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Physiology

Body weight and food intake at early estrus of rats on a high-fat diet

(puberty/vaginal opening/caloric intake/weanling/critical fatness)

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ABSTRACT Body weight, food intake, and age at vaginal opening and estrus were studied for two groups of weanling rats (age 21 days), fed on high-fat (24.6% by weight) and low-fat (5.0%) diets. Fat was substituted isocalorically for carbohydrate in the high-fat diet. The high-fat rats had estrus at 33.3 ± 0.8 days, significantly earlier ($P < 0.001$) than the age at estrus, 37.4 ± 0.7 days, of the low-fat rats. Estrus was simultaneous with vaginal opening in 81% of the high-fat rats, in comparison to 48% of the low-fat rats. The caloric intake per 100 g of body weight of the high-fat and low-fat rats did not differ at vaginal opening or at estrus, whereas the two groups differed significantly at both events in age, body weight, absolute food intake (g/day), and relative food intake (g/100 g of body weight per day) and absolute caloric intake (calories/day). Caloric intake/100 g of body weight as a function of chronological age first increased and then decreased steadily before estrus for both high-fat and low-fat rats. The findings support Kennedy's hypothesis that a food intake signal, now further defined as caloric intake/100 g of body weight, is a signal for puberty, and are in accord with the hypothesis that a critical body composition of fatness is essential for estrus in the rat, as in the human female.

Puberty in the rat (defined by vaginal opening and estrus) is more closely related to body weight than to age (1, 2). Food intake "appropriate for body weight" of well grown, retarded, and chronically underfed rats has a constant relation to the onset of ovarian activity, independent of age (1). Kennedy concluded that "food intake, or its correlate metabolic rate may act as the normal signal to initiate puberty" (1).

Weight growth in the rat has been reported to be more rapid on high-fat diets than on normal or low-fat diets (3–5). Female rats on high-fat diets, therefore, should have vaginal opening and estrus earlier than on a low-fat diet. Diets differing in the calories contributed by fat may also provide information on the finding of a constant food intake per unit of body weight at puberty in the rat (1, 2), since food intakes would differ between the two diets if rats were "eating to calories" in relation to body weight.

A positive relation between rate of sexual development in the rat and the amount of fat contained in the diet up to 20–40% has been reported (4, 6). However, detailed observation of weight, food intake, vaginal opening, and estrus were not made in these or other (5) studies.

This paper reports the age, body weight, food intake, and caloric intake at vaginal opening and at estrus of two groups of weanling rats fed on high- and low-fat diets. These relations between weight, fat and puberty in the rat are of special interest now because of the findings in the human female that a critical weight (7), representing a critical level of fatness (8, 9), is closely related to menarche and to the continued maintenance of regular ovulatory cycles (10). Amenorrhea follows weight loss below a minimal level of fatness and cycles resume with weight gain above that minimum level (10).

MATERIALS AND METHODS

Forty-two weanling Charles River (CD) female rats, age 21 days, were divided into two groups, randomized for body weight. The two groups were fed a high-fat (24.6%) and low-fat (5.0%) diet ad libitum. Fat was substituted isocalorically for carbohydrate in the high-fat diet. The amount of protein, salt mix, and vitamins were the same in both diets (Table 1). Rats were housed in a temperature-controlled room (22°) with automatically controlled lighting (14 hr light daily). Each rat was weighed daily. Food jars were weighed daily, and any spilled food was collected and also weighed. After day 25, rats were checked daily for vaginal opening and estrus. Estrus was determined by vaginal smears; the criterion was full cornification, stages II and III of Long and Evans (11). On the day of estrus rats were killed by etherization and frozen for future determination of body composition.

RESULTS

Rats fed the high-fat diet had vaginal opening and estrus significantly earlier than the rats fed the low-fat diet (Table 2, Fig. 1). Estrus was simultaneous with vaginal opening in 17 (81%) of the high-fat rats, in comparison to 11 (48%) of the low-fat rats. The mean interval between vaginal opening and estrus was 0.9 ± 0.4 days for the high-fat rats and 2.8 ± 0.7 days for the low-fat rats ($P < 0.05$) (Fig. 1).

