Dietary-induced obesity: effect of dietary fats on adipose tissue cellularity in mice

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1. Male and female mice, 4 weeks old, were fed *ad lib.* diets containing various amounts of lard (0–300 g/kg) or various kinds of dietary fats (300 g/kg) for 13 weeks. Fat cell number and size were determined by a histological method in three different adipose sites.

2. Lard at 200 g/kg diet (43% energy from lipids) was sufficient to promote fat cell hyperplasia in the parametrial fat. Hyperplasia was also observed in the subcutaneous fat in males. The relationship between fat cell hypertrophy and the level of lard in the diet was dependent on site and sex.

3. Obesity was produced whatever the kind of dietary fat eaten: lard, beef tallow, sunflower oil or soya-bean oil. In the subcutaneous depot of males given lard, fat cell size and number were increased, but only cell hypertrophy was observed in those given soya-bean oil. In the female groups of mice fat cell hyperplasia or hypertrophy or both were related to the adipose site but not the kind of dietary fat.

4. It is concluded that dietary fats of different origin can induce obesity in mice. The effects on adipose tissue cellularity depend on the levels and kind of fat eaten, the adipose site and sex.

One of the simplest ways of producing obese animals is to feed high-fat diets.

High dietary levels of lipids induced either adipose cell enlargement or fat cell hyperplasia or both, depending on the site and sex in adult mice and rats (Lemonnier, 1972). Furthermore, the extent of obesity attained in rats (Schemmel *et al.* 1970; Faust *et al.* 1978; Miller, 1979) and mice on high-fat diets (Fenton & Carr, 1951) varied with genetic constitution.

In most studies concerning dietary-induced obesity in adult animals, large quantities of lipid, between 400 and 600 g/kg, were fed. Although the type of fat used varied (lard, rich in mono-unsaturated and saturated fatty acids, was employed the most frequently) it has been suggested that obesity can be induced easily with diets enriched with solid fats but only infrequently with diets containing vegetable oils rich in polyunsaturated fatty acids (Dryden *et al.* 1956; Barboriak *et al.* 1958). According to certain authors, the nature of dietary fats has an effect on adipose tissue cellularity. Lemonnier *et al.* (1973) found that in adult rats, diets rich in saturated fatty acids promoted fat cell hyperplasia, while diets rich in unsaturated fatty acids promoted fat cell hypertrophy. The reverse was reported by Raulin *et al.* (1974).

The purposes of this investigation were to determine (1) the minimum level of dietary fat which induces fat cell hyperplasia and (2) the effect of different kinds of dietary fat on cellularity at several adipose sites.

NMRI mice were used in this study since increases in fat cell number produced by a high-fat diet had been reported to occur much earlier in these animals than in other strains of mice (Herberg *et al.* 1974).

METHODS

Diets and animals

Male and female NMRI mice were weaned at 4 weeks of age and housed five to a polypropylene cage, in a room with an ambient temperature of $22 \pm 1°$ and 12 h light.

Table 1. *Composition of diets (g/kg)*

Ingredient	Diets†				
	C	L 50	L 100	L 200	L 300
Bran	30	32	35	39	42
DL-methionine	4·0	3·0	2·0	1·0	0·5
Mineral salts	39	43	46	52	56
Vitamin mixture	21	24	25	29	31
Lard	0	50	102	205	300
Casein	121	140	170	219	270
Wheat flour	785	708	620	455	301
Percentage energy as					
Protein	21·6	21·3	21·8	21·8	22·3
Lipid	6·1	16·9	26·8	43·5	55·9
Carbohydrate	72·3	61·8	51·5	34·7	21·8
Energy (MJ/kg)‡	13·96	14·83	15·71	17·43	19·15

† The diets contained the same quantity of proteins, added vitamins, and mineral salts/J.
‡ Calculated values.

Animals had *ad lib.* access to food and water. Diets were distributed every 2 d and body-weights were recorded weekly.

In the first experiment, from weaning to 17 weeks of age, mice of both sexes were given either a control diet (C) or diets enriched with 50, 100, 200 or 300 g lard/kg. In the second experiment, also from weaning to 17 weeks of age, four high-fat diets (300 g/kg) were employed containing lard (L 30), or beef tallow (B 30) or soya-bean oil (SO 30) or sunflower oil (SF 30). These particular fats were used because lard and beef tallow are rich in both saturated and mono-unsaturated fatty acids, while soya-bean oil (which contains greater quantities of tri-unsaturated fatty acids than sunflower oil) and sunflower oil are rich in polyunsaturated fatty acids. The control diet (C) which was prepared in the laboratory contained approximately 30 g lipids/kg.

