Episodic Luteinizing Hormone Secretion in Man

PULSE ANALYSIS, CLINICAL INTERPRETATION, PHYSIOLOGIC MECHANISMS

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ABSTRACT The demonstration that luteinizing hormone (LH) release from the pituitary is episodic rather than constant raises fundamental questions regarding the physiologic control of pulsatile LH secretion and its possible alteration in patients with gonadal disorders. To evaluate this mode of LH secretion, quantitative means of analyzing LH pulse amplitude, frequency, shape, and area were established and utilized to study normal subjects and patients with disorders of gonadotropin secretion. Similar patterns of LH secretion were observed in normal men, in women during the follicular phase of the menstrual cycle, and in patients with hyper- and hypogonadotropism, hirsuitism, and amenorrhea (mean pulse amplitude 39–179% from nadir to peak, frequency 2.7–3.9 secretory spikes/6 h). These observations suggested that the pattern of LH secretion is similar in both normal individuals and in those with a variety of pathologic conditions. By contrast, the pattern of pulsatile secretion appeared to differ in the following conditions. LH pulses of higher amplitude (333±170%) and lower frequency (1.6±0.24 SEM/6 h) characterized the secretory patterns of women during the luteal phase of the menstrual cycle, suggesting that gonadal steroids may modulate LH pulses. LH pulses of low amplitude (26±2.1%) and frequency (1.3±0.36/6 h) were observed in women with anorexia nervosa.

Either integrated LH levels or a *mean* LH level determined from multiple samples provided a more accurate reflection of gonadotropin secretion than the use of single LH measurements. With multiple sampling over 6 h, it was possible to reduce the 95% confidence limit of LH estimates from ±50–90 to ±12%. This allowed normal subjects to be distinguished from patients with low or moderately elevated LH levels in whom gonadotropin levels in single samples were often in the "normal range."

Several aspects of the physiologic control of pulsatile LH secretion were studied. The concordance of follicle-stimulating hormone (FSH) with LH pulses progressively increased as LH pulse height increased ($P < 0.01$) suggesting possible hypothalamic mediation of gonadotropin pulses. Measurement of the "apparent half-life" of LH after secretory spikes revealed half times of 34–233 min. It is likely that this variability was attributable to at least two phenomena: (*a*) constant low level LH secretion that continued after certain secretory episodes but not others; (*b*) variable mixing of newly secreted LH into at least two pools. The alpha adrenergic-blocking agents, chlorpromazine and phentolamine, failed to block LH secretory spikes at doses sufficient to result in a 30 mm drop in systolic blood pressure in normal men.

INTRODUCTION

Following the development of methods for measuring gonadotropins in serum, it was assumed that luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were secreted continuously in man with resultant constant serum levels. However, several investigators have recently demonstrated that LH is secreted in a pulsatile fashion in man as well as in other species (1–14). Studies in monkeys (11) suggested that the stimuli inducing LH pulses originate in central nervous system centers which mediate these effects through neurons utilizing catecholamines as neurotransmitters.

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In view of these findings, it was necessary to develop a new approach for the quantification of gonadotropin secretion that would allow comparison between patients, even though LH levels were extremely variable in individual subjects. In addtion, it was pertinent to determine whether patients with a variety of disorders of the pituitary gonadal axis have unique patterns of pulsatile gonadotropin secretion that distinguish them from normal individuals. And finally, it was of interest to ascertain whether catecholamine-mediated neural events control the pulsatile secretion of LH and FSH in man. Quantitative means of analyzing LH pulse amplitude, frequency, shape, and area were established and utilized in this study to examine these questions.

METHODS

Quantitative parameters of LH pulses

Several descriptive parameters were established to compare the patterns of pulsatile LH secretion in normal subjects and patients with a variety of disorders of the pituitary-gonadal axis. These parameters included the coefficient of variation of LH levels over a 6 h period, the number of secretory pulses (increment from nadir to peak of greater than 20%) per 6 h, the percent and absolute increment in LH per secretory spike, the area under curves described by LH levels determined over a 6 h period, and the "apparent LH half-life" after each secretory spike. The "apparent LH half-lives" were determined from the log linear decrements of LH in serum that lasted at least 40 min and that followed preceding secretory spikes. The regression lines describing these decrements were drawn by computer,[1] using least squares analysis. Regression lines were excluded if nonlinear or if any point on the line differed from the computed line by more than could be explained by assay variability at the 99% confidence limits. All secretory spike parameters were calculated by IBM 360 computer, using a program designed for this purpose.[1]

Hormone assays

Serum LH levels were measured by a double antibody radioimmunoassay system similar to that previously described, utilizing reagents (anti-LH batch no. 1 antisera and LER 960 HLH for radioiodination) supplied by the National Pituitary Agency (15). With this system the lower limit of detectibility using 200 μl of plasma was 9 ng of LER 907/ml.[2] The limit of detectibility represents the lowest amount of LH that can be distinguished from zero LH in samples containing buffer only, with 95% confidence limits based on median assay variance. The precision of the assay was determined by assaying 20 aliquots of three plasma samples with low, intermediate, and high LH levels. In this study, the within assay coefficients of variation of duplicate samples were respectively 6.7, 3.8, and 4.9% at 76, 55, and 20% binding on the standard curve. Between assay variability at 50% binding was 13.3%. During initial studies anti-HCG batch no. 1, supplied by the National Pituitary Agency, was utilized in place of the anti-LH batch no. 1. Similar potency estimates were obtained for samples from 20 normal men using anti-HLH as antisera (63±36 [SD] ng/ml LER 907) compared with the estimates on these samples using anti-HCG batch no. 1 (79±57 ng/ml).

Serum FSH levels were measured by a similar double antibody radioimmunoassay system (15) using reagents supplied by the National Pituitary Agency (antihuman FSH batch no. 3 as antibody and LER 1,366 as human FSH for radioiodination). The sensitivity (defined as above) of this system using 200 μl of plasma is 30 ng/ml of FSH in terms of LER 907.[3] At 20, 35, and 74% binding on the assay standard curve, within assay coefficients of variation were 3.9, 3.1, and 4.3%, respectively. Between assay variability at 50% binding was 12.3%. All samples obtained from a single study were run in the same assay to avoid interassay variability.

