The Influence of High-Fat Diets on Estrous Cycles, Sperm Production and Fertility of Rats[1] (37253)

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Numerous studies have related nutrition to reproduction (1–4), but only a few dealt with the effect of high-fat diets on reproduction and these were confined to the requirement of essential fatty acids for reproductive performance. Deuel *et al.* (5) and Sheer *et al.* (6) reported that rats fed diets containing high levels of fat had more rapid growth and more efficient reproductive and lactational performances than rats fed diets containing low concentrations of fats. However, French *et al.* (7) found decreased reproductive performance of rats when the diet contained 23% corn oil compared to 4.4%; the animals fed the high-fat diet produced smaller offspring, but lactational capacity was similar in the two groups of animals.

Richardson *et al.* (8) reported that the number of young weaned by female rats was essentially the same when they were fed diets containing 3–18% fat, but was decreased when the diet contained 25% fat.

In the work reported herein, several reproductive parameters were measured in male and female rats fed a high (40%) fat diet *ad libitum* or restricted to give body weights similar to control rats fed a grain diet. In another experiment female rats were fed diets containing either 2.7 or 60% fat.

Procedures. Rats in three experiments were housed individually in an animal quarter maintained at about 22° with 12 hr of light and 12 hr of darkness. Weanling female Osborne–Mendel rats were used in Expts 1 and 2, and weanling male Sprague–Dawley in Expt 3. Water was available at all times. The duration of the dietary treatments and numbers of rats used for the first and second experiment are indicated in Figs. 1 and 2, respectively.

In the first experiment, rats were assigned at random to one of three groups, and fed a 40% fat diet[4] *ad libitum*, or the same diet *ad libitum* until the rats reached 250-g body weight when feed intakes were restricted or a grain diet[5] was offered on an *ad libitum* basis. Rats fed the restricted high-fat diet were fed to maintain body weight gain similar to grain-fed rats; usually this required 8–9 g of high-fat diet/rat/day.

In the second experiment, rats were fed *ad libitum* a diet[6] containing 2.7% fat or a

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[4] 40% high-fat diet composition (in %): Fat, 40 (Crisco); casein, 25; mineral mix (Wesson, General Biochemicals, Chagrin Falls, Ohio), 5; vitamin mix (vitamin fortification mixture, Nutritional Biochemicals, Cleveland, Ohio), 2.2; non-nutritive fiber, 2; aureomycin, 0.01; liver powder, 2; DL-methionine, 0.25; sucrose, 23.54. For those fed restricted amounts of this diet the % of casein was increased to 30 and sucrose decreased to 18.54. This was to assure that the restricted rats had adequate protein intake.

[5] Grain diet is a nutritionally adequate diet made primarily from a corn-soy mixture (composition described in this journal, Vol. 130, p. 1146, footnote 1, 1969).

[6] 2.7% low fat diet composition (in %): Corn oil, 2.7; casein, 25; mineral mix (Wesson, General Biochemicals, Chagrin Falls, Ohio), 5; vitamin mix (vitamin fortification mixture, Nutritional Biochemicals, Cleveland, OH), 2.2; nonnutritive fiber, 2–7.5 (by replacing sucrose, see text); aureomycin, 0.01; liver powder, 2; DL-methionine, 0.25; sucrose, 60.34.

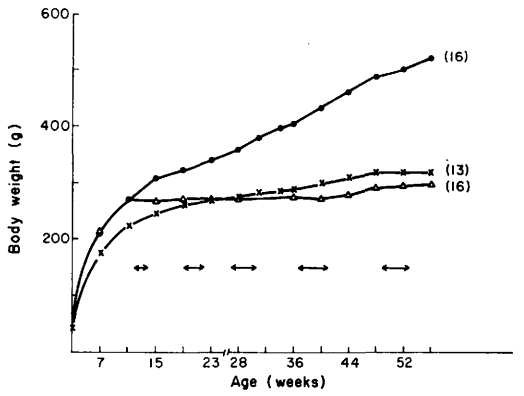

FIG. 1. Body weights of rats fed a grain diet *ad libitum* (×) or a 40% high fat *ad libitum* (●) or restricted (△). Arrows indicate time when vaginal smears were taken for determination of estrous stages. Values in parentheses indicate number of rats completing Expt 1.

diet[7] with 60% fat. To prevent rats fed low-fat diet from eating excessive amounts of food, cellulose in the diet was increased from 2 to 5% starting at age 5 weeks and then to 7.5% at age 10 weeks. The 7.5% cellulose was maintained until the completion of the experiment.

