

# Effect of Age, Sex, and Site on the Cellularity of the Adipose Tissue in Mice and Rats Rendered Obese by a High-Fat Diet

DANIEL LEMONNIER

*From the Laboratoire de Nutrition Humaine, Institut Scientifique et Technique de l'Alimentation, Paris 3ème, France*

**ABSTRACT** Cell size and number of parametrial fat pads were determined in Swiss mice made obese by means of a high-fat diet (40% lard w/w) given ad lib. This diet and a control were introduced to two groups of mothers during gestation and lactation, and sucklings were given the same diets as their mothers at weaning and throughout life.

2-wk old mice suckled by mothers fed a high-fat diet have fatter parametrial pads. This difference is due solely to an increase in fat cell size. After weaning, until the 18th wk, the two groups differed with a striking fat cell enlargement seen in the obese group. Later on, whereas cell numbers did not change in the control group, a constant and uninterrupted increase in number is shown in those of obese mice until the 52nd wk. Hyperplasia was observed only in adults. When the high-fat diet was introduced to adult rats it also triggered an increase in fat cell number.

Three sites of fat pads were compared in both sexes at 32 wk of age. All sites increased in weight in the high-fat fed group. This was due to: hyperplasia in perirenal site, hypertrophy in epididymal and subcutaneous sites, and hyperplasia plus hypertrophy in the parametrial one. So, in each sex, adipose sites in the obese mice reacted to the diet in a site-specific way.

It was concluded that the level of fat in a diet is involved in both formation and maturation of new fat cells and in the regulation of fat cell lipid content. The two processes may be separated or may act together according to the adipose tissue site.

This work was presented in part at the 1st International Congress on Energy Balance in Man, Paris, France, 16-18 September 1971.

Address requests for reprints to Dr. Lemonnier, I.S.T.A.-C.N.A.M., 292 Rue Saint Martin, Paris 3ème, France.

Received for publication 29 October 1971 and in revised form 10 July 1972.

## INTRODUCTION

Studies on the metabolism of adipose tissue are numerous but little is known about its growth either in experimental or in human obesities.

It seems that in obese human adults, an increased number of adipose cells is observed in early onset obesity whereas there is only fat cell hypertrophy when obesity appears later (1, 2).

In animals, most of the research was carried out on epididymal fat. This fat does not present any detectable modification in the number of fat cells in genetic (3-7), hypothalamic (8, 9), or nutritional (10) obesities in rats or mice, although obesity appears during the normal phase of fat cell multiplication. Yet, there may be an increased number of fat cells in other sites (7). When female mice are made obese by a high-fat diet, hyperplasia has been found in the parametrial fat (11). A similar hyperplasia is observed in genetically obese female rats (6).

Early dietary manipulations can effect permanent changes in the adipose cell number and size of the epididymal fat pads of the rat (12). An increase in these cell parameters can be induced in adult mice by preweaning overfeeding (13).

Our purpose was to determine whether hyperplasia seen in female mice made obese by a high-caloric diet was due to an increase in the number of adipose cells during the first weeks of life. Secondly, we have studied the cellularity of three of the most important adipose sites in male and female mice fed on a control or a high-fat diet.

A histological method has been chosen for measurement of cell size and number (10). This method has the advantage of being available whatever the cell size, and of allowing the measurement of the relative contribution of undifferentiated fat tissue in pad weight. Rapid meth-

ods using fat cell suspensions were thought to be inadequate for the present study: very small fat cells cannot be counted (14–16) and the collagenase treatment used in most of these techniques may lead to adipose cell destruction when large cells occur (14).

## METHODS

### Diets and animals

Adult male and female Swiss mice (Centre de Sélection des Animaux de Laboratoire, F-45, Orléans-la-Source) received one of the two following diets from mating:

**Control diet.** Per cent w/w: wheat flour 77.2; bran 3.0; casein 12.1; DL-methionine 0.4; lard 1.1; mineral salts 4.0; vitamin mixture 2.2. This diet contains 3% lipid and 18% protein (w/w), and in calories, respectively, 9 and 22%.

**High-fat diet.** Per cent w/w: wheat flour 7.7; bran 4.5; casein 32.1; lard 41.5; water 4.9; mineral salts 6.0; vitamin mixture 3.3. This diet contains: 41% lipids and 28% proteins (w/w), and in calories, respectively, 72 and 22%.

**Composition of the vitamin mixture (mg per 2.2 g):** thiamine 1.5; riboflavine 1.5; pyridoxine 1; Ca-pantothenate 5; niacin 5; B<sub>12</sub> 0.005; biotin 0.1; choline chloride 200; inositol 50;  $\alpha$ -tocopherol 30; menadiolone 1; *p*-aminobenzoic acid 30; retinol 800 IU; vitamin D<sub>3</sub> 200 IU; corn oil 700 mg.

**Composition of the salt mixture (mg per 4 g):** CaCO<sub>3</sub> 1,160; Na<sub>2</sub>C<sub>2</sub>H<sub>3</sub>O<sub>7</sub> · 5 H<sub>2</sub>O 1,020; K<sub>2</sub>HPO<sub>4</sub> 600; Na<sub>2</sub>H<sub>2</sub>PO<sub>4</sub> · H<sub>2</sub>O 480; MgSO<sub>4</sub> · 7 H<sub>2</sub>O 320; Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> · H<sub>2</sub>O 256; Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> 88; MnSO<sub>4</sub> · H<sub>2</sub>O 12; NaCl 64; CuSO<sub>4</sub> 1.6.

These two diets contain the same quantity of proteins, added vitamins, and mineral salts per calorie.