Plasma cortisol and compound S levels were determined using modifications of the competitive protein-binding assay described by Jubiz, Meikle, West, and Tyler (16) and Murphy, Engleberg, and Pattee (17). Progesterone was measured with a radioimmunoassay system (18). Human growth hormone (HGH) was estimated using a solid-phase radioimmunoassay system supplied by Abbott Laboratories (Abbott Scientific Products Div., South Pasadena, Calif.) and standardized in our laboratory against the National Pituitary Agency HGH double antibody system.

Collection of samples

In studies involving frequent sampling, a heparin well scalp vein needle was inserted into an arm vein and taped in place. Small amounts of heparinized saline were injected into the polyethylene line after each blood collection and removed immediately before sampling. Blood was allowed to clot, and the serum was separated and frozen before assay. Blood samples were obtained in normal volunteers at varying time intervals as described below. In all patients, samples were collected at 20-min intervals for 6 h.

Normal volunteers

Multiple blood samples were obtained from each of 24 normal males, ages 19–40. Four men were sampled daily for 60 days, and the others were sampled at hourly, 20-min, or 10-min intervals throughout a 10 h period. Five normal women (ages 24–28) with cyclic menses were also studied. Blood samples were obtained every 20 min for 8 h on days 8 and 22 after the onset of menses. In four of these women, blood was sampled daily throughout the cycle to document the midcycle surge of LH and FSH and to establish the time interval between day of (20 min) sampling and midcycle peak. In two women, midcycle gonadotropin peaks, luteal phase progesterone levels of 11.6 and 13.6 ng/ml, and duration of the luteal phases of 12–14 days were indicative of normal ovulatory menstrual cycles. Two additional women exhibited short luteal phases (19), as evidenced by the onset of menses 8 and 9 days after the midcycle LH peak and peak progesterone levels of only 6.4 and 3.8 ng/ml. Ovulation was not documented in the fifth patient

[1] Copies of this program are obtainable from the National Auxilliary Publications Service (NAPS). Order NAPS Document no. 02147 from ASIS/NAPS, Microfiche Publications, New York 10017.

[2] 1 mg of LER 907 equals 263 IU of Second International Reference Preparation of human menopausal gonadotropin in terms of LH.

[3] 1 mg of LER 907 equals 35.8 IU of Second International Reference Preparation of human menopausal gonadotropin in terms of FSH.

studied. None of these individuals had received oral contraceptives for at least 4 mo before study.

Hypothalamo-pituitary function tests

When there was clinical evidence of hypothalamo-pituitary dysfunction, the following group of provocative tests were used to establish the extent of hypofunction: HGH was measured during arginine infusion (0.5 g/kg) and during insulin-induced hypoglycemia (20). Plasma LH and FSH were measured during clomiphene citrate administration (200 mg/day for 6 days) (15, 21). ACTH reserve was tested by the administration of Metopirone, 2.0 or 3.0 g in a single dose followed by the measurement of 11-deoxycortisol in serum 8 h later (16). Serum thyroxine was measured as an index of thyroid-stimulating hormone (TSH) secretion.

Patients

28 patients with various abnormalities of the pituitary-gonadal axis were studied.

Hypogonadotropism. Gonadotropin secretion was examined in five patients with gonadotropin deficiency. Two women, one with anosmia (V. B.) and another without anosmia (K. M.), exhibited selective gonadotropin deficiency. Panhypopituitarism was present in a third woman (E. L.). The two men with hypogonadotropism included one patient with acromegaly (R. S.) and a 19-yr old patient with delayed puberty secondary to regional enteritis.

Hypergonadotropin. Seven women with high gonadotropin levels including five postmenopausal patients, one surgical castrate and one patient with Turner's syndrome (45,X karyotype in blood lymphocytes with bilateral streak ovaries documented by laparoscopy) were studied. No patient in this group had received hormonal therapy for at least 2 mo before study.

Secondary amenorrhea. Six nonhirsute patients with secondary amenorrhea of 6–24 mo duration were studied. Three of these patients developed amenorrhea after extremely stressful situations and were classified "psychogenic amenorrhea." The other three patients developed amenorrhea in association with galactorrhea (galactorrhea-amenorrhea). Evaluation revealed normal pituitary function as judged by the above outlined provocative tests and normal sella turcica size and configuration. In addition, 1 mg of dexamethasone suppressed a.m. plasma cortisol in a normal fashion. Prolactin levels were not measured in these patients.

Hirsutism. Seven patients with facial hirsutism without other evidence of virilism were examined. Two of these patients had enlarged ovaries observed at laparoscopy, whereas in the remaining five the ovarian size as determined by pelvic examination and laparoscopy (in four patients) were normal. Hirsutism was graded 1 to 4+ (22). Plasma testosterone levels varied from 44–147 ng/100 ml with 6/7 values above 65 ng/ml.[4] Plasma cortisol levels suppressed to < 3 μg/100 ml after 1 mg of dexamethasone.

Anorexia nervosa. Three girls, ages 19–22, with anorexia, marked weight loss, and amenorrhea were examined. Each of these subjects had normal anterior pituitary function as judged by the above outlined hypothalamo-pituitary function testing. In each patient, the diagnosis of anorexia nervosa was arrived at by two physicians and a psychiatrist. Two responded to intensive inpatient psychiatric therapy, and the third has responded to outpatient therapy.

[4] Patients with hirsutism and normal serum testosterone levels generally have elevated testosterone production rates (23).

