The Development of White Adipose Tissue

EFFECT OF LITTER SIZE ON THE LIPOPROTEIN LIPASE ACTIVITY OF FOUR ADIPOSE-TISSUE DEPOTS, SERUM IMMUNOREACTIVE INSULIN AND TISSUE CELLULARITY DURING THE FIRST YEAR OF LIFE IN MALE AND FEMALE RATS

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(1.) Male and female rats reared in litters of four gained body weight more rapidly than animals reared in litters of 16. The differences were more marked in males than females and became less marked in both sexes with advancing age. (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 animals from large litters. A sex-related difference in the distribution of adipose tissue between the four sites was seen in animals reared in litters of both four and 16. (3.) Although at 30 days of age all the animals had more numerous and larger fat-cells in their white-adipose-tissue depots than animals reared in large litters, the pattern of change thereafter was both site- and sex-specific. During the post-weaning period (30–300 days), although detailed differences were apparent between sites, a general pattern of increased cell size in males and increased cell numbers in females emerged as being the important determinants responsible for the differences in depot sizes seen when animals from litters of four and 16 were compared. (4.) Lipoprotein lipase activities, expressed as units/g fresh wt. of tissue, in the depots of animals reared in groups of four were unaltered compared with those reared in groups of sixteen during the post-weaning period (47–300 days of age), and enzyme activities expressed per depot merely reflected differences in tissue weights. (5.) Lipoprotein lipase activities per 10^6 cells were higher in males reared in fours compared with those reared in sixteens of equivalent age, but were unaltered for females. (6.) The persistent hyperinsulinaemia of animals reared in litters of four is discussed in relation to the observed differences in enzyme activity and white-adipose-tissue cellularity.

The changes in adipose-tissue lipoprotein lipase (clearing-factor lipase, EC 3.1.1.34) activity that occur during the period from birth to adulthood in the rat have now been characterized (Pequignot-Planche *et al.*, 1977; Hietanen & Greenwood, 1977; Cryer & Jones, 1978). The role of lipoprotein lipase in allowing the regulated uptake of circulating lipoprotein triacylglycerols by the extrahepatic tissues (Rogers & Robinson, 1974; Scow *et al.*, 1976; Linder *et al.*, 1976) and by adipose tissue in particular (Cryer *et al.*, 1976) indicate it as a potential control point in fat-cell development (Hietanen & Greenwood, 1977; Cryer & Jones, 1979). This potential involvement of the enzyme in fat-cell and ultimately adipose-tissue development led us to investigate the process of adipose-tissue enlargement, particularly with respect to tissue cellularity

and lipoprotein lipase activity, in rats rendered relatively obese by litter-size adjustment during the suckling period (Cryer & Jones, 1979). The animals studied previously were reared in litters of either four or 16 individuals, and the aspects of adipose-tissue metabolism in question were studied in animals up to the fourth week of life (Cryer & Jones, 1979). The purpose of the present study was to consider the relationships between body and adipose-tissue enlargement, tissue cellularity, lipoprotein lipase activity and circulating immunoreactive insulin concentrations in similar animals when they were studied during, approximately, the first year of life. The work was undertaken in an attempt to help resolve conflicting data relating to the mode of adipose-tissue enlargement, the growth stages in the tissue and the susceptibility of the metabolism and growth

of the tissue to environmental influences, the effects of which may or may not be persistent (Pitkin, 1978).

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. The inorganic salts and biochemicals used were of the highest purity available and were obtained from the sources given by Cryer & Jones (1978, 1979).

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, managed and had the size of litters adjusted as described by Cryer & Jones (1978, 1979). The animals of both sexes studied here were up to 300 days old and were reared as members of litters of four ('Fours') or 16 ('Sixteens') individuals. They were maintained normally after weaning (Cryer & Jones, 1978). Animals studied were killed between 09:00 and 10:00h and were in the fed state. Blood, serum and white-adipose-tissue samples from the perigenital (epididymal and uterine), perirenal, subcutaneous and intramuscular sites were collected and treated as described previously (Cryer & Jones, 1978).

