

The effect of different dietary fats on fat cell size and number in rat epididymal fat pad

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1. Rats were given diets containing different fats (200 g/kg) from weaning to 8 months of age. The fats were sunflower-seed oil (SFO), lard (L) and an equal mixture of sunflower-seed oil and lard (mixed-fat diet; MF). A control group was given the basal diet unsupplemented with fat. Energy intake and body-weight of individual animals were recorded weekly.

2. Three animals from each dietary group were killed after 5, 13, 17 and 29 weeks on the experimental diets. At slaughter epididymal fat pads were excised, weighed and the number and size of fat cells in the pad was determined.

3. There were no significant differences between the energy intake in the whole experimental feeding period for rats given the different fat diets. However, when energy intakes were examined on a monthly basis animals given MF had a significantly higher energy intake in the first 2 months of feeding than those animals given SFO. In the third month of feeding (when animals were over 12 weeks old) the group given L had a significantly higher intake than the group given SFO.

4. Body-weight, epididymal-fat-pad weight, cell size and cell number did not differ significantly between the 'fat-fed' animals at any individual slaughter period. Analysis of pooled values from the four slaughter periods revealed that animals given MF had significantly more fat cells than those given SFO.

5. We conclude that the nature of the dietary fat has no effect on the cellularity of developing adipose tissue but that the number of fat cells formed is sensitive to energy intake before 12 weeks of age in the rat.

Certain diseases prevalent in affluent countries have been attributed to changes in dietary patterns (see Vergroesen, 1975). Particular emphasis has been placed on dietary fats and vascular disease and where recommendations have been made by governmental or medical organizations these invariably include a paragraph advising modification of the fat composition of the diet. In the Netherlands, for example, it is recommended that saturated fat intake should be replaced to a considerable extent by polyunsaturated fat, of which linoleic acid is the major component.

It was recently reported by Widdowson and her colleagues (Widdowson, Dauncey, Gairdner, Jonxis & Pelikan-Filipkova, 1975) that the depot fat of Dutch babies had a very high linoleic acid content compared with their British counterparts. This difference might be attributed to the different fatty acid composition of the proprietary brands of infant formula foods commonly available in the two countries (Widdowson, 1973).

The findings of Raulin & Launay (1967) suggested that a high intake of linoleic acid early in life stimulated the production of fat cells in the rat. Because of the obvious implications for man, we have investigated the effects of feeding different fats on fat cell size, number and fatty acid composition in young rats and guinea pigs. The experiments with rats are described here and show that, although the fatty acid composition of fat in the epididymal fat pad depends on the composition of dietary fat, the number of fat cells is not influenced by the nature of the fat fed but on the energy intake in early life.

Table 1. *Fatty acid composition (g/100 g) of dietary and adipose tissue triglyceride of animals given diets containing different fats for 29 weeks*

(Values for adipose tissue are mean values from three animals with standard errors in parentheses)

Dietary group*	Fatty acid (g/100 g)									
	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	Others
Basal (control)										
Diet	4.6	4.6	21.7	2.1	5.3	27.1	27.3	3.4		3.9
Adipose tissue	2.0 (0.05)	2.0 (0.03)	26.2 (0.22)	2.6 (0.13)	3.2 (0.07)	32.9 (0.42)	26.6 (0.29)	1.2 (0.08)		3.3 (0.21)
MF										
Diet	0.8	1.5	18.6	1.4	10.4	28.4	35.5	1.4	0.1	1.9
Adipose tissue	0.7 (0.03)	1.0 (0.03)	19.6 (0.38)	2.0 (0.10)	4.6 (0.14)	32.8 (0.17)	37.1 (0.25)	0.3 (0.07)	0.6 (0.10)	1.3 (0.44)
L										
Diet	0.8	2.1	27.4	2.4	16.5	36.4	10.0	1.8	0.3	2.3
Adipose tissue	0.5 (0.03)	1.2 (0.05)	24.8 (0.45)	4.2 (0.77)	6.2 (0.58)	49.0 (0.61)	11.3 (0.43)	0.5 (0.05)	0.4 (0.07)	1.9 (0.14)
SFO										
Diet	0.8	0.8	9.8	0.3	4.3	20.4	61.0	1.0		1.6
Adipose tissue	0.1 (0)	0.6 (0.05)	11.2 (0.28)	0.9 (0.27)	3.0 (0.19)	21.8 (0.31)	59.2 (0.87)	0.4 (0.07)	1.2 (0.12)	1.6 (0.30)

MF, mixed fat; L, lard; SFO, sunflower-seed oil.

