

## Selective inhibition of follicle-stimulating hormone secretion by estradiol. Mechanism for modulation of gonadotropin responses to low dose pulses of gonadotropin-releasing hormone.

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### Research Article

Prepubertal girls and gonadotropin-releasing hormone (GnRH)-deficient females secrete follicle-stimulating hormone (FSH) preferentially in response to intravenous GnRH. With continued pulsatile GnRH stimulation, FSH secretion is reduced when plasma estradiol (E2) is increasing. To delineate the mechanisms involved in these changing gonadotropin responses, we studied the effect of low dose (0.025 micrograms/kg) pulsatile injections of GnRH in females with varying degrees and/or duration of endogenous GnRH deficiency (idiopathic panhypopituitarism, PHP; isolated growth hormone deficiency, IGHD; isolated gonadotropin deficiency, IGD; and anorexia nervosa, AN; both at low body weight and after weight regain). In patients presumed to have the most severe GnRH deficiency (PHP), responses of both FSH and luteinizing hormone (LH) were small and delayed, and no increase in plasma estradiol occurred during the 5 d of GnRH injections. In patients previously exposed to prepubertal or adult levels of endogenous GnRH secretion (IGHD, IGD, AN at low body weight), a rapid initial FSH response occurred that subsequently declined when plasma estradiol rose to concentrations greater than 40-50 pg/ml. Prior therapy with estrogen (micronized estradiol, Estrace) abolished FSH responses but LH responses were only slightly impaired. The degree of FSH response was dependent upon the time of initiation of estrogen relative to the onset of GnRH injections. Administration of estrogen after the first GnRH injection inhibited gonadotropin responses, whereas later estrogen therapy [...]

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# Selective Inhibition of Follicle-stimulating Hormone Secretion by Estradiol

## MECHANISM FOR MODULATION OF GONADOTROPIN RESPONSES TO LOW DOSE PULSES OF GONADOTROPIN-RELEASING HORMONE

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**ABSTRACT** Prepubertal girls and gonadotropin-releasing hormone (GnRH)-deficient females secrete follicle-stimulating hormone (FSH) preferentially in response to intravenous GnRH. With continued pulsatile GnRH stimulation, FSH secretion is reduced when plasma estradiol ( $E_2$ ) is increasing. To delineate the mechanisms involved in these changing gonadotropin responses, we studied the effect of low dose (0.025  $\mu\text{g/kg}$ ) pulsatile injections of GnRH in females with varying degrees and/or duration of endogenous GnRH deficiency (idiopathic panhypopituitarism, PHP; isolated growth hormone deficiency, IGHD; isolated gonadotropin deficiency, IGD; and anorexia nervosa, AN; both at low body weight and after weight regain). In patients presumed to have the most severe GnRH deficiency (PHP), responses of both FSH and luteinizing hormone (LH) were small and delayed, and no increase in plasma estradiol occurred during the 5 d of GnRH injections. In patients previously exposed to prepubertal or adult levels of endogenous GnRH secretion (IGHD, IGD, AN at low body weight), a rapid initial FSH response occurred that subsequently declined when plasma estradiol rose to concentrations  $>40$ – $50$  pg/ml. Prior therapy with estrogen (micronized estradiol, Estrace) abolished FSH responses but LH responses were only slightly impaired. The degree of FSH response was dependent upon the time of initiation of estrogen relative to the onset of GnRH in-

jections. Administration of estrogen after the first GnRH injection inhibited gonadotropin responses, whereas later estrogen therapy (after 1 d of GnRH pulses) blunted the GnRH induced FSH secretion without significantly impairing the LH response. In weight-regained anorexic patients who had spontaneous pulsatile LH secretion and a mean basal plasma estradiol concentration of  $53 \pm 15$  pg/ml, administration of GnRH pulses did not change plasma LH and a minimal FSH response was seen. The data indicate that the pattern of gonadotropin responses to low dose GnRH injections depends upon the degree of previous exposure of the pituitary to endogenous GnRH. Furthermore, estradiol selectively inhibits FSH secretion by a direct action on the pituitary gland. This action of estradiol provides an explanation for the selective reduction in FSH responses to GnRH seen during pubertal maturation in girls and during the mid-follicular stage of the menstrual cycle.

## INTRODUCTION

Puberty is thought to result from increased secretion of gonadotropin-releasing hormone (GnRH)<sup>1</sup> by the hypothalamus with consequent increased secretion of pituitary gonadotropins and gonadal steroids. During this process, the pattern of pituitary responsiveness to GnRH changes. Results from both cross-sectional (1, 2) and longitudinal (3) studies in girls indicate that

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<sup>1</sup> Abbreviations used in this paper: FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; IGD, gonadotropin deficiency; IGHD, isolated growth hormone deficiency; LH, luteinizing hormone; PHP, idiopathic panhypopituitarism; TRH, thyrotropin-releasing hormone.

follicle-stimulating hormone (FSH) responses decrease and luteinizing hormone (LH) responses increase as maturation progresses. In an effort to elucidate the nature of these changing responses we have previously administered low doses of GnRH in a pulsatile manner, to mimic the presumed physiological pattern of GnRH secretion, to female patients with absent or greatly reduced endogenous GnRH secretion. Our results (4, 5) and those of others (6, 7) have shown that the initial response to GnRH is one of predominant FSH secretion that later declines, while LH responses gradually increase. This pattern seen over 5 d of pulsatile GnRH injections is similar to that present during sexual maturation in girls. Prolonged administration of GnRH pulses to GnRH-deficient females results in changes in plasma FSH and LH that closely resemble those seen during the follicular phase of the normal menstrual cycle (8, 9). The initial rise in serum FSH declines after 4 or 5 d, when estradiol secretion from the developing follicle is increasing. This suggests that FSH secretion is selectively inhibited by estradiol.