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

White-adipose-tissue samples were delipidated by using acetone and diethyl ether (Cryer *et al.*, 1976), and the dried powders so produced were assayed for lipoprotein lipase as described previously (Cryer & Jones, 1978).

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 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). The data from between seven and 15 sections from each tissue sample were pooled 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 1×10^3 – 4×10^3 . From these data, fat-cell size was determined and the number of cells per depot was calculated as described previously (Cryer & Jones, 1979).

Results and Discussion

Effect of litter size on the body weight of male and female rats

Fig. 1 indicates that throughout the 300 days of study both male and female animals reared in litters of four were heavier ($P < 0.05$) than those reared in litters of 16. The sex-related differences in body weight for both 'Fours' and 'Sixteens' became discernible at approximately the same age (i.e. 40

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

Male and female rats were reared in litters of either four or sixteen individuals. The body weights of animals are shown as means, the bars indicate $\pm$ s.d. The number of animals in each group was between 6 and 24. Before 40 days of age the body weights of male and female animals were not significantly different and groups consisting of both sexes reared in litters of four or 16 are shown. Between 40 and 300 days of age, groups of males from litters of four ($\bullet$) and 16 ($\circ$) and females from litters of four ($\blacktriangle$) and 16 ($\triangle$) are shown separately.

days). The differences in body weights between 'Fours' and 'Sixteens' became less marked with advancing age for both sexes. Also the magnitude and percentage differences in weights between the groups were smaller in the females than in the males at all times. Rats that have been undernourished for a short period may or may not be as large as control animals whose growth has not been restricted, depending on the timing of the nutritional 'insult' (Widdowson & McCance, 1963). The relative 'insult' of being suckled in a litter of 16 compared with a litter of four, as in the present study, resulted in a persistent lowering of body weight throughout development, which was more extensive in males than females. The greater resistance to post-weaning nutritional rehabilitation seen when males reared as 'Sixteens' are compared with similarly treated females is in agreement with previous studies (Widdowson & McCance, 1960, 1963; Widdowson & Kennedy, 1962; Smart *et al.*, 1974; Williams *et al.*, 1974a,b). The 'Sixteens' in the present study were smaller and the 'Fours' were larger than animals of the same ages reared in normal-sized litters (i.e. 8–12 individuals; $P < 0.05$) (Cryer & Jones, 1978, 1979).

Effect of litter size on the changes in white-adipose-tissue weights during the development of male and female rats

For all the white-adipose-tissue depots studied at every time point (with the exception of two) in both sexes the tissues from 'Fours' weighed more than the tissues from 'Sixteens'. The differences in weight were not, however, always statistically significant. When the tissue weights were expressed as a proportion of total body weight (Fig. 2), differences in the age-related pattern of change were evident, between depots, between sexes and also when 'Fours' and 'Sixteens' were compared. For the perigenital depots the relatively larger depot of females grew continuously as a proportion of body weight throughout, as did the epididymal depot of the males. In both sexes, a not always significant difference between the relative depot sizes of 'Fours' compared with those of 'Sixteens' was observed in these perigenital depots. The perirenal depot of females (both 'Fours' and 'Sixteens') followed a similar overall pattern of change to the uterine depot, although the relative weights remained stable after 129 days of age. In both the male 'Fours' and 'Sixteens', however, the post-weaning decline in the relative size of the perirenal depot was merely recovered during the remainder of the period of study. A post-weaning decline in the relative size of the remaining depots (subcutaneous and intramuscular) was seen in the 'Fours' of both sexes with only comparatively small changes in size thereafter. In comparable 'Sixteens', changes in the size of these

two depots were less extensive than in the 'Fours.' A distinct sex difference in the distribution of adipose tissue among the four sites was apparent for animals reared as either 'Fours' or 'Sixteens.' Thus, although the perigenital and intramuscular depots represented similar proportions of body weight in both sexes, the contribution of the perirenal depot to body weight was much greater in females than males and conversely the contribution of the subcutaneous depot was much greater in males than females. The growth of the epididymal fat depot, for both normal animals (Greenwood & Hirsch, 1974; Cleary *et al.*, 1977; Cryer & Jones, 1978) and those made obese by litter adjustment (Knittle & Hirsch, 1968), has been studied quite extensively, but less attention has been paid to other white-adipose-tissue depots. This is despite the known metabolic variations that exist between such sites (Durham *et al.*, 1971; Persico *et al.*, 1975; Hartman & Christ, 1978) and the overall relative minor contribution of this site to the total adipose organ weight of the rat (Reed, 1930).

