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Caloric Restriction Inhibits Steroid-Induced Gonadotropin Surges in Ovariectomized Rhesus Monkeys

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We recently reported that caloric restriction inhibited ovulation in rhesus monkeys. The objective of the current study was to determine if caloric restriction affected the positive feedback response to ovarian steroids in non-human primates. Studies were conducted in four long-term ovariectomized rhesus monkeys. Animals were given an estrogen/progesterone challenge while maintained on a normal diet and on a diet that reduced body weight by approx 20%. In all cases, animals were maintained at the desired weight [based on a calculation of body mass index (BMI)] for a minimum of 4 wk before initiating the steroid challenge. Caloric restriction reduced BMI from 23.3 ± 0.3 to 18.9 ± 0.2 kg/m^2. The estrogen/progesterone challenge elicited an LH and FSH surge in each animal maintained at a normal BMI. By contrast, gonadotropin surges were significantly compromised when monkeys were challenged at a low BMI. In addition to affecting the reproductive axis, caloric restriction stimulated cortisol release and suppressed T_3 secretion. These endocrine effects of caloric restriction are consistent with our findings in ovary-intact monkeys. In summary, our previous reports in ovary-intact animals confirmed an effect of caloric restriction on tonic gonadotropin secretion leading to anovulation. Our current results suggest the effects of caloric restriction on the reproductive axis extend beyond inhibition of tonic gonadotropin secretion to include a disturbance of phasic gonadotropin secretion.

Key Words: Caloric restriction; positive feedback; LH; FSH; rhesus monkey.

Introduction

A negative energy balance characteristic of anorexia nervosa, intense athletic training, and/or severe dieting is associated with anovulation *(1)*. Nutritional amenorrhea results primarily from decreased secretion of gonadotropin-releasing hormone (GnRH). Gonadotropin secretion reverts to a pre-pubertal pattern of secretion *(2)*, while pituitary responsiveness to GnRH is significantly reduced *(3)*. This decreased drive by GnRH neurons results in low levels of circulating gonadotropins, which subsequently decreases production and secretion of ovarian steroids *(4)*. In addition to reproductive dysfunction, nutritional amenorrhea is accompanied by several metabolic disturbances including increased activity of the hypothalamic–pituitary–adrenal (HPA) axis and suppression of the thyroid axis *(5)*.

We recently reported that chronic caloric restriction leading to significant weight loss inhibited ovulation in rhesus monkeys *(6)*. Moreover, we showed that increased food intake and weight gain restored normal ovulatory function in these animals. Caloric restriction reduced basal levels of follicle-stimulating hormone (FSH), which likely impaired follicular development and contributed to anovulation. Although tonic gonadotropin secretion is essential to follicular development and ovulation, ovulation ultimately requires a gonadotropin surge induced by the positive feedback action of 17-β estradiol from the dominant follicle *(7)*. In order to elucidate possible neuroendocrine mechanisms involved in this primate model of nutritional amenorrhea, we compared the positive feedback responses of luteinizing hormone (LH) and FSH to an estrogen/progesterone challenge under normal dietary conditions and following chronic caloric restriction.

Results

The effect of caloric restriction on the LH surge in response to an estrogen/progesterone challenge in four ovariectomized rhesus monkeys is depicted in Fig. 1. A robust LH surge occurred in all four animals in response to a steroid challenge during normal BMI conditions. By contrast, only one animal had an LH surge during caloric restriction (panel 1D). Plotted in a similar fashion, Fig. 2 shows the effect of caloric restriction on the positive feedback response of FSH in the same four ovariectomized rhesus monkeys. All animals exhibited an FSH surge in response to a steroid challenge during normal BMI conditions. Caloric restriction inhibited the FSH surge in two animals, but had no effect on the FSH surge in the other two animals despite the LH surge having been inhibited in one of these animals (compare panels 1C and 2C).

Estrogen and progesterone levels for both normal and low BMI conditions are compared in Fig. 3. Progesterone