To examine the factors governing gonadotropin responses in females, we have studied the effects of low dose GnRH pulses in patients with different hypothalamic-pituitary disorders or anorexia nervosa at low body weight. Girls with panhypopituitarism, isolated growth hormone deficiency, and isolated gonadotropin deficiency were studied in order to assess the effects of different degrees of previous exposure of the pituitary to endogenous GnRH. Although it is not possible to accurately determine the level of endogenous GnRH secretion in humans, panhypopituitary patients would be expected to have the most severe deficiency of GnRH; girls with isolated growth hormone deficiency should have GnRH secretion similar to that present in normal prepubertal girls; and patients of adult age with isolated gonadotropin deficiency would represent prolonged exposure to prepubertal levels of GnRH.

In addition, as earlier studies have shown a close temporal relationship between declining FSH responses and a rising serum estradiol concentration, we assessed the effects and time course of action of estrogens on gonadotropin responses to GnRH. Gonadotropin responses were measured in adult patients with isolated gonadotropin deficiency or anorexia nervosa (at low body weight) who had received estrogens before and at different times after GnRH pulses were begun. In these patients, endogenous GnRH secretion is low and unlikely to change, and as the exogenous GnRH stimulus was constant, any effects of estrogen would result from a direct action of the steroid at the level of the pituitary gland. Patients with anorexia nervosa who had recently regained weight were also studied to determine the effects of GnRH pulses in a situation

where endogenous GnRH secretion was present and gonadotropin and gonadal steroid concentrations were within the normal adult range. In these patients, definite conclusions regarding the site of estrogen feedback cannot be drawn. However, by comparison of responses to those in GnRH deficient females, we hoped to gain insight into the relative importance of estrogen feedback at the hypothalamic and pituitary levels in normal females.

## METHODS

**Patients studied.** The clinical characteristics of the patients studied are shown in Table I. The diagnoses were made on the basis of clinical history and examination, skull roentgenograms, visual fields, buccal smear, or karyotype, and standard tests of pituitary, adrenal, thyroid, and gonadal function. Pituitary hormone responses to a standard GnRH test (2.5 µg/kg) and to thyrotropin-releasing hormone (TRH) were measured in patients 1–6. Patients 1 and 2 with idiopathic panhypopituitarism (PHP) were receiving replacement doses of thyroxine and hydrocortisone and were also being treated with human growth hormone (0.1 IU/kg im, three times per week) at the time of study. Patients 3 and 4 with isolated growth hormone deficiency (IGHD) were studied before treatment with human growth hormone therapy was begun. Subsequent follow-up of these patients has shown evidence of spontaneous sexual maturation. Patient 3 has not developed clinical evidence of puberty but biochemical evidence of adrenarche was present (plasma dehydroepiandrosterone sulphate of 80 mg/dl) 1 yr later. Patient 4 developed clinical signs of puberty (stage P2) 2 yr

TABLE I  
Clinical Characteristics of Patients Studied

Patient	Diagnosis	Age		Height	Weight	
		CA	BA			
		yr			cm	kg
1	PHP	16.5	10–11	138	29.6	93
2	PHP	16.7	12	155	42	85
3	IGHD	7.8	5	106	23.1	116
4	IGHD	9.4	3.5	101	16.6	101
5	IGD	18.4	14	157	57	114
6	IGD	26	—	180	66.3	95
7	AN	25	—	165	35.5	65
8	AN	17.4	—	152	31.8	67
9	AN	26	—	171	47	75
10	AN	18	—	163	43	76
11	AN	35	—	163	37	66
12	AN	19	—	158	35.3	67
13	AN	32	—	160	32.2	59
14	AN	22	—	167	50.9	84
15	AN	26	—	160	44.4	82
16	AN	20	—	162	47.5	85
17	AN	19	—	160	52.6	96
18	LBW	28	—	162	47.5	85

LBW, low body weight.

after completion of her study while her bone age had advanced to 10 yr. Patient 6 with isolated gonadotropin deficiency (IGD) had received 2 mo of oral contraceptive therapy that was discontinued 2 mo before study. Patients 7–13 with anorexia nervosa were all at their lowest body weight and had experienced secondary amenorrhea of between 2 and 3 yr duration of the time of this study. These patients experienced varying degrees of weight gain during psychotherapy following these studies but menstruation did not resume. Patients 14–17 all had a history of previous weight loss to <80% of ideal body weight and had previously been hospitalized for treatment of anorexia nervosa. All of these patients had gained weight (range, 4.5–7 kg) during the years prior to this study but had not resumed spontaneous menstruation. Patient 18 had primary amenorrhea and there was no clear history of acute weight loss. However, she had always been underweight and had gained 2.5 kg during the preceding 6 mo and was at her greatest weight at the time of the study. Patients 14–18 had all experienced withdrawal bleeding after 5 d of oral medroxyprogesterone acetate 2–3 mo before study.