The mothers were fed one of the two diets during gestation and lactation. Approximately 24 hr after birth, all litters were adjusted to about eight pups per litter in each of the two groups. Sucklings were given at weaning and until sacrifice the same diets as to their mothers, so that experiments were carried out with mice fed ad lib. and throughout their lives a control or a high-fat diet.

In the first experiment six groups of female mice fed the two diets were killed by decapitation at various intervals: 2, 6, 8, 18, 32, and 52 wk of age. The cell size and number of the parametrial pads were determined. In the second experiment, the cellularity of three main adipose sites, perigenital, perirenal, and abdominal subcutaneous, was determined in 32-wk old male and female mice fed the two diets throughout life. In a third experiment, 5-month old female rats (a cross between Sherman and Merck stock M), previously fed a commercial diet were randomly allowed to eat ad lib. one of the two described diets for 7 months. These rats were the control *lean* animals of our breeding stock of genetically obese Zucker rats (17).

### Dissection of tissues

The pairs of fat pads in each of the three most important sites were carefully and completely removed, weighed together, then treated by histological procedure, after their density had been measured. The abdomen was opened following the sagittal plane. Epididymal fat pads were cut close to the epididymis and part of the major blood vessels removed. The parametrial pads were completely separated from the ovaries and from the uterus, but not in 2-wk old mice. All the fat around the kidneys and adrenals was removed. The subcutaneous abdominal fat depot is well separated from the other subcutaneous site

lying dorsal to the scapular region. The pads were carefully dissected away from the skin and from the underlying muscle on the left and right sides as a triangular section extending up to the back as far as visible. For the rats, only the perirenal sites were removed.

### Histological procedure

Microscopic procedures of adipose tissue allows counting of small fat cells. A fixed morphological picture was necessary in the suckling animals to measure undifferentiated and differentiated tissues.

All the pairs of pads in the control group and 5–10 portions from the largest tissues of the obese group were treated according to the method of Bjurulf (18). 15- $\mu$  paraffin sections were cut and stained for mast cells<sup>1</sup> (toluidine 0.75%, phenol 1.50% in water) and for fat cell membranes (acetic acid 1%, sulfogreen 0.3% in water). This treatment does not modify cell size. As stated by Bjurulf none of the steps in the preparation of tissue and slides causes significant shrinkage (18). It was verified that the thickness of paraffin sections had no effect on cell size from 10 to 150  $\mu$ . 15- $\mu$  sections were chosen for convenience.

### Measurement of fat cell volume

Portions of slides were projected (19) onto the ground glass (area: 249 cm<sup>2</sup>) of a Projectina microscope (Altstaetten SG, Switzerland) at a magnification of 130 $\times$ . The number of clear areas (about 100–600 according to the tissue), each representing a space previously occupied by fat, was recorded. However, all cells beyond the ground glass by more than half of their area were excluded. At least five portions were recorded to get at least 1000 fat cells counted for each pair of fat pads.

The surface  $S'$  of the areas was calculated from the ratio of area of ground glass to number of clear areas counted in a portion of slide. Calculation of fat cells was made on the assumption that cells were spherical, leading to insignificant error (15).

It is known that if one cuts, at random, a sphere by a plane, the section obtained has an area which is normally distributed around a mean  $S' = \frac{4}{3}S$ ,  $S$  being the area of the largest section possible, i.e. that of the planar section passing by the very center of the sphere. The formula which gives the volume of a sphere from the area of its largest section  $S = 4/3 S'$  is:

$$V = \frac{4}{3\sqrt{\pi}} S^{\frac{3}{2}}$$

But another source of variation is the dimension of the spheres themselves. The preceding estimation of  $V$  is then skewed if one assumes only the variation to be attributed to the effects of the randomized planar sections. Let then  $s^2_{(S)}$  be the variance of the distribution of  $S$ , as estimated from the five or more replications of our countings, and  $s^2_{(V)}$  the variance of the distribution of  $V$ . The preceding formula is:

$$V \pm s_{(V)} = \frac{4}{3\sqrt{\pi}} [S \pm s_{(S)}]^{\frac{3}{2}}$$

<sup>1</sup> Mast cells were not counted because of their small number, less than that observed in rats (10).

A simplified formula was proposed<sup>2</sup> and calculations were made with the following one:

$$V = \frac{4S}{3\sqrt{\pi S}}$$

When areas are measured on the ground glass, not only the areas previously occupied by lipid are taken into account but those occupied by cytoplasm and membranes as well. But, areas (and volumes) are overestimated by extracellular supporting and vascular structures. However, this error does not appear to be significant: (a) In adult animals the measured density of the pads was 0.92 in the two groups. This density was very close to that of pure trioleine (0.915), i.e. oleic acid represented about 50% of the total fatty acids in the three sites of adipose tissue (4). (b) The same high percentage of lipid was found in the tissues:  $92.00 \pm 0.09$  and  $91.7 \pm 0.11$  ( $n = 5$  in each group) for obese and control groups, respectively. (c) Histological examination (Fig. 1) of the two groups showed no difference except for cell size. As previously described for epididymal fat of rats made obese by means of a high-fat diet (10), there was no apparent intersite or intergroup differences in supporting and vascular structures.

### Measurement of cell number

The fat cell number was calculated from the ratio of fat pad volume to adipose cell volume. This ratio has the advantage to be independent of the lipid content. As shown by Goldrick the percentage of lipid in isolated fat cells is not constant. Thus, when the cell number is calculated with the use of the total lipid content of the pads, instead of their volume, there is an underestimation of cell number for small fat cells from young animals (20). Furthermore, the range in cell size in our mice was more important than in those measured by Goldrick, and large site-to-site variations have been described in per cent of lipid in adipose tissues of young animals (16).

