The Early Development of White Adipose Tissue

EFFECTS OF LITTER SIZE ON THE LIPOPROTEIN LIPASE ACTIVITY OF FOUR ADIPOSE-TISSUE DEPOTS, SERUM IMMUNOREACTIVE INSULIN AND TISSUE CELLULARITY DURING THE FIRST FOUR WEEKS OF LIFE IN THE RAT

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1. Newborn rats were reared in litters of either four or sixteen individuals. The animals from the small litters gained body weight more rapidly than those from large litters during the first 29 days of postnatal life studied. 2. The relative weights of the perigenital, perirenal, subcutaneous and intramuscular white-adipose-tissue sites in the animals from small litters indicated their relative obesity compared with controls. 3. The adipose depots from animals reared in small litters had a greater proportion of lipid present, by weight, and had a greater number of larger fat-cells present in them compared with the depots of animals reared in large litters. 4. Compared with both normal-sized litter controls and animals reared in sixteens, during the period of study the animals from small litters were hypertriacylglycerolaemic but normocholesterolaemic. 5. During suckling the blood glucose concentrations of animals reared in fours were increased, as were the concentrations of circulating immunoreactive insulin. 6. During the 29 days of life studied, in general, the lipoprotein lipase activity of adipose depots from animals reared in fours was greater than for animals in large litters when expressed as μmol of non-esterified fatty acid released from the substrate/h per g fresh weight of tissue, per depot, or per million fat-cells, but were similar per cm^2 of fat-cell surface area. 7. The previously noted [Cryer & Jones (1978) *Biochem. J.* 172, 319-325] pattern of mid-suckling elevation, late-suckling decline and post-weaning increase in the lipoprotein lipase activity of the four white-adipose depots studied was not obliterated by the nutritional manipulations employed. 8. The relation of the enzyme-activity changes and their hormonal stimuli to triacylglycerol accumulation in fat-cells of animals from large and small litters is discussed in relation to the possible significance they may have to our understanding of neonatally induced obesity.

The changes in lipoprotein lipase (clearing-factor lipase, EC 3.1.1.34) activity that occur in the white adipose tissue of rats during development have received much attention (Péquignot-Planche *et al.*, 1977; Hietanen & Greenwood, 1977; Cryer & Jones, 1978). The function of the enzyme in promoting the hydrolysis and consequent uptake of circulating triacylglycerols (Robinson, 1970) make it potentially an important determinant of adipose-tissue lipid accumulation and indicate it as a potential control point in fat-cell development. Numerous studies have documented the normal development of adipose-depot cellularity in both control and genetically obese rodents (Knittle & Hirsch, 1968; Hirsch & Han, 1969; Johnson *et al.*, 1971; Johnson & Hirsch, 1972), but, despite the interest shown in the lipoprotein lipase activity and cellularity of adipose tissue from adult obese animals (Enser, 1972; de Gasquet & Péquignot, 1972; de Gasquet *et al.*,

1973), no work has appeared that has attempted to relate the two during the development of the obese state. It was the purpose of the present study to redress this situation somewhat by studying the development of white-adipose-tissue sites in the rat with respect to their cellularity and lipoprotein lipase activity during the induction of relative obesity by litter-size adjustment during the suckling period. It was a further aim to relate the observed changes to changes in circulating insulin concentrations and their effects on triacylglycerol transport. The effect of early nutrition on the immediate and delayed onset of obesity in the human has been the subject of much speculation, and, although James (1976) produced a number of plausible hypotheses concerning the development of obesity, these have not as yet been adequately investigated. The work reported here with experimental animals may have predictive utility in this context.

Materials and Methods

Materials

^{125}I -labelled insulin ($50\text{ }\mu\text{Ci}/\mu\text{g}$) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Reagents used in microscopy, and the clinical assay set for the determination of serum total cholesterol, were obtained from BDH Chemicals, Poole, Dorset, U.K. Other materials were obtained from the sources given by Cryer & Jones (1978).