* For details, see below.

EXPERIMENTAL

Animals

Male litter mate rats of the Colworth Wistar strain were assigned to one of four different dietary groups when weaned at 3 weeks of age. Each animal was housed individually with free access to food and water. The body-weights of the animals, food and water intake were recorded weekly.

Diets

The basal diet was compounded by Tyrell Byford and Pallet Ltd, Norfolk, and had the following crude composition (g/kg): crude protein (nitrogen $\times 6.25$) 257, fat 48, carbohydrate 518, ash 72, moisture 105. Three high-fat diets contained (g/kg) 800 crushed basal diet and 200 lard (L), sunflower-seed oil (SFO) or 100 L and 100 SFO (mixed-fat; MF). The energy values of the basal and high-fat diets were 0.80 and 1.07 MJ/g respectively. The fatty acid compositions of the diets are given in Table 1.

Procedure

Three animals in each dietary group were killed after 5, 13, 17 and 29 weeks on the experimental diets. Epididymal fat pads were excised and weighed, and tissue from the middle of one pad from each animal was taken for determination of fat cell size. In the 32-week-old animals another piece of tissue was taken for extraction of triglyceride. Isolated fat cells were prepared from the remaining tissue (Rodbell, 1964) and the DNA extracted by the method of Hollenberg & Vost (1968). DNA was determined by the diphenylamine method (Burton, 1956) using calf thymus DNA as a standard.

Fat cell size and number

Fat cell diameter was measured by a modification of the method of Sjöström, Björntorp & Vrána (1971) as described by Kirtland, Gurr, Saville & Widdowson (1975). From these values the mean cell volume, surface area and triglyceride content/cell were calculated by

Table 2. Monthly energy intakes (MJ) of rats given diets containing different fats

(Mean values adjusted for litter differences and standard deviations for each observation)

Dietary group*	Period on diet (weeks)							
	1-5	6-9	10-13	14-17	18-21	22-25	26-29	1-29
Basal (control)	8.51 ^a	9.16 ^c	8.34 ^c	8.90 ^d	9.33 ^e	9.16 ^e	8.04 ^e	60.46 ^e
MF	9.99 ^b	10.85 ^b	9.61	8.43	10.12	10.69	10.23	74.03
L	9.90	10.53	10.12	10.15	10.81	11.06	10.47	74.41
SFO	9.13	10.05	9.23 ^e	10.24	10.46	11.00	10.58	70.63
SD	0.82	0.60	0.68	0.76	0.69	0.41	0.41	3.09
No. of animals (per dietary group)	9	6	9	6	3	3	3	12

Superscripts: *a*, different from L and MF ($P < 0.05$); *b*, different from SFO ($P < 0.05$); *c*, different from L, MF and SFO ($P < 0.05$); *d*, different from L and SFO ($P < 0.05$); *e*, different from L ($P < 0.05$).

MF, mixed-fat; L, lard; SFO, sunflower-seed oil.

* For details, see p. 20.

computer, assuming the adipocytes to be spherical (Sjöström *et al.* 1971; Lemonnier, 1972). Fat cell number was determined by dividing triglyceride content/pad by triglyceride content/cell (Kirtland *et al.* 1975). For comparison, the number of cells was also calculated from the DNA content of isolated fat cells prepared from whole tissue (Rodbell, 1964) assuming that 10^6 cells contain $6.2 \mu\text{g}$ DNA (Enesco & Leblond, 1962).