The use of pads volume in the ratio to calculate the number of cells, is based on the assumption that adipose tissue sites are built only with adipose cells. The same assumption was made for calculation of cell volume from areas projected on the ground glass. In other words, both numerator and denominator were overestimated by the same factor, and it was shown in the calculation of cell volume that this overestimation was unimportant. Therefore, it was thought that determined values of cell number were a close estimation of the actual number.

Two determinations of adipose cells areas from the same tissues were made at intervals of several months to calcu-

<sup>2</sup> A series expansion of  $[S \pm s(s)]^{\frac{1}{2}} = S^{\frac{1}{2}} \left[ 1 \pm \frac{s(s)}{S} \right]^{\frac{1}{2}}$ , limited

to its second term, gives:  $S^{\frac{1}{2}} \left[ 1 \pm \frac{2}{3} \frac{s(s)}{S} + \frac{3}{8} \frac{s^2(s)}{S^2} \right]$ . It follows,

that:  $V \pm s(v) \simeq \frac{4}{3\sqrt{\pi}} S^{\frac{1}{2}} \left[ 1 + \frac{2}{3} \frac{s(s)}{S} \right] \pm \frac{2}{\sqrt{\pi}} S^{\frac{1}{2}} \cdot s(s)$ . The ex-

periments showed that the second term of the expression between square brackets was negligible. We then propose the

simplified formula:  $V \pm s(v) = \frac{4S}{3\sqrt{\pi S}} \pm \frac{2s(s)}{\sqrt{\pi S}}$

late the standard deviation:

$$SD = \sqrt{\frac{\sum d^2 - \frac{(\sum d)^2}{n}}{n-1}}$$

where  $d$  is the difference between the pairs, and  $n$  the number of pairs.

Determinations were made on a first group of tissues from six young animals (mean of the number of areas counted by portion = 394; five portions were recorded for each of the two determinations;  $n = 6$ ). A second group was composed of tissues from adult obese mice; data were, respectively, 149, 7, and 6. Results are expressed as a percentage of the mean of cell determinations in each group (coefficient of variation,  $C_v$ ). The errors of the determination, respectively, for the first and second group were, for area counts: 4.3 and 4.7%; errors for fat cell volume: 7.1 and 7.2%; errors for cell number: 5.7 and 3.5%.

When comparison was possible with other methods excellent agreement was found: results obtained with our histological method have been recently confirmed with a procedure including electronic counting of osmium-tetroxide-fixed fat cells in a suspension (14). In 2.5-month old control and *fafa* obese rats, Johnson, Zucker, Cruce, and Hirsch (7) found values differing from ours (6) by about 7 and 1.5% for fat cell volume and by no more than 3% for cell number in both groups, respectively.

### Measurements in suckling mice

In the parametrial adipose tissue of the pups it was found in two zones in slides: one with normal fat cells filled up with one large droplet of fat, and the other, called undifferentiated area, which contained small cells with or without several small droplets of fat. The volumes of these two areas were measured according to the volumetric method of Gepts (21). All the fixed tissues were completely cut in 10- $\mu$  sections. By projection on a paper and planimetry, the undifferentiated and differentiated areas were measured on 8-13 slices, i.e. one section for 10 and one for 20 control and high-fat diet groups, respectively. The total volume of each zone was  $V = abS/M$ ;  $a$  was 10 or 20 accordingly as one section for 10 or 20 were recorded;  $b$  was the thickness of one section;  $S$  was the total surface of measured areas for one of the two zones; and  $M$  was the magnification. The volume of the adipocytes was determined in the differentiated area as mentioned above. Their number was calculated from the ratio of volume of the differentiated tissue to mean volume of adipocytes in this zone. At 6 wk of age, the undifferentiated areas of the parametrial pads represented, respectively, 10 and 5% of the total areas on slides in the obese and in the control group. These percentages were subtracted from the pad weights to count cell number. In older mice such undifferentiated areas were not seen.

## RESULTS

*Growth and development of parametrial fat.* Table I and Fig. 2 show that there was a large increase in weight in the adipose tissue of the control group as it grew older; this growth was the result of an increase in cell size and number. After 18 wk of age, the weights of body and fat pads, as well as the cell size and number were plateaued.