Diet compositions are shown in Table 1. The salt mixture (US Pharmacopea 14) was purchased from ICN Nutritional Biochemicals (Hersham, Surrey) and the vitamin mixture was prepared in the laboratory (Lemonnier & Alexiu, 1974).

Cellularity determinations

All animals were killed by decapitation at 17 weeks of age. Genital, retroperitoneal and abdominal subcutaneous fat pads were dissected out, weighed and prepared using a histological procedure (as described by Lemonnier, 1972) for determination of fat cell size and number. Paraffin sections of adipose tissue (15 μm thick) were stained according to Bjurulf (1959) and placed onto the ground glass of a Projectina microscope. The adipose cells were then counted at a magnification of $\times$ 130. Mean fat cell volume (V) was calculated from the mean fat cell surface area (S) determined on the ground glass (taking account of the magnification) by the formula $V = 4\,S^{3/2}/(3\sqrt{\pi})$. In this method it is assumed that cells are spherical and that their distribution was symmetrical and similar in all groups and tissues. The results showed that this histological method agrees very closely with other methods in the literature (Lemonnier, 1981), although no account is taken of the fact that larger fat cells are more likely to be intersected than small cells. Fat cell number was estimated from the ratio, fat pad weight : mean adipose cell volume $\times$ 0·92 (0·92 was the measured density of the pads).

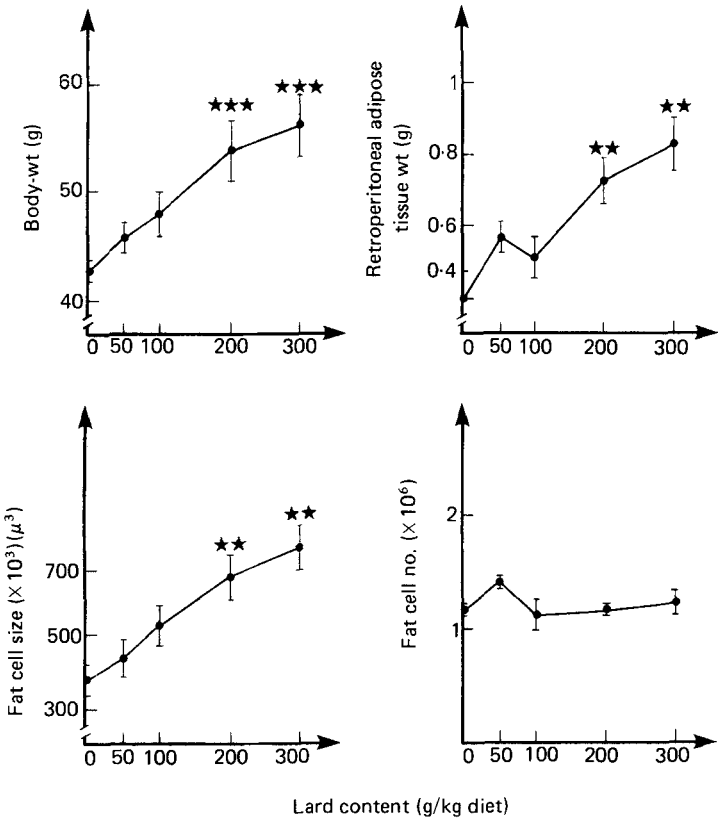

Fig. 1. Body-weight and retroperitoneal adipose tissue cellularity of male NMRI mice fed *ad lib.* diets differing in lard content (0–300 g/kg) from weaning to 17 weeks of age. Points are mean values with their standard errors represented by vertical bars. Seven to nine mice per group. Values were significantly different from those for the control diet (C) 30 g lipid/kg: ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

Mean values and their standard errors were calculated for each group of animals. Statistically significant differences between groups were determined by analysis of variance.

RESULTS

Expt 1. Body-weight and adipose tissue cellularity of mice fed different percentages of dietary fat.

Body-weight. The body-weights of mice aged 17 weeks given different amounts of lard are shown in Fig. 1 (males) and 2 (females). Increases in body-weight were proportional to the increasing level of dietary fat. Differences in body-weight compared to controls given diet C were highly significant ($P < 0{\cdot}001$) for male mice given 200 and 300 g lard/kg and in females given 100 g lard/kg or more.