Fatty acid analysis

Triglycerides were converted into fatty acid methyl esters by refluxing with toluene-methanol-conc. sulphuric acid (20:10:1, by vol.). Esters were extracted into diethyl ether, dried over anhydrous magnesium sulphate and analysed by gas-liquid chromatography (GLC, Pye model 104, Pye-Unicam Ltd, Cambridge, UK) using a column of 100 g DEGS-PS/900 g Supelcoport (Supelco, Chromatography Services Ltd, Bebington, Merseyside, UK) at 200° . Output was analysed using a chromatographic data processor (Digital Equipment Corporation Inc.).

Analysis of results

Values obtained from weekly records of body-weight, energy and water intake were analysed on a monthly basis, except that the values for the first month were obtained by pooling results from the first 5 weeks of feeding.

The results were analysed by normal analysis of variance. Initial analysis of values obtained for cell size and number showed that some values did not have a normal distribution. Therefore a non-parametric method of analysis was used that made no assumption about the distribution of results but considered only their relative rankings (Bernard & Van Elteren, 1973).

RESULTS AND DISCUSSION

Fatty acid composition of adipose tissue

It is well established that the fatty acid composition of the diet can affect the fatty acid composition of the body (Carroll, 1965). As shown in Table 1, after the animals had been fed on the experimental diets for 29 weeks, the fatty acid composition of epididymal fat was very similar to that of their respective diets (the notable exception in each dietary group being the apparent transformation of dietary stearic acid into oleic acid). Thus linoleic acid comprised 59.2 g/100 g adipose tissue fatty acids after feeding SFO, but only 11.3 g/100 g after feeding L.

Table 3. *Weights of animals and epididymal fat pads after feeding diets containing different fats for different time periods*

(Mean values and standard deviations for each observation. Body-weights are shown for the slaughtered animals (a), and for the whole group of animals on each diet (b), in the latter case adjustment was made for litter differences. No. of rats/group is given in parentheses except for the slaughtered animals where there were three animals/dietary group)

Dietary group†	Period on diet (weeks)											
	0	5		13		17		29				
	Body-wt (g) (b)	Wt of fat pad (g)	Body-wt (g) (a) (b)		Wt of fat pad (g)	Body-wt (g) (a) (b)		Wt of fat pad (g)	Body-wt (g) (a) (b)			
Basal (control)	50.5 (12)	1.03	194	203 (27)	4.14	361	329 (18)	2.52	329	331 (12)	5.49	374
MF	50.2 (12)	1.89*	229*	232* (12)	5.98	370	353* (9)	5.11*	365*	372* (6)	10.52*	440*
L	49.6 (12)	1.56	211	224* (12)	6.42	382	352* (9)	4.90*	344	363* (6)	10.89*	453*
SFO	50.2 (12)	1.97*	220	225* (12)	4.92	369	354* (9)	4.82*	367*	379* (6)	12.7*	463*
SD	1.7	0.24	14	15	1.37	22	20	0.76	17	19	1.45	22

MF, mixed fat; L, lard; SFO, sunflower-seed oil.

* Values were significantly different from the control group: $P < 0.05$.

† For details, see p. 20.

Table 4. Measurements of fat cell size in epididymal fat pads from rats given diets containing different fats

(Mean values for twelve rats/group and standard deviations for each observation. Pooled values obtained from animals killed after 5, 13, 17 and 29 weeks on the experimental diets)

Dietary group†	Cell size parameter			
	Diameter (μm)	Volume (μl)	Surface area (cm^2 per 10^6 cells)	Triglyceride ($\mu\text{g}/\text{cell}$)
Basal (control)	98.2	0.52	305	0.47
MF	100.9	0.58	327	0.53
L	107.3	0.74*	375*	0.68*
SFO	108.4*	0.72	377*	0.66
SD	10.8	0.23	78	0.21

MF, mixed fat; L, lard; SFO, sunflower-seed oil.

* Values were significantly different from control group: $P = 0.05$.

† For details, see p. 20.

Food and water intake

Generally control animals given the basal diet consumed a greater weight of food than 'fat-fed' groups, but when the energy value of the food was taken into account, the control group had a significantly lower ($P < 0.05$) energy intake than the 'fat-fed' groups in the whole experimental feeding period. The mean (\pm SE) values were (MJ): 60.5 ± 5.3 for the control group and 71.8 ± 0.92 for the 'fat-fed' groups. Animals given the basal diet drank significantly more ($P = 0.05$) water in each of the four time periods than those given 'fat-containing' diets (values not shown), presumably because the basal diet was drier and less palatable.