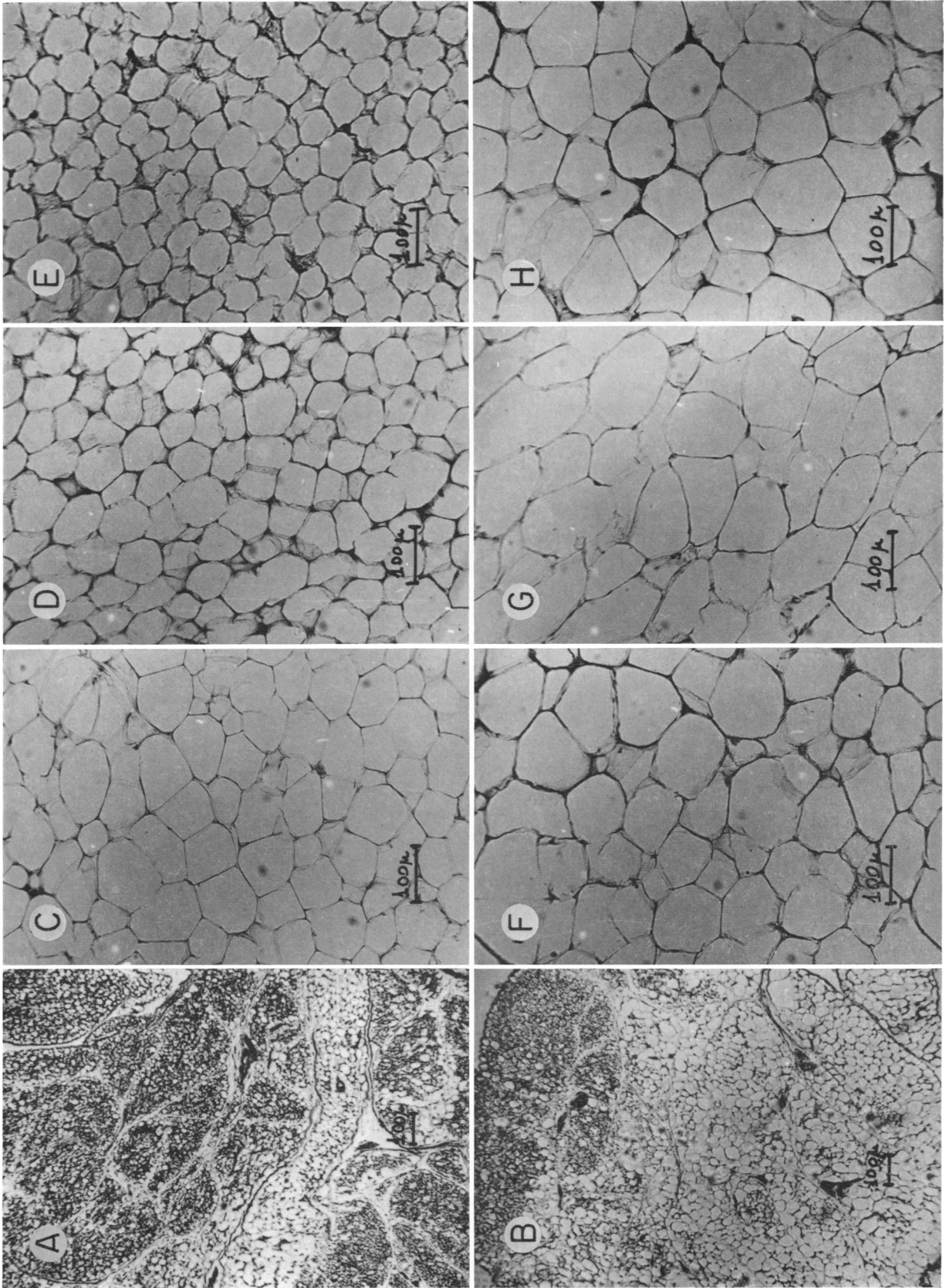


FIGURE 1 Aspect of adipose tissues in 2-wk old control (A) and "obese" (B) sucklings and in 32-wk old control (C, D, E) and obese mice (F, G, H). A, B, parametrial tissue; C, epididymal tissue; D, G, parametrial tissue; E, H, abdominal subcutaneous tissues. (D, G, E, H, female mice.)

TABLE I  
Cellularity of Parametrial Fat Pads of Swiss Mice Fed a Control (T) or a High-Fat Diet (S) (Mean  $\pm$  SEM)

Age	Diet	No. of mice	Body weight	Weight of two pads	Adipose cell volume ( $\times 10^3 \mu^3$ )	Adipose cell number ( $\times 10^6$ )
wk			g	mg		
2	T	5	7.64 $\pm$ 0.220 *	23.6 $\pm$ 4.13 *	25.4 $\pm$ 6.09 *	0.259 $\pm$ 0.0621
	S	5	9.16 $\pm$ 0.426	51.5 $\pm$ 7.96	144 $\pm$ 37.0	0.244 $\pm$ 0.0262
6	T	5	21.7 $\pm$ 0.374 *	366 $\pm$ 46.8 **	209 $\pm$ 20.7 *	1.568 $\pm$ 0.0794
	S	5	27.7 $\pm$ 0.673	1,037 $\pm$ 21.8	585 $\pm$ 88.3	1.621 $\pm$ 0.131
8	T	5	24.6 $\pm$ 0.314 **	572 $\pm$ 57.6 *	227 $\pm$ 9.3 *	2.73 $\pm$ 0.237
	S	5	28.2 $\pm$ 1.05	1,053 $\pm$ 163	463 $\pm$ 80.9	2.50 $\pm$ 0.109
18	T	5	35.9 $\pm$ 1.11 *	2,367 $\pm$ 166 *	534 $\pm$ 39.5 *	4.85 $\pm$ 0.265
	S	4	49.1 $\pm$ 2.47	5,039 $\pm$ 529	982 $\pm$ 58.5	5.57 $\pm$ 0.371
32	T	11	37.8 $\pm$ 1.44 *	2,460 $\pm$ 362 *	420 $\pm$ 37.2 *	6.16 $\pm$ 0.618 *
	S	8	64.2 $\pm$ 2.47	9,770 $\pm$ 1,070	953 $\pm$ 75.0	11.42 $\pm$ 1.250
52	T	7	40.0 $\pm$ 1.72 *	2,840 $\pm$ 328 *	493 $\pm$ 96.7 *	6.82 $\pm$ 0.608 *
	S	14	79.5 $\pm$ 3.61	17,500 $\pm$ 161	1,157 $\pm$ 48.2	17.00 $\pm$ 0.767 *

Significant differences: \*  $P < 0.01$ ; \*\*  $P < 0.05$ ; NS,  $P > 0.05$ .

The situation was quite different in the group of female mice fed on the high-fat diet: there was no plateau for body and fat pad weights nor for the number of fat cells. However, there was a trend toward reduced fat cell enlargement beginning at 18 wk.

