Chronic Underfeeding Increases the Positive Feedback Efficacy of Estrogen on Gonadotropin Secretion (44188)

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Abstract. **Reduced caloric intake has been shown to inhibit reproductive cycles in females of several mammalian species. Previous studies have shown that increased negative feedback efficacy of estrogen on gonadotropin secretion may be responsible. The present study was designed to test the alternate hypothesis that caloric restriction alters the positive feedback efficacy of estrogen on gonadotropin secretion. Adult, cycling female rats were placed on reduced food intake (R) equal to 50% of that consumed by *ad libitum*-fed controls (C). When R rats stopped cycling, both R and C rats were ovariectomized (OVX) and immediately implanted subcutaneously with a Silastic capsule containing 100 μg 17β-estradiol (E_2). Blood samples were obtained at 0900–1000 hr and 1600–1730 hr on Days 2, 4, 6, 8, 10, 12, and 14 after OVX and implantation. Follicle-stimulating hormone (FSH), luteinizing hormone (LH), and E_2 were measured by radioimmunoassay in duplicate aliquots. Results indicate that underfed female rats retain the ability to respond to elevated estrogen levels with an afternoon surge of gonadotropin which is present for at least 14 days for LH. By contrast, FSH surges in R rats became progressively smaller and were no longer significant after Day 10. The present results also demonstrate that the response of R rats to elevated estrogen levels is significantly greater than that of C rats on Days 2–4 for FSH and 2–14 for LH. It is concluded that an inability to respond to elevated estrogen levels with an afternoon LH surge is not the cause of the cessation of normal estrous cycles. The progressive decrease in the afternoon surge of FSH may be, at least partly, responsible for the decreased follicular development observed in underfed rats. Possible explanations of the enhanced LH response to the positive feedback of estrogen are discussed.** [P.S.E.B.M. 1997, Vol 216]

Caloric restriction has been demonstrated to inhibit reproductive cylicity in females of several species of mammals (for reviews see Refs. 1–3). Although the endocrine mechanisms mediating the nutritional effects have not been unequivocally established, in the adult female rat an enhanced negative feedback by estrogen on luteinizing hormone (LH) secretion has been shown to be at least partly responsible for the diet-induced suppression of cyclicity (4–7). Similar results implicating increased negative feedback have also been observed in the prepubertal female rat (8, 9). Increased positive feedback efficacy by estrogen has also been demonstrated in the prepubertal (10), but not the adult female rat, although response to exogenous gonadotropin-releasing hormone (GnRH) has been demonstrated (11, 12). The purpose of the present study was to investigate the effects of reduced caloric intake on the positive feedback of estrogen in adult female rats.

Materials and Methods

Animals. Female rats (Holtzman/Sprague-Dawley) were obtained at the age of 45 days. They were housed in plastic cages on a bedding of pine shavings in a controlled environment of 21° ± 1°C temperature and a 14-hr photoperiod (lights on 0500–1900 hr). Water was provided *ad libitum* to all animals throughout the study. Purina Lab Chow (Ralston Purina Co., St. Louis, MO) was provided *ad libitum* until normal estrous cycles could be ascertained

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through daily vaginal smears. At this time, half of the total number of rats were placed on a reduced (R) food intake of 50% of normal daily consumption. The other half were used as controls (C) and remained on *ad libitum* intake. Daily normal food consumption was established by previous measurements and by continued periodic measurement of the daily intake of the C group. The chow contains an average of 26% protein and is fortified with essential vitamins and minerals. Total energy content is 4.25 kcal/g. R rats were considered acyclic when they exhibited a lack of vaginal cornification for eight consecutive days. Most rats began exhibiting this condition 2–3 weeks after they were placed on the reduced diet.

Experimental procedures were performed in compliance with the NIH *Guide for the Care and Use of Laboratory Animals* and were approved by the Marquette University Institutional Animal Care and Use Committee. All surgical procedures and blood sample collections were performed under ether anesthesia.