The caloric intake/100 g of body weight of high-fat and low-fat rats did not differ at vaginal opening, or at estrus, although the two groups differed significantly at both events

Table 1. Composition of low- and high-fat diets

	Low fat		High fat		
	Calories	g/100 g	Calories	g	%
Casein	80	20.0	80	20.0	24.6
Starch	281	70.3	146	36.5	45.0
Fat (Spry)	45	5.0	180	20.0	24.6
Salt Mix (25)	—	4.0	—	4.0	4.93
Vitamin Mix (26)	2.0	0.5	2.0	0.5	0.62
Choline chloride	—	0.2	—	0.2	0.25
	408	100.0	408	81.2	100.0

All references to calorie are to the large or kilocalorie = 4.184 kilojoules.

Abbreviations: HF, high fat; LF, low fat; v.o., vaginal opening.

Table 2. Mean (±SEM) age, weight, food, and caloric intake at vaginal opening and at estrus of rats on high-fat and low-fat diets

			For differences high − low fat	
	High fat*	Low fat†	*t*	*P*
Initial weight (g) (at weaning)	42.5 ± 0.9	42.5 ± 0.8	0	n.s.
At vaginal opening				
Age (days)	32.4 ± 0.6	34.6 ± 0.6	2.59	<0.05
Weight (g)	101.9 ± 2.9	111.0 ± 2.7	2.30	<0.05
Food intake (g/day)	9.2 ± 0.3	12.6 ± 0.3	8.01	<0.001
g/100 g body weight	9.1 ± 0.2	11.4 ± 0.3	6.38	<0.001
Caloric intake/day	46.2 ± 1.5	51.4 ± 1.2	2.71	<0.01
Calories/100 g body weight	45.7 ± 1.0	46.5 ± 1.2	0.51	n.s.
At estrus				
Age (days)	33.3 ± 0.8	37.4 ± 0.7	3.86	<0.001
Weight (g)	107.0 ± 4.0	126.9 ± 4.0	3.52	<0.01
Food intake (g/day)	9.4 ± 0.3	13.4 ± 0.3	9.43	<0.001
g/100 g body weight	8.9 ± 0.3	10.7 ± 0.3	4.71	<0.001
Caloric intake/day	47.2 ± 1.5	54.7 ± 1.2	3.90	<0.001
Calories/100 g body weight	44.7 ± 1.5	43.7 ± 1.2	0.52	n.s.

n.s. = not significant.
* 1 gram = 5.02 calories.
† 1 gram = 4.08 calories.

in age, body weight, absolute food intake (g/day) and relative food intake (g/100 g of body weight) and absolute caloric intake (cal/day) (Table 2) (Figs. 2–4). At estrus, the caloric intake/100 g of body weight of the high-fat and low-fat rats were 44.7 ± 1.5 calories, and 43.7 ± 1.2 calories, respectively. In contrast, the mean weights of the high-fat and low-fat rats were 107.0 ± 4.0 g and 126.9 ± 4.0 g, respectively ($P < 0.001$); their food intakes were 9.4 ± 0.3 g and 13.4 ± 0.3 g, respectively, ($P < 0.001$), and their caloric intakes were 47.2 ± 1.5 cal and 54.7 ± 1.2 cal ($P < 0.001$), respectively. The difference in body weight between the two groups at estrus was greater than at vaginal opening, reflecting the longer interval between vaginal opening and estrus of the low-fat rats (Table 2) (Figs. 1 and 2).

As expected, weight growth of high-fat rats from weaning to estrus was faster than that of low-fat rats (Fig. 2). Velocity curves of weight growth show that vaginal opening and estrus in both high-fat and low-fat rats was preceded by an acceleration of weight growth and then a deceleration, (Fig. 5), similar to the acceleration to weight peak velocity and the deceleration found in girls before menarche (12), though of course on a much smaller scale.

The food intake/100 g of body weight also first increased and then decreased before vaginal opening and estrus in both groups of rats (Fig. 4), as had been found by Kennedy (1). Calorie intake/100 g of body weight plotted versus chronological age (Fig. 4) and versus days before estrus (Fig. 3) also shows an increase and then a steady decrease before estrus for both high-fat and low-fat rats. Fig. 3 shows the convergence to a similar caloric intake/100 g at estrus of high- and low-fat rats.

In addition to the difference found in body weight at estrus between high- and low-fat rats (Table 2), body weight at estrus increased with increasing age of estrus for high-fat rats and for low-fat rats (Fig. 6).