As soon as they were 2 wk old, the two groups of suckling mice differed in their body weight and in their embryonic fat pad weight (Table II). The respective contributions of the undifferentiated and differentiated zones to pad weight were different in the two groups. The zone differentiated into fat cells was increased six-fold in the sucklings whose mothers ate the high-fat diet, but their undifferentiated zone showed little changes. In this group, the considerable enlargement of the fat cells account for almost all the increased pad weight. Until the age of 18 wk, there was only hypertrophy of the adipose cells in the high-fat diet group when a comparison was made with the control group of the same ages (Table I). Later on, the number of fat cells continued to rise throughout the period of observation in the high-fat fed group. Therefore, in the obese group about 60% of the weight of the pads was due to new fat cells hypertrophied.

This hyperplasia in adults may be induced by an increase in caloric intake during gestation and lactation. Thus, subsequent hypercellularity could have resulted from the filling up of fat cells formed at this time. Furthermore, this strain of mice is known to develop a mild adiposity in a small number of animals. This was the case in our control group, so the high-fat diet could have accelerated or triggered an inherited process. Thus, in order to know if adults animals are able to produce fat cell hyperplasia by feeding a high-fat diet at that stage of

life, rats were used. Lean control Zucker female rats were chosen because of the homogeneity of their adipose tissue and body weights. None of the rats of this strain

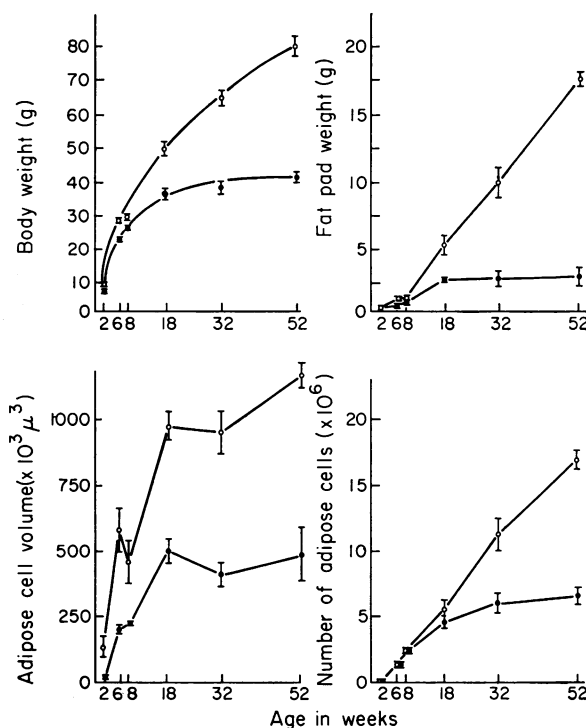


FIGURE 2 Weights of the body and pairs of parametrial fat pads (top of figure) and adipose cell size and numbers of female mice fed a control (●) or a high-fat diet S (○), at different ages. Vertical bar indicates mean  $\pm$  SEM.

TABLE II  
Weight and Cellularity of Embryonic Parametrial Fat Pads of 2-wk old Mice, whose Mothers were Fed a Control (T) or a High-Fat Diet (S) (Mean  $\pm$  SEM; five Mice in each Group)

Diet of the mothers	Body weight	Embryonic fat pad weight	Undifferentiated volume	Differentiated volume	Adipose cell volume ( $\times 10^3 \mu^3$ )	Number of adipocytes ( $\times 10^3$ )
	g	mg	mm <sup>3</sup>	mm <sup>3</sup>		
T	7.64 $\pm$ 0.220	23.6 $\pm$ 4.13	13.8 $\pm$ 0.722	6.95 $\pm$ 3.28	25.4 $\pm$ 6.10	259 $\pm$ 62.1
S	9.16 $\pm$ 0.426*	51.5 $\pm$ 7.96*	17.6 $\pm$ 1.32**	30.9 $\pm$ 8.55*	144 $\pm$ 37.0*	244 $\pm$ 26.2 (NS)

Significant differences: \*  $P < 0.01$ ; \*\*  $P < 0.05$ ; NS,  $P > 0.05$ .

show any tendency to a spontaneous adiposity over a 2 yr period. As shown in Table III cell number did not change in the control group between 5 and 12 months. The high-fat diet produced a slight increase in body weight but perirenal site was more than doubled. The cellularity of this site was affected by an increase in fat cell size and number. Caloric intake, measured daily during 11 days when the rats were 6.5 months old did not show any significant difference between the control and high-fat fed group.

*Sex and site differences.* Fig. 3 shows the results obtained in 32-wk old male and female mice in three sites of adipose tissue.

In the male obese group, perirenal and subcutaneous pad weights were increased about 2.7-fold, while the epididymal pads were increased only 1.9-fold. Therefore at 32 wk all sites in the male group weighed the same. The size of the adipose cells was different according to the site in the control group. The high-fat diet brought about an increase in size of epididymal and subcutaneous sites, but not of the perirenal pads which showed an increase in number ( $\times 2.4$ ).

In female obese mice, the most important increase in pad weight can be observed in the parametrial site

( $\times 5.1$ ). In this group adipose cells were, in the three sites, threefold larger than those of the control group. There was no difference in cell size between the sites of each of the two groups. There was no hyperplasia in the subcutaneous pads, while the most significant increase in cell number was observed in the parametrial pads ( $\times 2.4$ ). This excess of weight in parametrial fats was the ef-

TABLE III  
Cellularity of Perirenal Adipose Tissue (P-RAT) of Female Rats Fed *ad lib.* the Control (T) or High-Fat Diet (S) when 5 months old and until the Age of 12 months

	5 months old, fed commercial pellets	12 months old	
		Diet T	Diet S
Number	8	9	9
Body wt, g	250 $\pm$ 12.3	275 $\pm$ 12.6	344 $\pm$ 11.6*
Wt of the 2 P-RAT, g	2.89 $\pm$ 0.187	4.22 $\pm$ 0.437	9.69 $\pm$ 1.04*
Adipocyte volume ( $\times 10^3 \mu^3$ )	579 $\pm$ 47.7	856 $\pm$ 82.4	1249 $\pm$ 94.3*
Adipocytes number (millions)	5.57 $\pm$ 0.403	5.43 $\pm$ 0.439	8.29 $\pm$ 0.563*
Caloric intake/24 hr	—	61.0 $\pm$ 1.22	64.3 $\pm$ 1.82 (NS)

\* Very significant ( $P < 0.01$ ) differences with the control group of the same age. NS,  $P > 0.05$ .