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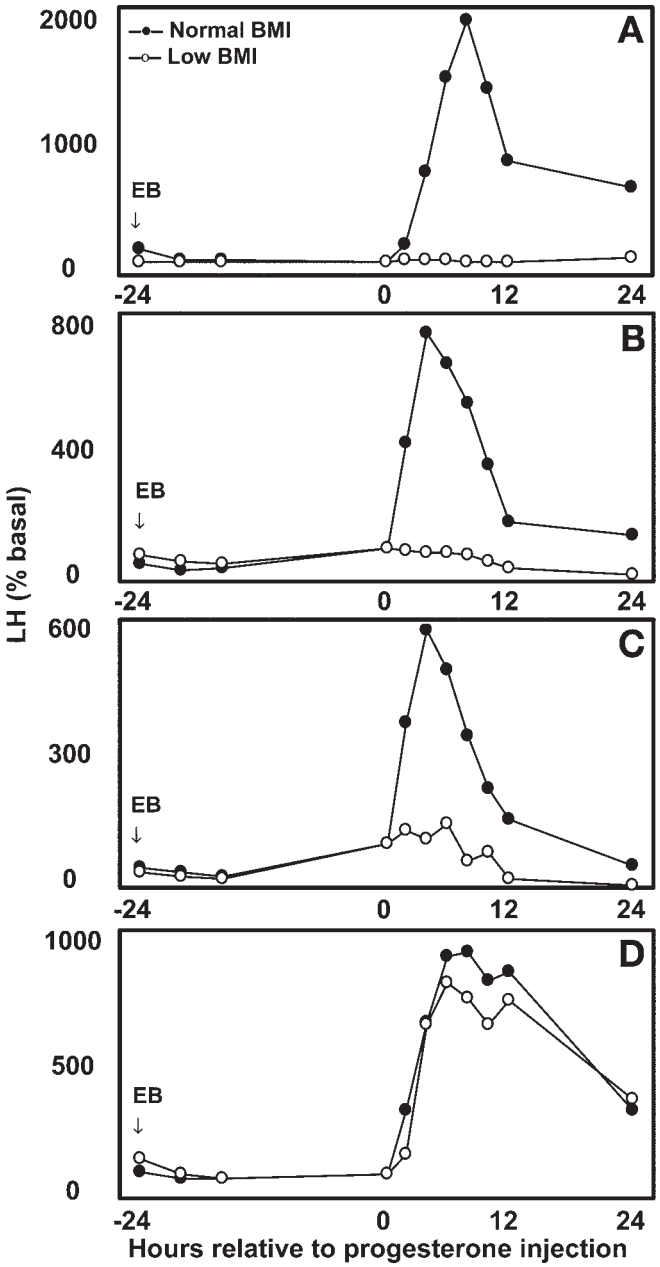

Fig. 1. Effect of caloric restriction on the positive feedback response of luteinizing hormone (LH) to estrogen and progesterone in ovariectomized rhesus monkeys. Individual LH responses following an estrogen/progesterone challenge at a normal or low BMI are compared. Two animals received a challenge while maintained at a normal BMI followed by a low BMI (panels **A** and **D**). The remaining animals were challenged initially at a low BMI followed by a normal BMI after re-alimentation (panels **B** and **C**). Values are expressed as a percentage of baseline. Basal levels are defined as LH levels at the time of progesterone injection. Caloric restriction inhibited LH surges in three of four animals.

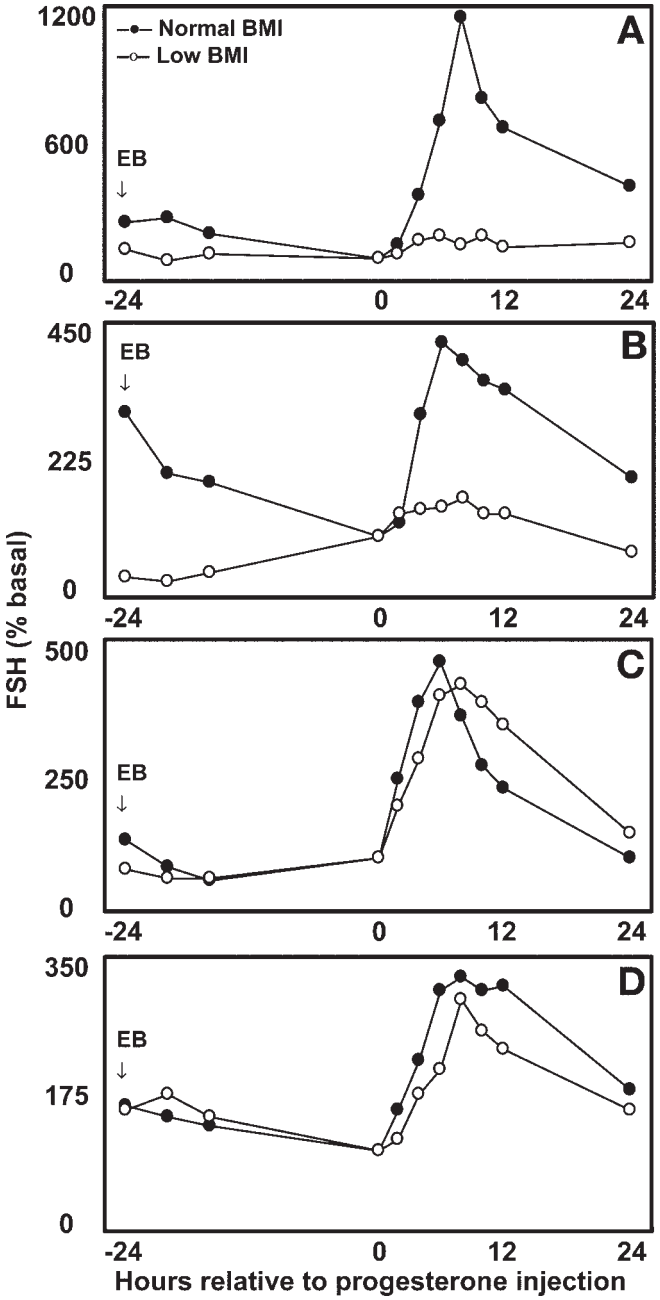

Fig. 2. Effect of caloric restriction on the positive feedback response of follicle-stimulating hormone (FSH) to estrogen and progesterone in ovariectomized rhesus monkeys. Details are the same as those described for Fig. 1. Caloric restriction inhibited the FSH surge in two animals (panels **A** and **B**), but had no effect on the FSH surge in the other two animals despite the LH surge having been inhibited in one of these animals (compare panels 1**C** and 2**C**).