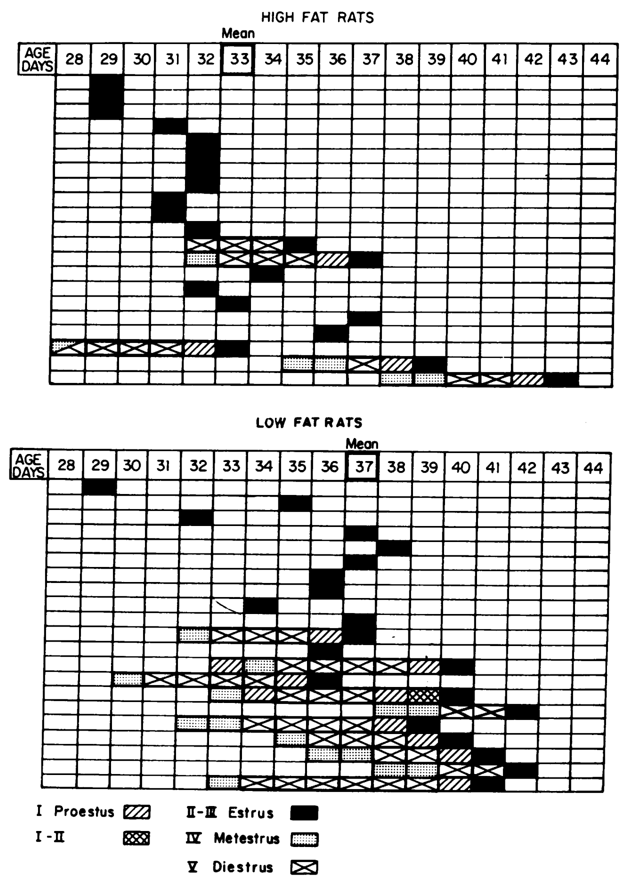

FIG. 1. Age at vaginal opening, and classification (11) of vaginal smears to estrus of high- and low-fat rats. First vertical line of vaginal smear record denotes day of vaginal opening. High-fat rats and low-fat rats are in order of increasing weight at estrus.

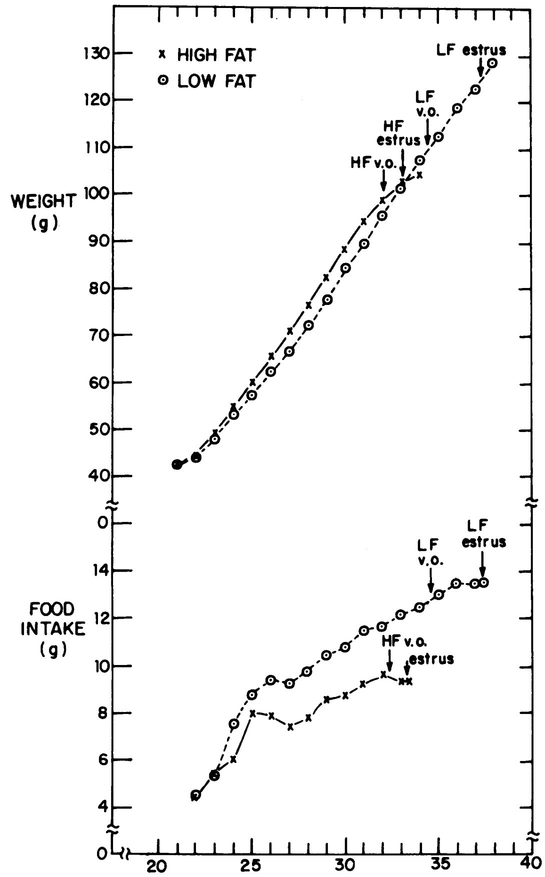

FIG. 2. Weight versus age, and food intake/day versus age of high fat (HF) (×) and low-fat (LF) (⊙) rats up to their respective mean ages of estrus. v.o. = vaginal opening.

$$\text{High fat: Weight}_{\text{estrus}} = -49.7 + 4.7(\pm 0.1)\cdot \text{Age}_{\text{estrus}}$$

$$\text{Low fat: Weight}_{\text{estrus}} = -54.1 + 4.8(\pm 0.1)\cdot \text{Age}_{\text{estrus}}$$