**Study protocol.** The nature of the study was explained in detail to the patients and their families and written consent was obtained on admission to the Clinical Research Center. On the following day (day 0) 2 ml of normal saline was injected every 2 h through a heparin lock, to act as a control for the subsequent GnRH injections, and base-line plasma gonadotropins and gonadal steroids were measured. Plasma LH and FSH were determined in samples drawn every 20 min during the day (1200–1500 h) and during sleep (2400–0300 h). Plasma estradiol was measured in the 0800 and 1800 h samples. Starting on day 1, GnRH (0.025  $\mu$ g/kg) was injected every 2 h for 5 d. Plasma LH and FSH were measured in preinjection samples at 0800, 1000, 1200, 1600, 1800, 2400, and 0400 h, and estradiol in the 0800 and 1800 h samples. The acute gonadotropin responses to intravenous GnRH pulses were assessed by measurement of LH and FSH every 30 min after the 0800 and 1800 h injections. In patient 1 the acute gonadotropin response was also measured at 1200 h and in patients 3 and 4 acute responses were only measured at 0800 h due to blood volume restrictions.

Patients 1–8 and 14–18 were studied on the above protocol. The protocol was repeated on patients 2 and 5, after they had been taking micronized estradiol (Estrace) 0.5 or 1 mg b.i.d. for 2 and 1 mo, respectively. Estradiol was continued throughout the 5 d of GnRH injections. Patients 9 and 10 were started on micronized estradiol (2 mg loading dose at 1000 h followed by 1 mg b.i.d. at 1000 and 2200 h)

after the first intravenous injection of GnRH on day 1. Patients 11, 12, and 13 were started on the same estradiol regimen beginning at 1000 h on day 2 (after 24 h of intravenous GnRH injections).

**Assays.** Plasma GnRH, LH, FSH and estradiol were measured by previously reported radioimmunoassay (RIA) (10, 11–13). Gonadotropin concentrations are reported as milli-International Units of the second international reference preparation of human menopausal gonadotropin after conversion from LER 907, which was used as the assay standard. The limit of detectability of the estradiol assay depended upon the volume of plasma available and was 10–15 pg/ml when 2 ml were used, and 20–30 pg/ml when only 1 ml was available.

**Calculations and statistics.** Mean LH and FSH on the control day were calculated from all samples obtained on day 0. Mean preinjection LH and FSH was the mean of samples drawn immediately before the GnRH injections. The daily LH and FSH increments were the mean of the maximum increments seen after the 0800- and 1800-h GnRH injections. Estradiol concentrations were the mean of measurements at 0800 and 1800 h. Results are shown as mean  $\pm$  SE. The significance of the differences between groups was assessed by the Wilcoxon-Mann-Whitney Rank Sum Test.

## RESULTS

### PHP

Plasma LH, FSH, and estradiol concentrations in the two patients studied are shown in Fig. 1 (patient 1) and Fig. 3 (patient 2, who was also studied while taking estrogens). In patient 1, mean LH, on the control day  $4.1 \pm 0.15$  mIU/ml did not increase significantly during the days of GnRH injections (day 5,  $4.3 \pm 0.3$  mIU/ml), and acute LH responses to GnRH remained small (day 1, 1.3 mIU/ml; day 5, 2.0 mIU/ml). Plasma FSH was below assay sensitivity on the control day (1.3 mIU/ml) and began to rise on day 2 ( $1.6 \pm 0.24$ ). Thereafter, FSH increased to  $4.6 \pm 0.15$  on day 3 and  $9.4 \pm 0.5$  mIU/ml on day 5. Patient 2 showed a similar pattern but the hormone responses were less marked. Serum LH increased slightly on days 3–5 and mean plasma FSH was not measurable until the third day of GnRH in-

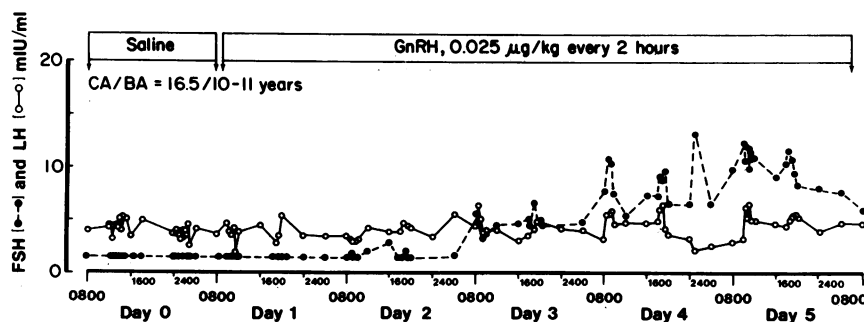


FIGURE 1 Plasma LH (○) FSH (●) and estradiol responses to pulsatile intravenous injections of GnRH for 5 d in patient 1 (PHP). Plasma estradiol was below assay detectability, 15 pg/ml, throughout the study. CA, chronological age, BA, bone age.