Alpha adrenergic blockade

Five normal men were sampled at 20-min intervals for 6–10 h on 3 successive days. On day 1, no medication was given. On day 2, phentolamine was administered intravenously at a rate of 0.5–0.75 mg/min for 6–8 h (total dose of 240 mg) according to the procedure of Porte (24). Standing and supine blood pressures were monitored at 15- to 30-min intervals, and the rate of administration of phentolamine was increased as necessary to insure adrenergic blockade that would result in at least a 30 mm Hg drop in diastolic blood pressure upon standing. On day 3, chlorpromazine (50 mg) was administered intramuscularly 2 h before the initiation of sampling and again 6 h later. All subjects remained in bed during the study periods, and two meals were allowed since previous studies have demonstrated that food ingestion does not influence pulsatile LH secretion (3).

LH half-life

To estimate the half-life of LH in man under conditions of negligible endogenous LH secretion (exogenous LH half-life), unlabeled hormone was administered to four patients with very low or undetectible LH levels. Three of these subjects, ages 20–27, had recently undergone hypophysectomy for pituitary tumor, and the fourth was a patient with hypogonadotropism and anosmia. 1.5 mg of unlabeled human LH (LER 1,417) supplied by the National Pituitary Agency was administered intravenously, and LH levels were then measured by radioimmunoassay techniques in blood samples collected at frequent intervals for 6 h.

RESULTS

Pattern of LH and FSH secretion

Normal men. Serial measurements of LH in normal men were extremely variable (coefficient of variation 21–43%) irrespective of whether samples were collected

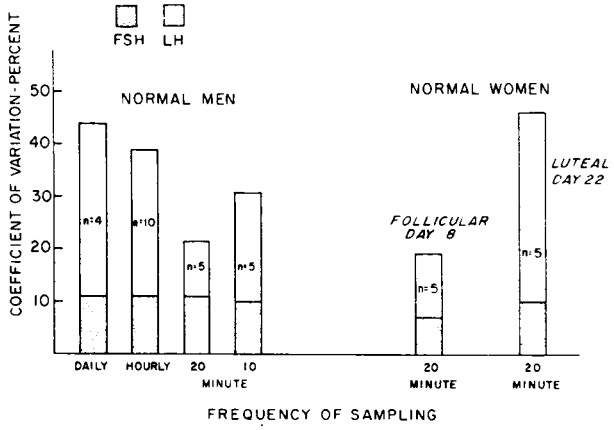

FIGURE 1 The coefficient of variation of FSH (shaded bars) and LH (total height of shaded and open bars) in normal men and women observed in serum samples obtained at daily, hourly, and 20 min sampling. Studies in women were carried out on day 8 (follicular phase) and day 22 (luteal phase) after the onset of menses.

TABLE I
*Pattern of Secretion of Gonadotropins**

Category	Patient	Serum LH: Mean LER 907	Serum LH: Area	Serum LH: Coefficient of variation	Serum LH: Secretory spikes/ 6 h	Serum LH: Mean secretory spike increment: %	Serum LH: Mean secretory spike increment: LER 907	Serum LH: "Apparent LH half-life	Serum FSH: Mean LER 907	Serum FSH: Coefficient of variation
		ng/ml	*ng/min* $\times 10^{-3}$	%			*ng/ml*	*min*	*ng/ml*	%
Normal men	(n = 10)	42±3.3	15.2±1.2	25±3.2	3.7±0.44	68±12	15±1.4	129±24	182±19	10.1±1.2
Normal women										
Follicular	(n = 5)	67±7.6	24.0±3.8	19±3.5	3.2±0.36	56±12	26±5.0	116±21	312±42	7.2±1.4
Luteal	(n = 5)	58±8.3	20.8±2.9	46±8.8‡	1.6±0.24§	333±170¶	73±15§	82±14	262±62	10.8±1.6
Secondary amenorrhea										
Psychogenic	C. L.	68	23.9	50	3	128	49	58	233	14
	J. G.	34	12.1	45	3	44	16	70	181	9.7
	D. G.	28	9.2	49	1	300	40	152	217	18
Galactorrhea-amenorrhea	W. P	31	12.3	34	3	191	36	68	208	4.2
	A. M.	30	10.2	20	4	50	16	72	109	14
	Y. M.	17	6.2	24	2	67	8	85	209	7.9
	Mean	35±7.0	12.0±2.5	37±5.3	2.7±0.41	130±41	28±6.7	84±14	192±18	11.0±2.1
Hirsutism										
Idiopathic	J. V.	57	19.7	23	2	29	14	115	171	8.6
	R. L.¶	99	36.2	20	3	56	23	70	240	7.5
	R. L.¶	34	10.5	102	2	1,000	100	44	215	13
	J. D.	37	13.4	31	6	97	22	69	150	14
	C. W.	24	8.1	48	0	0	0	142	91	15
	V. H.	123	44.3	25	4	91	75	50	204	7.4
PCO**	E. W.	45	16.1	35	1	71	20	95	134	9.2
	M. D.	131	46.6	31	4	91	80	39	190	9.0
	Mean	69±15	24±5.6	39±9.6	2.8±0.68	179±116	42±12	78±13	174±17	10.5±1.1

* Results expressed as mean±SEM.
‡ $P < 0.05$ by paired comparison, follicular vs. luteal.
§ $P < 0.01$ by paired comparison, follicular vs. luteal.
¶ $P < 0.01$ by paired comparison of log transformed, follicular vs. luteal.
‖ Patient studied on day 11 and day 4 of menses.
** PCO, polycystic ovarian syndrome.