Animals and tissues

Rats of the MRC hooded strain, from the colony of this Department, were used in all the experiments. The animals were bred and managed as described by Cryer & Jones (1978). Within 16 h of birth neonatal animals were distributed among the available dams in litters of either four or sixteen individuals. Equal numbers of male and female neonatal animals were placed in each litter. This rearrangement procedure was tolerated well by both the dams and neonatal animals. Animals in all the litters were weaned at 21 days of age. 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 and adipose tissue from the perigenital (epididymal or uterine), perirenal, subcutaneous and intramuscular sites were collected as described previously (Cryer & Jones, 1978). The adipose-tissue samples collected from male and female animals were studied separately. Serum, collected after centrifugation of the blood samples at $10000g$ for 5 min, was stored at -20°C until used.

Preparation of white-adipose-tissue samples for determination of lipoprotein lipase activity

White adipose tissue from particular sites was collected in pools of material from up to eight animals, depending on age and body weight. These samples were homogenized in 5 ml of 2.5% (w/v) casein solution in aq. $50\text{ mM-NH}_3/\text{HCl}$ buffer, pH 8.1, by using a motor-driven glass/Teflon Potter-Elvehjem homogenizer. The material was delipidated by using acetone and diethyl ether (Cryer *et al.*, 1976), and the resultant vacuum-dried powders were homogenized in $50\text{ mM-NH}_3/\text{HCl}$ buffer, pH 8.1, for the determination of lipoprotein lipase activity as previously described (Cryer & Jones, 1978). The assay of enzyme activity utilized a non-radioactive triacylglycerol emulsion (Intralipid; Vitrum, Stockholm, Sweden) activated by preincubation with ovine plasma. The linear rate of non-esterified fatty acid release from this substrate was determined by titration (Salaman & Robinson, 1966), and the enzyme activity in all preparations was characterized

by its inhibition by 0.6 M-NaCl (over 85%) and its requirement for the inclusion of plasma in the assay. One unit of enzyme activity is defined as $1\text{ }\mu\text{mol}$ of non-esterified fatty acid released from the substrate/h.

Determination of the chemical composition of serum

Serum triacylglycerol, cholesterol and glucose concentrations were determined by the methods of Martin (1975), Richmond (1973) and Uete *et al.* (1976) respectively, as described previously (Cryer & Jones, 1978). Serum immunoreactive insulin concentrations were determined by using the double-antibody technique of Hales & Randle (1963) with rat insulin (Novo Ltd., Copenhagen, Denmark) as standard.

Determination of fat-cell size and number

Fat-cell sizes in the four white-adipose-tissue depots were assessed by microscopic measurement of the diameter of fat-cells seen in fixed thin sections of tissue prepared as described by Ashwell *et al.* (1976). For each distinct tissue sample studied, data from at least six different microscopic sections were pooled in order to calculate the average corrected mean cell diameter as defined by Ashwell *et al.* (1976). The total number of cells observed for each determination was within the range 900–2600. Fat-cell volume was calculated from the average cell diameter by using conventional geometrical formulae. The number of fat-cells in a particular fat-depot was calculated by dividing the total tissue lipid content (determined by the method of Folch *et al.*, 1957) by the mean fat-cell mass (mean fat-cell volume $\times$ lipid density). Lipid density was assumed to be that of triolein (0.915; Björntorp & Sjöström, 1971). With the sample of cells defined above for the determinations of cell size and number, the occasional pockets of uncharacteristically small cells observed in these sections (and those of others, e.g. Ashwell *et al.*, 1975; Kirtland *et al.*, 1975) had no discernible effect on the average cell-diameter values obtained. The sections prepared in these experiments showed much less evidence of the heterogeneity in cell size and degree of brown-adipose-tissue infiltration previously reported (Kirtland *et al.*, 1976) for the intrascapular, subcutaneous and perirenal white-adipose-tissue depots of young guinea pigs.