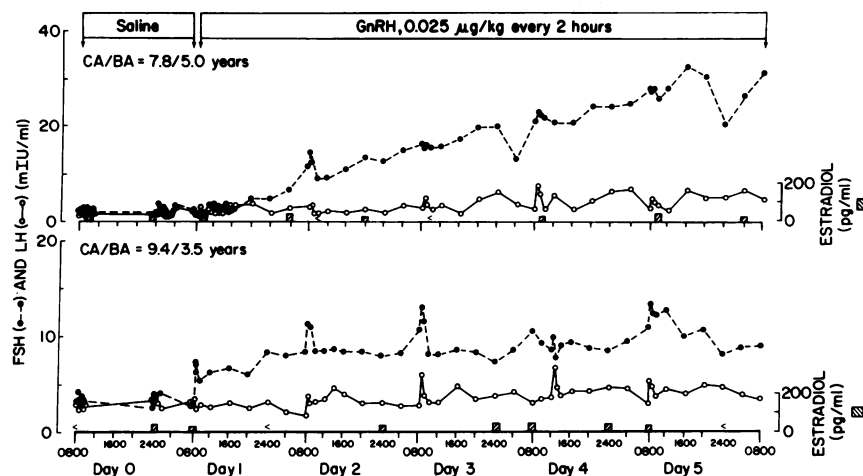


FIGURE 2 Plasma LH (○) FSH (●) and estradiol responses (hatched bars) to intravenous injections of GnRH for 5 d in patient 3 (upper panel) and patient 4 (lower panel) with IGHD. The symbol (<) indicates where estradiol was below assay detectability. The scale of the axis for LH and FSH is expanded  $\times 2$  in the lower panel for clarity. The transient falls in plasma FSH on days 3 and 5, in patient 3 were due to the inadvertent omission of a GnRH injection during the night.

jections after which FSH rose to a mean of  $5.3 \pm 0.3$  on day 5. In both patients, plasma estradiol remained below assay detectability throughout the study.

### IGHD

Plasma hormone responses in the two prepubertal girls (patients 3 and 4) with IGHD are shown in Fig. 2. Plasma LH responses were small in both patients and mean LH rose from  $1.6 \pm 0.13$  on day 0 to  $4.4 \pm 0.65$  mIU/ml on day 5 in patient 3, and from  $2.7 \pm 0.11$  to  $4.0 \pm 0.25$  mIU/ml in patient 4. In contrast to the panhypopituitary patients, both subjects with IGHD showed earlier responses in plasma FSH. In patient 3, mean FSH increased from  $2.2 \pm 0.17$  on day 0 to  $3.7 \pm 0.67$  mIU/ml on day 1 and then rose markedly to  $27.3 \pm 1.4$  mIU/ml on day 5. Plasma estradiol concentrations were at or below assay sensitivity until day 5 when estradiol (mean 32 pg/ml) was measurable in both samples. Patient 4 showed an immediate increase in plasma FSH after the first GnRH pulse and the mean FSH rose from  $3.5 \pm 0.12$  (day 0) to  $6.1 \pm 0.71$  mIU/ml on day 1. By day 2, FSH had risen to  $8.2 \pm 0.7$  and then plateaued through day 5 ( $10.1 \pm 0.51$  mIU/ml). Plasma estradiol was at or below the limit of detectability on day 0 and 1 but rose to 40 and 34 pg/ml on days 3 and 4, respectively. The evening estradiol sample on day 5 was undetectable however, and the small plasma sample available did not allow repeat assay.

### Effects of estrogen therapy on gonadotropin responses to GnRH pulses in GnRH-deficient patients

**Preceding estrogen therapy.** Two patients, one with IGD (patient 5) and one with PHP (patient 2), were studied both before and during therapy with Estrace and hormone responses are shown in Fig. 3. In patient 5, the immediate increase in plasma FSH seen during the initial study was completely abolished by estrogen therapy. In patient 2 FSH was undetectable throughout and the small FSH increase seen during the initial study did not occur. In contrast, plasma LH responses were reduced to a lesser degree by estrogen treatment and were not significantly lower on days 4 and 5 in either patient. Plasma estradiol remained fairly constant during Estrace therapy and mean estradiol was  $147 \pm 18.5$  pg/ml in patient 5 and  $51 \pm 3.2$  pg/ml in patient 2.

**Estrogen therapy initiated during GnRH injections.** In view of the fact that prior estrogen therapy abolished the FSH responses to GnRH, we performed studies to determine the effects of estrogen given during the course of GnRH injections. Patients 5–8 (2 IGD, 2 AN) represent a control group (group 1) who had no endogenous GnRH secretion as evidenced by absence of spontaneous peaks of LH secretion on the control day and who received only the GnRH injections. The individual data in these patients has previously been reported [patients 5 and 6, (5) patients