TABLE I—(*Continued*)

Category	Patient	Serum LH							Serum FSH	
		Mean LER 907	Area	Coefficient of variation	Secretory spikes/ 6 h	Mean secretory spike increment		"Apparent LH half-life	Mean LER 907	Coefficient of variation
						%	LER 907			
		ng/ml	*ng/min* $\times 10^{-3}$	%			*ng/ml*	*min*	*ng/ml*	%
Hypogonadotropism	V. B.	22	7.6	19	4	56	8.6	160	34	—
	E. L.	12	4.5	21	4	49	4.8	—	51	—
	R. S.	17	6.3	18	6	51	7.0	66	201	9.5
	K. M.	10	3.7	—	0	0	0	—	51	—
	R. P.	<9	—	—	—	—	—	—	<30	—
	Mean	15±2.7	5.48±0.88	19±1.0	3.5±0.92	39±6.9	5.1±1.3	113	77±39	
Hypergonadotropism										
Castrate	M. K.	591	213	17	5	53	239	—	2,891	15
Menopausal	B. E.	385	139	9.3	3	25	70	166	920	6.7
	E. P.	519	176	16	3	39	166	—	1,220	11
	M. T.	464	169	18	4	51	123	92	1,645	8.4
	N. B.	1,108	395	31	2	86	570	—	1,881	6.5
	S. A.	119	43.1	28	4	77	67	—	1,019	5.8
Turner's	J. P.	179	64.3	19	6	46	60	54	2,043	7.4
	Mean	481±125	171±45	20±2.8	3.9±0.51	54±8.1	185±70	104±34	1660±266	8.7±1.3
Anorexia nervosa	M. T.	11.2	4.0	12	2	29	3.0	103	105	9.3
	C. H.	11.4	4.1	7.1	1	22	2.2	165	121	6.5
	T. G.	24	8.3	13	1	27	7.0	—	83	6.8
	Mean	15.5±4.2	5.5±1.4	11.2±1.8	1.3±0.36	26±2.1	3.4±1.6	134	103±8.0	7.5±0.90

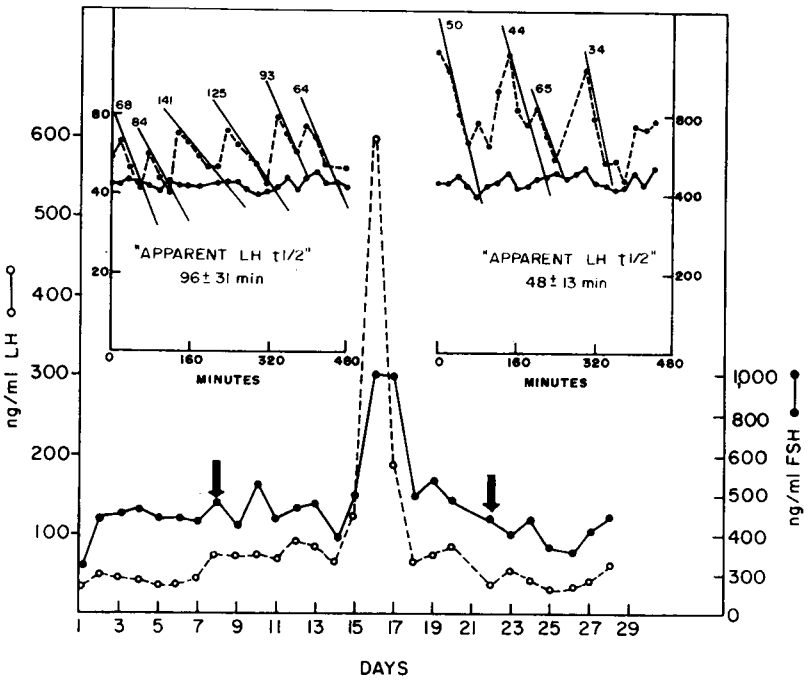

FIGURE 2 Pattern of LH and FSH levels (linear scale) in daily samples of blood collected from a single patient during a menstrual cycle. The inserts represent the LH and FSH levels (log scale) in serum samples collected at 20-min intervals for 8 h on days 8 and 22 (solid arrows) after the onset of menses. The solid lines represent the decrements of LH after secretory spikes (drawn by least squares analysis) which meet the criteria of linearity (see text). The numbers above each solid line represent apparent LH half-life calculated from that decrement.

at daily, hourly, 20- or 10-min intervals (Fig. 1). The variability was due to periodic rapid increases (secretory spikes) followed by slow decreases in plasma level of this gonadotropin. The mean peak LH level of each increase was 68±12% (range 20–400%) above the preceding nadir, and the secretory spike interval was 3.7 times/6 h (Table I). The mean apparent LH half-lives calculated from the LH decline after each secretory spike was 129±24 min (Table I). In comparison to LH, serum FSH levels were relatively constant (coefficient of variation, 10%) and abrupt increments comparable in magnitude with those for LH were rarely observed.

Normal women. In women, the qualitative patterns of gonadotropin secretion were like those of men with serum FSH levels showing very small fluctuations as opposed to the frequent secretory spikes of LH. It was of interest to note the quantitative differences in the pattern of LH secretion between the luteal and follicular phases of the menstrual cycle (Table I, Fig. 2). The mean coefficient of variation of LH was significantly greater during the luteal (46±8.8%) than during the follicular phase (19±3.5%) even though mean serum levels of LH were similar (58±8.3 vs. 67±7.6 ng/ml). The larger coefficient of variation was due to the fact that luteal-phase LH pulses were significantly larger ($P < 0.01$) (333±170%) than those of the follicular phase (56±12%). In addition, the LH secretory spikes occurred ($P < 0.01$) less frequently in the luteal phase (1.6±0.24 vs. 3.2±0.36 secretory spike/6 h). Although not statistically significant, the apparent LH half-lives during the luteal phase (82±14 min) appeared to be somewhat shorter than those observed during the follicular phase (116±21 min).

Patients. A marked degree of variability in serum LH but not FSH was observed in most patients (Table I). In groups of women with amenorrhea and with hirsutism, the LH secretory patterns were more like those observed during the luteal phase of the normal menstrual cycle. In these patient groups, both the mean coefficient of variation (amenorrhea, 37±5.3%; hirsutism, 39±9.6%) and the mean percent LH increment (amenorrhea, 130±41%; hirsutism, 179±116%) were high relative to those of the follicular phase. In addition, the apparent LH half-lives in these patients (amenorrhea, 84±14 min and hirsutism, 78±13 min) were similar to those observed in the luteal phase (82±14 min).