Cellularity. The weights of retroperitoneal (Fig. 1) and parametrial (Fig. 2) adipose tissues also increased in proportion to the fat content of the diet. Weight gain in the retroperitoneal fat depots of male mice resulted exclusively from an increase in adipocyte size which increased in proportion to the level of dietary lard. This increase in adipocyte size was significant ($P < 0{\cdot}01$) for diets containing 200 and 300 g lard/kg. In the parametrial fat depots of female mice, however, weight gain was attributable to an increase in both fat cell size and number. Moreover, fat cell size in this tissue was at a maximum in females

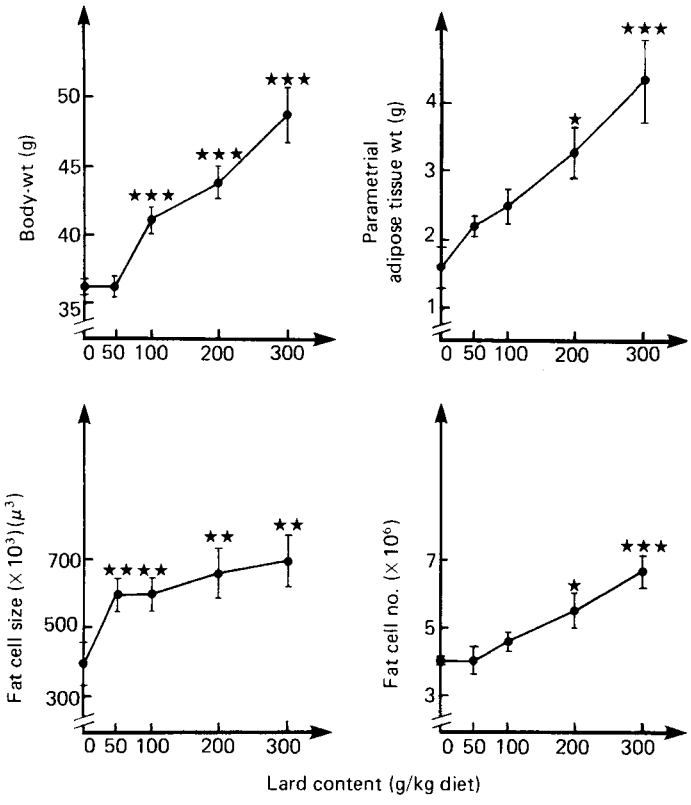

Fig. 2. Body-weight and parametrial adipose tissue cellularity of female mice fed *ad lib.* diets differing in lard content (0–300 g/kg) from weaning to 17 weeks of age. Points are mean values with their standard errors represented by vertical bars. Five to eight mice per group. Values were significantly different from those for the control diet (C) 30 g lipid/kg: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

given the 50 g lard/kg diet, while fat cell hyperplasia was significant for the 200 and 300 g/kg level ($P < 0{\cdot}05$). Therefore in this latter tissue, hypertrophy appeared first and hyperplasia followed.

The cellularity of three fat depots of male and female mice aged 17 weeks and given the 300 g lard/kg diet, is illustrated in Fig. 3. The results show that the extent of hypertrophy or hyperplasia or both varied according to the site or sex or both. In males given the high-fat diet, the greatest increase in pad weight was observed in the subcutaneous depot, due to an increase in both cell size and number but, as for control animals, fat cell size in this tissue was the smallest. As in retroperitoneal pads hypertrophy only was observed in male epididymal fat pads.

In females given the high-fat diet, weight gain was greatest at retroperitoneal and parametrial sites. In fact, parametrial adipose tissue represented 9% body-weight. Unlike parametrial tissue, retroperitoneal and subcutaneous adipose tissue displayed hypertrophy only. Mean cell size at the three sites in obese females was similar.