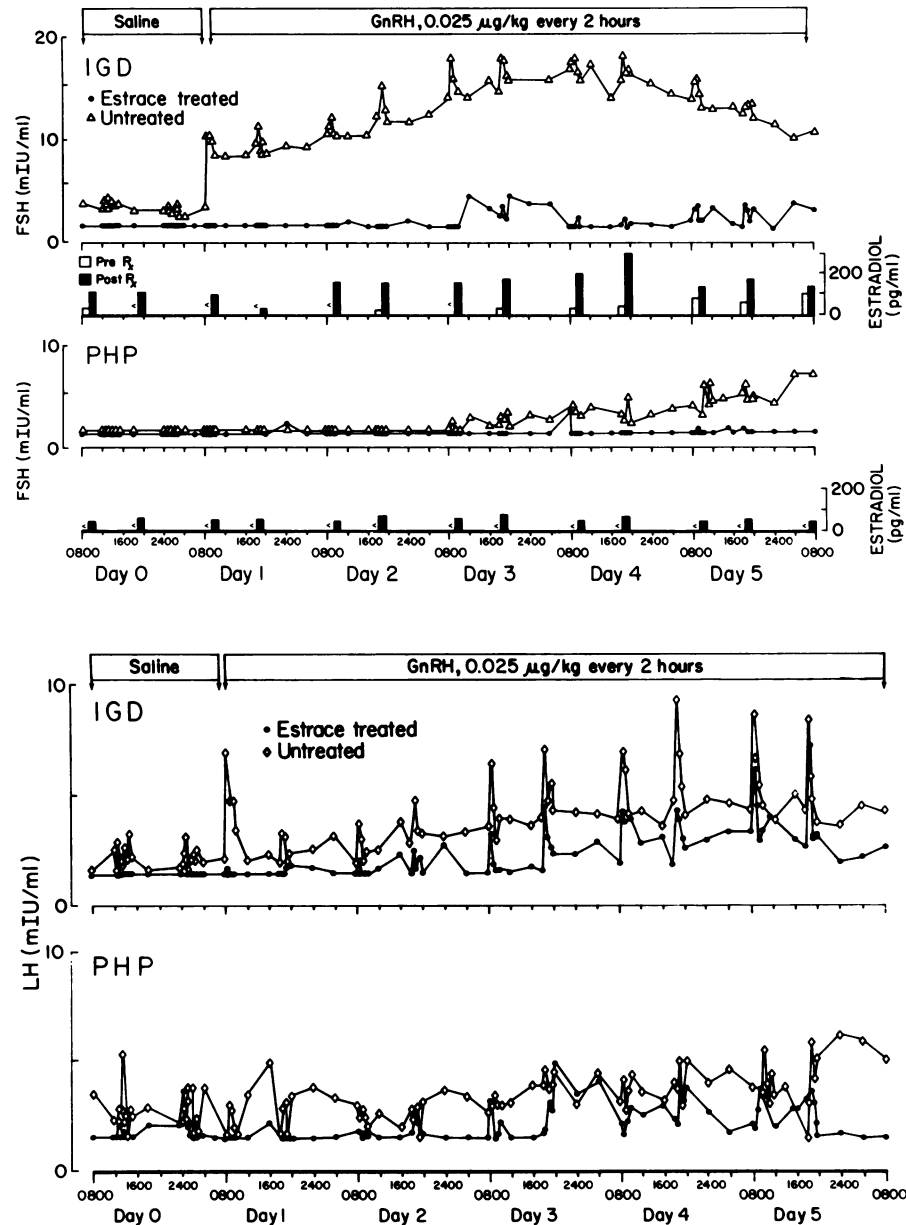


FIGURE 3 Plasma FSH and estradiol concentrations (upper panel) and LH concentrations (lower panel) in two patients (patient 5 IGD; patient 2 PHP) studied before and during administration of estrogen. Estrace (0.5 mg b.i.d.) was given for 2 mo in patient 2 and 1 mg b.i.d. for 1 mo in patient 5 before the second study and was continued throughout the GnRH injections. Plasma estradiol from the initial study, is shown as open bars, and from the repeat study, as closed bars. Symbols (<) indicate times when estradiol was below assay detectability during the initial study.

7 and 8, (4)] and only the group data are shown here. Two patients with AN (patients 9 and 10) were given estrace at 1000 h on day 1 (after the first intravenous GnRH injection) and constitute group 2. Three patients (patients 11–13) were given Estrace at 1000 h

on day 2 (after 1 d of GnRH injections) and formed group 3. In all patients (5–13) base-line samples on day 0 showed low gonadotropin levels and spontaneous peaks of LH secretion similar to that seen during early puberty (14) or in adult females (15) were not present

during the day or the night. Analysis of the base-line data did not reveal any significant differences between the three groups and thus day 0 data are combined in Fig. 4. In group 1, mean plasma FSH rose rapidly on day 1 and continued to increase to a plateau on days 3 and 4 before declining on day 5. Mean LH concentrations were also significantly elevated on day 1 ( $P$

$< 0.04$ ) and rose gradually to a plateau on days 4 and 5. Plasma estradiol increased gradually during the first 4 d of GnRH administration and rose more rapidly between days 4 and 5 (from  $52.6 \pm 8$  to  $99 \pm 20$  pg/ml) when mean FSH concentrations were falling. In group 2, administration of the loading dose of estrogen resulted in a rapid increase in plasma estradiol, which then remained constant in the range of 70 to 95 pg/ml. In the presence of this level of estradiol, GnRH injections did not result in a significant increase in mean FSH and responses of both hormones were markedly inhibited compared to group 1 ( $P < 0.001$ ). In group 3 patients, the later administration of estradiol produced a marked blunting of the FSH response and mean FSH concentrations were significantly lower than group 1 on days 3–5 ( $P < 0.0001$ ). LH responses were also reduced by 25 to 40% on days 2 to 5, but this reduction did not achieve significance when compared to group 1.