Body weights and vaginal smears were taken periodically as indicated in Figs. 1 and 2; stages of estrous cycles were determined by microscopic examination of stained (methylene blue) smears of vaginal flushings.

In the third experiment, 30 male rats were assigned 3 treatments with balanced body weight; one treatment was the 40% fat diet[4], another was the high fat diet restricted so that body weight gain was comparable to that in a third group of rats fed the grain diet[5] *ad libitum*. These dietary regimens were maintained until the rats were 8 weeks of age when both groups of rats fed the high fat diet were trained to eat only during the day; the rats fed the grain diet were offered their diet throughout the 24 hr. After 1 week of training each male rat was housed with four females in a breeding cage to allow mating during the night, but the males were transferred to their home cages to eat from 830 to 2030. Similarly the females were fed the grain diet only during the day to avoid any interference of the high-fat diet on the females. Mating was continued for 10 days, during which vaginal smears were examined to determine the presence of sperms. Sixteen days after sperms were found in the smear, the female was killed to determine the number of fetuses. On the 11th week, about 2 weeks after the mating period, the male rats were decapitated and testicular and epididymal number of sperms was determined (9). Data were analyzed statistically by means of Student's *t* test.

Results and Discussion. Due principally to respiratory infections, death rates in the first experiment were 14% for the rats fed the high-fat diet *ad libitum*, 7% for those fed restricted amounts of the high-fat diet, and 21% for those fed the grain diet *ad libitum*. The vaginal smear and body weight data in Figs. 1 and 3 include only rats completing the entire experiment without signs of respiratory infection. The difference in body weight between the rats fed the high-fat diet *ad libitum* and the grain diet *ad libitum* became significant at about age 6 weeks. At about 12 weeks of age, the body weights of the rats fed restricted amounts of diet plateaued so that these weights were comparable to those of the grain fed rats (Fig. 1).

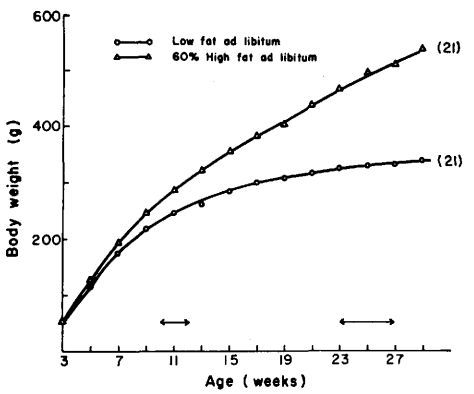

FIG. 2. Body weights of rats fed a low-fat 2.7 or a high fat 60% fat diet *ad libitum*. Arrows indicate time when vaginal smears were taken for determination of estrous stages. Values in parentheses indicate number of rats completing Expt 2. (△) 60% high fat *ad libitum*; (○) 2.7% low fat *ad libitum*.

[7] 60% high fat diet composition: similar to that described in footnote 6 except that diet contained 60% fat (Crisco) replacing sucrose and nonnutritive fiber remained constant at 2%.

In the second experiment, no death or respiratory infections occurred. Since the diet contained more fat in the second than in the first experiment, the body weight of the rats fed the high-fat diet *ad libitum* was considerably greater in the second than in the first experiment. Nevertheless, rats in both treatments should have contained large quantities of adipose tissues distributed as described (10).

