Carcass components at first estrus of rats on high-fat and low-fat diets: Body water, protein, and fat

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Contributed by D. M. Hegsted, November 1, 1976

ABSTRACT Carcass analysis data show that weanling rats fed high-fat (HF) and low-fat (LF) diets until the day of first estrus had similar body compositions at estrus, although the HF rats had estrus significantly earlier and at a lighter body weight ($P < 0.01$) than the LF-diet rats. Total water as percent of wet weight, protein as percent of wet weight, and the water/protein ratio of the HF- and LF-diet rats did not differ significantly, whereas the absolute amounts of body water and protein of the two diet groups differed significantly ($P < 0.01$), in accord with the difference in body weights of the HF and LF rats. The means (± SEM) of total body water as percent of wet weight were $66.2 ± 0.3\%$ for the HF rats and $66.4 ± 0.3\%$ for the LF-diet rats, whereas the mean absolute total body water for the two diet groups was $69.7 ± 2.2\, g$ and $81.1 ± 2.4\, g$, respectively ($P < 0.01$). Carcass fat of the HF rats, $15.6 ± 1.0\, g$, was identical with that of the LF-diet rats, $15.6 ± 0.9\, g$. The HF rats were therefore relatively fatter, $14.6 ± 0.6$ wet weight $\%$, than the LF-diet rats, $12.6 ± 0.4$ wet weight $\%$ ($0.05 > P > 0.01$). The percentage of water in the fat-free wet weight of the HF rats, $77.5 ± 0.3\%$, was significantly ($P < 0.01$) greater than that of the LF-diet rats, $76.1 ± 0.3\%$. These data and the high percentages of total water of body weight show that at first estrus the HF- and LF-diet rats had not as yet attained an adult body composition, similar to the human female at menarche. Within each diet group, the percentages of total water/body weight, protein/wet weight and fat/wet weight, or fat/dry weight, did not change significantly with increasing age of estrus, whereas each absolute carcass component—body water, protein, and fat—increased significantly with increasing age of estrus. The carcass data support C. C. Kennedy’s hypothesis that a metabolic signal, related to fat storage, is a signal for puberty in the rat and are in accord with the hypothesis that a critical body composition of fatness is necessary for estrus in the rat, as in the human female. The greater relative fatness of the HF-diet rats may be associated with higher levels of estrogen in the HF rats than in the LF-diet rats.

Weanling rats fed a high-fat (HF) diet had the first estrus significantly earlier than rats fed a low-fat (LF) diet (1). The caloric intake per $100\, g$ of body weight of the HF-diet and LF-diet rats did not differ at vaginal opening or at estrus, whereas the HF- and LF-diet rats differed significantly at both events in age, body weight, absolute and relative food intake, and absolute caloric intake (1). It was suggested that the constant caloric intake per unit of body weight at estrus may reflect the attainment of a particular body composition of fat/lean body weight, or fat/body weight (2), which may be essential for estrus and ovulatory cycles in the rat (1), as in the human female (3, 4).

This paper reports the body water, protein, and fat, determined by carcass analysis, of the HF-diet and LF-diet rats at first estrus. As far as we know, there have been no other published studies of the body composition of the rat at estrus or at vaginal opening. Reed et al. (5, 6) showed by carcass analysis that weanling female and male rats raised on a HF diet were absolutely and relatively fatter at various body weights ($50, 150, 250\, g$) than controls raised to the same weight on an equicaloric carbohydrate diet (5, 6).

MATERIALS AND METHODS

The HF and LF diets fed to the Charles River (CD) weanling female rats and the method of determination of first estrus (hereafter termed estrus) were described in a previous report (1).

There were 21 rats in each diet group. Rats were killed with ether on the day of first estrus, and kept frozen in small plastic bags until they were analyzed. Before analysis, the abdomen was opened and the stomach and cecum were excised and discarded. After weighing (wet weight), each carcass was chopped into small pieces, placed in a tared beaker, and dried at $95\degree$ to constant weight. The weight lost is considered to be body water. The dried carcasses were ground and aliquots were taken for fat determination (overnight Soxhlet extraction with ether) and total nitrogen (N) by the Kjeldahl method (7). Body protein was calculated as $N × 6.25$. The data for one HF rat (#7) were eliminated because the protein value (7.5 g) was unreasonable on two analyses.