Results and Discussion

Changes in animal weights

Fig. 1 shows the changes in total body weight for groups of young rats maintained in litters of either four or sixteen individuals. These changes are shown

Fig. 1. Effect of litter size on the changes in rat body weights during development

The weights of animals in litters of four (●), sixteen (○) or eight to twelve (△) individuals are shown as means; the bars indicated $\pm$ s.d. Each point represents the mean weight of between eight and sixteen animals. The male and female animals in particular groups were identical in weight and the means shown are for groups containing animals of both sexes. The animals were maintained and the litter sizes adjusted as described in the Materials and Methods section. Abbreviation: NB, newborn rat.

together with the growth curve for this strain of animals reared in normal-sized litters (eight to twelve individuals) (Cryer & Jones, 1978). The body weights of those animals in litters of four ('Fours') were significantly greater ($P < 0.001$), at all the times studied, than the weights of the animals from litters of sixteen individuals ('Sixteens'). The rate of increase in total body weight for the previously studied animals maintained in litters of eight to twelve was intermediate (mean of 1.35 g/day over the 30 days studied) to that of rats reared as 'Fours' (1.70 g/day) or 'Sixteens' (0.8 g/day). The body weights of rodents at weaning are generally believed to be inversely related to the number of animals in the litter during the birth-to-weaning period (Widdowson & McCance, 1960). Studies of the quantitative nature of this relationship have shown both species and strain differences (Wurtman & Miller, 1976; Kumersan *et al.*, 1967; Epstein, 1978), and particularly, although increase in litter size, with consequent relative under-nutrition, always causes a decline in growth rate for rats (for review see Watson *et al.*, 1978), animals in litters smaller than normal do not always exhibit persistently increased body weights at weaning (e.g. Wurtman & Miller, 1976). In the present study the

inverse relationship holds for the animals studied when compared with animals from the same colony raised in normal-sized (8–12) litters (Cryer & Jones, 1978). At weaning the animals, of both sexes, from litters of four had approximately twice the body weight of those from litters of sixteen.

Changes in fat-depot sizes

The enhanced rate of total body-weight increase exhibited by the 'Fours' was paralleled by a greater increase in the mass of the four white-adipose-tissue sites studied (upper panels of Fig. 2) when compared with the 'Sixteens'. The lower panels of Fig. 2 show that this increased adipose-tissue mass also represented a greater proportion of total body weight in the 'Fours' than in the 'Sixteens'. Fig. 3 shows that all the depots from the 'Fours' also contained a consistently greater proportion by weight of extractable lipid than those taken from 'Sixteens'.

Although the overall growth of the epididymal fat-depot has been studied extensively in normal animals (Greenwood & Hirsch, 1974; Cleary *et al.*, 1977; Cryer & Jones, 1978), in those made obese, by litter-size adjustment (Knittle & Hirsch, 1968), by pre-weaning supplementary feeding (Czajka-Narins & Hirsch, 1974), by lesion of the ventromedial hypothalamic nuclei (Hirsch & Han, 1969) and in a variety of genetically obese animals (e.g. Stern & Greenwood, 1974), less attention has been paid to the other white-adipose-tissue depots. This neglect is despite numerous observations in mature animals showing that the epididymal fat-depot is not always representative of tissue obtained from other sites (Durham *et al.*, 1971; Hahn, 1971; Persico *et al.*, 1975). Compared with the animals reared in litters of 8–12 (Cryer & Jones, 1978), although the overall weight gain in the 'Sixteens' was less extensive than normal the adipose organ was not preferentially or more extensively depleted in size than any other organ or tissue of these growing rats. In the 'Fours' during the period of study the adipose depots, singly or cumulatively, represent a greater proportion of body weight than in either the 'Sixteens' or the animals from the normal-sized litters described previously (Cryer & Jones, 1978). Thus, although other organ weights also increase in size in response to reduction in litter size (McCance & Widdowson, 1962; McCance, 1962), the adipose organ assumed a greater than proportionate role in the overall increase in body weight under the conditions described. In other words 'Fours' studied here can be considered as obese in relation to both the 'Sixteens' and the animals from litters of normal size (Cryer & Jones, 1978). That the period under study here is most active with respect to changes in the proportion of total lipid in the adipose depots confirms many previous reports initiated by the work of Hausberger & Gujot (1937).