The caloric intake/100 g of body weight *at estrus* decreased with increasing age of estrus for both high- and low-fat rats:

At estrus:

$$\text{High fat: Calorie intake/100 g} = 78.5 - 1.0(\pm 0.32)\cdot \text{Age}_{\text{estrus}}$$

$$\text{Low fat: Calorie intake/100 g} = 91.3 - 1.3(\pm 0.19)\cdot \text{Age}_{\text{estrus}}$$

Though the mean initial weights of the high-fat and low-fat rats were identical (Table 2), age of estrus and weaning weight were not correlated in the low-fat rats [regression coefficient −0.14 (±0.20)], but were slightly inversely correlated in the high-fat rats ($P = 0.05$):

$$\text{High fat: Age}_{\text{estrus}} = 50.4 - 0.40(\pm 0.16)\cdot \text{Weight}_{\text{weaning}}$$

However, as for all high-fat rats compared to the low-fat rats, the age of estrus of the 11 heavy (≥ 42.5 g at weaning) high-fat rats was significantly earlier, 31.6 ± 0.5 days, than that of the 11 heavy low-fat rats 36.5 ± 1.2 days ($P < 0.001$).

DISCUSSION

The finding that rats on a high-fat diet had an earlier age of estrus than did rats on a low-fat diet affirms previous findings on the effects of dietary fat on the age of sexual maturation in the rat (4) and is in accord with the apparent importance of fatness for menstrual function in the human female (8–10). These findings raise the possibility that an increase in the amount of calories from fat in the diets of developed countries in the last century may have contributed to the secular trend to an earlier age of menarche (13).

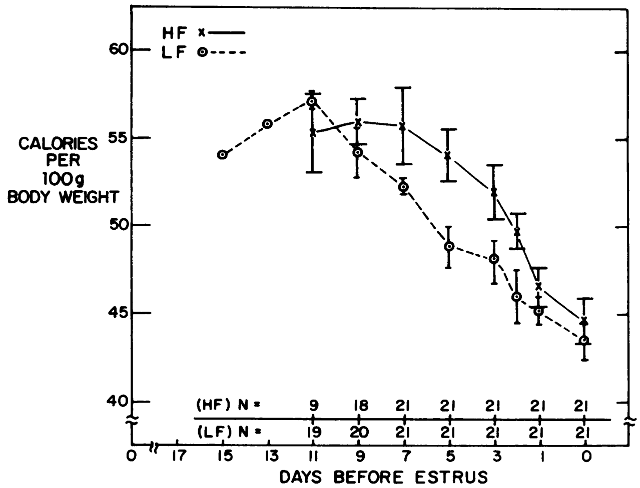

FIG. 3. Caloric intake/100 g of body weight versus *days before estrus* for high-fat and low-fat rats.

The important finding that rats on high-fat and low-fat diets consumed the same calories per unit of body weight at estrus, whereas their ages, body weights, and food intakes at estrus differed significantly, supports the hypothesis that a