Although the degree of adipose depot size enlargement was not extensive in the 'Fours', these animals were more fatty than 'Sixteens' and animals reared in normal-sized litters (Cryer & Jones, 1978). However, the sum of the weights of the four depots of 'Sixteens' was approx. 70% of that for 'Fours' in both male and female animals between 50 and 300 days of age.

Effect of litter size on changes in adipose-tissue cell number and size during the development of male and female rats

Although at 30 days of age all the animals reared as 'Fours' had more numerous and larger fat-cells in their relatively enlarged adipose depots (Figs. 3 and 4 and Cryer & Jones, 1979), the pattern of change thereafter appears both site- and sex-specific. Thus between 30 and 300 days of age the epididymal fat-depots of 'Fours' were enlarged over those of 'Sixteens', because in general they contained greater numbers of larger cells. For the uterine depot, however, the major distinction was the greater number of recognizable cells in the depots of 'Fours' compared with those of 'Sixteens.' In the perirenal depots of male 'Fours' the marginal differences in depot size compared with 'Sixteens' of the same sex could not be discerned clearly in cell size and number terms. However, in female 'Fours' an enlarged cell number contributed greatly to the observed enhancement of depot size compared with equivalent 'Sixteens.' In the subcutaneous depot of males the enhanced overall size of this depot in 'Fours' was a result primarily of an increase in the number of recognizable fat-cells over the first 200 days of life, which was maintained as a result of cell hypertrophy thereafter. In the females the difference in the subcutaneous depot size between 'Fours' and 'Sixteens' was

Fig. 2. Effect of litter size on the changes in white-adipose-tissue weights as a proportion of total body weight during development of male and female rats. The tissue weights are expressed as a proportion of total body weight. The upper and lower panels represent male and female animals respectively and data for the perigenital, perirenal, subcutaneous and intramuscular depots are shown in (a), (b), (c) and (d) respectively. The animals were reared in litters of either four (●) or 16 (○) individuals.

Fig. 3. *Effect of litter size on the changes in white-adipose-tissue cell mass occurring during development of male and female rats*
Adipose-tissue mean cell mass (ng) was determined as described in the Materials and Methods section. Each point indicates the mean of observations made on between seven and fifteen photomicrographic fields. The bars indicate $\pm$ s.e.m. The observations were made on (a) perigenital, (b) perirenal, (c) subcutaneous and (d) intramuscular white-adipose-tissue depots taken from male (upper panels) and female (lower panels) animals reared in litters of either four (●) or 16 (○) individuals.

Fig. 4. Effect of litter size on the changes in white-adipose-tissue cell numbers occurring during development of male and female rats. The total bilateral number of cells in each depot was calculated from the cell mass, depot weight and lipid content as described previously (Cryer & Jones, 1979). Each point indicates the mean of values calculated from mean cell masses derived from between seven and fifteen determinations carried out on each depot. The bars indicate $\pm$ s.e.m. The data are shown for (a) perigenital, (b) perirenal, (c) subcutaneous and (d) intramuscular white-adipose-tissue depots taken from male (upper panels) and female (lower panels) animals reared in litters of either four (●) or 16 (○) individuals.

almost exclusively as a result of an increase in cell numbers, apparently determined within the first few days of life. In the case of the intramuscular depot the early differences in cell numbers were replaced by cell-size enlargement as the major determinant of depot size increases seen in the 'Fours' versus the 'Sixteens', although the pattern was more clearly manifest in females than in males.