#### *Effects of pulsatile GnRH injections in anorexia nervosa patients after weight regain*

Previous studies (16–19) have shown that LH is secreted in a pulsatile manner, responsiveness to GnRH matures, and plasma estradiol concentrations increase, following weight regain in patients with anorexia nervosa. We therefore administered GnRH to five patients who had regained weight, but who remained amenorrhic, in an effort to determine the effects of increased endogenous estrogen concentrations on gonadotropin responses. The individual hormone concentrations are shown for patient 14 in Fig. 5, and mean data for patients 14–18 are shown in Fig. 6. All five patients showed evidence of pulsatile LH secretion on day 0, indicating the presence of endogenous GnRH secretion. The amplitude of the measured LH pulses on day 0 was  $7.3 \pm 2.2$  mIU/ml, which is similar magnitude to spontaneous LH pulses in normal women during the follicular stage of the cycle (unpublished observations) and to the LH increments in response to GnRH pulses ( $8.5 \pm 1.9$  mIU/ml on day 5). Plasma estradiol levels on day 0 were  $53 \pm 15$  pg/ml. After injection of GnRH, no significant changes in mean plasma LH occurred, and mean FSH showed a small transient increase on days 1 and 2 ( $P < 0.05$ , on day 2 compared to day 0). Responses of plasma estradiol were variable with two patients showing a two–threefold increase and three patients remaining unchanged over the 5 d of GnRH administration. Mean estradiol concentrations for the group were only increased on day 5 ( $P < 0.02$  compared to day 0).

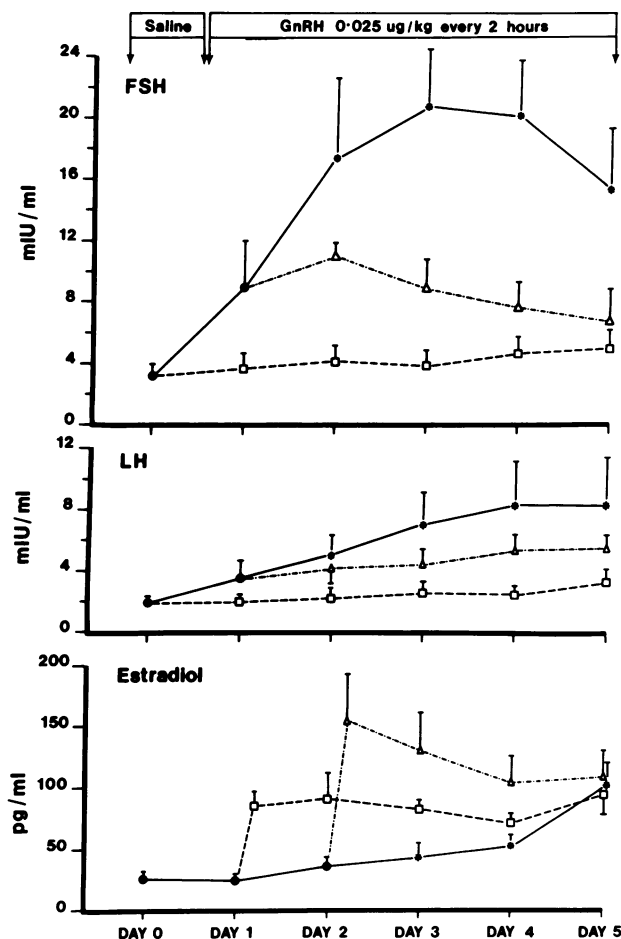


FIGURE 4 Effects of estrogen therapy initiated at different times during GnRH injections on mean plasma FSH (upper panel) LH (middle panel) and estradiol (lower panel) concentrations in patients with anorexia nervosa at low body weight. Data for these groups of patients are shown: group 1 (•) no Estrace; group 2 (◻) Estrace begun at 1000 h on day 1 (after the first dose of GnRH); group 3 (Δ) Estrace begun at 1000 h on day 2 (after 24 h of GnRH injections). Data from all groups are combined on the control day and groups 1 and 3 are combined on day 1 (both received GnRH only) and are shown as closed circles. Two values for estradiol are shown on day 1 (group 2) and day 2 (group 3), and the second value is the 1800 h measurement of estradiol 8 h after the loading dose of Estrace. Data are shown as mean ± SEM where the number of patients per group and not the number of hormone estimates were used to calculate the SEM.