By contrast, groups of patients with hypogonadotropism and with hypergonadotropism exhibited patterns

of LH secretion that were quite different from those of the luteal phase of the menstrual cycle. In these groups, both the coefficient of variation (19±1.0 and 20±2.8%) and the percent increases in LH per secretory spike (39±6.9, 54±8.1) were low and indistinguishable from those of the follicular phase. In addition, the apparent LH half-lives and frequency of secretory spike were also quite comparable with those of the first half of the normal menstrual cycle (Table I).

Three women with anorexia nervosa exhibited relatively little fluctuation of LH levels in serum. Not only was the mean coefficient of variation of LH the lowest observed in any patient group (11.2±1.8%), but the percent (26±2.1%) and absolute increments in LH per secretory spike (3.4±1.6 ng/ml) and the frequency of secretory spike observed over 6 h (1.3±0.36) were also low. That the reduction of pulses in these patients did not reflect methodologic errors inherent in measuring low gonadotropin levels was suggested by the fact that identical patient estimates were obtained when 200 or 400 μl of serum were used in the assay.

The pattern of LH secretion in patients with hypergonadotropism appeared to differ from those of other groups because of the large absolute increments in plasma levels of this gonadotropin during secretory spikes. These pulses (185±70 ng/ml) were two- to sevenfold greater than those exhibited by normal women (luteal, 73±15 ng/ml; follicular, 26±5.0 ng/ml). However, when analyzed according to the percentage rather than absolute increments, the LH secretory pulses in women with hypergonadotropism were no greater than those of any other patient group. Furthermore, the absolute LH increase during each secretory spike appeared related to the mean LH levels, as evidenced by the high correlation ($r = 0.94$) between secretory spike magnitude and mean LH level (Fig. 3). The single exception to this relationship occurred in the patterns found during the luteal phase in normal women (Fig. 2, Table I) whose secretory spikes were clearly of greater magnitude than those of other patients with comparable mean LH levels.

Clinical interpretation of inconstant LH levels

Integral vs. mean LH levels. Since serum LH levels were not constant, integral LH levels were examined as a better index of LH secretion. The integral levels was determined by calculating the area under curves described by LH levels at 20-min intervals over a 6 h period. We found, however, that the integral levels determined in such a fashion, correlated perfectly ($r = 1.00$) with the mean LH calculated from the individual LH determinations over the same 6 h period. This correlation indicated that in patients not receiving hormones, the mean plasma level might be just as useful as the integral level, provided enough samples were obtained.

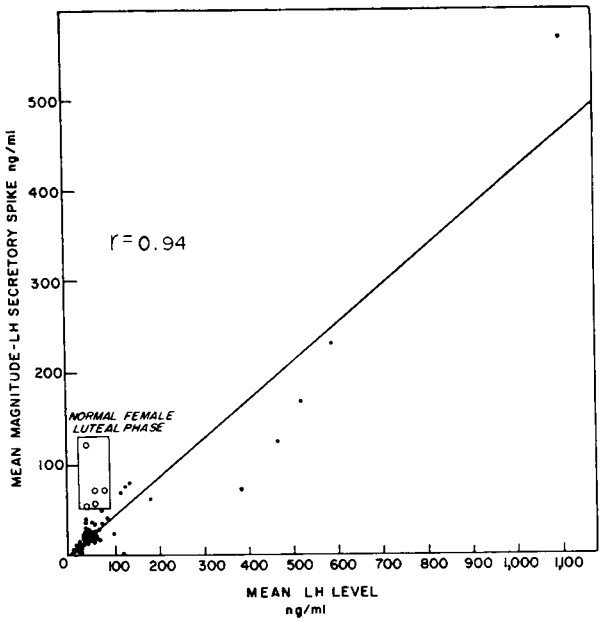

FIGURE 3 The correlation of the mean magnitude of each LH secretory spike, expressed as the nanogram increments from nadir to peak, with the mean levels of LH measured in blood samples collected at 10- or 20-min intervals for 6 h. The open circles represent the data obtained in women during the luteal phase of menses, whose secretory spikes are of greater proportionate magnitude than those of any other patient groups (see text).

The duration of sampling required for precise estimation of the mean plasma LH level was then determined. For this purpose, the cumulative mean LH levels and their 95% confidence limits were calculated for 1 through 6 h of consecutive sampling in each normal subject, as exemplified in a single patient in Fig. 4. In all subjects, the cumulative mean LH level had stabilized after 3 h of sampling because at least one secretory spike had occurred during this period. The 95% confidence limits of the cumulative mean LH for the normal subjects was ±18% at 3 h and ±12% at 6 h. These studies suggested that at least 3 h of observation (every 20 min sampling) are required to determine accurately the mean plasma level of LH in man.

Multiple sampling during the same day may not at times be feasible. A similar analysis of cumulative mean LH levels determined by daily sampling, revealed that nine daily samples must be obtained in order to reduce the 95% confidence limits of that mean to less than ±25%.

Usefulness of mean LH levels. The normal range of mean LH levels in men (range, 24–55 ng/ml) and women (range, 39–86 ng/ml) are shown in panel A of Fig. 5. Many of the LH levels determined on individual samples in these same subjects were well above (e.g. 157 ng/ml) or below (e.g. 9 ng/ml) the range for mean LH levels (Fig. 5, panel B). From these observations,

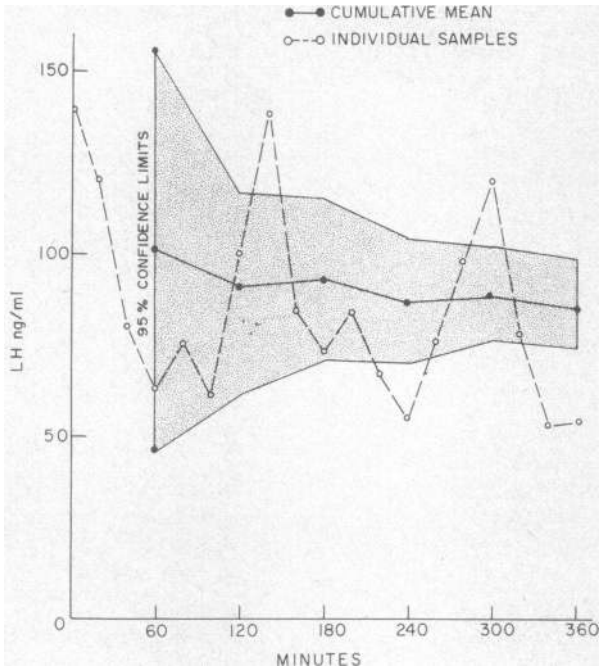

FIGURE 4 The solid line and shaded area are the cumulative mean LH level and the 95% confidence limits of that mean at hourly intervals for 6 h in a single patient. For comparison, the dotted lines and open circles represent the actual estimates of serum LH in samples obtained at 20-min intervals.

