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Physiological Properties of the Luteinizing Hormone Pulse Signal: Impact of Intensive and Extended Venous Sampling Paradigms on Its Characterization in Healthy Men and Women*

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ABSTRACT. The pulsatile nature of the gonadotropin signal is a critical determinant of physiological activation of the gonadal axis. Nonetheless, major uncertainties exist regarding the exact patterns of LH secretion that constitute normal physiological profiles in man. To assess possible bases for the discrepancies in the literature, we sampled blood at 5-min intervals for 24 h in eight normal men and eight normal women in the early follicular phase of the menstrual cycle. The constituent 5-, 10-, 15-, and 20-min immunoactive LH series and the consituent 6-, 12-, or 24-h sampling durations provided ranges of sampling intensities and durations for analysis of significant LH pulses. A technique for minimizing the influence of false positive immunoassay errors on peak detection was used to aid in estimating apparent true positive LH pulse frequency.

Nonlinear curve fitting of the relationship between sampling intensity and apparent true positive LH pulse frequency revealed a stable pulse frequency estimate at intensive rates of venous sampling, with values of 19.5 ± 1.9 (±SEM) pulses/24 h (periodicity, 73.8 ± 6.5 min/pulse) in men and 20.6 ± 3.6 pulses/24 h (periodicity, 70.0 ± 10 min/pulse) in women. Further analyses indicated that sampling every 3.1 and 2.0 min for 24 h would be required to capture 90% of the LH pulses in men and women, respectively. Moreover, even with a 5-min sampling rate, the statistical counting errors of the LH pulse frequency estimates varied markedly with sampling duration; for example, in men sampled in 6-, 12-, and 24-hr sessions, the values were, respectively, 49%, 35%, and 24% of the observed pulse frequencies. Similar variations were found in women. Counting errors were 30–50% higher for conventional 20-min sampling rates than for 5-min sampling intervals.

Measured interpulse intervals varied widely from 10–330 min (median, 60 min; n = 131 LH pulses) in men and from 10–340 min (median, 65 min; n = 125 LH pulses) in women. In addition, absolute LH pulse amplitudes varied from 1–28 mIU/ml (median, 4.1 mIU/ml) in men and from 1–24 mIU/ml (median, 3.6 mIU/ml) in women. These estimates were associated with a median number of points identified within each pulse of 6.0 in men and 4.0 in women.

In summary, the present exhaustive sampling studies demonstrate for the first time stable estimates of physiological LH pulsations in normal men and women, and document broad ranges of normal LH pulse amplitudes and interpulse intervals. We conclude that both intensive and prolonged sampling conditions are necessary to define stable and precise estimates of physiological LH pulse patterns and to disclose the physiological ranges of these properties in normal individuals. (*J Clin Endocrinol Metab* **62:** 881, 1986)

MANY endocrine glands signal remote target tissues by intermittent rather than continuous hormone secretion. For example, physiological function of the reproductive system is marked by pulsatile rather than constant gonadotropin release (1–6). This episodic pattern is essential for physiological activation of the gonadal axis (1–7). Moreover, pulsatile gonadotropin release is altered distinctively in certain pathophysiological states. For example, LH pulse frequency varies significantly during stages of the normal menstrual cycle (5, 6), during pubertal maturation (8), and in various secondary hypogonadotropic states, such as those associated with steroidal suppression of the gonadal axis (9, 10), hyperprolactinemia (11), anorexia nervosa (12), or acquired amenorrhea accompanying strenuous aerobic training (13). The importance of altered pulsatile LH release in these conditions is suggested by the empirical demonstration that pulsatile (but not continuous) administration of exogenous GnRH can restore normal

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gonadal function in hypogonadotropic individuals (14, 15).

Despite the pivotal importance of pulsatile LH release for normal gonadal function, major uncertainties exist regarding the exact patterns of normal LH secretion. These uncertainties reflect the following principal areas of controversy: 1) the dependence of LH pulse frequency estimates on sampling intensity, 2) the errors inherent in estimating LH pulse frequency in relation to varying sampling duration, 3) the physiological range of LH interpulse intervals in normal individuals, 4) the degree of nonuniformity of LH pulse amplitudes in normal men and women, and 5) the certitude of peak detection, for example as assessed indirectly by the number of points indentified within any single pulse (16). Thus, in reports to date, sampling intensities have varied from blood withdrawal every 1 min to sampling every 0.5 h, while sampling durations have ranged from 4–24 h (1–13). In these disparate and admittedly arbitrary sampling protocols, discrepancies as great as 5-fold have been described for estimated physiological LH pulse frequency in man (1–6, 17–19). In addition, further complexity has been introduced recently by results from intensive sampling paradigms of restricted total duration, which provided estimates of LH pulse frequency that increased continuously at more rapid sampling rates (16–20).