Fig. 2. *Effect of litter size on the changes in white-adipose-tissue weights during development of the rat*
All the sites studied were bilateral and all the weights represented are the sums of both sides. The points indicate the mean weights of tissues taken from animals in litters of four (●) or sixteen (○) and the bars indicate s.d. The number of independent observations used for each mean was between four and sixteen. No sex differences in tissue weights were noted during the period studied and therefore the means shown are for groups containing animals of both sexes. The epididymal adipose tissue of males and the uterine adipose tissue of female animals were not significantly different in weight and have been combined together as the perigenital adipose tissue (a). The perirenal, subcutaneous and intramuscular adipose-tissue sites are shown in (b), (c) and (d) respectively. Abbreviation: NB, newborn rat. The upper panels indicate the fresh weight of the depots, the lower panels show these weights calculated as a percentage of body weight.

Fig. 3. Effect of litter size on the changes in the extractable lipid content of white-adipose-tissue sites during development of the rat

Each point represents the result of a duplicate estimate of extractable lipid (determined as described in the Materials and Methods section) on an independent pooled sample of adipose tissue taken from animals reared in litters of either four (●) or sixteen (○). The panels represent data for the (a) perigenital, (b) perirenal, (c) subcutaneous and (d) intramuscular depots. Abbreviation: NB, newborn rat.

Changes in white-adipose-tissue cell size and number

Fig. 4 shows the changes in fat-cell mass (upper panels) and total bilateral fat-depot cell number (lower panels) for the four adipose-tissue sites of animals raised in both sizes of litter. For particular animals of the same age from litters of the same size, the perigenital, perirenal and subcutaneous mean fat-cell masses (upper panels of Fig. 4) were similar throughout the period of study. The mean masses of cells from equivalent intramuscular sites were, how-

ever, consistently, but not always significantly ($P < 0.05$), larger than cells from the other three sites. In animals from litters of four the progressive increase in cell size of the perigenital, perirenal and subcutaneous sites that occurred over the first 10–14 days of age was followed by a variably distinct hiatus in cell enlargement lasting between 4 and 8 days. The progressive increase in cell size (which was characteristic of cells from the intramuscular site throughout) was then reasserted during the remainder of the time studied. The increase in cell mass for the

Fig. 4. Effect of litter size on the changes in white-adipose-tissue cell mass and number during development of the rat. Adipose-tissue mean cell mass and number were determined as described in the Materials and Methods section. Each point indicates the mean of observations made on between four and eight photomicrographic fields (see the Materials and Methods section). The bars indicate s.d. The observations were made on (a) perigenital, (b) perirenal, (c) subcutaneous and (d) intramuscular white-adipose-tissue depots taken from animals reared in litters of either four (●) or sixteen (○) individuals. The mean mass of single cells in ng and the total number of cells in each bilateral depot are given. Abbreviation: NB, newborn rat.

adipose-tissue depots of 'Sixteens' was generally less extensive than that of the 'Fours'. Also, whereas there was a progressive increase in cell size for the perigenital and perirenal sites of the 'Sixteens' throughout the period of study, such increase occurred quite abruptly between days 10 and 19 of age in the subcutaneous and intramuscular depots. These generally smaller cells seen in the tissues taken from 'Sixteens' were complemented by the consistently smaller numbers of cells found in the total depots of these animals when compared with 'Fours'.