it is clear that precise discrimination of normal subjects from those with low or moderately elevated mean LH levels is difficult when single LH determinations are used. The mean LH levels determined from multiple samples, however, minimize the overlap between normal subjects and patients with mild abnormalities of gonadotropin secretion (Fig. 5, panel C).

Physiological studies of pulsatile LH secretion

Constant vs. discontinuous LH secretion. If LH secretion ceases completely between secretory pulses, the subsequent disappearance of endogenous hormone from blood should parallel the disappearance of exogenously administered LH. To investigate this hypothesis, LH half-lives were determined in four gonadotropin-deficient men after administration of highly purified human LH (LER 1,417). The disappearance of exogenous LH in blood was described by two linear exponentials with a mean initial phase half time of 39 min (range, 32–65) and a mean second-phase half time of 121 min (range, 90–145) (Fig. 6). By contrast, endogenous LH disappearance (after secretory spikes) was described by a single linear exponential with apparent half times which could be arbitrarily divided into three categories: (*a*) half times similar to those during the initial-phase disappearance of exogenous LH (32–65 min); (*b*) half times longer than those during the second-phase disappearance of exogenous LH (90–145 min), and (*c*) half times intermediate between those observed during the initial- and second-phase half-lives of exogenous LH. As an explanation for these observations, it appeared likely that short, apparent half-lives (32–65 min) reflect complete cessation of LH secretion between secretory spikes. Furthermore, continuance of low level LH secretion after secretory spikes can account for apparent LH half-lives of greater than 145 min. However, this simple comparison of endogenous and exogenous LH disappearances cannot explain whether continuous LH secretion could account for the large number of intermediate half-lives observed.

In an effort to determine whether factors other than discontinuance of LH secretion might also influence LH

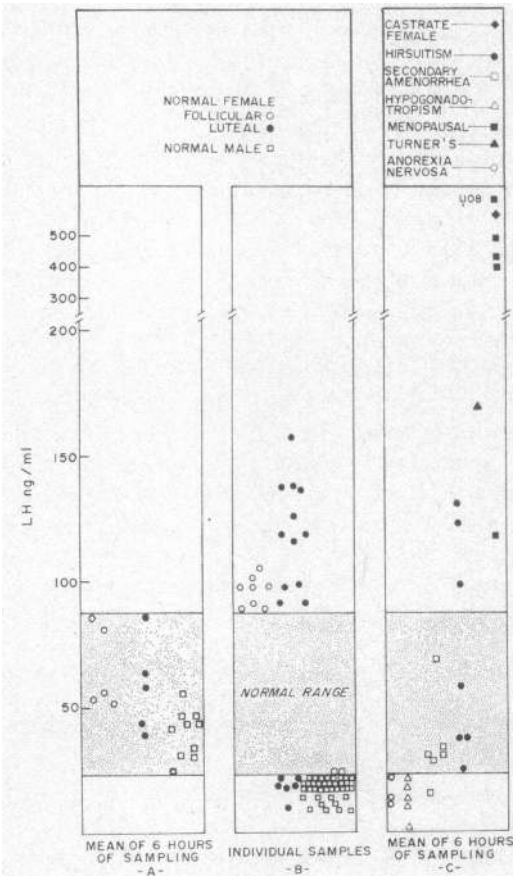

FIGURE 5 (A) The normal range of 6-h mean LH levels is represented by the shaded area. Each data point reflects the mean levels of LH observed in blood samples collected at 10- or 20-min intervals for 6 h. (B) Estimates of LH concentration in individual samples which were above or below the normal range established by calculating 6-h mean LH levels in these same normal subjects. (C) 6-h mean LH levels in patients with various abnormalities of the pituitary-gonadal axis.

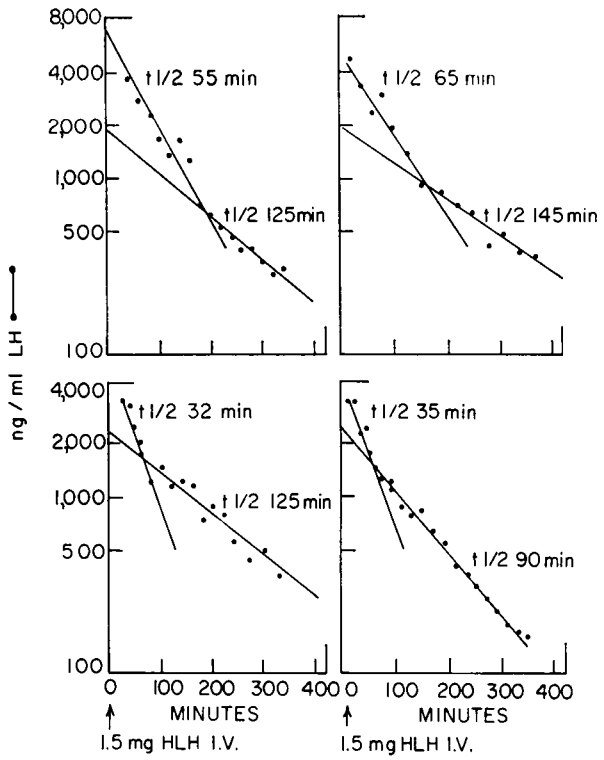

FIGURE 6 LH levels in the sera of four hypogonadal men after the injection of 1.5 mg of human LH intravenously. The solid lines represent the early and late phase half-life of LH in each individual subject. The regression lines were drawn after determining the best fit from least squares analysis after manual stripping of the late phase from the early phase exponential.

disappearance, we observed that apparent LH half-lives were related to the height of the secretory spikes; that is, short, apparent LH half-lives were observed after large secretory spikes and prolonged apparent half-lives were seen after small secretory spikes. A significant inverse correlation ($r = -0.87$) existed between the secretory spike height and apparent LH half-lives (Fig. 7). This latter observation suggested that the apparent LH half-life actually reflects components of initial and second-phase disappearance of LH, and the contribution of each of these phases to apparent half-life relates to the height of the preceding secretory spike. The wide range of apparent half-lives observed, therefore, results from variable contributions of the initial- and second-phase LH disappearance in addition to constant low level LH secretion between pulses.