As shown in Table 2, there was no statistical difference between the energy intake of rats given different fat diets in the whole experimental feeding period. However, when energy intake was examined on a monthly basis, different patterns of intake were observed between the three fat diets. In the first 2 months of feeding, no statistical differences ($P > 0.05$) were detected between the energy intake of groups given MF and L, or between those given L and SFO, although those given MF had a higher ($P < 0.05$) energy intake than rats given SFO during this period. In the third month of feeding animals given L had a significantly higher ($P < 0.05$) energy intake than those given SFO. Whether these patterns of intake occurred by chance or whether they represent some kind of preference by the young rat for a particular type of dietary fat is unknown.

Body-weight

The higher energy intake of 'fat-fed' animals was reflected in higher body-weights compared with the control group. This difference was noted from the first week of feeding, although it only became statistically significant ($P < 0.05$) from the fifth week of feeding onwards when the body-weights of all animals on each diet were considered.

Weights of fat pads

Weights of epididymal fat pads appeared to be related to body-weight (Table 3). At the end of the experiment, animals given the fat diets weighed more and had significantly heavier ($P < 0.05$) fat pads than those given the basal diet. There were no significant differences in either body-weights or fat pad weights between the 'fat-fed' groups after 29 weeks of feeding.

Table 5. *Fat cell number ($\times 10^{-6}$) in epididymal fat pads of rats given diets containing different fats for different time periods*

(Mean values and standard deviations for each observation)

Dietary group†	Period on diet (weeks)				
	5	13	17	29	1-29
Basal (control)	3.12	5.48	5.87	7.21	5.07
MF	5.70	6.91	11.36	12.62	8.94*
L	4.48	4.36	10.58	12.63	7.75*
SFO	4.63	5.82	10.71	9.65	7.44*
No. of animals per dietary group	3	3	3	3	12
SD	1.11	1.92	2.73	2.97	2.08

MF, mixed fat; L, lard; SFO, sunflower-seed oil.

* Values significantly different from control group: $P < 0.05$.

† For details, see p. 20.

Fat cell size

No significant differences in any measure of fat cell size between dietary groups could be detected at any of the experimental time periods. Only by pooling results for all four time periods could any differences be detected (Table 4). This analysis indicated that animals given SFO and L had significantly larger ($P < 0.05$) cells than control animals. Cell size in the groups given MF was not significantly different from that of the control group. Although differences failed to reach significance among the 'fat-fed' groups, the results suggest that the size of fat cells was in the order control $<$ MF $<$ L = SFO.

Fat cell number

Again, differences between diets failed to reach significance for any one time period, although there was a tendency for fat pads of animals on the control diet to have a smaller fat cell number than the 'fat-fed' groups (Table 5). Pooling the results for all time periods revealed that control animals had a significantly ($P < 0.05$) smaller number of fat cells than 'fat-fed' animals.

Values for cell size and number were examined by a non-parametric method of analysis, which showed that cell number differed ($P = 0.05$) between animals given MF and SFO. No other treatment differences were apparent between fat-fed groups.

In summary no significant difference was detected between the number of fat cells in epididymal fat pads of rats given diets containing L or SFO, or between those animals given MF or L, although there was a difference in fat cell number between animals given MF and those given SFO. Similar results were obtained when the energy intake of the 'fat-fed' groups in the first 2 months of feeding was examined: no significant difference was detected between the energy intake of rats given L or SFO, or between those given MF and L, although there was a difference in energy intake between MF- and SFO-fed groups. Animals given L consumed more food than the group given SFO during the third month of feeding (i.e. when the animals were over 12 weeks of age), yet there was no difference between the final number of cells in the epididymal fat pads of these two groups.

We conclude that: (1) the nature of the dietary fat has no effect on the cellularity of developing adipose tissue; (2) the number of fat cells formed is sensitive to energy intake; (3) this sensitivity to energy intake only occurs before 12 weeks of age in the rat.

