Pubertal Food Intake, Body Length, Weight, and Composition in the Well Fed Female Rat

RICHARD WILEN⁽³⁹⁾ AND FREDERICK NAFTOLIN

Department of Obstetrics and Gynecology, McGill University, Royal Victoria Hospital, Women's Pavilion, Montreal, Quebec, Canada

Summary

Pubertal age, food intake, body length, weight, and composition were determined by direct measurements in 29 well fed female rats studied from birth to first estrus. The average birth and weaning weights of the 12 early maturing rats were 6.99 ± 0.13 g and 50.13 ± 1.16 g, respectively, and did not differ significantly from those of the 11 late maturers (6.97 ± 0.16 g and 49.72 ± 1.42 g, respectively). Mean values for all pubertal measurements are included in Table 1. At puberty, the late maturing animals were heavier, longer, and had attained a greater quantity of total body water than the early maturers. Although both groups ate the same total amounts of food, late maturing rats at vaginal opening and at first estrus consumed relatively less food per 100 g body weight than did early maturers. Estrus was simultaneous with vaginal opening in 10 (83%) of early and 4 (36%) of the late maturing rats. Despite the similarity in the proportions of total body water, fat, and

protein in early and late maturers, a regression analysis, shown in Figure 1, indicates a significant decrease in the proportion of body water ($P < 0.001$), and a significant increase in the proportion of body fat ($P < 0.05$) with increasing age at first estrus. The percentage of body protein does not change with increasing age at first estrus (regression coefficient = 0.13).

Speculation

Our findings in female rats are consistent with those in monkeys and domestic animals which show pubertal age to be positively correlated with body weight. These observations are at variance with results reported for girls who show an unchanging average body weight at all ages of puberty. The differences in metabolic and somatic development observed in pubertal rats suggest that the attainment of a "critical" body weight, level of fat, and food intake may not be the principal factors initiating puberty.

Studies on female rodents (24,35), domestic animals (6,21), and humans (7) have shown that at sexual maturation, average body weights are the same for well- and undernourished subjects. The undernourished, however, attain maturity at a greater age. Studies on well-nourished early and late maturing girls show that average weight at menarche is the same, but that the late maturers are taller (11). Food or caloric intake per 100 grams of body weight is the same at puberty for rats that are well- or undernourished (24) or fed a high or low fat diet ad libitum (9). Nonetheless, as age at first estrus increases within groups of rats, body weight increases while food intake per 100 grams of body weight decreases (9).

In rats (24) and in humans (11) the attainment of a certain body weight and associated metabolic rate has been hypothesized to be a signal for the initiation of puberty. More recently, studies based on estimated total body water in pubertal girls have suggested that, in addition to acquiring a "critical" body weight and metabolic rate at puberty, individuals must reach a "critical" level of fatness (10). Unfortunately, studies with direct measurements of body composition are not available. Because of the absence of studies which report the relationships of pubertal age with food intake and body length, weight and composition in animals and humans at sexual maturation, we have investigated these variables in well-fed pubertal female laboratory rats.

MATERIALS AND METHODS

Rats (Sprague-Dawley, Charles River Strain), obtained at a time at which they were pregnant for no more than two weeks, were individually caged in our laboratory. Temperature (21°C) and light (14 hr. daily) were controlled. At birth, pups were separated from their mothers and male pups discarded. Litters of ten female pups were randomly placed with mothers that had just given birth and had their pups removed. Mothers were fed Purina Laboratory Chow and watered ad libitum, and individually caged with their pups. Pups were weaned at 21 days of age and placed in individual cages. Twenty-nine pups were fed an optimal diet ¹ ad libitum. This optimal diet, although considered to be low in fat by Frisch et al. (9) contains percentages of fat and protein similar to those found in Purina Laboratory Chow. Each pup was weighed (to the nearest 0.1 g.) every other day from birth to first estrus and at vaginal opening. Food intake was determined at 24 hour intervals by weighing the food containers (to the nearest 0.1 g.) from which the weaned pups ate. From time of weaning rats were observed daily for vaginal opening. Thereafter, vaginal smears were taken daily to determine first estrus (first appearance of completely cornified epithelial cells). Pubertal food intake was taken to be the average daily amount of food consumed during the 72 hours prior to first estrus or vaginal opening. At first estrus, rats were sacrificed by ether anesthesia and weighed; nose-rump length was recorded with a standard sliding compass. Rats were then immediately sealed into individual plastic bags and frozen for future body composition analysis. The frozen carcasses were chopped into small pieces and placed in tared aluminum dishes. They were dried at 95°C, to constant weight. The weight loss was taken to equal total body water. The dried carcasses were then crushed and ground. Soxhlet extraction for a minimum of four hours using petroleum ether on 5 g. portions was used to determine percentage of fat. Two gram portions of the powdered fat-free carcasses were heated with 50 ml. of 50% sulfuric acid for 3-4 days at approximately 100°C. The hydrolysate was quantitatively transferred to a 500 ml. volumetric flask with water and made up to volume. The total nitrogen content of this solution was determined by the micro-Kjeldahl method and was corrected to protein content by multiplying by a factor of 6.25 (17). Rats were specified as early or late maturers by using the median age at first estrus (38.0 days) as the dividing point. Six animals attained first estrus at the median age of 38 days and thus were not included in either group. The statistical significance of mean values and regression slopes were determined by a Student t-test (30).