During the initial feeding period, the estrous cycle lengths of rats in Expt 1 were within normal ranges of 4–5 days (Fig. 3). However, the rat fed the high-fat diet *ad libitum* had the shortest cycle length among the three groups. After the 31st week of age the lengths of estrous cycles increased in all rats. Undoubtedly, part of the increase was associated with increasing age of the rats. Nevertheless, there was a distinctly sharper increase for those fed the high-fat diet *ad libitum* (Fig. 3). Statistically, only those fed high-fat diet *ad libitum* had a significantly greater estrous cycle length in comparison to the other groups. Thus, the high-fat diet prolonged the estrous cycle when the rat had reached approximately 37–41 weeks old and not at younger ages.

In Expt 2, no significant difference in estrous cycle lengths exists between the two groups of rats from 10 to 12 weeks of age. However, during the 23–27 weeks of age, the estrous cycle was prolonged significantly for the high-fat *ad libitum* rats compared to the low-fat fed rats (Table I). Prolongation of the cycles occurred at an earlier age for the rats in this experiment than those in the first

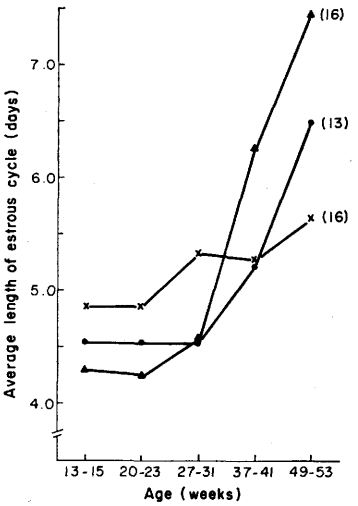

FIG. 3. Average length of estrous cycle at different times after feeding rats a grain diet *ad libitum* (●) or a 40% high-fat diet *ad libitum* (△) or restricted (×). Values in parentheses indicate number of rats completing Expt. 1.

TABLE I. Lengths of Estrous Cycles of Rats Fed a Low or High-Fat Diet *ad Libitum* (Expt. 2).

Diet	No. of rats	Length of cycle in days	Vaginal smears at 10–12 wk	Vaginal smears at 23–27 wk
			(% of cycles)	
2.7% fat, *ad libitum*	21	4	15.0	13.3
		5	75.0	53.3
		6	6.7	14.5
		7	3.3	18.9
		Av length (days) of cycle	5.28 ±0.34[a]	6.22 ±0.66
60% fat, *ad libitum*	21	4	12.7	7.6
		5	72.7	48.5
		6	5.5	9.1
		7	9.1	34.8
		Av length (days) of cycle	5.80 ±0.64	8.71 ±1.41[b]

[a] ± Standard errors.

[b] Significantly higher than low fat group ($p < 0.01$).

TABLE II. Distribution of Lengths of Estrous Cycles of Rats Fed a Grain Diet or High Fat Diet Either *ad Libitum* or Restricted (Expt. 1).

Diet	Length of cycle in days	Vaginal smears at 13–15 wk	20–23 wk	27–31 wk	37–41 wk	49–53 wk
				(% of cycles)		
Grain, *ad libitum*	4	54.5	66.7	62.5	41.3	29.5
	5	42.2	22.2	26.8	36.0	33.3
	6	—	4.4	7.1	9.4	9.2
	7	3.1	6.7	3.6	13.3	28.0
	Av length (days) of cycle	4.55 ±0.21[a]	4.53 ±0.26	4.52 ±0.21	5.21 ±0.52	6.48 ±1.12
40% fat, *ad libitum*	4	75.0	78.3	50.7	18.2	12.1
	5	22.7	20.0	42.0	54.5	34.5
	6	—	1.7	5.8	7.8	13.8
	7	2.3	—	1.5	19.5	39.6
	Av length (days) of cycle	4.30 ±0.14	4.23 ±0.12	4.58 ±0.17	6.26[bc] ±0.86	7.45[d] ±1.11
40% fat, restricted	4	57.9	25.5	30.5	20.0	23.5
	5	36.8	66.7	50.8	60.0	38.2
	6	—	3.9	6.8	11.8	16.2
	7	5.3	3.9	10.2	8.2	22.1
	Av length (days) of cycle	4.84 ±0.50	4.86 ±0.22	5.32[e] ±0.54	5.25 ±0.39	5.63[f] ±1.14

[a] ± Standard errors.
[b] Significantly higher than those fed grain diet at 37–41 weeks ($p < 0.02$).
[c] Significantly higher than at 27–31 weeks ($p < 0.01$).
[d] Significantly higher than at 27–31 weeks ($p < 0.01$).
[e] Significantly higher than those fed 40% fat *ad libitum* ($p < 0.01$).
[f] Significantly lower than those fed 40% fat *ad libitum* ($p < 0.05$).

experiment, possibly because the fat content of the diet was increased from 40% in the first experiment to 60% in the second.