Expt. 2. Body-weight and adipose tissue cellularity as affected by the nature of dietary fats

Body-weight. Tables 2 (females) and 3 (males) show that different types of dietary lipids (lard, beef tallow, soya-bean and sunflower oils) induced obesity in mice. Differences in

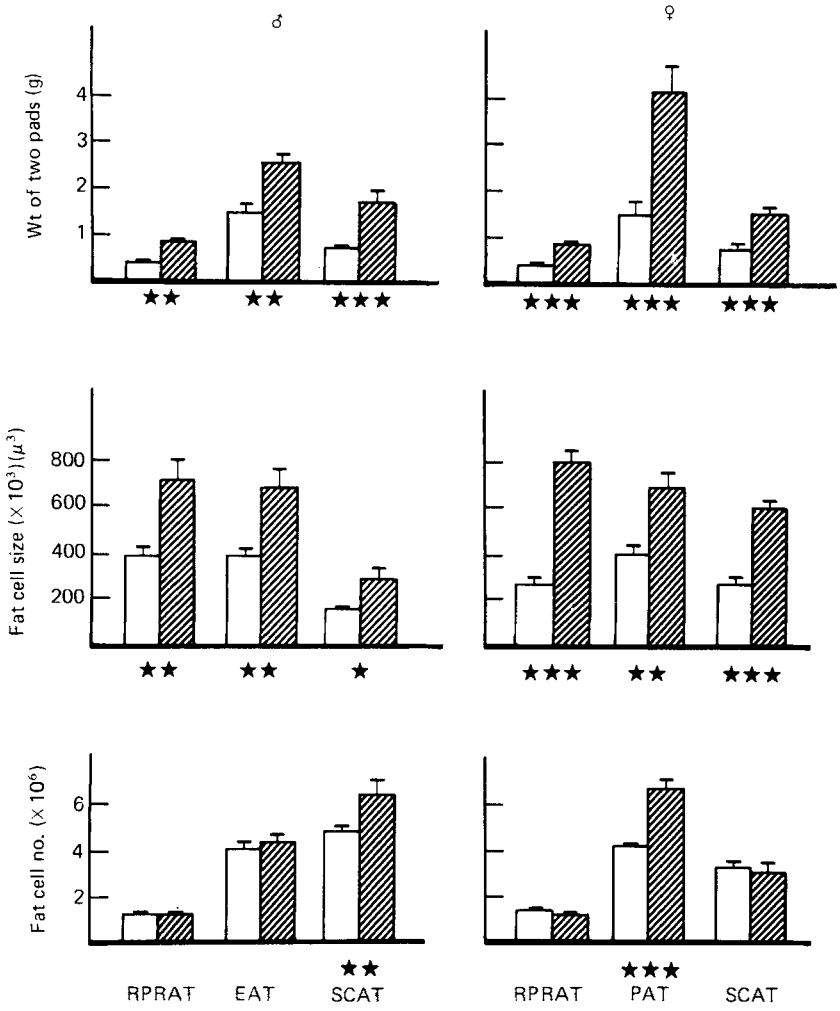

Fig. 3. Cellularity of three adipose tissues of 17 week-old male and female NMRI mice fed *ad lib.* either a control diet (C) 30 g lipid/kg □ or a high-fat diet (L) 300 g lard/kg ▨ from weaning. Points are mean values with their standard errors represented by vertical bars for seven to twelve males per group and six to eight females per group. RPRAT, retroperitoneal adipose tissue; EAT, epididymal adipose tissue; PAT, parametrial adipose tissue; SCAT, abdominal subcutaneous adipose tissue. Values were significantly different: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

body-weight between mice given these high-fat diets (300 g/kg) were observed in females only. Compared to control animals, females given the beef-tallow diet gained much more weight (+44%) than those given the sunflower-oil diet (+22%). The differences in weight gain between the latter two high-fat groups were significant ($P < 0{\cdot}01$). Weight gains of females given lard or soya-bean oil diet were comparable (+33%).

Adipose tissue. Weight gain in female parametrial adipose tissue (Table 2) was greatest (+242%) in animals given the beef-tallow diet and least (+160%) in animals given the sunflower-oil diet. All high-fat diets induced fat cell hyperplasia associated with significant fat cell hypertrophy, except the sunflower diet where hypertrophy was insignificant. The increase in fat cell number was greatest on the beef-tallow diet and fat cell size was signi-

Table 2*a*. *Expt. 2. Body-weight and parametrial adipose tissue cellularity in the female mice given either a control diet (C) or high-fat diets (300 g/kg) containing lard (L 30) or beef tallow (B 30) or soya-bean oil (SO 30) or sunflower oil (SF 30) from weaning to 17 weeks of age*

(Mean values with their standard errors)

Diets†	Animals (no.)	Body-wt (g)		Adipose tissue wt (g)		Fat cell volume ($\times 10^3$) (μ^3)		Fat cell no. ($\times 10^6$)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
C	6	36	0·6	1·53	0·329	388	66·2	3·98	0·107
L 30	8	48	2·0**	4·15	0·580***	681	75·4**	6·63	0·453***
B 30	7	52	2·1***	5·23	0·584***	771	49·4***	7·32	0·713***
SO 30	7	48	2·7***	4·35	0·658***	842	78·0***	5·60	0·655*
SF 30	7	44	0·6***	3·08	0·195*	567	25·7	5·90	0·410**

Mean values were significantly different from those for the group given diet C: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

† For details, see Table 1.