Like other aspects of white-adipose-tissue development the preweaning changes in fat-cell size and number have been neglected hitherto, especially in depots other than the epididymal site (Greenwood & Hirsch, 1974). The data on cell size and number changes in the perigenital depot of 'Sixteens' reported here were qualitatively and quantitatively similar to the more limited data reported by Greenwood & Hirsch (1974), and confirmed that during this early phase of development the perigenital adipose depot increased in size as a result of both cellular hyperplasia and hypertrophy. These combined data are, however, at variance with those of Cleary *et al.* (1977), where no increase in cell size was observed between days 12 and 21 after birth, although there was a gain in cell numbers. This discrepancy cannot be explained by differences in the methodology of cell-size determinations, since Greenwood & Hirsch (1974) and Cleary *et al.* (1977) both used the same method, i.e. that of Hirsch & Gallian (1968), whereas the present series of experiments used a distinct procedure (see the Materials and Methods section). As in the case of the perigenital fat-depot of 'Sixteens', the growth of the other fat-depots studied proceeded by a combination of hypertrophy and hyperplasia. In the intramuscular depot the hyperplastic element of the increase was less distinct than for the other three sites. The overall increase in the relatively enlarged size of all the depots studied in animals raised as 'Fours' was also a combination of cell hypertrophy and hyperplasia during the 30 days of postnatal life studied. This situation would naturally precede the combined increase in cell size and number noted by Knittle & Hirsch (1968) when the epididymal adipose tissue of similar groups of animals were compared at ages ranging from 5 to 20 weeks. However, there were different detailed patterns of this interrelationship between increase in cell size and number for the four adipose-tissue sites in the present experiments. For example, cell-number increases were more pronounced than cell-size differences during the suckling period when the perigenital and perirenal depots of 'Fours' and 'Sixteens' were compared, whereas size difference became more important quantitatively than increase in cell numbers after weaning in all the sites. How these early changes relate to later develop-

mental changes remains to be investigated, but it is clear that during suckling both the size and number of cells in all four depots studied are susceptible to change induced primarily by a nutritional stimulus. However, despite the fact that elevation of plasma insulin concentrations is characteristic of a number of genetically inherited obese states in animals, where adipose-tissue cell sizes and numbers are increased (Johnson *et al.*, 1973; Hunt *et al.*, 1976; Bray, 1977) and elevations of the plasma hormone concentrations by injection into young post-weaned animals can stimulate fat-cell proliferation (Kazdova *et al.*, 1974), the serum insulin concentrations in the present studies could not be closely related to adipose cell dimensions other than that all three variables were higher in 'Fours' than in 'Sixteens'.

Changes in serum triacylglycerol, cholesterol, glucose and insulin concentrations

Figs. 5(a), 5(b), 5(c) and 5(d) show the serum concentrations of triacylglycerol, cholesterol, immunoreactive insulin and glucose respectively for animals reared in the large and small litters previously defined. The 'Fours' were significantly hypertriacylglycerolaemic compared with the 'Sixteens' during suckling but not thereafter. The 'Fours' also had higher concentrations of circulating triacylglycerol during this period when compared with the previously studied animals taken from normal-sized litters (eight to twelve individuals) (Cryer & Jones, 1978). 'Fours' and 'Sixteens' both showed similar plasma cholesterol concentration changes during development. These were comparable with those previously reported for animals in litters of eight to twelve (Cryer & Jones, 1978) (Fig. 5b). On seven of the eleven date points studied the 'Fours' had plasma immunoreactive insulin concentrations significantly higher ($0.02P < 0.01$) than those observed for the 'Sixteens' (Fig. 5c). Animals reared in litters of either four or sixteen individuals had plasma insulin concentrations during suckling that were significantly lower than those observed for neonatal animals ($P < 0.001$) or weaned individuals ($P < 0.01$). The immunoreactive insulin concentration measured in the sera of a group of six 24 h-starved adult male rats during the period of the present study was $17.7 \pm 11.1 \mu\text{units/ml}$, which compared well with previously reported values for such animals (Cryer *et al.*, 1976). The glucose concentrations measured in the plasma of the developing animals are shown in Fig. 5(d). The glucose concentrations in the plasma of 'Sixteens' were similar to those previously reported for animals in litters of eight to twelve (Cryer & Jones, 1978). The 'Fours' by comparison had significantly ($0.02 < P < 0.001$) elevated plasma glucose concentrations between 2 and 21 days of age but not thereafter.