profiles were identical under both experimental conditions. There was no difference in peak serum progesterone levels (paired *t*-test; $p = 0.24$) or the time to peak progesterone levels between groups (paired *t*-test; $p = 0.60$). Serum estradiol levels in the two groups were different in that the time to peak estradiol levels was delayed in caloric-restricted animals compared with controls (paired *t*-test; $p = 0.002$). Peak estradiol levels and estradiol levels at the time of the progesterone challenge were not significantly different between groups (paired *t*-test; $p = 0.54$ and $p = 0.40$, respectively).

The effects of caloric restriction and re-alimentation on cortisol and free triiodothryonine (T_3) secretion are presented

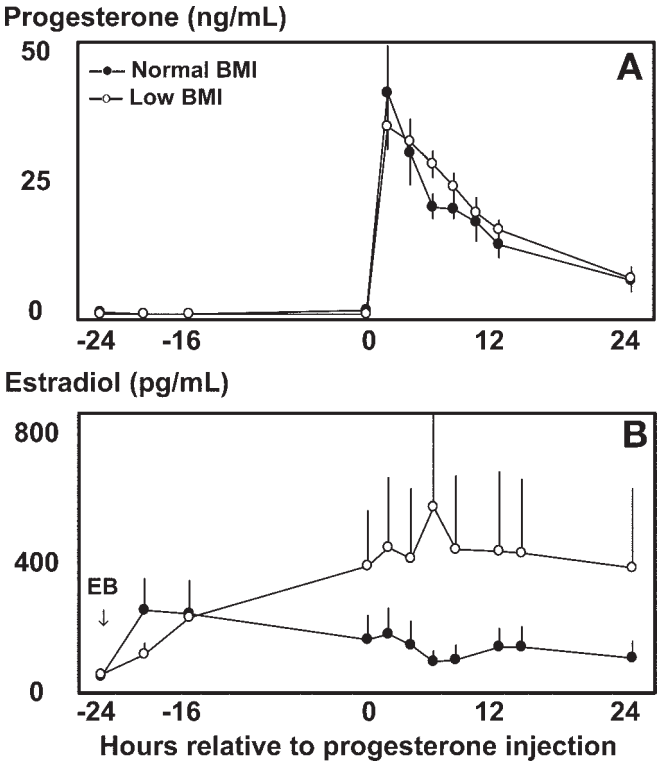

Fig. 3. Comparison of serum progesterone and estradiol levels following an estrogen/progesterone challenge under normal and low BMI conditions. Mean progesterone levels (± SE) in four ovariectomized rhesus monkeys following 2.5 mg of progesterone are shown for normal and low BMI conditions in panel **A**. Peak serum progesterone levels and time to peak progesterone levels were comparable between groups (paired *t*-test: $p = 0.24$ and 0.60, respectively). Mean estradiol levels (± SE) in four ovariectomized rhesus monkeys following 30 µg of estradiol benzoate are shown for normal and low BMI conditions in panel **B**. Peak estradiol levels were not different between groups (paired *t*-test; $p = 0.54$), whereas the time to peak estradiol levels was delayed in the low BMI group (paired *t*-test; $p = 0.002$).