Our findings and conclusions differ from those of Raulin and her co-workers (Raulin &

Launay, 1967; Launay, Vodovar & Raulin, 1968; Launay, Dauvillier & Raulin, 1969; Desnoyers, Vodovar, Lapous & Raulin, 1971; Raulin, Lapous, Dauvillier & Loriette, 1971; Launay, Richard, Alavoine & Raulin, 1972). These workers presented few statistical values relating to body-weight and food intake of the animals or indeed of fat cell size and number. The importance of measuring food intake cannot be over-emphasized since the findings of the French workers could be explained if under their laboratory conditions animals given SFO had a higher energy intake in the early weeks of feeding than the animals given L.

Alternatively, the discrepancies might be accounted for by differences in methodology. Raulin & Launay (1967) based their estimates of cell number on DNA measurements. We found that the recovery of fat cells from adipose tissue depended very much on the composition of fat in the tissue, a higher recovery being obtained from tissues of animals given SFO (80%) as compared with those given L (40%). This may be related to the greater stability of 'SFO cells' as opposed to 'L cells', with respect to temperature changes and shear forces, an hypothesis in agreement with two reports of the French workers themselves (Goureau & Raulin, 1970; Desnoyers *et al.* 1971).

Before accepting as a general biological principle that energy intake at a specific time and not dietary composition alone influences fat cell number it is essential: (1) to study the effect of dietary manipulation during the 'sensitive period' when adipose tissue is dividing; (2) to monitor food and energy intake accurately; (3) to subject methodology to critical examination.

Regarding point (1), Greenwood & Hirsch (1974) used the incorporation of [³H]thymidine to study the development of adipocyte cellularity in rat epididymal fat pad. They found that fat cell synthesis occurred in rats aged 7 weeks and younger but not in mature animals of 12 and 20 weeks of age. Thus our proposal that the 'sensitivity' to energy intake only occurs before 12 weeks of age in rat epididymal fat pad agrees with the period of cell division proposed by Greenwood & Hirsch (1974). The sensitive period cannot be expected to be the same for all species. (DiGirolamo & Mendlinger, 1971; Lemonnier, 1972) or for all tissue sites within a species (Johnson, Zucker, Cruce & Hirsch, 1971; Lemonnier, 1972).

The guinea pig, unlike the rat, is born with 10–16% of its body-weight as fat (Widdowson, 1950). The epididymal fat pads are scarcely visible at birth but grow rapidly, the total cell number increasing by over twelvefold (DiGirolamo & Mendlinger, 1971). These findings could be interpreted as evidence for the existence of different sensitive periods for adipose tissue within the guinea pig, epididymal fat having a somewhat late-developing character. Whether the same holds true for different sites of adipose tissue deposition in the rat, or indeed in the human, is unknown.

Without detailed knowledge of food and energy intake we could have concluded that dietary fat composition did indeed influence cellularity and it seems probable that some discrepancies in the literature (Lemonnier, 1969; Raulin *et al.* 1971) could be explained by the authors' failure to record energy intakes.

Finally, not only may alternative methods yield different results, but the same method may give results that differ according to diet because of the influence of depot fat composition on cell stability.

The 'sensitive period' for human adipose tissue development is not known: it could occur entirely before birth. Unlike the rat, which is born in a foetal state with very little adipose tissue, the human baby is born with a considerable amount of fat (Widdowson, 1950). Therefore a proportion of fat cell formation must occur in utero.

If the proliferation of fat cells in the human can be stimulated by energy intake, as we have shown here happens in the young rat, then this would imply that an increased energy

intake by the foetus or young baby would lead to an increase in the total number of fat cells in the body, compared with those individuals with more average intakes. A causative association between the possession of a 'larger-than-average' number of fat cells and obesity remains unproven. In many respects the guinea pig represents a more relevant model of adipocyte development in the human than does the rat and current studies with guinea pigs (Kirtland, Gurr & Widdowson, 1976; Pavey, Widdowson & Robinson, 1976; Gurr, Robinson, Pavey & Widdowson, 1976) in conjunction with the present results with rats should give more indication of the likely effects of early fat feeding in infants.

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