Changes in lipoprotein lipase activities

The upper panels of Fig. 6 show the activities (expressed as units/g fresh weight of tissue) of lipoprotein lipase determined in the white-adipose-tissue depots of animals taken from litters of either four or sixteen during the first 30 days of life. As noted previously, for animals taken from normal-sized litters (Cryer & Jones, 1978) the increased activity observed during the mid-suckling period declined to very low values toward the end of the period with the mothers. The original mid-suckling activities were

either totally or partially recovered in all the depots studied after weaning of both the 'Fours' and 'Sixteens'. The total lipoprotein lipase activity (units/g fresh weight of tissue) of the perigenital, perirenal and subcutaneous adipose tissue taken from 'Fours' was significantly higher throughout the suckling period, than that of equivalent tissue samples obtained from 'Sixteens'. These differences between 'Fours' and 'Sixteens' became less obvious after weaning.

The lower panels of Fig. 6 show the pattern of these changes in lipoprotein lipase activity in the adipose tissue of 'Fours' and 'Sixteens' when ex-

Fig. 5. Effect of litter size on the changes in the concentrations of (a) triacylglycerol, (b) cholesterol, (c) immunoreactive insulin and (d) glucose in the serum of rats during development

Blood was collected as described in the Materials and Methods section from rats of different ages reared in litters of either four (●) or sixteen (○). (a) Triacylglycerol, (b) cholesterol, (c) immunoreactive insulin and (d) glucose were measured as described in the Materials and Methods section. Each point represents the mean of between four and eight independent sera. The bars indicate S.D. There were no apparent sex differences. Abbreviation: NB, newborn rat.

Fig. 6. *Effect of litter size on the changes in the total lipoprotein lipase activity of the (a) perigenital, (b) perirenal, (c) subcutaneous and (d) intramuscular white adipose tissue of male and female rats during development*

Samples of white adipose tissue collected from the (a) perigenital, (b) perirenal, (c) subcutaneous and (d) intramuscular sites of male and female rats of different ages, reared in litters of either four (●) or sixteen (○), were pooled and treated as described in the Materials and Methods section. Lipoprotein lipase was then determined in acetone/ether-dried preparations of the tissue. The lines join the mean enzyme activities [units/g fresh wt. of tissue (upper panels) and units/total bilateral depot (lower panels)] measured in both male and female animals and each point indicates the mean of between four and eight independent observations; the bars indicate s.d. The patterns of change were not significantly altered when male and female animals were considered separately. Abbreviation: NB, newborn rat.

pressed as total activity per adipose-tissue depot per unit body weight. Throughout the period in all the adipose-tissue depots studied, enzyme activity expressed in this way was significantly ($0.02 < P < 0.001$) higher in the 'Fours' than in the 'Sixteens'. The general pattern of enzyme-activity change was unaltered when this means of expression was employed, but the difference between the activities of 'Fours' and 'Sixteens' was, however, more pronounced than previously.

Fig. 7 shows the lipoprotein lipase activities expressed per million fat-cells (upper panels) and per cm^2 of fat-cell surface area (lower panels). The patterns of enzyme activity/cell in all the depots of 'Fours' were similar to those shown in Fig. 6. In general the enzyme activities calculated per cell for 'Fours' was higher than for 'Sixteens'. But, although the differences were significant throughout the period of study for the perigenital and intramuscular depots, no differences existed for the preweaned perirenal and post-weaned subcutaneous depots. When the lipoprotein lipase activities were expressed per unit of cell surface area (lower panels of Fig. 7) the pattern of differences between 'Fours' and 'Sixteens' became less obvious than for the other means of expression, although the late-suckling decline and post-weaning recovery persisted.

The developmental changes that occur in the activity of lipoprotein lipase in the epididymal adipose depot of the male rat, both perinatally (Péquignot-Planche *et al.*, 1977) and throughout the first 7 months of life (Hietanen & Greenwood, 1977; Cryer & Jones, 1978), are now well established. The observations on this tissue have been extended to include other white-adipose-tissue depots of both male and female rats by Cryer & Jones (1978), who observed the remarkable similarity between the pattern of enzyme-activity change during development in all the depots. The present experiment indicates that nutritional influences induced by changes in litter size are unable to obliterate the characteristic pattern of enzyme-activity change that occurs in the first 30 days of life (Fig. 6; Cryer & Jones, 1978), but are able to alter the magnitude of such changes.