Although therapy of hypogonadotropic patients with exogenous GnRH pulses has been moderately successful using fixed pulse frequency and fixed pulse amplitude regimens, more refined treatment strategies may require an exact recapitulation of the physiological pulse properties of gonadotropin release. To that end, we combined both intensive and prolonged venous sampling conditions to characterize the nature of the physiological LH pulse signal in normal man.

Materials and Methods

Eight normal young men (age range, 21–27 yr) and eight normal young women (age range, 21–36 yr) were studied. All women had a history of normal menstrual function and were studied 3–5 days after the onset of menses. All subjects had normal serum concentrations of LH, FSH, PRL, T_4, TSH, total and free testosterone, and estradiol as well as a normal history and physical examination. After giving written informed consent, the subjects were admitted to the Clinical Research Center, and an iv heparin lock was inserted. One hour later, venous sampling was undertaken by withdrawing blood at 5-min intervals for 24 h. During this time, the subjects were permitted to eat three meals.

The blood was permitted to clot at room temperature, and the serum was separated and frozen for subsequent RIA of LH (discussed below). All samples from an individual subject were analyzed in the same assay. In addition, to assess more precisely the degree of intraassay measurement error, 45 replicates from the individual's serum pool (prepared by removing 50 μl serum from each of the 288 individual samples) were assayed as 15 replicates each at the beginning, middle, and end of the corresponding assay. The pooled coefficient of variation from these 45 replicates was used in pulse analysis. The individual samples were assayed in triplicate for immunoactive LH content with the exact reagents and procedures described previously (21). In the 8 men, the individual pooled intrassay coefficients of variation averaged 8.04 ± 1.38% (± SD), with an absolute range of 7.0–10.2%, while the 24-h mean LH concentrations ranged between 7.11 and 14.7 mIU/ml [mean, 10.91 ± 4.08 (±SD)]. In the 8 women, the individual pooled intrassay coefficients of variation averaged 9.38 ± 0.37%, with an absolute range of 8–11%, while the 24-h mean LH concentrations ranged between 7.0 and 12.6 mIU/ml (mean, 8.76 ± 1.73). The mean LH values were not correlated with the intrassay coefficients of variation derived from the corresponding replicate pools for either men or women.

The presence of LH pulses was evaluated by a modification of the method of Santen and Bardin (6), as described previously (19, 20). This modification requires that a significant LH pulse represent a minimum (threshold) increase of 4 times the relevant individual intrassay coefficient of variation (19). A 4-fold multiple results in an estimated false positive rate of 1.53% (22). The relevant individual intrassay coefficients of variation were calculated from the 45 within-assay replicates developed from the pool of the individual's own experimental series and run within the same assay, as described above.

The curvilinear relationship between sampling intensity and apparent LH pulse frequency was analyzed by nonlinear least squares curve fitting of the relationship $y = kX/(1 + kX)$, where k is a slope parameter, with calculated confidence intervals for the precision of fit (23).

The statistical counting error in the estimate of LH pulse frequency was expressed as the SD of the number of pulses divided by the corresponding mean pulse frequency, *i.e.* the coefficient of variation of the observed LH pulse frequency estimate. This value was termed a coefficient of variation for the LH pulse frequency estimate.

The number of points contained within a pulse was counted, beginning with the first value within a peak that followed the preceding nadir. Counting included consecutive points up to the onset of the upstroke of the next pulse (as defined by the appearance of a new nadir), the first of three consecutively increasing values, when the increasing values did not create a significant pulse; or the first of eight consecutively stable (± 10% variation) values which formed neither a nadir or a peak. The interpulse interval (expressed in minutes) was taken as the time separating two consecutive peaks. The amplitude (as a percentage or milliinternational units per ml) was calculated from immediately preceding nadir to peak (19).