1. Optimal diet: casein 20%, starch 70.3%, fat (hydrogenated vegetable oil-Spry, Lever Brothers, New York) 5%, vitamin mix 0.5% (16), salt mix 4% (15), choline chloride 0.2%.

RESULTS

Of the 23 experimental animals not having first estrus on the median (the 38th) day for the total population, 12 rats in which first estrus occurred before 38 days of age were specified as early maturers and 11 rats whose age at first estrus exceeded 38 days were designated as late maturers. The average ($\pm$ S.E.) birth and weaning weights of early maturing rats were 6.99 ± 0.13 g. and 50.13 ± 1.16 g., respectively, and did not differ significantly from those of late maturers (6.97 ± 0.16 g. and 49.72 ± 1.42 g., respectively). Estrus was simultaneous with vaginal opening in 10 (83%) of the early and 4 (36%) of the late maturing rats. Late maturing rats were significantly heavier and longer than early maturers (Table 1). The use of the mean day of first estrus as a dividing point resulted in an uneven distribution of early and late maturers. Despite this, the calculations for food intake and somatic variables at first estrus and at vaginal opening using the mean age at first estrus (39.6 days) as the dividing point for defining early versus late maturers yielded the essentially the same findings.

Although late maturing rats have a significantly greater amount of total body water at first estrus, their average percentage of total body fat, protein and water are the same as in the early maturers (Table 1). Nonetheless a regression analysis using all twenty-nine rats shows that the percentage of total body water, which is the inverse of body fatness (17), decreases ($P < 0.001$) while the percentage of total body fat increases ($P < 0.05$), with increasing age at estrus (Fig. 1). The percentage of body protein does not change with increasing age at estrus (regression coefficient = 0.13).

Early and late maturing rats at vaginal opening and at first estrus consume the same amount of food; however, food intake/100 grams of body weight is lower in the late maturing animals (Table 1).

DISCUSSION

The finding that early and late maturing laboratory rats attain a different body weight and percentage of fat at first estrus is apparently discordant with the hypothesis that a "critical" body weight representing a critical level of fatness and metabolic rate may trigger the initiation of puberty (10). Our observation that body weight at puberty increases with age in the rat is at variance with reports on girls showing early and late maturing subjects attaining the same average body weight at menarche (11,37). The disparity of these observations may be explained by variations in the adolescent weight spurt prior to menarche (32). Furthermore, different racial groups have different weights at menarche (20). In the American girls reported, group heterogeneity may mask a more racially defined, positively correlated, age and weight relationship at menarche.

Differences in pubertal age-weight distributions may occur between humans and other mammals. A recent study of the well-fed pubertal rhesus monkey (36) shows that the rhesus at menarche, like the ewe (7) and the heifer (1) at first estrus has a pubertal age-weight distribution similar to the rat at first estrus.

Deceleration of skeletal growth is highly correlated with the timing of sexual maturation in mammals (32). That well-fed, late maturing rats are longer than their well-fed, early maturing controls (Table 1) suggests a greater capacity for additional bone growth in late maturers. Furthermore, both well- and undernourished late maturing girls are taller than their earlier maturing counterparts (8,11).

The proportion of total body fat increases from birth to adulthood in female rodents (5,14) and humans (4,10). In humans, estrogen formation from androgen precursors has been demonstrated *in vitro* in adipose tissue (13,29). Moreover, levels of estrogen excretion and metabolism in adult women may vary directly with increasing body weight (3) and percentage of estimated fat (10). Estrogen formation by rodent body fat has, however, yet to be demonstrated. Furthermore, the attainment of early puberty in light lean female rats and other species (36) suggests that a high level of body fat associated with the attainment of a "critical" body weight may not be the principal factors initiating sexual maturation. To this extent, the lower percentage of simultaneous vaginal opening and estrus found in heavy, fat, late maturing rats may reflect characteristically diminished pituitary function associated with low levels of food intake and metabolic rate (Table 1) (24). Chronically underfed late maturing rats that are gonadotropin deficient display a prolonged interval between onset of pubertal events (24). In the sexually maturing female rat estrogen begins acting to suppress food intake and rate of weight gain (33) as its ability to induce pubertal events increases (31). In the late maturing well-fed rat, a low but rising sensitivity to increasing levels of circulating gonadotropin and estrogen would offer a partial explanation for the increased growth observed at vaginal opening and at first estrus. The local production of estrogen from androgen precursors by the limbic and hypothalamic tissues controlling gonadotropin secretion has already been raised as having a possible role in the development of sexual maturation (28).