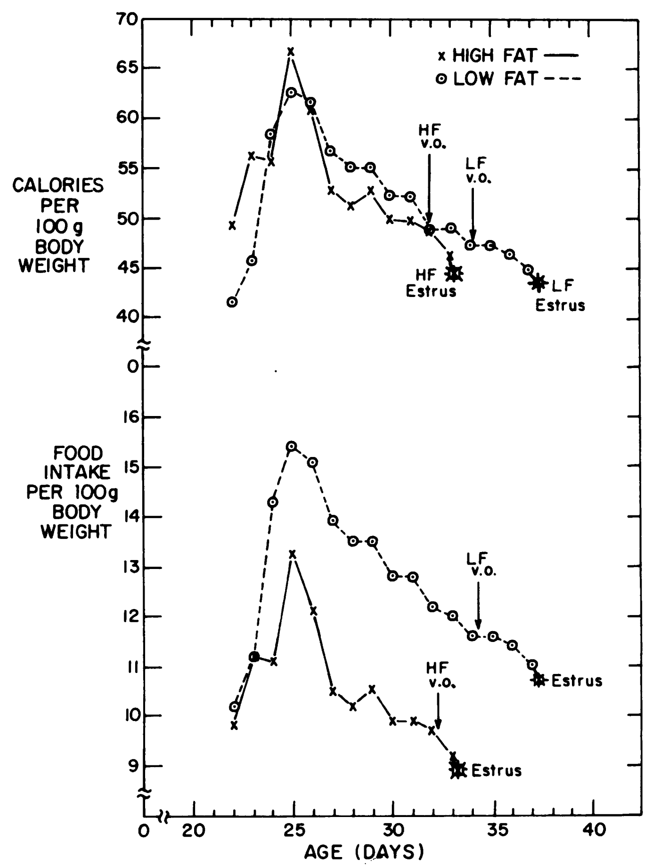

FIG. 4. Food intake/100 g of body weight versus age, and caloric intake/100 g of body weight versus age for high-fat and low-fat rats up to their respective mean ages of estrus.

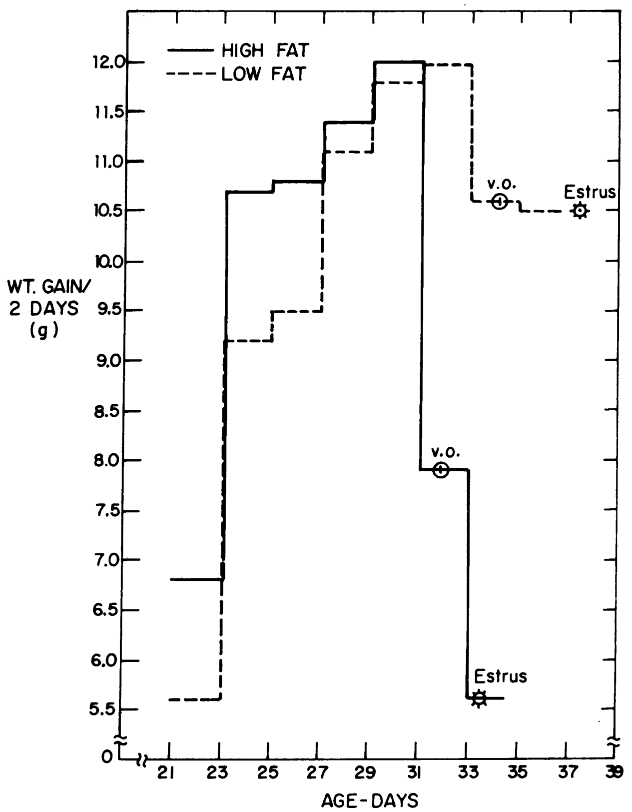

FIG. 5. Weight gain/2 days versus age for high-fat and low-fat rats up to their respective mean ages of estrus.

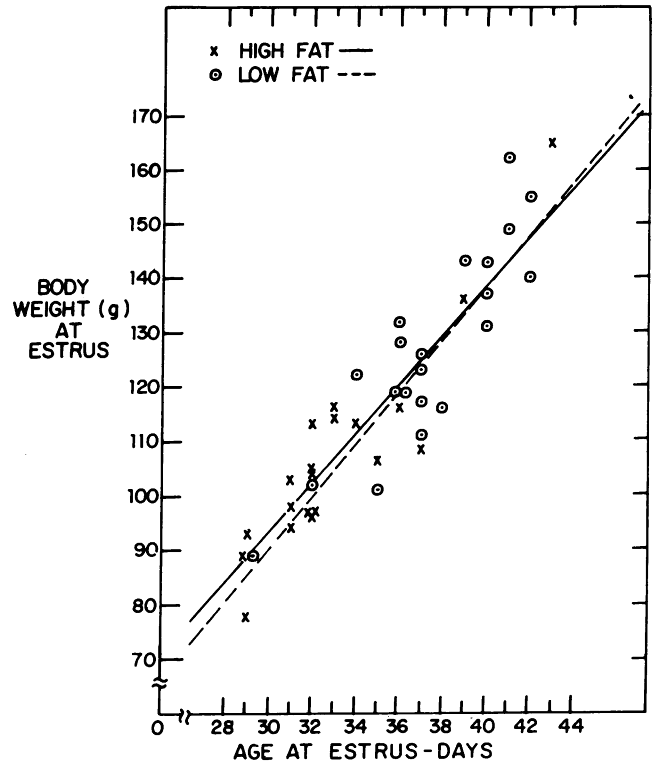

FIG. 6. Weight at estrus versus age at estrus for high-fat and low-fat rats.

signal of metabolic rate or energy balance is a signal for puberty (1, 2). In Kennedy's experiments fast and slow growing rats were given diets of the same caloric value; estrus was at differing ages, but at a constant food intake per unit of body weight. Kennedy hypothesized that the food intake signal, or its correlate metabolic rate, caused a change in the hypothalamic control of gonadotropin secretion (1).