Correlation of FSH with LH spiking. In all patients studied, LH levels varied greatly in contrast to the intermittent increments in FSH levels of lesser magnitude. Although FSH and LH secretory bursts were often independent, concordant increases in serum FSH levels during LH secretory spikes were observed significantly ($P < 0.001$) more frequently than would be expected to occur by chance. Furthermore, the concordance of FSH with LH pulses significantly increased ($P < 0.01$) as a function of the height of the LH pulses.

In contrast, there was no concordance between LH spikes and those of ACTH as reflected by serum cortisol levels (25) even when a 20 min lag in rise of cortisol (to compensate for time of ACTH effect) was allowed (Table II).

Alpha adrenergic blockade. Intravenous administration of the alpha adrenergic-blocking agent, phentolamine, in amounts sufficient to produce marked orthostatic hypotension in five normal men, resulted in no alteration of LH secretory spike activity as reflected by coefficient of variation (control, 19±2.7%, phentolamine, 20±2.7%), incremental magnitude of each secretory pulse (Control, 17±2.4, vs. 25±8.0 ng/ml), or apparent LH half-life in the five normal men studied. Another alpha blocker, chlorpromazine, did not reduce, and to our surprise slightly increased the magnitude of LH secretory pulses in the five subjects studied. Mean LH secretory spike increment during chlorpromazine administration was 31±3.3 compared with 17±2.4 ng/ml during the control period even though the frequency of secretory spikes did not change (Control, 2.8±0.2 vs. chlorpromazine, 2.8±0.4). These responses were divergent from the effects noted in castrated monkeys receiving larger doses of these same medications (11). Present data do not allow speculation whether these differences are due to dosage or species effects.

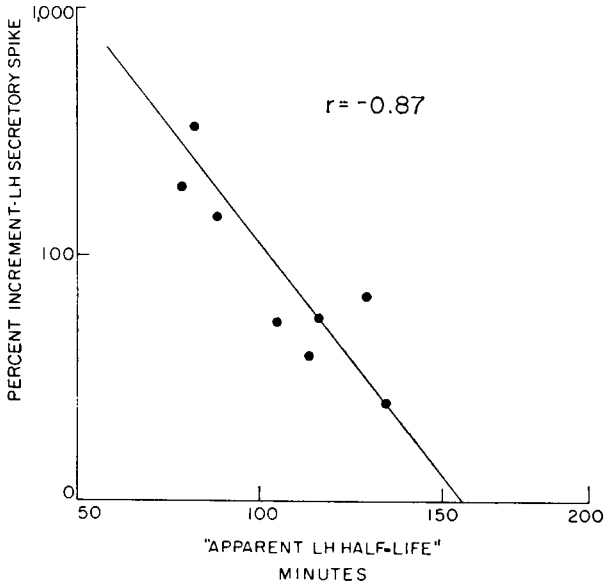

FIGURE 7 Correlation between the mean LH secretory spike magnitude in percent and apparent LH half-life. Each data point represents the mean of a group of patients listed in Table I.

TABLE II

Concordance of FSH and Cortisol Increments with LH Secretory Spikes

Mean LH secretory spike increment-Percent	Simultaneous FSH levels‡			Simultaneous cortisol levels			Cortisol levels 20 min after LH spike		
	Increase	No change	Decrease	Increase	No change	Decrease	Increase	No change	Decrease
20% or greater	125* (63%)	17 (9%)	57* (28%)	15	3	18	15	4	17
40% or greater	66 (65%)	8 (8%)	28 (27%)	9	1	10	7	3	10
60% or greater	48 (69%)	4 (6%)	17 (25)%	6	1	6	6	2	5
80% or greater	38 (78%)	3 (6%)	8 (16%)	6	1	6	4	0	5
100% or greater	31 (78%)	3 (7%)	6 (15%)	4	1	4	3	1	5
200% or greater	8 (89%)	0	1 (11%)	2	0	2	1	0	3

* $P < 0.001$ by X^2 (increase vs. decrease).

‡ $P < 0.01$ by Kendall's Rank correlation (increasing concordance FSH with LH spikes of increasing magnitude).

An incidental observation in this study was the constancy of the mean LH levels in individual men over 3 days of study. Even though major pharmacologic manipulations were performed, the mean LH levels for the initial day differed from those on the following 2 days by only 13% (range, 2–31%) in the five patients studied.

DISCUSSION

Patterns of secretory spiking. The rhythmic secretion of LH at 1- to 3-h intervals has been described for several species (1–14). In man, pulsatile LH secretion has been observed in normal men and women and in individuals with gonadal failure (1–10, 14). In the present study similar patterns of LH secretion were detected in a large number of patients with hypo- and hypergonadotropism, amenorrhea, and hirsutism. These observations suggest that pulsatile LH release occurs in the presence or absence of gonadal steroids and that the pattern of LH secretion is similar in both normal individuals and in those with a variety of pathological conditions. In addition, they emphasize that a common mechanism for initiation of LH secretion is operative in all patients.