Serum total calcium and protein concentrations were measured with the Technicon RA 1000 analyzer (Technicon Instruments Corp., Tarrytown, NY), as described previously (24, 25). Significant differences within subjects were determined by paired intrasubject t testing, and group differences were evaluated using analysis of variance (26).

Results

To test the relationship between LH pulse frequency and sampling intensity, the number of LH pulses iden-

tified per 24 h was plotted against the number of samples analyzed per 24 h. For example, at a sampling rate of every 5 min, 288 samples were analyzed, while sampling every 10, 15, or 20 min yielded, respectively, 144, 96, or 72 samples/24 h. The curvilinear relationships depicting 24-h LH pulse frequency *vs.* sampling intensity for men and women are shown in Fig. 1. These results document a highly significant dependence of estimated LH pulse frequency on sampling intensity for both men and women ($P < 0.001$) and disclose the apparently asymptotic nature of the LH pulse frequency estimate. The calculated asymptote (maximal LH pulse frequency) was 19.5 ± 1.9 (±SE) pulses/24 h in men and 20.6 ± 3.6 pulses/24 h in women.

The relationship between sampling intensity and LH pulse frequency could be analyzed further to assess optimal rates of sampling by using nonlinear least squares curve fitting. The curvilinear relationship between sampling intensity and observed LH pulse frequency indicated that 50% and 90% of the maximal estimate of pulse frequency would be attained by sampling every 21.6 and 3.1 min in men and every 18.9 and 2.0 min in women, respectively.

The influence of sampling duration on the LH pulse frequency estimate was tested by comparing the number of LH pulses observed at 1, 2, 4, 6, 8, 12, 16, 20, and 24 h for sampling rates of either every 5- or 20 min. In this analysis, the mean number of LH pulses increased in a linear fashion for both 5- and 20-min sampling intervals, but this rate of increase (assessed by the slope) was significantly greater for the 5-min than for the 20-min sampling paradigm. For men, the mean (and 67% confidence limits) for the slope of the linear regression of sampling duration on the number of LH pulses was 0.757 (0.738–0.775) pulses/h for 5-min sampling, and 0.407 (0.404–0.432) pulses/h for 20-min sampling rates ($P < 0.001$). For women, the values were 0.293 (0.286–0.301) pulses/h for 5-min sampling and 0.749 (0.731–0.767) pulses/ h for 20-min sampling rates ($P < 0.001$). The corresponding periodicity (interval between pulses) for the men was 79.3 (77.4–81.3) min/pulse for 5-min sampling compared with 147.4 (139–155) min/pulse for 20-min sampling ($P < 0.01$). For the women, the corresponding periodicities were 80 (78–82) min/pulse for 5 min sampling and 205 (201–209) min/pulse for 20-min sampling ($P < 0.001$).

To test the influence of LH pulse detection threshold on the stability of the observed LH pulse frequency, data from the eight men and eight women were analyzed for LH pulses using thresholds of 20% (original method of Santen and Bardin), 30%, 40%, 60%, or 100%. The corresponding LH pulse frequency estimates per 24 h were then plotted in relation to sampling intensity (every 90 min through every 5 min. Fig. 2. These results indicate