Table 2*b*. *Analysis of variance: Statistical comparisons between groups of mice given different high-fat diets (L 30, B 30, SO 30, SF 30)*

	Body-wt	Adipose tissue wt	Fat cell volume	Fat cell no.
L 30 *v.* B 30	—	*	—	*
L 30 *v.* SO 30	—	—	—	—
L 30 *v.* SF 30	—	—	—	—
B 30 *v.* SO 30	—	*	—	***
B 30 *v.* SF 30	*	***	*	***
SO 30 *v.* SF 30	—	—	**	—

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

ficantly larger in females given diets rich in beef-tallow or soya-bean oil than in those given sunflower oil.

Weight gain in the male retroperitoneal pad (Table 3) was similar and significant for mice given sunflower oil (+94%) or soya-bean oil (+121%) or lard (+97%) but insignificant (compared to controls) for animals given the beef-tallow diet (+52%).

The observed differences in pad weight were due to fat cell hypertrophy, cell number remaining unchanged. Fat cells from the soya-bean oil group were consistently larger than those of the sunflower oil ($P < 0·01$) and lard groups ($P < 0·05$). Adipocyte size, however, did not differ significantly in males given control or beef-tallow diets.

It was observed in this experiment (although the findings are not presented here) that cellular response to a high-fat diet, in the male epididymal pad and in the female retroperitoneal pad, does not depend on the nature of the fat ingested, and that excessive development at these sites was due to hypertrophy alone. In contrast, the changes in cell number produced by a high-fat diet in the subcutaneous pad varied with the nature of the constituent fat and sex.

Fig. 4 shows comparisons of subcutaneous adipose cellularity in mice given high-fat diets (300 g/kg) containing lard or soya-bean oil. Male animals given the lard diet showed an increase in both fat cell size and number, while only hypertrophy occurred in males given

Table 3*a*. *Expt. 2. Body-weight and retroperitoneal adipose tissue cellularity in the male mice given either a control diet (C) or high-fat diets (300 g/kg) containing lard (L 30) or beef tallow (B 30) or soya-bean oil (SO 30) or sunflower oil (SF 30) from weaning to 17 weeks of age*

(Mean values with their standard errors)

Diets†	Animals (no.)	Body-wt (g)		Adipose tissue wt (g)		Fat cell volume ($\times 10^3$) (μ^3)		Fat cell no. ($\times 10^6$)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
C	7	43	1·1	0·414	0·0593	392	55·1	1·15	0·076
L 30	8	56	3·1***	0·815	0·0838**	742	70·4**	1·22	0·128
B 30	10	55	3·0***	0·629	0·0863	523	57·7	1·29	0·114
SO 30	8	53	1·1***	0·915	0·0874**	908	62·6***	1·12	0·110
SF 30	9	51	2·6*	0·804	0·109 **	656	93·5**	1·36	0·112

Mean values were significantly different from those for the group given diet C: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

† For details, see Table 1.

Table 3*b*. *Analysis of variance: statistical comparisons between groups of mice given different high-fat diets (L 30, B 30, SO 30, SF 30)*

	Body-wt	Adipose tissue wt	Fat cell volume	Fat cell no.
L 30 *v.* B 30	—	*	—	—
L 30 *v.* SO 30	—	—	*	—
L 30 *v.* SF 30	—	—	—	—
B 30 *v.* SO 30	—	**	***	—
B 30 *v.* SF 30	—	—	—	—
SO 30 *v.* SF 30	—	—	**	—

* $P < 0·05$. ** $P < 0·01$, *** $P < 0·001$.

the soya-bean oil diet. On the other hand, in female mice the nature of the dietary lipids fed had no effect on subcutaneous fat cell number; the observed weight gains resulting only from increases in fat cell size.