Since the pattern of LH secretion appeared, by inspection, to be qualitatively similar in most subjects, it was pertinent to determined whether there were quantitative differences between patients and normal men and women. Several parameters were selected which could be used to describe the pulsatile secretory patterns in quantitative terms. These included frequency (number of secretory spikes/6 h), amplitude (percent and absolute secretory spike increment), and apparent LH half-life. It is appreciated that the limits set in the definition of these parameters are arbitrary and relate to methodologic rather than physiologic criteria. For example, while LH pulses not sufficient to produce a 20% increment in serum LH may be physiologic, they are not considered for analysis since they cannot be detected with 99% confidence limits by our assay system.

From analysis of these parameters in normal women, the pattern of LH secretion in the first half of the menstrual cycle was shown to differ from that of the second half of the cycle. High amplitude, low frequency pulses characterized the luteal portion of the menstrual cycle, whereas low amplitude, high frequency pulses were observed in the follicular phase. To our surprise, most patients secreted LH in patterns that resembled those of either the follicular or the luteal phase. This observation emphasizes that particular patterns of LH secretion are not unique to certain pathological states.

Patients with anorexia nervosa may be an exception to this general statement. Decreased gonadotropin secretion as a consequence of starvation is known to produce amenorrhea and delayed puberty. In the present study, patients with caloric and protein restriction secondary to anorexia nervosa had not only reduced mean LH levels but also low frequency, low amplitude patterns that differed from all others examined. Although other patients with a variety of psychiatric disorders have amenorrhea, such patients examined to date have not

had low amplitude secretory spikes. From the available data, we tentatively conclude that restricted food intake may modify gonadotropin secretion by a different method than is operative in any other disease states.

One of the most striking changes in LH secretion occurs between the follicular and the luteal phases of the menstrual cycle when there is a shift from high frequency, low amplitude to low frequency, high amplitude secretory spikes. It is of interest that similar low frequency, high amplitude pulses were observed in both castrate monkeys (12) and in patients with gonadal dysgenesis recovering from estrogen-induced gonadotropin suppression (26). In these studies, estrogen infusions resulted in blood levels similar to or exceeding those which occur before the midcycle LH peak in women. It is possible that in normal women, the high estradiol levels at midcycle sensitize the hypothalamus or pituitary to facilitate the high amplitude secretory spikes observed during the luteal phase (27). The progesterone increment during the luteal phase could also account for the characteristic high amplitude luteal-phase LH patterns. However, the administration of progesterone to castrate monkeys did not alter LH pulses even when luteal-phase progesterone levels were achieved (12). In addition, two women in the present study with short luteal phases associated with reduced progesterone levels (3.8–6.4 ng/ml) exhibited spiking patterns identical with those observed in the two women with luteal-phase progesterone levels of 11.6–13.6 ng/ml. Although it is assumed that gonadal steroids do alter the magnitude and frequency of the LH pulses between the follicular and the luteal phases of the menstrual cycle, further studies are required to establish which hormones are involved and how they interrelate with the central nervous system and the pituitary.

Clinical interpretation of inconstant LH levels. Since all subjects in this study exhibited wide variations in serum LH levels, an integrated value was determined as an index of LH secretion. It was found, however, that mean LH levels calculated from samples drawn at 20-min intervals for 6 h provided a method for evaluating LH secretion that was as useful as the integrated value. Without multiple sampling, a single measurement of LH in a normal man allows an estimate of mean LH with an accuracy of 50% and in a normal woman during the follicular and luteal phases, ±38 and ±92% respectively. The mean LH level from 6 h of samples has an error of ±12%. The only situation in which mean LH levels would not be useful would be during periods of rapid increase or decrease in LH secretion as might be observed during stimulation or suppression tests. Under these conditions, integrated values would be a better index of LH secretion.

Several well-defined clinical circumstances required the determination of mean LH levels by frequent sampling. Individuals with low LH levels cannot be accurately distinguished from normal subjects on the basis of single samples (Fig. 5) because of the overlap resulting from the low levels of LH often exhibited by normal subjects at the nadir of secretory spikes. Similarly, individuals with moderately elevated levels, such as women with hirsutism, cannot be distinguished from normal women on the basis of single determinations. In addition, studies of gonadotropin secretion in which one wishes to demonstrate 20–40% changes in hormone concentrations require multiple sampling. For example, the demonstration of LH increments after clomiphene or the suppression of LH after testosterone in individual patients necessitates that the error resulting from fluctuating levels be reduced to less than 20%. In contrast, the measurement of LH in a single blood sample will usually be sufficient to discriminate subjects with gonadal failure with resultant fivefold or greater elevations of LH from eugonadal subjects (Fig. 5).

Studies were carried out during nonsleep periods in all patients since it is practical to study a large number of patients in this fashion. It is recognized that various anterior pituitary hormones may have different patterns of secretion during sleep and samples collected during sleep may provide additional information relevant to the pathogenesis of certain clinical disorders.

Origin of LH pulses. Insufficient data has been reported at present to clarify the site of origin of stimuli resulting in pulsatile LH secretion. Possible sites include the pituitary, the hypothalamus, catecholamine-mediated stimulatory neurons abutting on the median eminence, or higher central nervous system centers. Whereas the initiation of pulses strictly at the pituitary level is possible, no evidence exists to support this possibility. It is more likely that the stimuli for pulsatile secretion reside above the pituitary level and act through a LH-releasing factor. If this were the case, FSH and LH increments might then be expected to occur simultaneously under certain circumstances, since LH-releasing hormone is also known to stimulate FSH release. In this regard, FSH increased during LH pulses in our patients significantly more frequently than could be ascribed to chance (Table II).

Since the administration of exogenous LH-releasing factor to humans results in FSH rises only when relatively large LH increments are produced, FSH secretory pulses should occur only during large LH secretory pulses (28–31). In this regard, the concordance of FSH with LH secretory spikes increased as the magnitude of LH pulses increased in our patients. Although compatible with the possibility that LH secretory spikes are LRF mediated, these observations in no way ex-

clude the possibility that two separate gonadotropin-releasing factors may be released simultaneously to result in concordant LH and FSH pulses.

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