were estimated from the time of fertilization, indicated by the presence of a copulation plug, and were accurate to within 12 h. Foetuses (17–21 days old) were delivered by caesarian section with the mother under diethyl ether anaesthesia, and foetal weights were used to confirm gestational age (Lang, 1965). Foetuses and young animals up to 20 days of age were killed by decapitation; older animals were anaesthetized with ether and killed by exsanguination from the abdominal aorta. Only pups from litters containing eight to twelve individuals were used. All the animals studied were killed between 09:00 and 10:00 h, and were considered to be in the fed state, since the stomachs were invariably full.

Blood was collected into polyethylene centrifuge tubes of 400 μl capacity and left for 30 min on ice before centrifugation at 10000 g for 5 min in a microcentrifuge (Microfuge B; Beckman Instruments, Palo Alto, CA, U.S.A.). The serum was aspirated and stored at −20°C until used. After blood collection, 30 μl of sheep blood. The lipoprotein lipase activities measured with sheep recalified citrated plasma were 95±6% (mean ± S.D. for 18 determinations) of those with rat material; therefore the material from sheep was considered a suitable source of the activator apoprotein for the artificial substrate used in the routine assay of the enzyme. The assay incubations were carried out at 30°C for between 1.5 and 2.5 h in the presence of 1 unit of heparin (as defined by the manufacturers)/ml. The rates of non-esterified fatty acid (free fatty acid) release in the assays were linear over these times, and the non-esterified fatty acid release was measured as described previously (Salaman & Robinson, 1966). Enzyme activity was expressed as μmol of non-esterified fatty acid released/h at 30°C. The enzyme studied at all stages of the animal’s development was characterized by its inhibition by 0.6 M NaCl (over 85%) and its requirement for serum in the assay.

**Determination of the chemical composition of serum**

Triacylglycerol concentrations were measured in duplicate on 0.15 ml samples of serum by the method of Martin (1975). Serum glucose was measured in 5 μl samples by the method of Uete et al. (1976). Total serum cholesterol was measured in 0.01 ml samples with a clinical assay set, based on the cholesterol oxidase method of Richmond (1973), kindly given by Dr. B. Sturgeon (BDH Chemicals). Student’s t test was used to estimate the significance of differences (Fisher & Yates, 1957).
Results

Changes in animal and fat-depot sizes

Fig. 1 shows the changes in body weight for the rats studied. Fig. 2 shows how the five adipose-tissue sites studied during the development of the rat up to 145 days of age changed in weight. For the subcutaneous, perirenal and intramuscular fat-pads the changes were identical in both male and female animals and the means shown are for groups made up of animals of both sexes. Of the sites studied, the discrete axial subcutaneous depot was the largest during the early part of life, but, after remaining as a relatively constant proportion of body weight throughout suckling, it declined considerably during the remaining period of study. The intramuscular adipose tissue remained a constant proportion of total body weight throughout the period of study and was the smallest in weight at 145 days. The perirenal,

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Fig. 1. Changes in rat body weights during development
Each point represents the mean weight of between six and twelve animals; the bars indicate s.d. Male and female animals were identical in weight up to 65 days of age and the means shown are for groups containing animals of both sex. At 145 days the sexes were significantly different in weight and are shown separately (●, male; ○, female). Abbreviation: NB, newborn.

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Fig. 2. Changes in white-adipose-tissue weights during development of the rat
All the sites studied were bilateral and all the weights represented are the sum of both sides. The points indicate the means and the bars indicate s.d. The numbers of observations for each mean are indicated in parentheses for the following sites: subcutaneous (dissectable for the axial site; see the Materials and Methods section), ▲, (6-12); perirenal, ▲ (4-11); epididymal, ● (4-12); uterine, ○ (4-12); intramuscular, □ (4-8). Abbreviation: NB, newborn. At 43 and 145 days of age the subcutaneous-adipose-tissue depot weighed 390±220mg and 427±160mg respectively. At 145 days of age the perirenal-, epididymal- and uterine-adipose-tissue depots weighed 681±276mg, 1054±300mg and 641±116mg respectively.

epididymal and uterine adipose-tissue depots increased in absolute weight only slowly up to 30 days of age but more rapidly thereafter, becoming significantly greater as a proportion of body weight at 43 (P<0.01) and 145 (P<0.001) days for the epididymal fat-pad and at 145 days (P<0.002) for the perirenal and uterine sites.