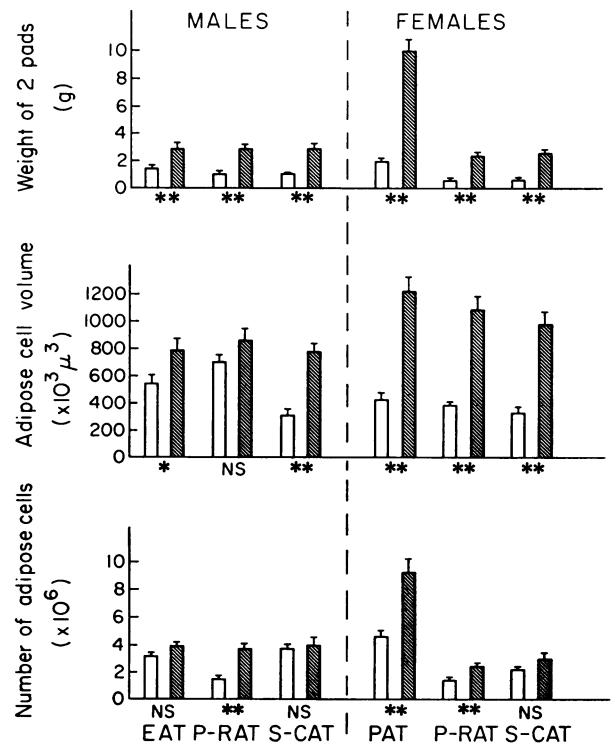


FIGURE 3 Cellularity in three sites of adipose tissue of 32 wk old male and female mice fed a control (T, open bar) or a high-fat (lined bar) diet. EAT, epididymal adipose tissue; PAT, parametrial adipose tissue; P-RAT, perirenal adipose tissue; S-CAT, abdominal subcutaneous adipose tissue. Vertical bar indicates mean  $\pm$  SEM; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; NS,  $P > 0.05$ . The body weight and number of animals were: for the males, group T, 48.2  $\pm$  1.81 (9), group S, 67.7  $\pm$  2.44 (10); for the females, group T, 34.6  $\pm$  0.70 (9), group S, 64.4  $\pm$  2.39 (11). (Mean  $\pm$  SEM.)

fect of both an increase in cell size and number, as observed.

## DISCUSSION

The growth of the adipose tissue of control Swiss mice is very similar to that of lean litter mates of the yellow obese mice (5) and of control rats (7, 9): postnatal growth results in both an increase in the size and number of the fat cells during the first 4 months of life. Later on, size and number appear to be stabilized. In the control rats, cell number was unchanged from the age of 5 to 12 months but cell size increased.

It is interesting to note the repercussion of a diet given to lactating mothers on the weight of their sucklings and on the growth of their sucklings adipose tissue when 2 wk old. At this age, the pups do not eat anything other than milk. These whose mothers receive the high-fat diet have doubly enlarged fat pads. There is a possibility that the observed changes in adipose tissue may reflect an increased caloric intake of the mothers and/or changes in milk production and composition induced by the high-fat diet. It is significant that this excess of weight is the effect of adipose cells enlargement not of hyperplasia. This nutritional obesity which appears very early is not produced by fat cell hyperplasia in young animals since one cannot find any difference in the number of fat cells in the two groups until the age of 4 months.

It has been claimed that after a predetermined age, the number of cells is fixed in most organs (22) and particularly in adipose tissue: the number of adipocytes cannot be changed in adults by dietary manipulations such as starvation or realimentation (9, 23). Male rats made obese by means of a high-fat diet lose some weight when returned to control diet; this affects epididymal fat cell volume but not number (10). This constancy is in agreement with the control group of mice. But a continuous and constant increase in fat cell number is seen in the group fed on a high-caloric diet. A similar figure is observed in the perirenal fat of the genetically obese Zucker (*fafa*) rat in which hyperplasia appears after the 14th week (7). In both cases there was a preceding fat cell enlargement followed by an increase in numbers suggesting formation of new fat cells in adults. But, the observed increase in fat cell number, induced genetically or by a diet, does not exclude that preformed cells were laid down in early life. The idea of the existence in early onset obesity of these preexisting fat cells (7, 9) has led to the categorization of human obesity according to the cellularity of adipose tissue, with early onset obesity characterized by a hyperplasia, and adult onset by a hypertrophy (1, 2).

The current studies with mice cannot determine whether new fat cells arise or whether preexisting cells deposit lipids in adult obese mice. The contribution of the

number of embryonic, or preexisting fat cells, to preadipocytes filled with small droplets of lipids has not been assessed in the undifferentiated zone of the 2-wk old mice. But, this zone was only slightly increased in the pads of mice suckled by mothers fed the high-fat diet, suggesting that fat cells might not be laid down in sufficient number at that early developmental stage. A more satisfactory explanation should be that a true hyperplasia is being observed in adults.

The following separate experiment supports this hypothesis: female mice given our high-fat diet when 6 wk old showed at 40 wk the same hyperplasia as those mice described in Table I; number of fat cells in control and high-fat diet groups were  $6.2 \pm 0.7 \times 10^8$  and  $12.3 \pm 0.9 \times 10^8$  respectively ( $n = 6$  in each group).