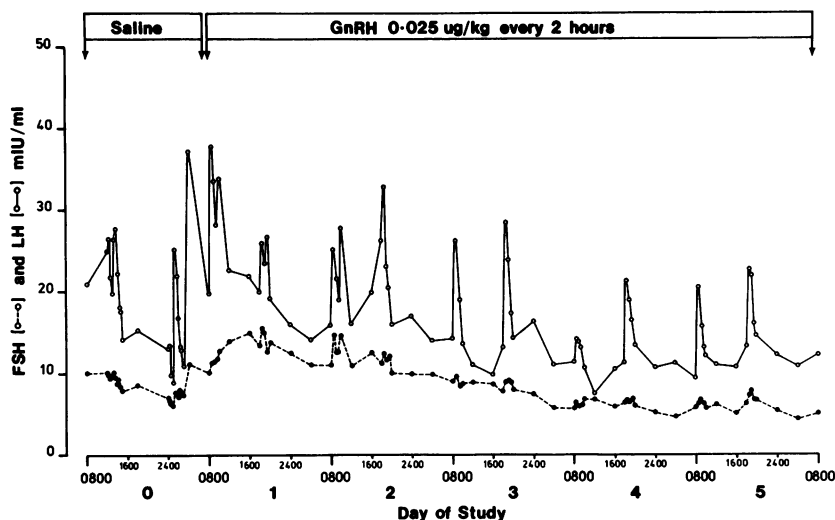


FIGURE 5 Plasma LH (○) and FSH (●) responses to GnRH injections in patient 14. Pulsatile LH secretion is clearly present on the control day 0. Mean plasma estradiol concentrations in this patient were 52, 44, 90, 111, 86, and 129 pg/ml on days 0–5, respectively.

## DISCUSSION

Previous studies have clearly documented that the predominant response to GnRH in normal prepubertal girls is one of FSH secretion, and LH responsiveness does not exceed that of FSH until the onset of puberty. The predominant FSH response occurs after both intravenous bolus injections of different doses of GnRH (1, 2) or after intravenous infusions (20) and does not appear to be related to the manner of GnRH stimulus. A similar pattern is seen in GnRH-deficient subjects of adolescent or adult age (4–7), and this has suggested that LH synthesis and secretion is more dependent upon an increased level of GnRH stimulation of the pituitary than is the synthesis of FSH. For both ethical and practical reasons, most studies using repetitive GnRH stimuli have been performed in children at or near the age of pubertal onset, and few data are available in young girls. Thus, in these earlier studies the pituitary had been exposed to prepubertal levels of GnRH secretion for several years and this may have been adequate to initiate and maintain FSH synthesis. In the first part of this paper, we report data on responses to low dose GnRH pulses in patients with different degrees of presumed previous exposure to GnRH secretion.

It is not possible to accurately assess the exact level of GnRH secretion in humans, but available evidence suggests that gonadotropin responses to a standard GnRH test (2.5 µg/kg i.v.) provides an indication of the degree of previous pituitary exposure of GnRH in hypopituitary girls. In a recent study (21) that ex-

amined serial GnRH tests, gonadotropin responses in PHP patients were significantly lower than those in IGHD or normal girls. Of particular importance is the observation that with advancing age PHP patients were unresponsive to GnRH, whereas LH responses in IGHD were indistinguishable from those in normal girls. FSH responses to GnRH in IGHD girls were smaller than in normal subjects, but declined with age

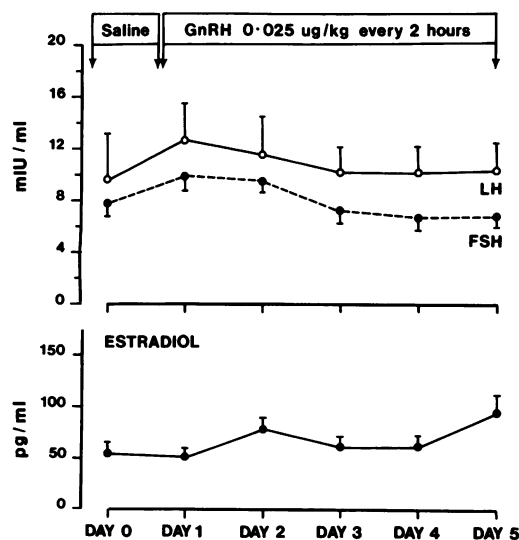


FIGURE 6 FSH, LH, and estradiol responses to GnRH injections in five amenorrheic patients with anorexia nervosa who had regained weight (patients 14 thru 18). Data are shown as mean±SEM.



in an identical manner to those seen during normal sexual maturation. Standard GnRH tests were performed in the patients studied and were included in the earlier report (21). These findings provide support for our contention that GnRH deficiency is both prolonged and severe in PHP patients and that GnRH secretion is similar to that of prepubertal girls in IGHD patients.

In two patients with PHP, a small rise in gonadotropins occurred in response to GnRH pulses and significant FSH secretion was not observed until after 3 d of GnRH therapy. Two girls with IGHD showed more rapid responses of plasma FSH with clear increases being present on the first day of GnRH injections. Older patients with IGD or AN at low body weight, showed immediate and marked FSH responses to GnRH pulse injections. The complexity of the protocol required for the studies, resulted in few patients being available and the results may not be applicable to all individuals with these disorders. However, taken together these data indicate that FSH responsiveness to GnRH is related to the degree or duration of prior pituitary exposure of GnRH, and that GnRH secretion is required to initiate and maintain FSH synthesis.