We can now further define the signal of food intake/100 g of body weight at estrus as caloric intake per unit of body weight. The constant caloric intake per unit of body weight found for high- and low-fat rats at estrus may reflect the attainment of a particular body composition of fat/lean body weight, or fat/body weight, which may be essential for estrus and ovulatory cycles in the rat, as in the human female (8–10). Changes in food intake during growth in the rat are closely correlated with changes in the size of the adipose tissue (2, 14). In the human female, loss of body weight in the range of 10–15%, which corresponds to a loss of body fat of 35–40%, is associated with loss of menstrual function (10, 15), and the ability to respond to luteinizing-hormone-releasing factor (16).

The differences in body weight of the high- and low-fat rats found at estrus differs from Kennedy's findings for optimally grown and retarded rats, whose weights did not differ at estrus, but is in accord with the heavier weight at estrus of his chronically undernourished rats (1). Interestingly, Charles River (CD) weanling rats fed Purina Chow ad lib. had estrus at 38.5 ± 1.1 days, and at a weight of 123.8 ± 2.8 g (unpublished data). The age and the weight at estrus of the Purina-fed rats are similar to those found for the low-fat rats (Table 2).

The earlier age of estrus of the high-fat rats, the larger percentage of high-fat rats that had simultaneous vaginal opening and estrus, and the longer interval between vaginal opening and estrus in the low-fat rats (Fig. 1) indicate that estrogen levels were higher sooner in the high-fat rats than in low-fat rats. The prolonged interval between vaginal opening and estrus in low-fat rats suggests that the pituitary function in these rats was not fully developed at the time of vaginal opening, and it took some time before sufficient amounts of gonadotropins for ovulation were secreted. Insufficiency of the pituitary function in chronically underfed rats was reported by Kennedy (1). These rats showed persistent vaginal cornification, and no sign of ovulation was observed. When such rats were injected with chorionic gonadotropin, all ovaries showed many corpora lutea (1).

The excretion and metabolism of estrogen in women are known to vary with body weight, and hence fatness (17–19). Conversely the growth of adipose tissue may be affected by increases in estrogen secretion, particularly after peak velocity (20), when a positive feedback may be established in the rat (21) and in the human female (22).

A rise in estrogen in the rat may also contribute to the fall in caloric intake observed after peak velocity in the rat (2, 23) and in adolescent girls (24).

Though one must be cautious about comparisons between species, there is a surprising similarity in the acceleration and deceleration of weight growth and food intake in the rat before estrus (Fig. 5) to the adolescent spurt of the human female before menarche (7).