Hormone Measurements. Plasma was assayed in Experiment 1 for luteinizing hormone (LH) by double antibody radioimmunoassay (RIA) (13) using antibody (Pool 15) provided by Dr. G. D. Niswender, Colorado State University, and hormone for iodination obtained from Dr. L. E. Reichert, Albany Medical College. In the rest of the study, LH was assayed using RIA kits obtained from the NIADDK. All potencies are expressed as NIADDK-rLH-RP-2 equivalents. Plasma FSH concentrations were measured with kits obtained from the NIADDK and potencies are expressed as NIADDK-rFSH-RP-2 equivalents. All samples from a single experiment were assayed in one assay. Estradiol was measured in 0.5-ml aliquots of plasma, extracted with anhydrous ether, by modification (5) of the RIA method of Wright *et al.* (14) using antibody supplied by Dr. D. C. Collins, Emory University School of Medicine.

Estrogen-Filled Capsules. Silastic tubing (Dow Corning) of 1.57-mm i.d. × 2.41-mm o.d. was cut into 1-cm lengths and packed with 100 μg of 17β-estradiol (Sigma Co., St. Louis, MO) diluted in crystalline cholesterol (Sigma). Both ends of the capsule were sealed with Silastic Adhesive (Dow Corning). The capsules were subcutaneously implanted in a "nurse" rat for 18–24 hr in order to establish a stable rate of release before implantation into the experimental rats. Capsules were removed from the nurse animal and immediately implanted into the experimental rats.

Statistical Analysis. Where applicable, data were evaluated by analysis of variance (ANOVA). Duncan's new multiple range test was used to compare means within treatments, while between treatment comparisons were made by Student's *t* test. Statistical significance is indicated at the $P < 0.05$ level.

Experiment 1. The ability of 17β-estradiol to produce daily afternoon elevations of gonadotropin secretion was compared between 10 acyclic R and 10 age-matched C female rats. A single blood sample was obtained by cardiac puncture into a heparinized syringe at 0900–1000 hr of Day 0. Ovariectomy (OVX) was performed and at the same time a Silastic capsule containing 100 μg E_2 was placed subcutaneously in a dorsal location. On Days 2, 4, 6, 8, 10, 12, and 14 blood samples were drawn from all rats at 0900–1000 and 1600–1730 h. Plasma samples were stored at −25°C until they were assayed for LH in duplicate 200-μl and FSH in duplicate 50-μl aliquots respectively. Plasma E_2 concentrations were determined in samples from Days 2, 8, and 14. All rats were examined for the presence of the capsule at the time they were sacrificed.

Experiment 2. This experiment was designed to investigate whether some of the results obtained in Experiment 1 were influenced by the repeated bleeding two times per day on alternate days. The experimental protocol was similar to that utilized in Experiment 1, except that samples were collected only on Day 0 and on Days 2 and 14 following OVX.

Results

Experiment 1. *Plasma FSH concentrations.* ANOVA indicated a significant effect on plasma FSH concentrations (Fig. 1) for time of day and for days post-OVX in both C and R rats. A significant interaction between these two main effect was found in R but not C rats. A significant effect of level of food intake was also observed for morning but not afternoon samples. However, a significant interaction between level of food intake and days post-OVX was noted in the concentration of the afternoon samples.

Morning values of C rats were 2- to 3-fold higher following OVX and did not change significantly throughout the experiment, except Day 12, which was significantly higher than values measured on Days 2 and 4. Afternoon values in C rats were approximately 100% higher than