A further credence is provided by another experiment in rats: adult control rats fed our control diet did not show any increase in perirenal fat cell number between 5 and 12 months of age. But, when given ad lib. our high-fat diet at 5 months, a more than 50% increase in fat cell number was observed in this site 7 months later. These findings demonstrate the ability of adipose tissue to grow by an increase in adipocyte number in adults, and that adult cell number can be affected by dietary manipulations. They also suggest that, whereas the number of fat cells appears to be fixed in control adult animals, this is not the case in obese ones. However, if these findings strongly suggest that a true hyperplasia is being observed in animals rendered obese by a high-fat diet, radioactive methods are required to settle this important point. Indeed, the increase in cell number, as observed in rats, might be due to the development of preformed fat cells. This hypothesis should mean that preexisting fat cells exist not only in early onset obesity, but also in control animals, and that particular nutritional conditions, such as a high level of dietary fats, are able to trigger deposition of lipids in these preformed cells.

An other attempt to obtain experimental obesity in adults was conducted by Salans, Horton, and Sims in human volunteers. They did not observe any increase in adipocyte number (2). This discrepancy with our results may be the particular effect of the very high level of fat used in our experimental diet which was probably not the case for their human volunteers. Another source of explanation may be in the special difficulties encountered for the calculation of the total number of fat cells in human subjects: the principle of the method assumes an identical development of cell adipose sites, since only the size of the subcutaneous fat cells is taken into account to calculate cell number (1, 2). As long as the relative contribution and cellularity of main fat sites of the body to the total body mass is not defined, total numbers of adipocytes calculated from subcutaneous site will be a poor estimation. Moreover, a great site-to-site vari-

ability in the cell size of the subcutaneous site occurs in control and in obese humans (2, 18). As emphasized by the above-named authors, conclusions about cellularity in humans must be drawn with caution. This is particularly suggested by our observations in mice showing that intraabdominal and subcutaneous fat depots may be influenced differently in obesity (Fig. 3).

Knittle and Hirsch have shown that rats bred in litters of 4 and 22 differed in the size and number of epididymal adipose cells when 5 wk old (2 wk after weaning) and the differences became more marked with time (12). This method when used with rats, produced low body weight in the large litters (24). Furthermore, it is known that animals undernourished in early life show striking reductions in cell number in many kinds of tissues (22). Thus, these differences in fat cell number and size may be the effect of undernutrition. However, control animals of the same strain (9), as described by the same laboratory, closely approximate those of large litters in body weight, fat cell number, and size.

Aubert, Suquet, and Lemonnier have specified that mice, bred in litters of four, were overfed sucklings when compared with pups bred in nines. Although the mice were fed ad lib. since weaning, differences in body lipids in the groups increased from weaning to the age of 18 wk. At that age parametrial fat cells were enlarged and more numerous in mice from small litters when compared with those raised in group of nine. A third group of mice raised in litters of 20 showed only a cell size reduction (13). Increasing litter size did not reduce the fat cell number in these mice; this result may be due to the less striking effect of this experimental device in mice than in rats. These two works show that early nutritional manipulations induce changes in adult adipose cell number, although they do not demonstrate whether these effects occur before weaning, i.e., at the time of the manipulations. But, because differences increased later on, all these findings suggest that these changes observed in adults cannot be exclusively attributed to early nutrition, but must also be attributed to later feeding pattern and behavior which may be modified by varying litter size (25).

A similar situation was observed in obesity produced in our mice by a high-fat diet. Obesity increased with time: the excess of weight was due solely to hypertrophy in the first 4 months and then was amplified by hyperplasia of the fat cells. These obese mice demonstrate a marked caloric hyperphagia when adults (26). Therefore, one may suggest that the observed hyperplasia of adipose cells might be more a consequence of the recent level of food intake induced by early feeding pattern, than a direct effect of early nutrition.

However, hyperphagia does not necessarily induces fat cell hyperplasia. As shown by Hirsch and Han (9),

in the hypothalamic obesity of rat, there is no fat cell hyperplasia, although these animals are known to show the most striking hyperphagia. In contrast, rats fed a high-fat diet when adults showed a mild excess in their body weight and apparently a normal caloric intake, but adipose tissue weights were more than doubled and an increase in fat cell number was observed (Table III). Thus, the described hyperplasia was a specific effect of the level of fat in the diet. This effect on adipose tissue may be magnified in rats when the high-fat diet is introduced in early age; in these conditions they became, as the herein mice, markedly obese and hyperphagic (10, 26).

An other aspect of this study was the demonstration of sex and site differences in adipocyte cellularity induced by dietary manipulations.

In control male mice the three sites, epididymal, perirenal, and subcutaneous, differed as to their cell size. The high-fat diet produces hyperplasia limited to the perirenal site and hypertrophy in the two others, so that cell size is the same for the three sites. In the females, the high-caloric diet demonstrated more striking cell hypertrophy and hyperplasia. This may explain why growth curves are plateaued in males but not in females (27). These findings suggest also that in certain specific conditions there might be a maximum cell size, since for obese males as well as for obese females, the three adipose sites showed the same cell size. So, in each sex, adipose sites in the obese react to the diet in a site-specific way. The same is observed in the genetic obesity of the rat (6, 7); thus, mutant gene and diet produce similar effects on adipose tissue site cellularity.

The regulation of the adipose tissue mass is determined by two processes: formation of new fat cells and regulation of fat cell size. It is surprising to see that a single factor such as the fat diet content governs both formation and maturation of new fat cells as well as regulation of fat cell lipid content. These two processes may be separated as to the site or act on the same site.