As noted above, although the epididymal site has been the subject of considerable interest in relating cell size and number changes to adipose-tissue enlargement during development (Greenwood & Hirsch, 1974; Hietanen & Greenwood, 1977; Cleary *et al.*, 1977), other depots have been severely neglected in this regard. The cell-size and number changes reported here for the epididymal fat-depots of both 'Fours' and 'Sixteens' were consistent in pattern with those reported by Greenwood & Hirsch (1974), and the differences noted in this depot between 'Fours' and 'Sixteens' were similar to those described by Knittle & Hirsch (1968). For the other depots the data reported here for animals up to 300 days of age can be correlated with that previously reported for the period up to 30 days of age (Cryer & Jones, 1979). For male animals the final cell number in the perirenal depot was unaffected by litter size, but the period over which that final number was achieved was altered by the early nutritional influence. Thus in the 'Fours' the stable final cell number was reached within the first 20 days of life [Fig. 3 and Fig. 4 of Cryer & Jones (1979)], whereas in the 'Sixteens' the increase in numbers continued to the same final total over the first 80 days of life. In this depot of female animals, however, the number of recognizable fat-cells continued to increase to a maximum at approx. 130 days in 'Fours' and probably even later for 'Sixteens'. Thus in this depot both nutritional and sexual influences appear to affect the progression of tissue development, but not the final cell number. This was also the case with the subcutaneous depot, where, in females reared in litters of four, the increase in fat-cell number was completed within the first 20 days of life, but continued to approx. 100 days in the males reared under similar conditions. In the subcutaneous depot of males from large litters equivalence of cell numbers with animals reared as 'Fours' took 200 days. In the intramuscular fat-pads of both males and females reared as 'Fours' the final number of cells in the depot seemed to be determined within the first 10 days of life, and this number of cells was eventually equalled in the 'Sixteens', but over a considerably longer period of development (50–100 days). This study indicates the heterogeneity in the growth patterns of different adipose-tissue depots and indicates the susceptibility of the patterns to changes induced by both genetic and environmental factors. This further indicates the inadequacy of a single adipose-tissue sample for the

definition of obesity types in adults and indicates the need for other longitudinal studies useful to the definition of the cellular aetiology of obesities induced by different genetic and environmental factors.

Effect of litter size on the changes in white-adipose-tissue lipoprotein lipase activity and on serum concentrations of triacylglycerol, cholesterol, glucose and immunoreactive insulin during the development of male and female rats

The lipoprotein lipase activities of the four depots studied were unaffected by litter-size adjustment during the post-weaning period (47–300 days of age) when the activities were expressed per unit weight of tissue (Fig. 5), although during the earlier part of life the differences were substantial and statistically significant (see Cryer & Jones, 1979). The general post-weaning decline in the lipoprotein lipase activity expressed in this way was a common feature of all the depots studied in both male and female animals. The activities determined in the depots are similar to those previously reported for tissues taken from animals of similar ages but reared in normal-sized litters (Cryer & Jones, 1978). When expressed per total depot the enzyme activities in the tissues of 'Fours' were elevated over those of equivalent 'Sixteens' in direct relationship with the already noted differences in tissue weights. When, however, the lipoprotein lipase activity was expressed on a cellular basis a distinction between the sexes became apparent. Thus in the males, where the depot-size enhancement apparent during the post-weaning life of 'Fours' was mostly contributed by cell-size enlargements, the lipoprotein lipase activity per million cells in the depots studied was elevated in the 'Fours' over the activities seen in the 'Sixteens' (upper panels of Fig. 6) ($P < 0.05$, after 100 days of age). In the females, on the other hand, where cell-size differences between 'Fours' and 'Sixteens' were less distinct, no significant differences were discernible in the lipoprotein lipase activities in the depots of the two groups when expressed on this cellular basis (lower panels of Fig. 6). When the enzyme activity was expressed per unit of fat-cell surface area no consistent differences between the adipose-tissue depots of both male and female 'Fours' and 'Sixteens' were observed. This invariant depot-specific activity of lipoprotein lipase per unit of cell surface area is a common feature of observations made in a number of animal models of obesity (de Gasquet & Pequignot, 1972; de Gasquet *et al.*, 1973; Cryer & Jones, 1979).