DISCUSSION

In this study, the importance of quantity and composition of dietary fat on middle-term adipose tissue development in mice was emphasized.

The findings of Expt 1 show that moderate levels of fat in the diet, administered from weaning to 17 weeks of age, were sufficient to promote fat cell hyperplasia in mice. We have defined a level of dietary fat at which fat cell hyperplasia occurred significantly in parametrial adipose tissue: 200 g lard/kg diet, representing 43% energy from lipids. Also, hypertrophy occurred first, followed by hyperplasia at this site. Fat cell number increased once adipocyte size had reached a maximum, which suggests that a critical adipocyte size might be a stimulus for the appearance of new adipocytes (Lemonnier & Alexiu, 1974; Kral, 1976; Häger *et al.* 1977; Faust *et al.* 1978). The cellular response of adipose tissue to the high-fat diet differed according to site and at the same site according to sex (Fig. 4), supporting previous observations in Swiss mice (Lemonnier, 1972). An increase in fat cell number was not observed in all fat pads studied. This occurred only in parametrial adipose

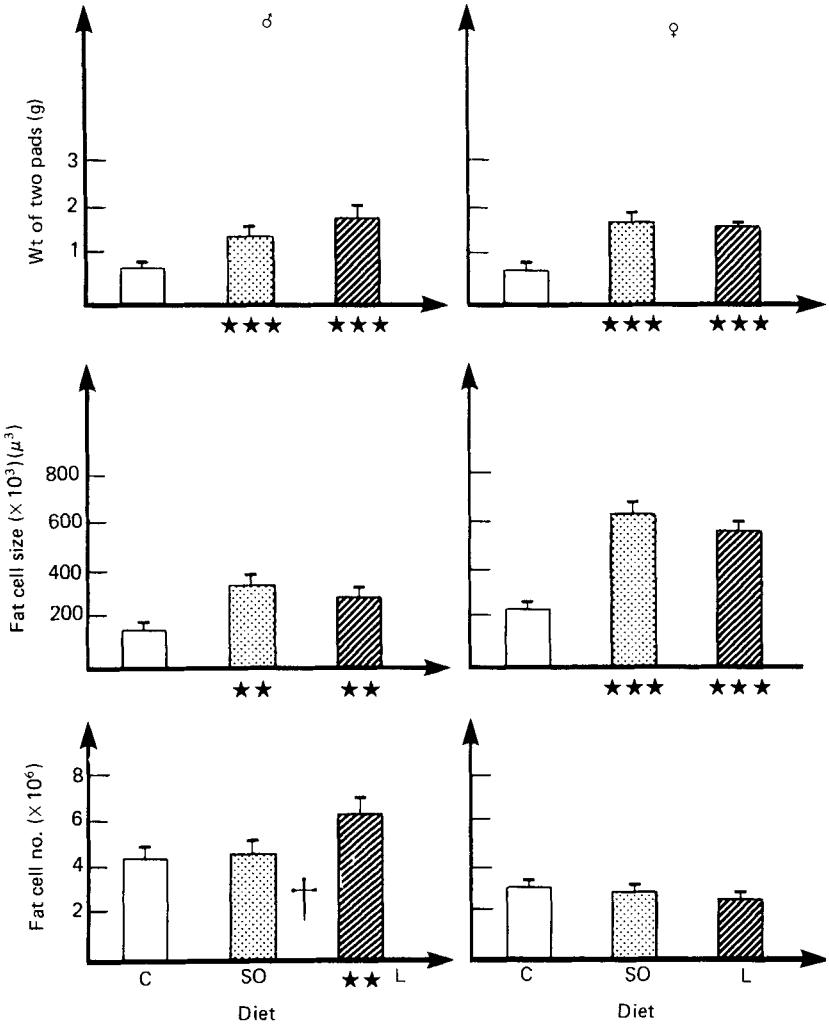

Fig. 4. Abdominal subcutaneous adipose tissue cellularity of male and female NMRI mice fed *ad lib.* either a control diet (C) 30 g lipid/kg □ or high-fat diets (300 g lipid/kg) enriched with soya-bean oil (SO) ▨ or lard (L) ▩ from weaning to 17 weeks of age. Points are mean values with their standard errors represented by vertical bars for six to twelve mice per group. Values were significantly different from those for the control diet: ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$. Values of the group (SO) were significantly different from those for the group (L): † $P < 0{\cdot}05$.

tissue of female mice and subcutaneous adipose tissue of male mice. Weight gains at all other depots resulted from an increase in fat cell size. In male retroperitoneal tissue, the increase in fat cell size was proportional to the lipid concentration of the diet given. We also found that epididymal weight gain was due to hypertrophy rather than an increase in cell number. In general, the epididymal pad is considered to be least sensitive to stimuli known to induce fat cell hyperplasia in adipose tissue.