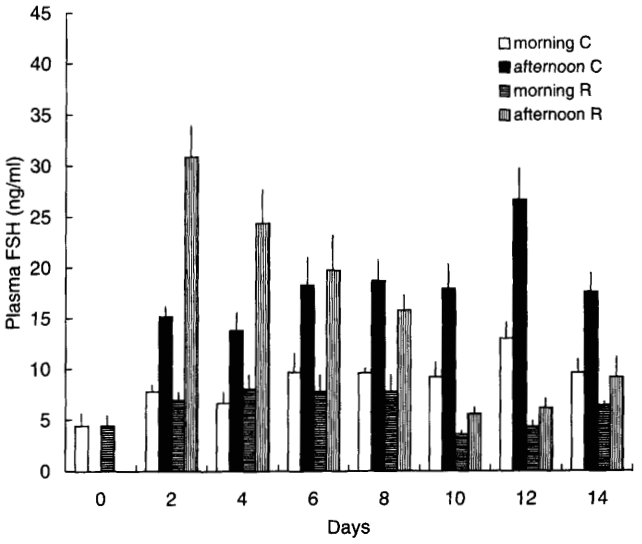

Figure 1. Morning and afternoon plasma FSH concentrations (mean ± SEM) of *ad libitum*-fed (C) and underfed (R) female rats on Days 2, 4, 6, 8, 10, 12, and 14 after ovariectomy and implantation of 100 μg 17β-estradiol.

morning values, and this difference was significant on all days of sampling.

Morning values in R rats also exhibited a rise following OVX, but the increase was significant only on Days 4–8 and did not differ from pre-OVX values on Days 10–14. The pattern of the afternoon values in R rats was different from those of the C group. An exaggerated (433%) increase was seen on Day 2. This was followed by a progressive decline between Days 2 and 8, and on Days 10–14 afternoon concentrations were lower than those observed in C rats.

Plasma LH concentrations. ANOVA indicated a significant effect on plasma LH concentrations (Figs. 2 and 3) for the time of day and for days post-OVX in both C and R groups. A significant interaction between these two main effects was found in R but not C rats. Level of food intake was found to exert a significant effect on both morning and afternoon values, and a significant interaction was observed between level of food intake and days post-OVX. No significant difference was found between pre-OVX concentrations and the morning concentrations on Days 2–8 in C rats and 2–10 in R rats. No significant changes in morning values of R rats were detected between Days 2 and 14. However, in C rats morning concentrations rose significantly on Day 12 when compared with values on Days 2–8 and remained elevated on Day 14. Afternoon concentrations in C rats showed an approximate 2-fold increase over morning values on Days 2 through 8. This difference declined on Days 10–14. By contrast, in R rats afternoon concentrations on Day 2 were 40 times as high as morning values. The difference between morning and afternoon values declined between Days 4 and 14, with a low value of a 2.3-fold increase on Day 12. The ratio of afternoon to morning values was higher in R (mean = 10.5) than in C (mean = 1.9) rats on all days sampled.

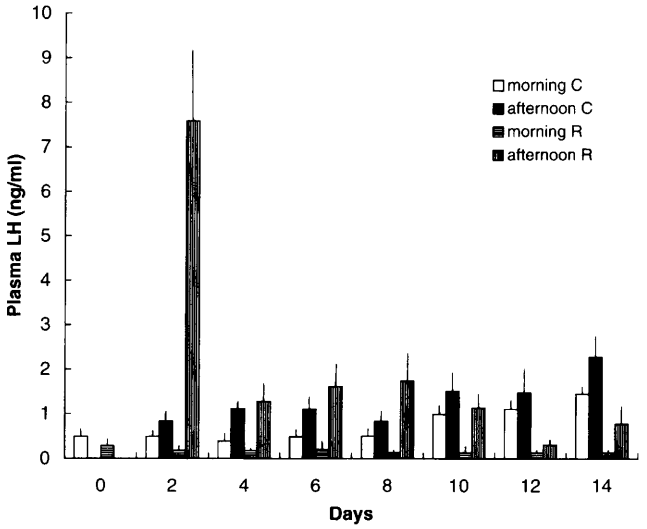

Figure 2. Morning and afternoon plasma LH concentrations (mean ± SEM) of *ad libitum*–fed (C) and underfed (R) female rats on Days 2, 4, 6, 8, 10, 12, and 14 after ovariectomy and implantation of 100 µg 17β-estradiol.