RESULTS

Total Body Water. At estrus, total body water (total water, TW) as percent of wet weight ($100 \times$ the ratio of total water (g): wet weight (g)) of the HF-diet rats, $66.2 ± 0.3\%$, (SD 1.5$-$), did not differ significantly from that of the LF-diet rats, $66.4 ± 0.3\%$ (SD 1.4$-$) (Fig. 1), whereas the absolute value of total water of the HF rats, $69.7 ± 2.2\, g$ (SD 9.8$-$) differed significantly from that of the LF-diet rats, $81.1 ± 2.4\, g$ (SD 10.8$-$) (Table 1, Fig. 2).

Total water increased significantly with increasing age of estrus for both HF- and LF-diet rats (Fig. 2).

HF: $TW(g) = -16.7 \pm 2.58 \times \text{Age}_{estrus}$

LF: $TW(g) = -26.9 \pm 2.89 \times \text{Age}_{estrus}\,$

Total water as percent of wet weight (percent water) did not change significantly with increasing age of estrus for LF-diet rats (Fig. 1).

LF: Percent water $= +72.68 \pm 0.17 \times \text{Age}_{estrus}\,$

For HF-diet rats, percent water decreased significantly with age of estrus (Fig. 1).

HF: Percent water $= 73.85 \pm 0.23 \times \text{Age}_{estrus}\,$

Abbreviations: HF, high fat; LF, low fat.
However, if HF rat #5, an exceptional, late-maturing, heavy rat (1) was omitted, percent water also did not change significantly with increasing age of estrus for the HF-diet rats (Fig. 1).

HF: Percent water = $69.27 - 0.08 \times \text{Age}_{estrus}$

Protein (N \times 6.25). Protein as percent of wet weight (100 \times the ratio of protein (g): wet weight (g) of the HF-diet rats, 14.6 \pm 0.6\% (SD 1.7\%), did not differ significantly from that of the LF-diet rats, 15.1 \pm 0.3\% (SD 1.5\%) (Fig. 3), whereas the absolute total protein of the HF-diet rats, 15.4 \pm 0.6 g (SD 2.8 g), was significantly less than that of the LF-diet rats, 18.5 \pm 0.7 g (SD 3.0 g), as would be expected from the lighter body weight of the HF-diet rats at estrus (1) (Table 1).

Protein (g) increased significantly with increasing age of estrus for both HF- and LF-diet rats (Fig. 4).

HF: Protein (g) = \(-6.7 + 0.66 \times \text{Age}_{estrus}\)

LF: Protein (g) = \(-4.2 + 0.61 \times \text{Age}_{estrus}\)

Protein as percent of wet weight did not change significantly with increasing age of estrus for either HF- or LF-diet rats (Fig. 3).

HF: Protein/wet weight (%) = 12.66 + 0.05 \times \text{Age}_{estrus}

LF: Protein/wet weight (%) = 18.01 + 0.08 \times \text{Age}_{estrus}

The water/protein ratio of the HF-diet rats, 4.6 \pm 0.1 (SD 0.5), did not differ significantly from that of the LF-diet rats, 4.4 \pm 0.1 (SD 0.5) (Table 1).

Body Fat. The mean body fat of the HF- and LF-diet rats at estrus was identical, 15.6 \pm 1.0 g (SD 4.5 g) and 15.6 \pm 0.9 g (SD 4.1 g), respectively (Table 1). However, the HF-diet rats were relatively fatter, 14.6 \pm 0.6 wet weight % (SD 2.6\%) and 42.9 \pm 1.3 dry weight % (SD 5.9\%), than the LF-diet rats, 12.6 \pm 0.4 wet weight % (SD 2.0\%) and 37.5 \pm 1.1 dry weight % (SD 4.9\%) (Table 1), because the body weight of the HF rats was significantly lighter (at their earlier age of estrus) than that of the LF rats (1).

Body fat increased significantly with increasing age of estrus for both HF- and LF-diet rats (Fig. 5), as would be expected from the fact that body weight increased significantly with increasing age of estrus for both diet groups (1).