Figs. 3(a), 3(b), 4(a), 4(b) and 4(c) respectively show the changes in lipoprotein lipase activity of white adipose tissue from the epididymal site in males, the uterine site in females and the perirenal, subcutaneous and intramuscular sites of both male and female animals during postnatal development. In general, although the absolute enzyme activity was different in various sites, the patterns of activity change over the 145 days studied were remarkably similar. In all the white-adipose-tissue sites studied lipoprotein
Fig. 3. Changes in the lipoprotein lipase activity of (a) the epididymal white adipose tissue of male rats and (b) the uterine white adipose tissue of female rats during development

(a) Samples of epididymal white adipose tissue from male rats of different ages were collected and pooled as described in the Materials and Methods section. Lipoprotein lipase was then determined in acetone/ether-dried preparations of the tissue. Each point indicates the mean of between four and eight independent observations; the bars indicate s.d. Activity is shown as units/g fresh wt. of tissue (●) or as units/depot (bilateral) (○). (b) Samples of uterine white adipose tissue from female rats of different ages were collected and treated as described in (a) above. Abbreviation: NB, newborn. At 37 and 145 days respectively the enzyme activity (units/depot) in epididymal adipose tissue was 5.5 ± 1.8 and 44.9 ± 12.4. At 145 days the enzyme activity (units/depot) present in uterine adipose tissue was 12.3 ± 4.6.

Fig. 4. Changes in the lipoprotein lipase activity of the (a) perirenal, (b) subcutaneous and (c) intramuscular white adipose tissue of male and female rats during development

Samples of white adipose tissue collected from the (a) perirenal, (b) subcutaneous and (c) intramuscular sites of male and female rats were treated as described in the legend of Fig. 3. The lines join the mean enzyme activities measured in both male and female animals (4–13 independent observations). The patterns of changes were not significantly altered when male and female animals were considered separately. Activities are shown as units/g fresh wt. of tissue (●) and as units/depot (bilateral) (○). Abbreviation: NB, newborn. At 43 and 145 days respectively the enzyme activity (units/depot) was 3.3 ± 2.0 and 14.6 ± 6.9 in perirenal adipose tissue. In subcutaneous adipose tissue, at 145 days the activity (units/depot) was 4.7 ± 2.0 and in the intramuscular depot was 1.8 ± 0.8.
subcutaneous depots, where a consistent decline occurred earlier, i.e. after 43 days. The activity as expressed per total depot increased progressively in all the sites studied between 20 and 145 days of age.

At the end of the study (145 days) the enzyme activity in all the depots expressed per g of tissue were significantly ($P = 0.02–0.001$) lower than those observed during mid-suckling and early post-weaning life. The adult values were, however, significantly higher ($P = 0.02–0.001$) than the minimum values observed during late suckling (i.e. at 20 days of age).

Figs. 5(a), 5(b) and 5(c) indicate respectively the patterns of change in the serum concentrations of triacylglycerol, cholesterol and glucose in the same developing rats. The triacylglycerol, cholesterol and glucose concentrations in the serum of foetal animals were not significantly different from those measured in animals over 30 days of age. The concentration of all three serum components remained constant between 30 and 145 days of age. However, significant changes occurred during the late foetal to early post-weaning period of life. First, serum cholesterol concentrations (Fig. 5b) rose and fell progressively during the period, with a significantly ($P<0.001$) elevated maximum between 17 and 19 postnatal days. Triacylglycerol (Fig. 5a) showed a broader (but significantly elevated) peak of serum concentration during the suckling period. The changes in serum glucose concentrations shown in Fig. 5(c) exhibited the characteristic, transient, postnatal hypoglycaemia previously observed by Yeung & Oliver (1968), followed by a return to relatively stable values thereafter.

Discussion

From Figs. 1 and 2 it can be shown that, except for the intramuscular site (previously unstudied in this regard), all the fat-depots altered in relative size during development, but in a non-uniform manner.

In particular, the subcutaneous fat-pads declined as a proportion of body weight throughout, which was in contrast with the epididymal, uterine and perirenal sites, although, as previously noted (Hahn, 1970), the subcutaneous adipose tissue developed earlier than any of the abdominal sites. However, no significant changes in relative sizes occurred during the critical period of lipoprotein lipase activity changes between 1 and 30 days of age. Previous studies of adipose-tissue cell size and numbers have shown that, for the epididymal adipose tissue at least (Greenwood & Hirsch, 1974), the most pronounced increase in the rate of cell growth occurs after 21 days. By contrast, cell division and increases in cell number proceed most rapidly before weaning. It appears therefore that lipoprotein lipase activity first emerges during a period of active cell proliferation, then declines to a low value near the end of suckling and subsequently re-emerges during a period when active cell growth and lipid accretion is occurring. Overall the changes in enzyme activity were consistent from depot to depot, but the relative sizes of the depots changed in different ways such that no significant correlation between depot growth, as assessed by a crude weight measurement, and lipoprotein lipase activity were apparent, at least during the pre-weaning part of life. This is not unexpected, since many other factors, including the rates of lipogenesis de novo and lipolysis, would also affect the progress of fat accretion by the tissue, and in addition many factors are known to influence the rate of cell proliferation in adipose tissue (Hahn & Novak, 1975). This lack of a direct relationship between lipoprotein lipase activity and adipose-tissue growth during particular periods of development has also been noted and commented on by Jones (1976) in studies on foetal guinea pigs, in which perirenal-adipose-tissue enzyme activity fell during late gestation at a time when tissue growth was considerable. In this instance a decline in the response to lipolytic hormones and an increase in lipogenesis de novo were concluded to account for the rapid
lipid accumulation by the tissue when lipoprotein triacylglycerol fatty acid uptake from the plasma was presumably low. A similar situation could exist in rat adipose tissue during suckling, since, in contrast with the situation in the liver (Villee & Hagerman, 1958; Carroll, 1964), fatty acid synthesis in adipose tissue is higher at this time than in adults (Schnell et al., 1961; Hahn et al., 1968). Also, although hormone-sensitive lipase (triacylglycerol lipase, EC 3.1.1.3) activity is high in rat white adipose tissue during late suckling (15–20 days of age) (Hahn, 1965; Hahn & Drahota, 1966), the high unesterified fatty acid concentration found in the plasma of fed suckling rats is probably of dietary origin, since it declines on starvation (Hahn, 1970), in contrast with the change in lipolytic rate mediated by hormone-sensitive lipase seen in adults. Thus a high lipogenic rate together with a low rate of triacylglycerol turnover from the tissue could contribute to fat accumulation by adipose tissue when lipoprotein lipase activity was low in the second half of the suckling period.