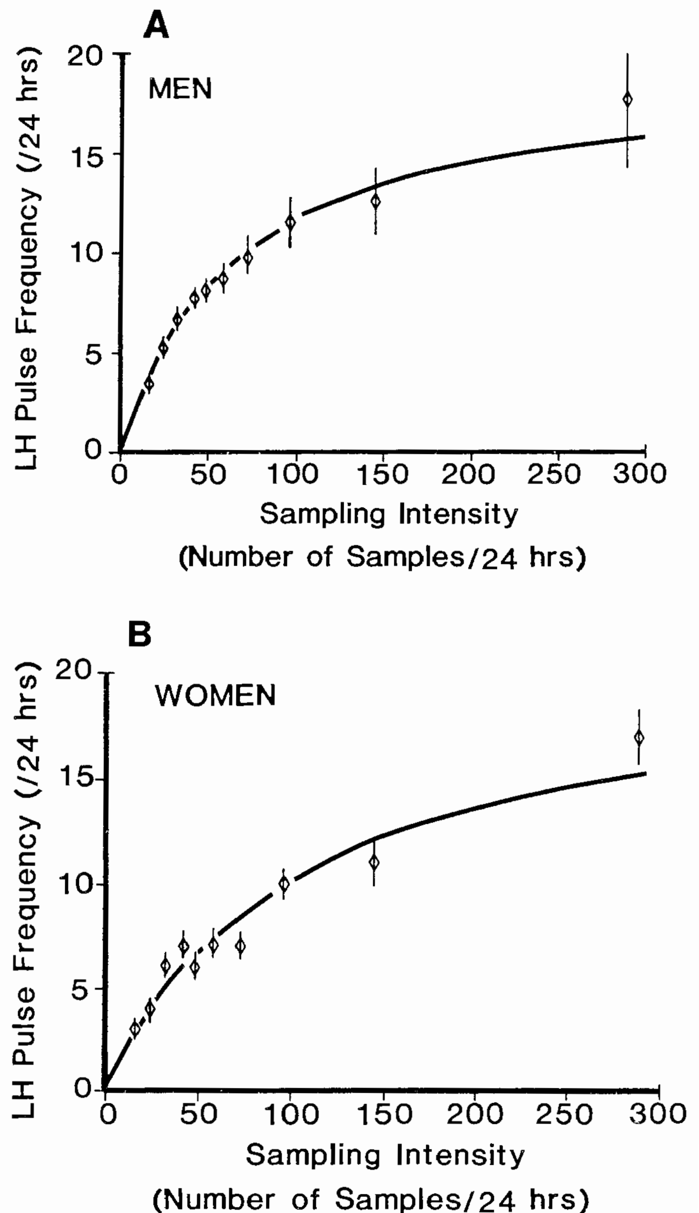

FIG. 1. Curvilinear relationship between sampling intensity (number of samples analyzed per 24 h) and LH pulse frequency (number of LH pulses detected per 24 h). LH pulse frequencies (mean ± SEM) are shown for eight normal men (A) and 8 normal women (B) sampled at 5-min intervals for 24 h (288 samples/24 h). The parent 5-min series were analyzed further in relation to their 10 min constituents (144 samples/24 h), and their 15 through 45 min constituents as indicated. Significant LH pulses were identified using the criterion of 4 times the individual intraassay coefficients of variation as threshold. Nonlinear curve fitting of the relationship sampling intensity *vs.* 24-h pulse frequency disclosed asymptotic curves in both men and women.

that independently of threshold, in both men and women, there was a progressive increase in the apparent LH pulse frequency, and that this increase approached a plateau at sampling intervals of 5–10 min. The plateau estimates in relation to a range of thresholds are given in Table 1. At low thresholds, the plateau value was