The pattern of FSH response on the latter days of GnRH injections is also of interest and again indicates a continuum; the response pattern being dependent upon prior GnRH secretion and hence the state of maturation of the hypothalamic-pituitary-ovarian axis. In PHP patients, FSH concentrations continued to rise throughout the 5 d of GnRH injections and estradiol was not detectable in plasma. A similar consistent increase in FSH occurred in the younger patient with IGHD (patient 3) in whom estradiol was consistently measurable only on day 5. In contrast, in the older IGHD patient, estradiol was in the range of 30 to 40 pg/ml after the 2nd d of GnRH injections and the rapid initial rise in plasma FSH was blunted on subsequent days. In the adult aged GnRH-deficient patients, plasma FSH rose rapidly to a plateau, and then declined at a time when estradiol concentrations were increasing rapidly. These data indicate that in the presence of estradiol, FSH responses to GnRH are blunted and plasma FSH falls. Moreover, as the patients had absent or low endogenous GnRH secretion, and the exogenous GnRH stimulus was constant, this action of estradiol is exerted at the level of the pituitary gland. Thus, estradiol feedback in females appears to be similar to steroid feedback in males where testosterone therapy directly inhibits FSH secretion at the pituitary level (22). This conclusion is supported by the results of estradiol administration to GnRH-deficient patients before and during GnRH injections. Prior administration of estradiol, or initiation of therapy early in the course of GnRH pulses abolished FSH

responses; later introduction of estrogen resulted in a selective inhibition of FSH secretion.

In all these studies, the concentrations of estradiol achieved also tended to reduce LH responses to GnRH, though the effect was much less marked than that on FSH secretion. Estradiol concentrations in the range of 40 to 100 pg/ml appear to inhibit both FSH and LH responses to GnRH and no augmentation of LH responsiveness to repetitive GnRH stimuli were noted. This observation is in agreement with previous studies of the effects of estradiol concentrations on LH secretion and the "self-priming" effect of GnRH. Estrogen induced positive-feedback was not evident in adult females unless plasma estradiol exceeded 300 pg/ml (23). Similarly, augmented LH responses to GnRH normally occur after estradiol administration (24) or when estradiol is elevated in excess of 200 pg/ml during the menstrual cycle (25).

These observations provide an explanation for the changing patterns of gonadotropin responses after prolonged low dose GnRH administration to GnRH-deficient females. Plasma hormone profiles closely resemble those seen during the follicular phase of the normal menstrual cycle (8, 9, 26) with an initial predominance of FSH that later declines when estradiol levels increase to a range of 50 to 100 pg/ml (9). LH augmentation does not occur until estradiol has risen to concentrations of 200 pg/ml or higher. A selective feedback of estradiol on FSH may have important implications for the normal control of menstrual cycles. The FSH rise during the early follicular phase results in induction of LH receptors and aromatase activity in the maturing follicular (27, 28) and is essential for normal follicular development. Failure of FSH secretion results in inadequate follicular development and an ovulation, and reduced FSH secretion has been associated with a short luteal phase (29). While this initial FSH secretion is essential for normal menstrual cyclicity, the temporary nature of the FSH predominance may also be important. Administration of pre-ovulatory levels of estradiol to rhesus monkeys in the early follicular phase, leads to inhibition of FSH and prolongation of the follicular stage of the cycle (30). In a normal cycle, the majority of the follicular phase estradiol increase appears to be secreted by the dominant follicle, as ablation of that follicle in monkeys results in an acute fall in estradiol (31). Thus, increasing estradiol secretion from the dominant follicle would inhibit FSH secretion and the fall in FSH may be part of the mechanisms involved in inhibiting growth of other developing follicles. Growth of the dominant follicle may not be impaired by this reduction in FSH, as it has an increased blood supply (32), and a high estradiol milieu that is known to augment ovarian responsiveness to gonadotropins (33). Thus, the

selective action of estradiol at the pituitary level in reducing FSH secretion may be an important facet in the overall control of normal ovulation.

The results in amenorrheic patients after weight regain are also of interest. In these patients, pulsatile GnRH secretion was present as evidenced by spontaneous LH peaks and the administration of exogenous GnRH did not significantly change plasma LH and had minimal effects on FSH. In patients with an intact hypothalamic-pituitary-ovarian axis, the exact level of estradiol feedback (either hypothalamic or pituitary) cannot be determined. However, by analogy to the GnRH-deficient patients, the minimal rise in FSH was probably due to the preexisting plasma estradiol concentrations of 53 pg/ml exerting selective feedback on the pituitary. This has important implications for the use of pulsatile GnRH to induce ovulation in patients with hypothalamic amenorrhea. In previous studies GnRH administration has resulted in variable success in these patients (26, 34, 35) and short luteal phases have been reported. Numerous factors may contribute to the relatively poor results, and in some studies GnRH injections were not given in an appropriate dosage or in the pulsatile manner necessary for optimal gonadotropin responsiveness (36). However, our results suggest that pulsatile GnRH may only be fully effective in patients with low basal estradiol concentrations, so that the initial response is one of predominant FSH secretion.

In conclusion, our data indicate that the pattern of gonadotropin responses to pulsatile GnRH is dependent upon the degree of previous exposure of the pituitary to endogenous gonadotropin-releasing hormone. In gonadotropin-deficient patients, the rapidity of the initial FSH response is related to the amount and/or duration of earlier GnRH secretion, and similarly the ovarian response is more rapid in patients who have previously been exposed to GnRH. Estradiol exerts a direct feedback on the pituitary to selectively inhibit FSH secretion. This action explains the diminishing FSH secretion seen during sexual maturation and may also be an important mechanism in the control of normal menstrual cycles.

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