Although no development data on the lipoprotein lipase activity of white-adipose-tissue from genetically obese animals are available, studies have been carried out to compare the enzyme activity in the tissues of mature obese animals and their lean litter mates (Enser, 1972; de Gasquet & Péquignot, 1972; de Gasquet *et al.*, 1973; Schonfeld *et al.*, 1974). All the studies indicate an increased total lipoprotein lipase activity per depot in genetically obese adults when compared with lean litter mates, but this is entirely expected because of the massive increase in depot mass in such animals. Although some studies (de Gasquet & Péquignot, 1972; de Gasquet *et al.*,

1973) suggest that the enzyme activity per g fresh weight of tissue is the same in obese and lean litter mates, others (Enser, 1972; Schonfeld *et al.*, 1974) indicate an elevation in the tissues from the obese animals. Although early in the development of the nutritionally induced obesity activity per g is elevated (Fig. 6), in the 'Fours' this difference may not persist throughout life and preliminary studies indicate that such a difference is not permanent (A. Cryer & H. M. Jones, unpublished observations).

The need to distinguish between age and cell-size (and number) effects on adipose-tissue metabolism has been stressed previously (Holm *et al.*, 1975). In the present study the activity of lipoprotein lipase per fat-cell was elevated in the adipose-tissue depots of 'Fours' throughout the first 30 days of life when compared with 'Sixteens'. This overall picture of the larger cells (Fig. 4) of the 'Fours' having a greater total activity of lipoprotein lipase associated with them agrees with the previous observations on obese animals, where enzyme per cell in the perigenital and perirenal sites of genetically obese rats and mice demonstrated a similar enhancement (de Gasquet *et al.*, 1973; de Gasquet & Péquignot, 1972). Similarly in the present studies (lower panels, Fig. 7) and those of de Gasquet *et al.* (1973) and de Gasquet & Péquignot (1972), the increased lipoprotein lipase activity of the enlarged cells was related to an unaltered activity of the enzyme when it was expressed per unit of fat-cell surface area. These data together with similar data from Swiss mice made obese with a high-fat diet (de Gasquet *et al.*, 1973) suggest that the increase in lipoprotein lipase activity per fat-cell may be a common feature of both genetic and nutritionally induced obesities.

In Fig. 8 the percentage change in lipoprotein lipase per cell is compared with the percentage increase in cellular lipid in a manner previously adopted by Hietanen & Greenwood (1977) in studies on the epididymal fat-depot. The data in Fig. 8 suggest that for the perigenital, perirenal and subcutaneous depots of 'Fours' the significant periods of fat-cell lipid accretion are preceded or coincident with increases in lipoprotein lipase activity per cell. Similarly, in these three depots, during the phase of lipoprotein lipase activity (per cell) decline that occurred substantially between either day 10 or 14 and day 20, there is a hiatus in the rate at which the cells accumulate lipid. This relationship is not so clear in the intramuscular depot of 'Fours' and appears not to exist for cells from any of the depots of 'Sixteens' during this first 30 days of life. The relative contribution of lipogenesis *de novo* and lipoprotein triacylglycerol fatty acid uptake to total lipid accumulation in the fat-cell during development is not known, but the data in Fig. 8 may indicate a less important role for lipoprotein lipase in the lipid filling of the cells from 'Sixteens' than is the case in 'Fours'.

Fig. 7. Effect of litter size on the changes in lipoprotein lipase activity per cell of the (a) perigenital, (b) perirenal, (c) subcutaneous and (d) intramuscular white adipose tissue of male and female rats during development

The lipoprotein lipase activities shown in Fig. 6 have been expressed as units of activity per 10⁶ cells. The cell numbers are as shown in Fig. 4 (upper panels). The activities have also been expressed as units of activity per cm² of cell surface area (lower panels). Other details are as described in the legend to Fig. 6.