The increase in fat cell number induced by a high-fat diet could result from the production of new adipose cells rather than from the differentiation of pre-existing precursor cells. This was demonstrated by direct measurement of mitotic activity, i.e. the incorporation of tritiated thymidine into DNA of adipocytes was shown to increase in adult rats given a

high-fat diet (Klyde & Hirsch, 1979; F. Bourgeois, C. Doucet & D. Lemonnier, unpublished results).

The findings of Expt 2 show that obesity, defined as a great accumulation of adipose tissue (Mayer, 1970), develops as easily in NMRI mice given high-fat diets containing vegetable oils or diets containing lipids of animal origin. Nevertheless, the composition of the dietary fat can influence adipose tissue cellularity in various ways. Hyperplasia associated with hypertrophy was observed in the subcutaneous fat pads of male mice given a lard diet, while fat cell hypertrophy only occurred in the same tissue of male mice given the soya-bean oil diet. These results agree with findings in rat retroperitoneal adipose tissue reported by Lemonnier *et al.* (1973), who suggested that an excess of dietary polyunsaturated fatty acids would induce an increase in fat cell size, while an excess of dietary saturated fatty acids would induce an increase in fat cell number. However, such changes in cell number, varying with the nature of the lipids fed, were not observed in female subcutaneous adipose tissue nor at other adipose sites. All dietary fats used and particularly beef tallow, provoked fat cell hyperplasia in female parametrial adipose tissue. Thus, cellular response of adipose tissue to fat type depends on site or sex or both.

According to Kirtland & Gurr (1978) adipose cellularity was not influenced by the nature of the dietary fat but was sensitive to energy intake before 12 weeks of age in the rat. As the energy intakes of our animals were not measured, differences in the observable number of adipocytes could be due to differences in energy intake. However, several previous studies have shown that there was no systematic relationship between fat cell hyperplasia and an increase in energy intake in different kinds of obesity. Lemonnier & Alexiu (1974) reported that a marked hyperplasia and a marked increase in energy intake occurred in 10-month-old mice given a high-fat diet. In contrast, an increase in fat cell number was induced by a high-fat diet without any significant increase in energy intake in rats (Lemonnier, 1972) and in mice (Herberg *et al.* 1974). In ventro-medial hypothalamic-lesioned rats, known for their hyperphagia, a dramatic adipocyte hypertrophy without hyperplasia has been observed (Hirsch & Han, 1969). After an adrenalectomy in fa/fa obese rats energy intake and fat cell size decreased, while the adipocyte number still increased (Yukimura & Bray, 1978). Thus, fat cell hyperplasia does not seem necessarily to be related to energy intake. Moreover, it cannot explain the different effects of localization, sex and composition of fat diet on cell number in our present study.

At present, the relationship between the high-fat diet and fat cell number is unknown. However, it is well established that dietary-induced obesity results from direct accumulation of fatty acids, and that long-term administration of a high-fat diet promotes a decrease in lipogenesis and subsequently an increase in adipose tissue lipoprotein lipase activity (De Gasquet *et al.* 1974; Lemonnier *et al.* 1975). Lipoprotein lipase activity in several adipose tissues of normal rats has been shown to increase proportionally with dietary lipid concentration (Pascal *et al.* 1977).

Studies have shown that lipids rich in polyunsaturated fatty acids induce an increase in lipoprotein lipase activity in rat epididymal fat (Pawar & Tidwell, 1968*a*) and in retroperitoneal and epididymal fat of guinea pigs (Cryer *et al.* 1978). Moreover, it has been demonstrated that the capacity of the epididymal fat pad to liberate fatty acids and glycerol is decreased in rats given a diet rich in polyunsaturated fatty acids (Pawar & Tidwell, 1968*b*).

In order to understand why each adipose depot reacts differently to a high-fat diet, it would be necessary to evaluate the influence of environmental factors such as local blood supply, which might directly or indirectly affect cell proliferation and filling. In consequence, a better knowledge of adipocyte formation is paramount.

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