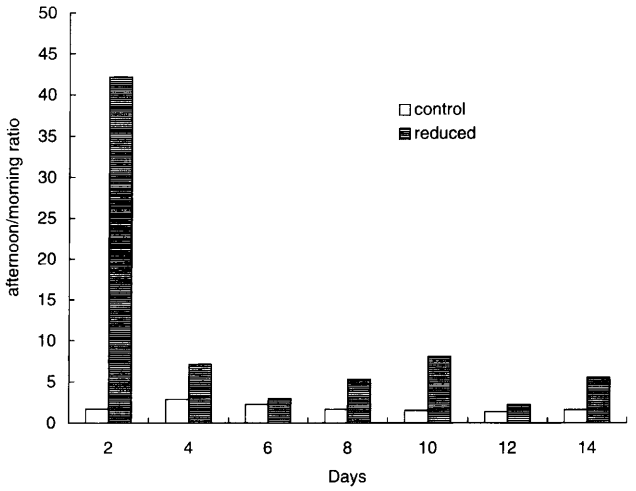

Figure 3. Ratios of afternoon to morning plasma LH concentrations in *ad libitum*–fed control and underfed female rats on Days 2, 4, 6, 8, 10, 12, and 14 after ovariectomy and implantation of 100 µg 17β-estradiol.

Plasma 17β-estradiol concentrations. ANOVA indicated no significant effects on plasma 17β-estradiol concentrations, (Fig. 4) for either level of food intake or days post-OVX.

Experiment 2. *Plasma FSH concentrations.* ANOVA revealed a significant effect on plasma FSH concentrations (Fig. 5) for time of day in both C and R groups and for days post-OVX in R rats. A significant interaction between these two main effects was also found in R rats. Level of food intake had a significant effect on afternoon, but not morning, values and the interaction between level of food intake and days post-OVX was also significant for the afternoon levels. The rise in afternoon concentrations on Day 2 was again greater in R than in C rats (192% vs 25% respectively). By Day 14, however, R rats showed no significant afternoon rise.

Plasma LH concentrations. ANOVA revealed a significant effect on plasma LH concentrations (Fig. 6) for time of day in both C and R animals, while days post-OVX had a significant effect only in R rats. The R group also showed a significant interaction between the two main effects. Level of food intake had a significant effect on both morning and afternoon values and a significant interaction was noted between level of food intake and days post-OVX for afternoon concentrations. The ratio of afternoon to morning concentrations was 26 and 3.1 in R and C rats, respectively.

Morning concentrations were significantly reduced in R rats when compared with C animals on both Days 2 and 14. By Day 14, the afternoon rise in R rats was reduced, but a significant, 2.4-fold increase was still present, while in C rats the afternoon rise was no longer significant.

Discussion

These studies demonstrate three important observations regarding underfed adult female rats, which have ceased cycling as a result of inadequate caloric intake.

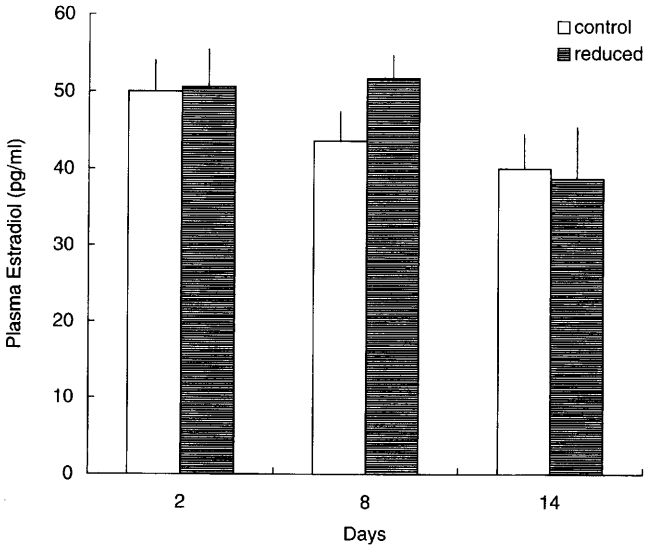

Figure 4. Plasma concentrations of 17β-estradiol (E_2) in *ad libitum*–fed control and underfed female rats 2, 8, and 14 days after ovariectomy and implantation of 100 μg E_2.