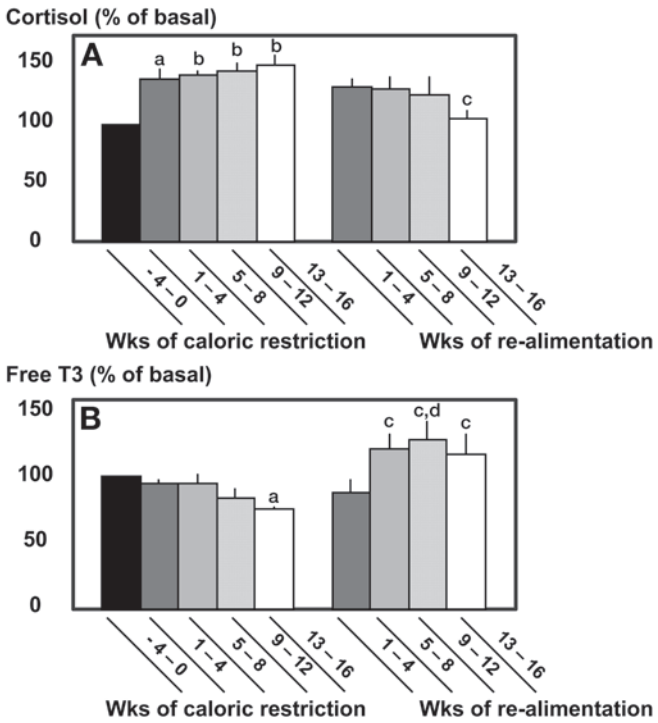

Fig. 4. Effect of caloric restriction and re-alimentation on cortisol and free T_3 secretion in ovariectomized rhesus monkeys. Mean integrated hormone levels (±SE, $n = 4$) at four successive time points during caloric restriction and re-alimentation are compared. Values are expressed as a percentage change from baseline. Basal levels are defined as mean levels in the 4 wk preceding caloric restriction. Cortisol secretion was significantly increased by caloric restriction (panel **A**: Tukey–Kramer; a = $p < 0.05$, b = $p < 0.01$), whereas re-alimentation decreased cortisol secretion to levels observed prior to caloric restriction (ANOVA; $p > 0.05$ compared to baseline; Tukey–Kramer; c = $p < 0.01$ compared with wk 13–16 of caloric restriction). Free T_3 levels were significantly decreased by caloric restriction (panel **B**: Tukey–Kramer; a = $p < 0.05$) and significantly increased by re-alimentation (Tukey–Kramer; c = $p < 0.05$ compared with wk 13–16 of caloric restriction; d = $p < 0.05$ compared with wk 1–4 of re-alimentation).

in Fig. 4. Cortisol secretion was significantly increased within 4 wk of caloric restriction and levels remained elevated throughout the 16 wk (Fig. 4A: Tukey–Kramer; $p < 0.05$). Re-alimentation decreased cortisol secretion to levels that were comparable to those observed prior to caloric restriction (ANOVA; $p > 0.05$). In contrast to cortisol, levels of free T_3 were significantly decreased following 13 wk of caloric restriction (Fig. 4B: Tukey–Kramer; $p < 0.05$). Within 4 wk of re-alimentation, T_3 secretion was increased to levels observed prior to caloric restriction (ANOVA; $p > 0.05$). T_3 levels continued to increase during re-alimentation and were significantly increased by wk 9–12 of re-alimentation (Tukey–Kramer; $p < 0.05$).

Table 1 summarizes nutritional and endocrine parameters at the time of the estrogen and progesterone challenge under control and caloric-restricted conditions. BMI and caloric intake (estimated from monkey chow alone) were significantly reduced in caloric-restricted animals (paired *t*-test: $p = 0.00005$ and $p = 0.03$, respectively). Cortisol levels at the time of the estrogen/progesterone challenge in caloric-restricted animals were significantly increased compared to control conditions (paired *t*-test: $p = 0.002$), whereas free T_3 levels were significantly reduced (paired *t*-test: $p = 0.005$). Peak estrogen and progesterone levels were comparable between groups (paired *t*-test: $p = 0.54$ and $p = 0.24$, respectively), as were estradiol levels at the time of progesterone injection (paired *t*-test: $p = 0.40$).

Discussion

Rhesus monkeys maintained at a normal BMI demonstrated unambiguous LH and FSH surges in response to an estrogen/progesterone challenge. By contrast, only one of four animals maintained at a low BMI demonstrated comparable gonadotropin responses to the steroid challenge. These data indicate that caloric restriction leading to significant

Table 1
Nutritional and Endocrine Parameters at the Time of the Estrogen and Progesterone Challenge Under Control and Caloric-Restricted Conditions

Monkey	BMI (kg/m2)	Caloric Intake (kJ/day)	LH/FSH Surge	Peak E_2 (pg/mL)	T_0 E_2 (pg/mL)	Peak P4 (ng/mL)	Cortisol (μg/dL)	Free T_3 (pg/mL)
				Control				
A	23.3	1769	Yes/Yes	500 (8)	349	59.4	41.8	3.9
B	23.8	2241	Yes/Yes	109 (4)	70	31.6	32.3	2.4
C	23.6	1769	Yes/Yes	128 (4)	95	54.8	47.7	2.7
D	22.6	1415	Yes/Yes	491 (4)	106	26.5	24.1	4.2
Mean	23.3 ± 0.3	1799 ± 169	4/4	307 ± 109	155 ± 65	43.1 ± 8.2	36.5 ± 5.2	3.3 ± 0.4
				Caloric Restriction				
A	19.2	1062	No/No	101 (26)	64	44.3	55.4	3.0
B	19.1	1651	No/No	1000 (30)	748	34.5	48.1	1.9
C	19.2	1062	No/Yes	998 (30)	492	40.5	59.3	1.9
D	18.2	1297	Yes/Yes	139 (24)	139	25.8	33.8	3.2
Mean	18.9 ± 0.2*	1268 ± 139*	1/4	560 ± 254	360 ± 159	36.3 ± 4.0	49.2 ± 5.6*	2.5 ± 0.3*
p value	0.00005	0.03		0.54	0.40	0.24	0.002	0.005

( ) = Indicates time (h) of peak estrogen concentration relative to EB injection.
T_0 E_2 = Estrogen concentration at time of progesterone injection (T_0).

weight loss interferes with the positive feedback response to ovarian steroids. These results complement a previous study in which we demonstrated that chronic caloric restriction inhibited tonic gonadotropin secretion and disrupted ovulation in ovary-intact rhesus monkeys *(6)*. Given that a gonadotropin surge is obligatory for ovulation, amenorrhea induced by chronic caloric restriction likely results from both a failure to stimulate follicular development and an inability to respond to signals that generate the gonadotropin surge.