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1. Kennedy, G. C. & Mitra, J. (1963) "Body weight and food intake as initiation factors for puberty in the rat," *J. Physiol.* **166,** 408–418.
2. Kennedy, G. C. (1969) "Interactions between feeding behavior and hormones during growth," *Ann. N.Y. Acad. Sci.* **157,** 1049–1061.
3. Greenfield, J. W. (1969) Discussion after Kennedy, G. C., "Interactions between feeding behavior and hormones during growth," *Ann. N.Y. Acad. Sci.* **157,** 1060–1061.
4. Deuel, H. J., Jr., Meserve, E. R., Straub, E., Hendrick, C. & Scheer, B. T. (1948) "The effect of fat level of the diet on general nutrition, :I," *J. Nutr.* **33,** 569–582.
5. Innami, S., Yang, M. G., Mickelsen, O. & Hafs, H. D. (1973) "The influence of high-fat diets on estrous cycles, sperm production and fertility of rats," *Proc. Soc. Exp. Biol. Med.* **143,** 63–68.
6. Scheer, B. T., Soule, D. F., Fields, M. & Deuel, H. J., Jr. (1948) "The effect of fat level of the diet on general nutrition, :II," *J. Nutr.* **33,** 583–592.
7. Frisch, R. E. & Revelle, R. (1971) "Height and weight at menarche and a hypothesis of menarche," *Arch. Dis. Child.* **46,** 695–701.
8. Frisch, R. E. (1974) "A method of prediction of age of menarche from height and weight at ages 9 through 13 years," *Pediatrics* **53,** 384–390.
9. Frisch, R. E., Revelle, R. & Cook, S. (1973) "Components of the critical weight at menarche and at initiation of the adolescent spurt: Estimated total water, lean body mass and fat," *Hum. Biol.* **45,** 469–483.
10. Frisch, R. E. & McArthur, J. (1974) "Menstrual cycles: Fatness as a determinant of minimum weight for height necessary for their maintenance or onset," *Science* **185,** 949–951.
11. Long, J. A. & Evans, H. M. (1922) "The oestrous cycle in the rat and its associated phenomena," *Mem. Univ. Calif.* **6,** 1–148.
12. Frisch, R. E. & Revelle, R. (1971) "Height and weight of girls and boys at the time of initiation of the adolescent growth spurt in height and weight and the relationship to menarche," *Hum. Biol.* **43,** 140–159.
13. Frisch, R. E. (1972) "Weight at menarche: Similarity for well-nourished and undernourished girls at differing ages and evidence for historical constancy," *Pediatrics* **50,** 445–450.
14. Liebelt, R. A., Ichinoe, S. & Nicholson, N. (1965) "Regulatory influences of adipose tissue on food intake and body weight," *Ann. N.Y. Acad. Sci.* **131,** 559–582.
15. Boyar, R. M., Katz, J., Finkelstein, J. W., Kapen, S., Weiner, H., Weitzman, E. D. & Hellman, L. (1974) "Anorexia nervosa: Immaturity of the 24-hour luteinizing hormone secretory pattern," *N. Engl. J. Med.* **291,** 861–865.
16. Warren, M. P., Jewelewicz, R., Dyrenfurth, I., Ans, R., Khalaf, S. & Vande Wiele, R. L. (1975) "The significance of weight loss in the evaluation of pituitary response to LH-RH in women with secondary amenorrhea," *J. Clin. Endocrinol. Metab.* **40,** 601–611.
17. Brown, J. B. & Strong, J. A. (1965) "The effect of nutritional status and thyroid function on the metabolism of oestradiol," *J. Endocrinol.* **32,** 107–115.
18. Nodate, Y. (1969) "Estrogen Metabolism," *Sanfujinka No Jissai* (Tokyo) **18,** 219–229.
19. Iizuka, R. & Kawakami, S. (1968) "Body weight change and sexual function in women," *Sanfujinka No Jissai* (Tokyo) **17,** 388–394.
20. Frisch, R. E. (1974) "Critical weight at menarche, initiation of the adolescent spurt, and control of puberty," in *Control of Onset of Puberty,* eds. Grumbach, M. M., Grave, G. D. & Mayer, F. E. (John Wiley & Sons, Inc., New York), pp. 403–423.
21. Smith, E. R. & Davidson, J. M. (1968) "Role of estrogen in the cerebral control of puberty in female rats," *Endocrinology* **82,** 100–108.
22. Kulin, H. E., Grumbach, M. M. & Kaplan, S. L. (1972) "Gonadal-hypothalamic interaction in prepubertal and pubertal man: Effect of clomiphene citrate on urinary follicle-stimulating hormone and luteinizing hormone and plasma testosterone," *Pediat. Res.* **6,** 162–171.
23. Wade, G. N. & Zucker, I. (1970) "Development of hormonal control over food intake and body weight in female rats," *J. Comp. Physiol. Psychol.* **70,** 213–220.
24. Mitchell, H. S., Reed, R. B., Valadian, I. & Hoff, M. (1966) "The adolescent growth spurt and nutrient intake," *Proc. Int. Congr. Nutr., 7th* **4,** 1–8.
25. Hayes, K. C., Rabin, A. R. & Berson, E. L. (1975) "An ultrastructural study of nutritionally induced and reversed retinal degeneration in cats," *Am. J. Pathol.* **78,** 505–524.
26. Hegsted, D. M., Gallagher, A. & Hanford, H. (1974) "Inositol requirement of the gerbil," *J. Nutr.* **104,** 588–592.