Kennedy (23) regarded food intake or its correlate, metabolic rate, as a trigger which might change hypothalamic control of gonadotropins and thereby initiate puberty (24). The hypothesis was formulated from observations on well- and underfed rats that attained puberty at differing ages even though they were eating the same amount per unit of body weight (24). In contrast, the presently studied late maturing rats, heavier and proportionately fatter than their early maturing controls, consume less food per unit of body weight (Table 1). This is consistent with observations on increasingly lower metabolic rate and food intake in growing animals (2,22) and humans (34) whose heat-producing body mass increases at a greater rate than their heat-radiating surface area (2). Moreover, it is suggested that some compensating effect of greater proportional body fat thought important in regulating energy balance by the hypothalamus (18,19,22) may play a role in lowering food intake/100 grams of body weight in the fatter, late maturing animals (Fig. 1). Although early and late maturing girls weigh the same, early maturers are estimated to be fatter (10) and may consume less calories daily (25).

In female rats (14) and humans (4) the percentage of total body protein to weight increases from birth to sexual maturation. Nonetheless, the proportion of body protein (an approximate measure of lean body mass or body cell mass and greatest contributor to basal metabolism (27)) is the same for early and late maturing rats (Table 1) or girls (22) at puberty.

Well-fed pubertal female rats with varying body weight, percent fat, or food intake may be generating a similar but yet to be defined metabolic signal which is received at the hypothalamic level and may be important in triggering the onset of sexual maturation.

CONCLUSION

Birth, weaning and pubertal body weights were studied in 29 rats. Food intake, body length, and body composition at sexual maturation (first estrus) were measured. Contrary to an existing belief based on evidence gathered from observations on rats and humans (references in the Introduction to the paper), that in a homogeneous group, sexual maturation is attained at an approximately constant level of body weight, body fat and food intake, we found that at estrus the later maturing rats are heavier, fatter and consume a lesser amount of food per unit of body weight than the early maturers. We present evidence that the average total body protein does not differ significantly for the early and late maturers at first estrus.

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39. Requests for reprints should be addressed to: Dr. Richard Wilen, Women's Pavilion, Royal Victoria Hospital, 687 Pine Avenue West, Montreal, Quebec, Canada. H3A 1A1.
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TABLE 1. Mean ($\pm$ SEM) age, weight, and food intake at vaginal opening and at first estrus. Mean nose-rump length, total body water, and percent body water, fat and protein at first estrus.

	Early Maturers	Late Maturers	All rats combined	Early and late Differences	
	N = 12	N = 11	N = 29	t	P
At Vaginal Opening					
Age (days)	34.2 $\pm$ 0.47	42.4 $\pm$ 1.60	37.9 $\pm$ 0.94	5.2	<0.001
Body Weight, Bwt (g)	122.7 $\pm$ 3.30	150.0 $\pm$ 7.70	135.3 $\pm$ 3.99	3.4	<0.005
Food Intake, FI (g/day)	14.3 $\pm$ 0.58	16.0 $\pm$ 0.87	15.1 $\pm$ 0.43	1.7	n.s.
FI/100 g Bwt/day (g)	11.7 $\pm$ 0.23	10.7 $\pm$ 0.27	11.1 $\pm$ 0.17	2.8	<0.01
At First Estrus					
Age (days)	34.6 $\pm$ 0.51	45.9 $\pm$ 2.01	39.6 $\pm$ 1.23	5.7	<0.001
Bwt (g)	124.8 $\pm$ 3.36	162.4 $\pm$ 9.60	143.3 $\pm$ 5.79	3.4	<0.005
Nose-Rump Length (cm)	15.2 $\pm$ 0.14	16.5 $\pm$ 0.30	15.8 $\pm$ 0.17	4.0	<0.001
Total Body Water, TBW (g)	83.9 $\pm$ 1.78	107.1 $\pm$ 4.96	94.6 $\pm$ 2.80	4.6	<0.001
TBW/Bwt. Percent.	67.7 $\pm$ 0.60	66.0 $\pm$ 0.90	66.9 $\pm$ 0.48	1.6	n.s.
Percent Fat	9.1 $\pm$ 0.35	10.6 $\pm$ 0.88	9.9 $\pm$ 0.47	1.6	n.s.
Percent Protein	17.9 $\pm$ 0.43	17.5 $\pm$ 0.56	17.6 $\pm$ 0.29	0.5	n.s.
FI/day (g)	14.4 $\pm$ 0.57	14.9 $\pm$ 0.89	14.7 $\pm$ 0.41	0.5	n.s.
FI/100 g Bwt/day (g)	11.5 $\pm$ 0.24	9.3 $\pm$ 0.62	10.5 $\pm$ 0.31	3.4	<0.005

n.s. = not significant.

Fig. 1. The percentage of total body water and fat at first estrus in the well-fed rat.