Estradiol benzoate (30 μg) alone induces an LH surge in ovariectomized rhesus monkeys that closely resembles the spontaneous gonadotropin surge at midcycle *(8,9)*. Estradiol's primary site of action is the anterior pituitary, and not the hypothalamus, because the estrogen-induced gonadotropin surge in rhesus monkeys is unaffected by hypothalamic lesions *(10)*, surgical disconnection of the medial basal hypothalamus *(11)*, morphine administration *(12)*, or barbiturate anesthesia *(7)*. We chose to use a combined estrogen/progesterone challenge to elicit a gonadotropin surge for several reasons. First, the combined steroid challenge advances the gonadotropin surge, increases its amplitude, and decreases its duration *(8)*. These characteristics allow for the gonadotropin response to be accurately documented with relatively few blood samples. Second, the estrogen/progesterone–induced gonadotropin surge is mediated by a hypothalamic component. Accordingly, the estrogen/progesterone–induced surge is temporally associated with increased hypothalamic electrical activity *(13)* and can be blocked by GnRH antagonism in women *(14)* and by pentobarbital in monkeys *(8)*. Although the physiological relevance of the abbreviated estrogen/progesterone–induced gonadotropin surge is uncertain *(9)*, there is significant support for preovulatory progesterone secretion as a facilitator of the spontaneous gonadotropin surge *(9,15)*. In primates, a preovulatory rise in progesterone occurs approx 12 h before the onset of the gonadotropin surge *(15)*. This increase in progesterone secretion (approx 1 ng/mL) appears essential to the elaboration of the gonadotropin surge, because RU486 (a progesterone antagonist) blocked the preovulatory gonadotropin surge and ovulation in both women *(16)* and monkeys *(17)*. Despite the obvious disparity between periovulatory progesterone levels and those achieved by the estrogen/progesterone challenge, we chose the combined challenge, as it had the potential to demonstrate an effect of caloric restriction on either the hypothalamic or pituitary component of the positive feedback response. The observation that gonadotropin surges were inhibited in most caloric-restricted animals suggests that caloric restriction compromised the hypothalamic and/or pituitary response to the positive feedback actions of ovarian steroids.

Our current findings are consistent with the report that anorexic patients challenged with ethinyl estradiol did not exhibit gonadotropin surges *(18)*. The mechanism whereby anorexia inhibits the positive feedback response to estradiol is unknown. One possibility is that chronic inhibition of GnRH and estradiol may reduce gonadotrope responsiveness *(19)*. It should be noted that the ability to respond to estrogen positive feedback appears to predict the resumption of menses in recovering anorexics *(18)*. Following improved nutrition and weight gain, those that resumed menses demonstrated positive feedback responses to exogenous

estrogen while weight-recovered anorexics that remained anovulatory were unresponsive to an estrogen challenge. These data underline the importance of a functional gonadotropin surge mechanism for ovulation in patients recovering from chronic undernutrition.

When challenged during a low BMI condition, one animal had an LH and FSH surge while a second animal exhibited only an FSH surge. The most probable reason that gonadotropin surges were not inhibited in Monkey D is that the level of negative energy balance was less extreme in this animal. This explanation is supported by several lines of evidence. First, only a modest reduction in caloric intake (1415 vs 1297 kJ/d) was required to reduce her BMI from 22.6 to 18.2 (Table 1). Second, this animal demonstrated the lowest cortisol concentration of the four animals during caloric restriction. This level was substantially lower than the average (33.8 μg/dL compared to 49.15 μg/dL). Moreover, this animal had the highest T_3 level of the four animals during caloric restriction (3.2 pg/mL compared to a mean of 2.5 pg/mL). These two endocrine responses to caloric restriction imply that this animal was less metabolically challenged by the level of caloric restriction employed. The presence of an FSH surge in the absence of an LH surge suggests that gonadotropins may be differentially affected by nutritional status. In anorexic women, the FSH response to GnRH was shown to be intact and even enhanced, whereas the LH response was significantly impaired *(3)*. Because a greater FSH response to GnRH is also reported in prepubertal girls *(20)*, and the activity of the GnRH/gonadotrope axis of anorexics is similar to that of prepubertal/peripubertal girls *(2)*, this phenomenon may explain why FSH responsiveness to GnRH was maintained during caloric restriction and may extend to the mechanism of positive feedback. This postulate requires further assessment.