Table 1 shows the serum concentrations of triacylglycerol, cholesterol, glucose and immunoreactive insulin for animals reared in the large and small litters. No differences between the serum concentrations of triacylglycerol, cholesterol and

Fig. 5. Effect of litter size on the changes in the total lipoprotein lipase activity per unit weight 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 (upper panels) and female (lower panels) rats of different ages, reared in litters of either four (●) or 16 (○), were treated as described in the Materials and Methods section. Lipoprotein lipase activity was determined in acetone/ether-dried preparations of the tissue. The points indicate mean enzyme activities (units/g fresh wt. of tissue) measured in independent samples taken from between four and six individuals; the bars indicate $\pm$ s.d.

Fig. 6. *Effect of litter size on the changes in lipoprotein lipase 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. 5 have been expressed as units of activity per 10^6 cells. The cell numbers are as shown in Fig. 4. The results are for the (a) perigenital, (b) perirenal, (c) subcutaneous and (d) intramuscular white-adipose-tissue of male (upper panels) and female (lower panels) animals reared in litters of either four (●) or 16 (○) individuals.

Table 1. *Effect of litter size on the concentrations of triacylglycerol, cholesterol, glucose and immunoreactive insulin in the serum of rats during development*

Blood was collected and triacylglycerol, cholesterol, glucose and immunoreactive insulin were determined as described in the Materials and Methods section. Means $\pm$ S.D. are given with the number of independent sera studied in parentheses. Male and female animals did not show any significant differences, so the groups shown here contain data from animals of both sexes.

Plasma component	Litter size	Age (days)	...	15	30	47	80	129	200	247	308
Triacylglycerol (mm)	4			4.0	1.8	1.8	1.6	1.3	2.6	2.1	3.8
				± 1.1 (6)	± 0.3 (6)	± 0.5 (8)	± 0.6 (8)	± 0.5 (8)	± 1.1 (6)	± 1.0 (6)	± 0.8 (6)
	16			3.1	1.7	1.8	1.1	1.7	1.9	2.3	2.3
				± 1.2 (6)	± 0.2 (6)	± 0.5 (7)	± 0.3 (8)	± 0.5 (8)	± 0.5 (6)	± 0.5 (6)	± 0.5 (6)
Cholesterol (mm)	4			5.0	2.0	2.3	2.2	1.3	1.4	1.4	2.2
				± 0.3 (6)	± 0.4 (6)	± 0.3 (8)	± 0.3 (7)	± 0.5 (8)	± 0.4 (6)	± 0.3 (6)	± 0.4 (5)
	16			4.9	2.6	2.5	2.1	1.2	1.2	1.2	1.8
				± 0.4 (6)	± 0.5 (6)	± 0.3 (8)	± 0.3 (8)	± 0.2 (8)	± 0.3 (6)	± 0.5 (6)	± 0.4 (6)
Glucose (mm)	4			8.0	7.5	9.9	9.7	7.7	9.1	7.6	8.9
				± 1.1 (6)	± 1.3 (6)	± 1.8 (8)	± 0.8 (8)	± 1.0 (8)	± 0.6 (6)	± 1.0 (6)	± 0.7 (6)
	16			6.4	8.0	8.6	10.4	7.6	8.8	8.6	9.3
				± 1.3 (6)	± 1.0 (6)	± 1.0 (8)	± 0.9 (8)	± 0.6 (8)	± 0.7 (6)	± 1.1 (6)	± 1.0 (6)
Insulin (μ -i.u./ml)	4			6.3	25.3	34.2	86.3	60.8	33.3	27.9	54.5
				± 4.3 (6)	± 9.0 (6)	± 25.9 (9)	± 42.4 (9)	± 35.1 (6)	± 8.86 (6)	± 18.7 (6)	± 29.0 (6)
	16			3.1	10.0	8.0	20.6	20.2	26.1	10.9	36.0
				± 2.0 (6)	± 3.5 (6)	± 6.2 (8)	± 26.4 (9)	± 16.6 (6)	± 23.2 (6)	± 4.1 (6)	± 16.0 (6)