In all the tissue sites studied from 1-day-old animals, lipoprotein lipase activity per g of tissue was in all cases at least 50% of the adult value. This observation is entirely consistent with the increase in inguinal adipose-tissue enzyme activity noted in neonatal rats during the first 24h of life by Pequignot-Planche et al. (1977). The coincidence of the increases in adipose-tissue triacylglycerol content and lipoprotein lipase activity noted by these authors, and substantiated in our own experiments, suggests that this enzyme could be of use as a marker enzyme for the differentiation and development of the putative pre-adipocyte suggested to be present in adipose tissue, particularly during such developmental periods when such cells cannot be recognized or counted by conventional methods (Hollenberg & Vost, 1968; Kirtland et al., 1975; Dodds et al., 1976).

The developmental situation studied here provides a further example of the reciprocity that normally exists between the activities of lipoprotein lipase and hormone-sensitive (mobilizing) lipase in adipose tissue (Robinson & Wing, 1970). Thus lipoprotein lipase activity is high and hormone-sensitive lipase activity is low during early suckling (Hahn, 1965), but the situation reverses between days 10 and 20 and reverses again after day 20–24. The observations were made on fed animals in both cases, and whether the reciprocity in activities would be maintained and/or reversed on starvation remains to be investigated.

The rapid rise in serum triacylglycerol concentrations soon after birth is primarily related to food intake, since if fat is removed from the neonatal diet the concentrations fall (Friedman & Byers, 1961). The triacylglycerol concentrations measured in the present study remained high throughout suckling, and were at their highest when adipose-tissue lipoprotein lipase activity was its lowest between 19 and 22 days of age. The concentrations fell coincidently with the rise in lipoprotein lipase activity that occurred after weaning, but exclusive causality is not implied, since the fat content of the diet also declined considerably at this time (Dymsza et al., 1964) and the activity of lipoprotein lipase in other extrahepatic tissues was not considered. However, the rise in total adipose-tissue lipoprotein lipase activity at weaning could be significant in the subsequent control of serum triacylglycerol concentrations, since it is at this time that hepatic lipogenesis becomes an important process and endogenous triacylglycerols are secreted into the serum (Hahn & Novak, 1975). The pattern of serum cholesterol changes noted for rats in the present study agrees with previous reports for other species (Carroll et al., 1973; Hamilton & Carroll, 1977) and the peak observed in suckling is known to be related to the intake of milk lipids (Harris et al., 1966). As with the serum triacylglycerols, the peak of cholesterol concentration is coincident with the minimum values of lipoprotein lipase activity in adipose tissue, and cholesterol in the serum declines in parallel with the post-weaning increase in the enzyme activity. As before, however, the change in diet occurring at this time must be considered the main determinant of change in cholesterol concentrations.

Hormonal factors can be shown to affect adipose-tissue lipoprotein lipase activity in the adult rat (Cryer et al., 1975) and in particular a close correlation exists between the serum insulin concentration and the activity of the enzyme in the tissue (Cryer et al., 1976). Changes in insulin concentrations (Blazquez et al., 1975), also known to affect developing adipose tissue in terms of its proliferation and cellularity (Kazdova et al., 1974), have also been implicated in the control of the increase in lipoprotein lipase activity seen by Pequignot-Planche et al. (1977) during the first 24h of life. Perhaps the subsequent post-natal changes in enzyme activity respond to the same signal, particularly since plasma insulin concentrations in rats are known to decline to low values during suckling and to rise rapidly to adult values between days 20 and 30 after birth (Blazquez et al., 1970).

Age and cell size are both variables that affect adipose-tissue metabolism (Holm et al., 1975). We have characterized changes in lipoprotein lipase activity in the tissue with age only, and, although the observations are consistent with changes in plasma constituents and tissue metabolic capacities, more information will be needed before the complete implications of these changes for adipose-tissue development can be assessed.

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