The serum progesterone profiles were identical under both normal and low BMI conditions. By contrast, peak estradiol concentrations tended to be higher in the low BMI group in addition to there being a significant delay in the time to peak estradiol levels. This was particularly the case in Monkeys B and C in which peak estradiol levels were almost 10-fold higher. We speculate that decreased body weight and fat content may have affected estrogen absorption and/or estrogen metabolism. Nevertheless, we have no reason to suspect that higher estrogen levels in the caloric-restricted animals might have inhibited the gonadotropin surge particularly because levels at the time of progesterone injection (T_0) were not significantly different between groups. Of the three animals in which the gonadotropin response was affected by caloric restriction, only one animal (Monkey B) demonstrated estradiol levels at T_0 that were inordinately higher than any of those reported under control conditions. In fact, no particular estrogen profile was associated with a particular gonadotropin response during caloric restriction. For example, despite similar peak estrogen levels, time to peak estrogen levels and estrogen levels at T_0, Monkeys A and D had very different gonadotropin responses during caloric restriction (no surge vs LH/FSH surge, respectively). Therefore, while the alterations in the estrogen profile during caloric restriction are curious, differences in estradiol levels do not appear to account for differences in the gonadotropin response.

Chronic caloric restriction stimulated the HPA axis as evidenced by a significant increase in cortisol secretion. Cortisol levels remained elevated throughout caloric restriction and were significantly higher at the time of the estrogen/progesterone challenge in caloric-restricted animals compared to controls. Given that cortisol secretion was normalized following re-alimentation and levels were significantly reduced in animals undergoing their second estrogen/progesterone challenge at a normal BMI, the re-establishment of gonadotropin surges may depend on normalization of cortisol secretion. In both human and non-human primates, long-term elevations in cortisol inhibit the reproductive axis *(21)*. Most evidence in primates suggests that cortisol inhibits GnRH secretion *(21)*. In human models of chronic energy restriction, such as anorexia and exercise-induced amenorrhea, basal cortisol levels are increased reflecting heightened activation of the HPA axis *(5)*. Although increased cortisol secretion in both athletes and anorexics reflects a metabolic state of undernutrition, activation of the HPA axis in these women, particularly in anorexics, is also associated with psychological stress *(22)*. Following nutritional rehabilitation and psychiatric treatment, the resumption of menses is accompanied by normalization of cortisol secretion suggesting cortisol may contribute to amenorrhea in these conditions.

Caloric restriction inhibited the thyroid axis while re-alimentation restored thyroid hormone secretion. Although these data infer a possible role for thyroid hormone in the maintenance of estrogen positive feedback, unlike cortisol, there is little support for thyroid hormone as a regulator of GnRH/LH secretion in primates *(23)*. Thyroid hormone did not reverse suppression of pulsatile gonadotropin secretion following food deprivation in monkeys indicating that decreased activity of the thyroid axis was not responsible for acute inhibition of gonadotropin secretion *(23)*. In fact, most evidence implicating thyroid hormone in the regulation of the reproductive axis indicates that thyroid hormone acts at the level of the ovary *(24)*. Recently, Williams and colleagues reported that changes in thyroid hormone levels paralleled changes in menstrual cyclicity in exercising monkeys *(23)*. Exercise-induced amenorrhea was accompanied by a decrease in thyroid hormone, while resumption of menses induced by increased caloric intake was associated with increased circulating thyroid hormone. We showed similar changes in free T_3 levels and ovulatory status in monkeys undergoing chronic caloric restriction followed by re-alimentation *(6)*. The current study extends these findings by demonstrating that changes in thyroid axis activity occur independently of changes in ovarian function.

In summary, caloric restriction inhibited steroid-induced gonadotropin surges in ovariectomized rhesus monkeys. This decrease in GnRH/pituitary responsiveness to ovarian steroid feedback suggests that disruption of phasic gonadotropin secretion is part of the neuroendocrine mechanism whereby nutritional deficits inhibit the reproductive axis.

Materials and Methods

Animal Husbandry

Studies were conducted in four ovariectomized female rhesus monkeys (*Macaca mulatta*). Animals were housed in groups of two or three animals in a light- and temperature-controlled environment (lights on: 0700 to 1900 h; temperature: 22°C). Monkeys ranged in age from 6 to 14 yr and weighed between 5.1 and 8.1 kg. BMI was calculated by dividing weight in kilograms by crown-rump length in meters squared *(25)*. The BMI recorded for each monkey was 28.5, 26.8, 26.4, and 23.0 kg/m^2. In order to achieve a uniform BMI between 23 and 24 kg/m^2, animals were separated for feeding (0900–1100 h and 1400–1600 h) and the diets were individualized. Adjustments to diets included: (1) increasing the vegetable component, (2) modifying the number of monkey chow biscuits allotted (Hi Protein Monkey Diet Jumbo, Ralston Purina, St. Louis, MO), and (3) eliminating high carbohydrate treats. Diets were supplemented twice daily with fruit. Water was available *ad libitum*. Monkeys were weighed weekly using a transfer box, which eliminated the need for sedation. All animal husbandry practices and experimental procedures conformed to regulations set forth by the Canadian Council on Animal Care and were approved by the Queen's University Animal Care Committee.