glucose between 'Fours' and 'Sixteens' were apparent, although the 'Fours' were consistently hyperinsulinaemic compared with 'Sixteens' throughout the period of study ($P = 0.1-0.01$). The data in Table 1 are for groups made up of both male and female animals. Such groups were used because, unlike the other variables measured, no significant sex differences in the serum composition were noted. The hyperinsulinaemia of the 'Fours' developed very early in life (Cryer & Jones, 1979) and was persistent (Table 1). The hyperinsulinaemia could therefore have a potential role in the subsequent metabolic changes associated with the development of the relative obesity seen in 'Fours', although direct causation cannot be implicated on this evidence alone.

Although for adult animals studied at a single time point differences in the size of adipocytes from different adipose depots are known (Hartman & Christ, 1978; Storck & Spitzer, 1974; Jamdar, 1978), little is known of the progression to final cell size in depots other than the epididymal fat-pads of

male rats. Furthermore, although such observations have now been made (see above), the relevance of cell size and number changes to alterations in cellular and tissue metabolism are a neglected but important area for future consideration, particularly since it is now becoming apparent that differential metabolic activities exist between fat tissue depots in the rodent, which may not be related to cell age or size.

The broad distinction between the enhanced enlargement of the adipose depots of 'Fours' over 'Sixteens' that exists between male and females and its relation to lipoprotein lipase activities, however, deserves attention here. Broadly, in males, where the enhanced final tissue size of 'Fours' relies mainly on cell-size differences, the lipoprotein lipase activity per cell was generally increased over that of equivalent 'Sixteens'. This observation is in keeping with the previously held view that increased activities of adipose-tissue lipoprotein lipase per cell are a common feature of both genetically and nutritionally induced obesities, as are enlarged cells (de Gasquet *et al.*, 1973; de Gasquet & Pequignot,

1972). Conversely very little comparative information exists for females, but in the present study at least a different picture from that exhibited by males emerges. In females, where cell-number enhancements are the major determinant of size difference between the depots of 'Fours' and 'Sixteens', the lipoprotein lipase activity per cell is not different in the two groups. Since plasma immunoreactive insulin concentrations have been correlated with total adipose-tissue lipoprotein lipase activity, for the epididymal depot at least (Cryer *et al.*, 1976) a potential relationship between the hyperinsulinaemia of 'Fours' and elevated enzyme activity per cell might be indicated for the male, which seems, however, not to apply so directly in the female. The possible involvement of oestrogenic steroids in the determination of the observed sex differences may be indicated by the observation that exogenous oestrogen administration to normal male rats depresses lipoprotein lipase activity in adipose tissue (Wilson *et al.*, 1976), and oophorectomized female rats have an increased enzyme activity in adipose tissue (Hamosh & Hamosh, 1975). Thus the female sex hormones may also depress lipoprotein lipase activity per cell, and, if the effect of 17β -oestradiol on the replication of human adipocyte precursors in culture (Roncari & Van, 1978) is operative *in vivo*, the same hormonal stimulus may be involved in the increase in recognizable fat-cell numbers during female adipose-tissue enlargement. Further experimentation will be required, however, to distinguish potential mechanisms responsible for the apparent sex-related differences.

From the studies reported here it is at present impossible to predict, for males at least, which emerges first, the enlarged cell or the enhanced lipoprotein lipase activity, but in studies of normal adipose-tissue enlargement (Hietanen & Greenwood, 1977) and that stimulated early in life by nutritional alteration (Cryer & Jones, 1979) changes in lipoprotein lipase activity seem to precede periods of rapid adipose-tissue fat accretion. What is more certain, however, is that, although such a relationship may be involved in the development of nutritionally induced obesity, many other factors, controlled hormonally or otherwise, are relevant to an overall understanding of the situation and await consideration.

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