HF: Fat (g) = \(-14.70 + 0.90 \times \text{Age}_{estrus}\)

LF: Fat (g) = \(-17.80 + 0.89 \times \text{Age}_{estrus}\)
However, relative fatness [fat/dry weight (%) and fat/wet weight (%)] did not change significantly with increasing age of estrus for either the HF- or LF-diet rats (Fig. 6):

HF: Fat/dry weight (%) = 40.30 + 0.076 (±0.40) · Ageestrus
Fat/wet weight (%) = 10.09 + 0.140 (±0.17) · Ageestrus
LF: Fat/dry weight (%) = 19.86 + 0.470 (±0.30) · Ageestrus
Fat/wet weight (%) = 4.28 + 0.220 (±0.14) · Ageestrus

Age of Estrus Versus Body Fat and Relative Fatness. As would be expected from the preceding equations, age of estrus increased significantly with increasing absolute carcass fat (g) for both HF- and LF-diet rats (Fig. 7):

HF: Ageestrus = 25.37 + 0.52 (±0.14) · Fat (g)
LF: Ageestrus = 28.20 + 0.59 (±0.07) · Fat (g)

Age of estrus did not change significantly as a function of relative fatness, either fat/wet-weight (%) or fat/dry weight (%), for HF- or LF-diet rats:

HF: Ageestrus = 32.39 + 0.026 (±0.20) · Fat/dry weight (%)
Ageestrus = 29.90 + 0.240 (±0.32) · Fat/wet weight (%)
LF: Ageestrus = 29.26 + 0.220 (±0.10) · Fat/dry weight (%)
Ageestrus = 29.50 + 0.630 (±0.36) · Fat/wet weight (%)

**DISCUSSION**

The carcass data show that at first estrus the HF- and LF-diet rats had similar body compositions, although their ages and body weights differed significantly (1). Total body water as percent of wet weight, protein as percent of wet weight, and the water/protein ratios did not differ significantly between the two diet groups, whereas the absolute amounts of water and protein differed significantly, in accord with the weight differences of the HF- and LF-diet rats.

The identical means for carcass fat (g) for HF- and LF-diet rats was unexpected. This finding does not necessarily mean the two groups had identical amounts of adipose tissue. Since
the mean body weight of the HF-diet rats at estrus was significantly lighter than that of the LF rats. The HF-diet rats were relatively fatter at estrus than the LF-diet rats (Figs. 6–8, Table 1). The most probable reason for this difference in relative fatness is that the HF-diet rats had a more rapid rate of growth to estrus (1). It is known that rapidly growing rats (8), sheep, cows and pigs (9, 10), and human beings (11, 12) have more fat per unit body weight than do their slower-growing counterparts. The reason for this difference is not known. A possible explanation is that the faster growers have a longer intestinal tract relative to body weight (8, 13, 14) and therefore digest food more efficiently.

The percentage of water in the body normally is an index of relative fatness (15, 16) because of the relative constancy of water, about 0.72, in the adult lean body mass (17–19). However, we found that the HF- and LF-diet rats did not differ in percent water and did differ in relative fatness. The explanation is a slight, but significant, difference between the two groups in the percentage of water in the fat-free wet weight (lean body mass): the HF-diet rats had 77.5 ± 0.3% water, the LF rats, 76.1 ± 0.3% water (F < 0.01) in the fat-free wet weight.

This finding is most probably explained by the difference in ages of the HF- and LF-diet rats at estrus. Normally there is a decrease of the percentage water in the fat-free body mass (i.e., the lean body mass which includes the skeleton) with increasing age because of the differential increase of the skeleton relative to the skin or muscle—both the latter tissues have a relatively high water content (19). The LF-diet rats were older than the HF rats at estrus. The LF-diet rats therefore would be expected to have a slightly larger skeletal component in the fat-free body mass than the HF-diet rats, and thus a slightly lower water content of the fat-free mass compared to the HF-diet rats (19). The finding of relatively high percentages of water in the lean body mass of both the high-fat and low-fat diet rats compared to the usual values observed in the adult rat (17, 18) shows that at first estrus the rats had not as yet attained an adult body composition.

The relatively high percentages of water as percent body weight at estrus of the HF- and LF-diet rats, 66.2 ± 0.3 and 66.4 ± 0.3%, respectively, also showed that the HF- and LF-diet rats had not as yet attained an adult body composition. Scheer et al. (20) reported that female rats raised on 5 and 20% fat diets (ad libitum) were 58.8 and 56.7% water/body weight, respectively, at the mean body weights of 202 and 223 g, respectively (no ages given, presumably adult ages at these weights). The 5% fat diet rats had 19.1% carcass fat at 202 g; the 20% fat diet rats were 21.7% carcass fat at 223 g (20). From these data of Scheer et al. (20) we can calculate that the water in the fat-free mass of the rats fed 5 and 20% fat diets was 0.73 and 0.72, respectively, in accord with other data for adult animals (18, 19).