Experimental Protocol

Caloric Restriction and Re-alimentation

Beginning at a BMI of 23–24 kg/m^2, animals were placed on a 16-wk regimen of caloric restriction. The goal was to achieve a 20% decrease from normalized weight over 16 wk by decreasing caloric intake by a maximum of 40%. This was accomplished by decreasing the amount of monkey chow allotted to each animal. Every 2 wk, one cube of monkey chow was removed, provided weight loss had not exceeded the desired rate of decline. The bulk of each animal's diet was maintained by increasing the amounts of low calorie vegetables included in a midday meal. An iron-rich vitamin supplement was also included. Once a BMI of 18–19 kg/m^2 was achieved, the animals were maintained at that weight for a minimum of 4 wk, at which time the gonadotropin response to an estrogen/progesterone challenge was tested. Following the steroid challenge, food intake was gradually increased over a 16-wk period. The rate of re-alimentation depended on the rate of weight gain in each animal. In each case, the aim of re-alimentation was to restore a normal BMI (23–24 kg/m^2) within 16 wk. Throughout caloric restriction and re-alimentation, food intake was monitored daily, animals were weighed weekly, and blood samples drawn (0900–1000 h) weekly by venipucture from conscious animals. Samples were assayed for LH, FSH, cortisol and free T_3.

Estrogen/Progesterone Challenge

Each animal was randomly assigned to undergo two estrogen/progesterone challenges while maintained at (1) a normal BMI followed by a low BMI or (2) a low BMI followed by a normal BMI. In all cases, animals were kept at the desired BMI for a minimum of 4 wk before initiating the estrogen/progesterone challenge. The model for inducing the gonadotropin surge involved a low dose of estrogen followed by progesterone. This method of eliciting a positive feedback response has been thoroughly characterized *(8)*. Ovariectomized animals received a subcutaneous implant of a Silastic® capsule (inner diameter 0.132 in., outer diameter 0.183 in., 3 cm in length; Dow Corning, Midland, MI) containing 17-β estradiol as previously described *(26)*. Following a 24-h incubation in phosphate-buffered saline, a capsule was implanted subcutaneously in the scapular region of anesthetized animals (Ketamine HCL 10 mg/kg, Rogarsetic, Montreal, QC, Canada; and Valium 25 mg/kg, Sabex, Boucherville, QC, Canada). Two weeks later, animals were injected subcutaneously with 30 μg estradiol benzoate in oil (EB; Steraloids, Newport, RI) followed 24 h later with 2.5 mg of progesterone in oil (Sigma Chemicals, St. Louis, MO). Blood samples were taken by venipucture every 2 h from h 0 to 12 and at h 24 relative to progesterone injection in order to characterize estradiol, progesterone, and gonadotropin concentrations.

Radioimmunoassays

Blood samples were refrigerated overnight. Serum was harvested following centrifugation (1280*g*) and stored at –20°C until assayed. LH and FSH were assayed in duplicate using reagents provided by the National Hormone and Pituitary Program. Standard curves used LH and FSH reference preparations AFP 6936A or AFP 6940A, respectively. Serum samples were incubated with LH (AFP 6936A) or FSH (AFP 782594) antibodies followed by the addition of ^{125}I-radiolabeled LH (AFP 6936A) and FSH (AFP 6940A). A sheep anti-rabbit γ-globulin (Prince Laboratories, Toronto, ON, Canada) was used to precipitate the antigen–antibody complex. Precipitation was facilitated by adding 12.5% Carbowax® (1.5 mL; Sigma Chemicals) prior to centrifugation. Assay sensitivity, defined as the concentration of reference preparation required to reduce counts per minute by 2 SD below the zero standard, was 0.6 ng/mL for LH and 0.1 ng/mL for FSH. The intraassay coefficient of variation (CV) was 7.4% and 9.6% for LH and FSH, respectively. All LH and FSH measurements were determined in a single assay. Progesterone and estradiol were assayed using commercial

RIA kits from Diagnostic Products Corporation (Los Angeles, CA). Assay sensitivity was 0.02 ng/mL for progesterone and 8.0 pg/mL for estradiol. Intraassay CV for progesterone and estradiol assays were 3.6% and 4.3%, respectively. All progesterone and estradiol measurements were determined in a single assay. Integrated cortisol and free T_3 were determined at 2 wk intervals. Serum pools were generated by combining equal volumes of two consecutive serum samples drawn 1 wk apart. Cortisol and free T_3 were assayed using commercial RIA kits from Diagnostic Products Corporation (Los Angeles, CA). Assay sensitivity was 0.3 μg/dL for cortisol and 0.2 pg/mL for free T_3. Intraassay CV for cortisol and free T_3 assays were 3.5% and 4.0%, respectively. All cortisol and free T_3 measurements were determined in a single assay.