In general, during the early phases of the experiments, the average length of estrous cycles was close to reported values for rats of comparable ages (11, 12). Later, when the rats had time to be affected by the diets or by advancing age, the prolongation of the estrous cycle was primarily caused by a prolongation of diestrus and secondarily by proestrus and estrus. Early in the experiments, a small proportion of the rats on each diet had 6- to 7-day cycles (Tables I and II), but the majority had cycles between 4 and 5 days duration. When the rats were older or fed high-fat diets *ad libitum* for extended periods, the proportion of 6- and 7-day cycles increased (Tables I and II).

The reasons for the prolonged estrous cycles are not clear. Several investigators have suggested that excessive fat may sequester estrogen, and thereby prevent or damp the fluctuations of estrogen necessary for estrous or menstrual cycles (13, 14). For example, obese women had slower excretion rates of estrogen into the urine than thin women (15, 16). Other investigators could detect no difference in estrogen release rate from adipose tissues from different subjects at various degrees of obesity (17).

In Expt 3, high-fat diets had no influence on the fertility of male rats as measured by the number of pregnancies and fetuses in the uteri of the females with which the males were mated (Table III). Furthermore, the number and concentration of sperms in the testes and epididymides were not significantly different among the males fed from weanling until 11 weeks of age the three different

TABLE III. Reproductive Performance of Male Rats Fed a Grain Diet, or a High-Fat Diet Either *ad Libitum* or Restricted (Expt. 3).

	Males tested (no.)	Females used for mating (no.)	Pregnant females (%)	Sterile male (no.)	Fetus per pregnant rat (no.)
Grain, *ad libitum*	10	40	65	0	11.3
High fat, *ad libitum*	10	40	65	0	11.5
High fat, restricted	10	40	55	1	10.7

TABLE IV. Average and Standard Errors of Weight and Sperm Count (Expt. 3).

		Testes	
Diet	Testes wt (g)	Total sperm count ($\times 10^8$)	Sperm count per gram testes ($\times 10^8$)
Grain, *ad libitum*	1.83 ± 0.10	3.59 ± 0.51	1.96 ± 0.30
High fat, *ad libitum*	1.78 ± 0.12	3.87 ± 0.40	2.18 ± 0.17
High fat, restricted	1.85 ± 0.09	3.40 ± 0.59	1.84 ± 0.31
		Epididymides	
Diet	Epididymides wt (g)	Total sperm count ($\times 10^8$)	Sperm count per gram tissue ($\times 10^8$)
Grain, *ad libitum*	0.55 ± 0.04	2.83 ± 0.45	5.18 ± 0.71
High fat, *ad libitum*	0.54 ± 0.03	2.72 ± 0.53	5.02 ± 0.92
High fat, restricted	0.56 ± 0.03	3.02 ± 0.41	5.35 ± 0.59

rations (Table IV). This was also true for the weights of the testes and epididymides. From these results, it can be concluded that the fertility of male rats was not altered by the 40% fat diet.

Summary. The estrous cycle lengths of rats fed high-fat diets *ad libitum* or restricted, a grain diet or a low-fat diet were determined in two long-term studies. In the first experiment, with a 40% fat diet, the rats had prolonged estrous cycles beginning at 37–40 weeks of age caused primarily by a prolongation of diestrus. During the early stages of feeding the high-fat diet did not prolong the estrous cycles. In the second experiment, the diet contained 60% fat and the prolongation of diestrus occurred at 23–27 weeks of age. In male rats, however, high fat diets from weanling to 11 weeks of age did not affect fertility or sperm production.

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