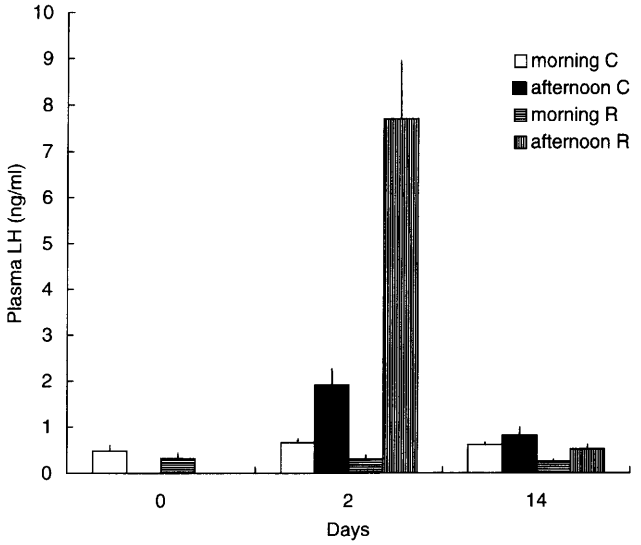

Figure 6. Morning and afternoon plasma LH concentrations (mean ± SEM) of *ad libitum*–fed (C) and underfed (R) female rats 2 and 14 days after ovariectomy and implantation of 100 μg 17β-estradiol.

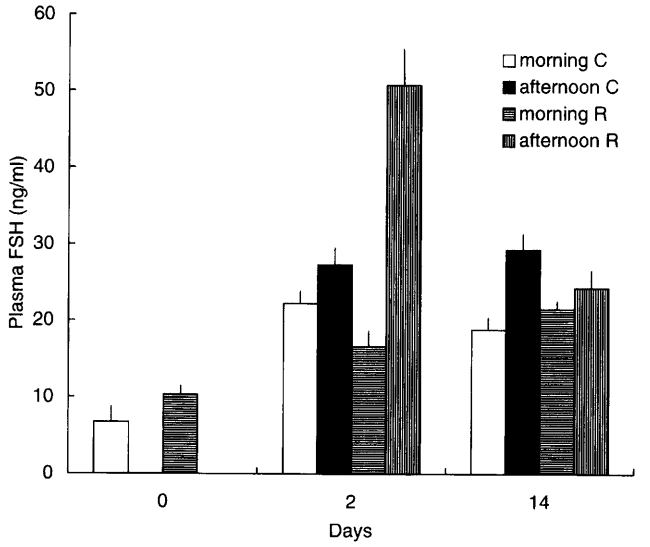

Figure 5. Morning and afternoon plasma FSH concentrations (mean ± SEM) of *ad libitum*–fed (C) and underfed (R) female rats 2 and 14 days after ovariectomy and implantation of 100 μg 17β-estradiol.

First, the results show for the first time that despite cessation of normal cycles, underfed adult rats are capable of responding to elevated estrogen levels with an afternoon rise in gonadotropin secretion. This observation, with respect to LH secretion, is consistent with results obtained in underfed prepubertal rats (10) and in underfed, ovariectomized lambs (15).

Second, the present study demonstrates that underfed female rats are capable of repetitive afternoon surges at least for 14 and 10 days for LH and FSH, respectively, following OVX and implantation of estrogen. In contrast, the afternoon LH rise in C rats was no longer significant after Day 10. This difference between the two groups may have been due to the fact that in R rats morning concentrations remained unchanged while in C rats they exhibited a consistent increase between Days 8 and 14. This increase in morning LH values of C rats suggests a partial "escape" from the negative feedback effect of E_2 since assayable levels of E_2 did not change significantly between Days 2 and 14, and vaginal smears remained cornified throughout the study. The E_2 levels are in agreement with proestrous concentrations of this hormone reported earlier (16) and measured in our laboratory (unpublished data). Morning concentrations of plasma LH in R rats remained below those observed in C rats throughout the study, indicating the increased negative feedback efficacy of estrogen reported in earlier studies (4–6).