Data Analysis

A gonadotropin surge was defined as a twofold increase relative to basal concentrations (gonadotropin value at the time of progesterone injection) that began within 6 h of progesterone injection and remained elevated above baseline at h 12. Changes in cortisol and free T_3 secretion following 16 wk of caloric restriction or re-alimentation were analyzed using a one-way repeated measures analysis of variance (ANOVA). A Tukey–Kramer multiple comparison test was performed when ANOVA resulted in $p < 0.05$. Peak progesterone and estradiol levels and time to peak levels were analyzed by paired *t*-tests. Mean BMI, caloric intake, cortisol, and free T_3 (±SE) at the time of the steroid challenge under both experimental conditions were compared by paired *t*-tests. Statistical significance was set at $p < 0.05$.

Acknowledgments

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References

1. Warren, M. P., Voussoughian, F., Geer, E. B., Hyle, E. P., Adberg, C. L., and Ramos, R. H. (1999). *J. Clin. Endocrinol. Metab.* **84,** 873–877.
2. Boyar, R. M., Katz, J., Finkelstein, J. W., et al. (1974). *N. Engl. J. Med.* **291,** 861–865.
3. Sherman, B. M., Halmi, K. A., and Zamudio, R. (1975). *J. Clin. Endocrinol. Metab.* **41,** 135–142.
4. Reid, R. L. and Van Vugt, D. A. (1987). *Fertil. Steril.* **48,** 905–913.
5. Laughlin, G. A., Dominguez, C. E., and Yen, S. S. (1998). *J. Clin. Endocrinol. Metab.* **83,** 25–32.
6. Lujan, M. E., Krzemien, A. A., Reid, R. L., and Van Vugt, D. A. (2004). The Endocrine Society 86th Annual Meeting, New Orleans, Louisiana, June 16–19, 2004. Abstract P1-215.
7. Knobil, E. (1988). *Hum. Reprod.* **3,** 469–472.
8. Terasawa, E., Noonan, J., and Bridson, W. E. (1982). *J. Endocrinol.* **92,** 327–339.
9. Hotchkiss, J. and Knobil, E. (1994). In: *The physiology of reproduction*, 2nd ed. Knobil, E. and Neill, J. D. (eds.). Raven Press: New York.
10. Nakai, Y., Plant, T. M., Hess, D. L., Keogh, E. J., and Knobil, E. (1978). *Endocrinology* **102,** 1008–1014.
11. Krey, L. C., Butler, W. R., and Knobil, E. (1975). *Endocrinology* **96,** 1073–1087.
12. Ferin, M., Wehrenberg, W. B., Lam, N. Y., Alston, E. J., and Vande Wiele, R. L. (1982). *Endocrinology* **111,** 1652–1656.
13. Yeoman, R. R. and Terasawa, E. (1984). *Endocrinology* **115,** 2445–2452.
14. Kolp, L. A., Pavlou, S. N., Urban, R. J., Rivier, J. C., Vale, W. W., and Veldhuis, J. D. (1992). *J. Clin. Endocrinol. Metab.* **75,** 993–997.
15. Ferin, M. (1999). *J. Clin. Endocrinol. Metab.* **84,** 1768–1774.
16. Batista, M. C., Cartledge, T. P., Zellmer, A. W., Nieman, L. K., Merriam, G. R., and Loriaux, D. L. (1992). *J. Clin. Endocrinol. Metab.* **74,** 565–570.
17. Collins, R. L. and Hodgen, G. D. (1986). *J. Clin. Endocrinol. Metab.* **63,** 1270–1276.
18. Wakeling, A., DeSouza, V. A., and Beardwood, C. J. (1977). *Psychol. Med.* **7,** 397–405.
19. Wang, C. F., Lasley, B. L., Lein, A., and Yen, S. S. (1976). *J. Clin. Endocrinol. Metab.* **42,** 718–728.
20. Marshall, J. C. and Kelch, R. P. (1979). *J. Clin. Endocrinol. Metab.* **49,** 712–718.
21. Saketos, M., Sharma, N., and Santoro, N. F. (1993). *Biol. Reprod.* **49,** 1270–1276.
22. Golden, N. H., Jacobson, M. S., Schebendach, J., Solanto, M. V., Hertz, S. M., and Shenker, I. R. (1997). *Arch. Pediatr. Adolesc. Med.* **151,** 16–21.
23. Williams, N. I., Helmreich, D. L., Parfitt, D. B., Caston-Balderrama, A., and Cameron, J. L. (2001). *J. Clin. Endocrinol. Metab.* **86,** 5184–5193.
24. Doufas, A. G. and Mastorakos, G. (2000). *Ann. NY Acad. Sci.* **900,** 65–76.
25. Jen, K. L., Hansen, B. C., and Metzger, B. L. (1985). *Int. J. Obes.* **9,** 213–224.
26. Lujan, M. E., Krzemien, A. A., and Van Vugt, D. A. (2003). *Endocrine* **21,** 109–114.