Fig. 8. Comparison of the changes in lipoprotein lipase activity per fat-cell mass and changes in fat-cell mass during the early development of rat perigenital, perirenal, subcutaneous and intramuscular white adipose tissue

Data taken from Figs. 4 (upper panels) and 7 (upper panels) has been recalculated as (●) lipoprotein lipase per fat-cell (percentage of peak value) and (○) lipid per fat-cell (percentage of peak value) in order to facilitate a temporal comparison of the two. The data have been recalculated for the (a) perigenital, (b) perirenal, (c) subcutaneous and (d) intramuscular depots taken from animals reared in litters of four (upper panels) or sixteen (lower panels). The points represent means; s.d. bars have been omitted for clarity. NB, Newborn rat.

That changes in plasma insulin concentrations may be related to the observed differences between the developing adipose tissue of 'Fours' and 'Sixteens' is a strong possibility. But the known developmental changes in hormone concentrations other than insulin are more difficult to relate to the observations. For example, glucagon (Hahn *et al.*, 1977), growth hormone (Blazquez *et al.*, 1974), thyroid-stimulating hormone, thyroid hormones (Dussault & Labrie, 1975) and corticosterone (Takeuchi *et al.*, 1977), all of which are known, from work on adult animals, to be effectors of adipose-tissue lipoprotein lipase activity changes either *in vivo* or *in vitro* (Nestel & Austin, 1969; Robinson & Wing, 1970; Borensztajn *et al.*, 1972, 1973; Hemon *et al.*, 1975; Bagdade *et al.*, 1976), change in plasma concentration during the period of early life studied. In particular, the serum concentrations of insulin (Fig. 5 and Blazquez *et al.*, 1970), corticosterone (Takeuchi *et al.*, 1977) and growth hormone (Blazquez *et al.*, 1974) decline from high pre- and peri-natal values to remain at relatively low concentrations through suckling and are followed by a rise to adult concentration at weaning. The data available suggest that for glucagon the relatively high serum concentrations of the hormone observed during suckling decline in response to weaning (Hahn *et al.*, 1977), and that the thyroid hormones increase progressively to adult concentrations during suckling (Dussault & Labrie, 1975). The overall pattern of change in lipoprotein lipase activity can only at this time be related in any way to changes in serum insulin concentrations, since corticosterone and growth hormone would be expected to affect the enzyme activity in a reciprocal manner to that of insulin. That the responsiveness of the tissue to the hormones mentioned may not be as expected from studies of adult animals should also be considered. Differences do exist and are high-lighted by the observation that glucagon has no effect on lipolysis in the white adipose tissue of suckling rats (Hahn, 1971), in contrast with its profound effects in adults. The relationship of cell size and age to hormone responsiveness, cell proliferation, lipoprotein lipase activity and other metabolically important processes deserves further investigation.

Finally, since the hyperinsulinaemia of the 'Fours' may have profound effects on the development of the metabolism and cellularity of white adipose tissue, it is of interest to speculate on the possible origin of the elevated serum insulin concentrations. Early indirect (Hahn *et al.*, 1961) and later more direct evidence (Snell & Walker, 1973; Ferré *et al.*, 1977a) suggests that during suckling blood glucose concentrations are maintained substantially by gluconeogenesis. It has also been shown that fat-feeding can partly reverse starvation-induced hypoglycaemia (Ferré *et al.*, 1977b), and that inhibition of the oxidative utilization of fatty acids induces a depres-

sion of gluconeogenesis and a hypoglycaemia in suckling pups (Ferré *et al.*, 1977a; Pégorier *et al.*, 1977). Thus it is possible that the enhanced availability, in the 'Fours' studied here, of fatty acid substrates for oxidation could contribute to their suckling hyperglycaemia. This relative hyperglycaemia compared with that in 'Sixteens' was lost at weaning. Further, this hyperglycaemia may contribute to the observed hyperinsulinaemia in the 'Fours', since it is known that the β -cells of the pancreas, even in early neonatal rats, are fully able to respond to changes in blood glucose concentrations (Girard *et al.*, 1974). The interrelationships of lipid and carbohydrate metabolism and their control by hormones in early development and particularly during the onset of obesity deserve further attention.

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