At first estrus, therefore, both the HF- and LF-diet rats, similar to the human female at menarche (21), have not as yet attained the adult body composition of percentage water, or percentage fat, of body weight. In the human female, the attainment of adult body composition is correlated with the attainment of a high level of fecundity (21) (R. E. Frisch, unpublished data). Girls have a period of adolescent sterility or subfecundity during the time interval from menarche to the completion of growth (21, 22). Similarly, the first estrus in the rat is usually followed by a period of partial infertility; litter size rises to a peak as the animal matures (23). Our findings suggest that full fecundity in the rat may also be associated with adult body composition.

Within each diet group of HF and LF rats, total body water as percent of wet weight, protein as percent of wet weight, and fat as percent of wet weight (or as percent of dry weight) did not change significantly with increasing age of estrus, whereas each absolute carcass component increased with increasing age of estrus, as expected from the increasing body weight with age in both groups (1).

The findings that percent water did not differ between the two groups of rats at estrus, and that it also did not vary within each group at estrus (Fig. 1) is especially interesting because of the importance of this parameter at sexual maturation in the human female (3, 4, 11, 21).

Also significant, relative to the adolescent growth of the human female, is the finding in the rat that fat as percentage of wet weight (or as percentage of dry weight) at estrus did not change significantly within each diet group with increasing age of estrus, although the later maturing rats in each group were significantly heavier than the earlier maturing rats. A late maturing rat apparently had to be at a heavier weight in order to be at the same relative fatness as an earlier maturing rat. In contrast, the human female adolescent growth spurt seems to make prepubertal fast and slow growers more equal in their efficiency of growth during the adolescent growth spurt, so at menarche they are at about the same relative fatness (earlier matures somewhat fatter than later matures), at about the same body weights (24, 25).

The finding that the HF-diet rats were relative fatter, at a lighter weight, than the LF-diet rats at estrus, whereas there was no significant difference in relative fatness within each diet group with increasing weight at estrus, indicates that the greater number of calories from fat eaten by the HF-diet rats had a greater effect on relative fatness than was shown by the early and late maturing HF or LF rats, who presumably differed genetically in tempo of growth.

The finding that the LF-diet rats were relatively less fat than the HF rats at estrus may be the explanation of the smaller percentage of LF-diet rats with simultaneous vaginal opening and estrus, and the longer interval between vaginal opening and estrus, observed in the LF rats (1). These differences had suggested lower levels of estrogen in the LF-diet rats than the HF-diet rats. Nimrod and Ryan (26) have found that aromatization of androgens to estrogen takes place in adipose tissue in the human female. Adipose tissue may therefore be a significant extragonadal source of estrogen (26). Also, the difference in relative fatness of the HF- and LF-diet rats may affect the metabolism of estrogen, which has been shown to vary with

![FIG. 8. Fat versus dry weight for HF and LF rats.](image-url)
body weight and fatness in women (1, 27), and/or the secretion of gonadotropins. Scheer et al. (28) have noted that the sexual development, subsequent fertility, and lactation performance of rats recovering from undernutrition were all greater on diets containing liberal amounts of fat (10–40%) than on diets containing little or no fat (0–5%). A difference from controls in the length of estrous cycles has also been observed in adult rats maintained on a very high fat diet (40%) (29).

The role of estrogen in modulating food intake (30) and fat storage (17), and the reverse relationships indicated above of the role of adipose tissue in the secretion and regulation of gonadal hormones remain to be elucidated. Two interesting questions are: (i) whether the rate of deposition of fat, which affects the degree of saturation (31), affects interactions, if any, with gonadal hormones; and (ii) whether specific fat depots, such as the gonadal and parametrial depots in the rat (5, 6, 32) have a special function.

The body composition data at first estrus of the HF- and LF-diet rats support Kennedy's hypothesis that some signal related to metabolic rate or food intake (1), determined by fat stores (33), is a signal for puberty in the rat. How the hypothalamus may be signaled, and the nature of the signal, are not known.

Finally, the data indicate that there are early and late maturing rats, which may differ endocrinologically as well as physiologically.

We thank George Wong for assistance with the statistical calculations, and Professor Robert Reed, Department of Biostatistics, Harvard School of Public Health, for helpful discussion of the statistical results. D.M.H. was supported in part by Public Health Service Research Grants HL-12399 and KS-AM18455, National Institutes of Health. R.E.F. was supported in part by Population Council Grant no. D74-94C. The paper was written while R.E.F. was a Fellow of the John Simon Guggenheim Memorial Foundation (1975–1976).