#### ACKNOWLEDGMENTS

The author is indebted to André Alexiu and Cyrille Keko Pintos for their expert technical assistance, and to Dr. Robert Lowy for his statistical advice.

This work was supported by a Research grant (Contrat libre No. 71.5.219.12.) from the Institut National de la Santé et de la Recherche Médicale (France).

#### REFERENCES

1. Hirsch, J., and J. L. Knittle. 1970. Cellularity of obese and nonobese human adipose tissue. *Fed. Proc.* 29: 1516.
2. Salans, L. B., E. S. Horton, and A. H. Sims. 1971. Experimental obesity in man: cellular character of the adipose tissue. *J. Clin. Invest.* 50: 1005.
3. Hellman, B., I. B. Täljedal, and S. Westman. 1962. Morphological characteristics of the epididymal adipose



- tissue in normal and obese-hyperglycemic mice. *Acta Morphol. Neerl-Scand.* **5**: 182.
4. Lemonnier, D., J. Winand, J. Furnelle, and J. Christophe. 1971. Effects of a high-fat diet on obese-hyperglycemic and non-obese Bar-Harbor mice. *Diabetologia.* **7**: 328.
  5. Hellman, B., L. Thelander, and I. B. Täljedal. 1963. Postnatal growth of the epididymal adipose tissue in yellow obese mice. *Acta Anat.* **55**: 286.
  6. Lemonnier, D. 1971. Sex difference in the number of adipose cells from genetically obese rats. *Nature (Lond.)*. **231**: 50.
  7. Johnson, P. R., L. M. Zucker, J. A. F. Cruce, and J. Hirsch. 1971. The cellularity of adipose depots in the genetically obese Zucker rat. *J. Lipid Res.* **12**: 706.
  8. Hellman, B., I. B. Täljedal, and B. Petersson. 1962. Morphological characteristics of the epididymal adipose tissue in mice with obesity induced by goldthioglucose. *Med. Exp.* **6**: 402.
  9. Hirsch, J., and P. W. Han. 1969. Cellularity of rat adipose tissue: effects of growth, starvation, and obesity. *J. Lipid Res.* **10**: 77.
  10. Lemonnier, D., and A. Alexiu. 1970. Cellularité et caractères morphologiques du tissu adipeux du rat rendu obèse par un régime hyperlipidique. *Arch. Anat. Microsc. Morphol. Exp.* **59**: 1.
  11. Lemonnier, D. 1970. Augmentation du nombre et de la taille des cellules adipeuses dans l'obésité nutritionnelle de la souris. *Experienta (Basel)*. **26**: 974.
  12. Knittle, J. L., and J. Hirsch. 1968. Effect of early nutrition on the development of rat epididymal fat pads: cellularity and metabolism. *J. Clin. Invest.* **47**: 2091.
  13. Aubert, R., J. P. Suquet, and D. Lemonnier. 1971. Effets à long terme de trois niveaux d'ingesta lactés sur les lipides corporels, la taille et nombre de cellules adipeuses de la souris. *C. R. Hebd. Séances Acad. Sci. Ser. D. (Paris)*. **273**: 2636.
  14. Hirsch, J., and E. Gallian. 1968. Methods for the determination of adipose cell size in man and animals. *J. Lipid. Res.* **9**: 110.
  15. Sjöström, L., P. Björntorp, and J. Vråna. 1971. Microscopic fat cell size measurements on frozen-cut adipose tissue in comparison with automatic determinations of osmium-fixed fat cells. *J. Lipid. Res.* **12**: 521.
  16. Di Girolamo, M., S. Mendlinger, and J. W. Fertig. 1971. A simple method to determine fat cell size and number in four mammalian species. *Am. J. Physiol.* **221**: 850.
  17. Zucker, T. F., and L. M. Zucker. 1963. Fat accretion and growth in the rat. *J. Nutr.* **80**: 6.
  18. Bjurulf, P. 1959. Atherosclerosis and body-build with special reference to size and number of subcutaneous fat cells. *Acta Med. Scand.* **166**: Suppl. 349.
  19. Zingg, W., A. Angel, and M. D. Steinberg. 1962. Studies on the number and volume of fat cells in adipose tissue. *Can. J. Biochem. Physiol.* **40**: 437.
  20. Goldrick, R. B. 1967. Morphological changes in the adipocyte during fat deposition and mobilisation. *Am. J. Physiol.* **212**: 777.
  21. Gepts, W. 1958. A propos des méthodes de détermination quantitative du tissu insulaire. *C. R. Soc. Biol.* **152**: 879.
  22. Winick, M., and A. Noble. 1966. Cellular response in rats during malnutrition at various ages. *J. Nutr.* **89**: 300.
  23. Hollenberg, C. H., A. Vost, and R. L. Patten. 1970. Regulation of adipose mass: control of fat cell development and lipid content. *Rec. Prog. Horm. Res.* **26**: 463.
  24. Widdowson, E. M., and R. A. McCance. 1960. Some effects of accelerating growth. I. General somatic development. *Proc. R. Soc. Lond. B. Biol. Sci.* **152**: 188.
  25. Seitz, P. F. D. 1954. The effects of infantile experiences upon adult behavior in animal subjects. I. Effects of litter size during infancy upon adult behavior in the rat. *J. Psychiatr. Res.* **110**: 916.
  26. Lemonnier, D. 1967. Aspects physiologiques de l'obésité expérimentale produite par des régimes hyperlipidiques. *Cah. Nutr. Diet.* **11**: 29.
  27. Lemonnier, D. 1967. Obésité par des régimes hyperlipidiques chez le rat et la souris. *Nutr. Dieta* **9**: 27.