In contrast to the maintained LH surges, the afternoon rise in plasma FSH concentrations of R rats appeared to diminish between Days 2 and 10, and was no longer significant on Days 12 and 14. On the other hand, in C rats, the afternoon to morning FSH concentration ratios remained constant throughout the study. Therefore, it is possible that such a gradual reduction and eventual loss of FSH surges in the intact rat may contribute to the reduced follicular development (17–19), which has been reported earlier as a result of reduced food intake (20). The reduced follicular development may result in inadequate estrogen levels to evoke a positive feedback effect and result in cessation of cyclicity. The lack of significant FSH surges on Days 12 and 14 in R rats were not due to the repetitive bleedings, since the animals in Experiment 2 showed a similar difference between Days 2 and 14.

Third, perhaps the most noteworthy observation resulting from this study is the exaggerated response of R rats to E_2. While the initial diet-induced increases in afternoon to morning ratios of plasma hormone concentrations is apparent for FSH, it is particularly enhanced for LH on Day 2 and remains elevated throughout the study when compared with

C rats. The reasons for this increase in the positive feedback efficacy of E_2 cannot be determined from the present data, but more than one possibility must be considered.

First, one must consider the possibility that circulating estradiol levels were different in R than in C rats. However, the present results indicate no differences at any of the three measured times. Therefore, this does not appear to be the reason for the increased response of R rats. Second, one must consider possible differences in progesterone levels between R and C rats. The role of progesterone in amplifying the LH surge has been documented (21). Although the rats in the present study were ovariectomized, differences in adrenal progesterone secretion may have accounted for the above observations. However, comparison of serum progesterone concentrations of OVX *ad libitum*–fed and underfed rats (22) did not reveal an increase as a result of reduced caloric intake.

The third explanation one must consider is the potential role of Neuropeptide Y (NPY) in the enhanced response of the LH secretory mechanism to E_2. The role of NPY in modulating LH secretion has been well documented (23). In particular, this substance has been shown to both stimulate gonadotropin-releasing hormone (GnRH) secretion from the medial basal hypothalamus and to increase pituitary response to GnRH in pentobarbital-blocked proestrous rats (24, 25). By contrast, in OVX rats NPY has an inhibitory effect on LH secretion (23). Studies in starved female rats have shown a significant increase in hypothalamic NPY concentrations (26), and in the underfed sheep an increase in the level of NPY mRNA has been demonstrated (27, 28). Therefore, the exaggerated response to exogenous E_2 shown by R rats in the present study may have been the result of increased NPY secretion, resulting in amplified GnRH secretion and/or increased pituitary gonadotrope response to GnRH.

The fourth possible explanation that one must consider is the role of corticosterone. Although there is general agreement on the ability of corticosterone to enhance the FSH surge in estrogen primed rats, the results of studies regarding the effects of corticosterone on the LH surge are equivocal (29, 30). Yet, the results of the present study show a more pronounced effect on LH than FSH secretion. Whereas the afternoon elevations of LH in R rats remain significant through the duration of the study, the afternoon elevations of FSH decline after Day 4 and are no longer significant on Days 12 and 14. Furthermore, measurements of corticosterone in female rats following 3–4 weeks of reduced food intake indicated no significant increases in either morning or afternoon plasma corticosterone concentrations when compared with *ad libitum*–fed controls (Piacsek and Becker, unpublished data). Therefore, altered corticosterone secretion does no appear to be responsible for the enhanced positive feedback efficacy of estrogen.

In summary, the present studies show that the positive feedback efficacy of E_2 in calorically restricted adult rats is not only preserved but, in fact, increased. Therefore, an inability to evoke an afternoon LH surge is not the cause of the cessation of normal estrous cycles.

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