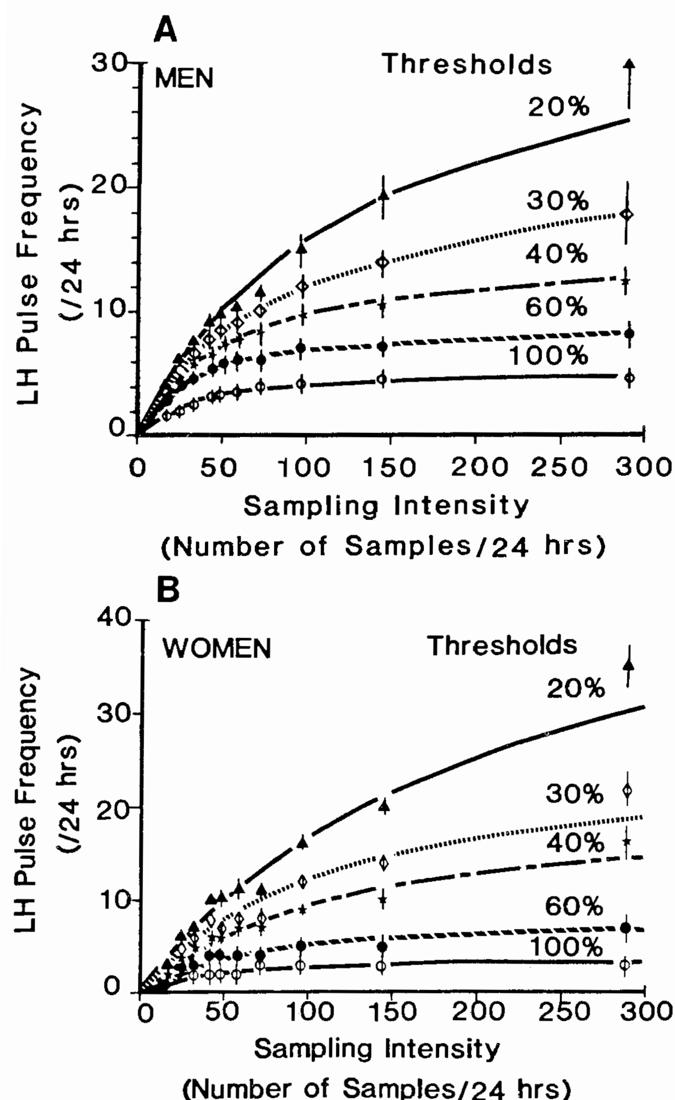

FIG. 2. Influence of pulse detection thresholds on estimates of LH pulse frequency as sampling intensity (frequency) is increased. The relationship between LH pulse frequency and sampling intensity is depicted as shown in Fig. 1, except that LH pulse frequency was computed for the range of pulse detection thresholds shown. Independent of threshold, at more intensive rates of sampling (higher numbers of samples analyzed per 24 h), there is a tendency to approach an asymptotic estimate of LH pulse frequency.

increased, presumably reflecting false positive errors as well as more true positive pulses (22). For higher thresholds, the plateau value was lower and was achieved at less intensive sampling rates. The latter presumably reflects underestimation of true LH pulse frequency (*i.e.* increased false negative error rate) associated with more restrictive thresholds as well as decreased false positives (22). Most significantly, the curvilinear relationships in Fig. 2 clearly indicate that a stable estimate of LH pulse frequency is possible at sufficiently intensive and prolonged sampling rates, independent of the exact threshold chosen.

TABLE 1. Asymptotic estimates of LH pulse frequency (number of pulses per 24 h) in men and women (early follicular phase) sampled at 5-min intervals for 24 h

Detection thresholds (%)	Maximal estimates of LH pulse frequency as a function of pulse detection threshold	
	Men	Women
20	36.9 (30.3–43.3)	54.4 (42.6–65.5)
30	20.1 (18.5–21.7)	26.5 (22.0–30.8)
40	14.5 (13.4–15.6)	20.1 (16.7–23.4)
60	9.5 (8.5–10.4)	8.40 (6.6–10.0)
100	5.7 (5.0–6.3)	3.8 (3.2–4.3)

The resultant LH series were analyzed for significant pulses using the indicated thresholds (expressed as percent increase above baseline). The asymptotes of the relationship sampling intensity *vs.* LH pulse frequency (as plotted in Fig. 2, *top and bottom panels*) are given with 67% confidence limits (n = 8 subjects in each group).

Further studies revealed that the LH pulses disclosed at 5-min sampling rates were not simply attributable to variations in sample processing and/or hydration, since serial serum protein and total calcium concentrations in such samples did not exhibit pulsatile fluctuations even when a 20% threshold was applied (Fig. 3). In addition, the reliability of the 24-h pulse frequency estimates was tested by separately measuring all interpulse intervals in the men (n = 131 interpulse intervals) and women (n = 125 interpulse intervals) The mean (±SEM) and median interpulse intervals were 76 ± 5.4 and 60 min (absolute range, 10–330 min) in men and 80 ± 6 and 65 min (absolute range, 10–340 min) in women (Fig. 4). These values do not differ significantly from the plateau estimates obtained by nonlinear curve fitting of the relationship sampling intensity *vs.* LH pulse frequency (*see above*). Furthermore, the certitude of peak detection was substantial with 5-min sampling intervals, at least as reflected by means (±SEM) of 7.6 ± 0.6 and 5.3 ± 0.3 points contained within each LH pulse in men and women, respectively (corresponding median values, 6.0 and 4.0).

These studies also permitted us to appraise the range of variation of LH pulse properties within individuals. For example, for interpulse intervals in the eight individual men, the coefficients of variation (individual SD/mean) for interpulse intervals ranged from ±31% to ±84% (median, ±58.5%). Similarly, the coefficients of variation for the interpulse intervals in the eight individual women studied ranged from ±63% to ±99% (median, ±75%). LH pulse amplitudes also exhibited a wide dispersion of values both within the same individuals and among different individuals. Figure 5 depicts the dispersion of pulse amplitudes for 131 LH pulses in men and 125 pulses in women. Similarly, the individual men and women had wide ranges of variation in LH pulse amplitude when expressed as coefficients of variation, from ±

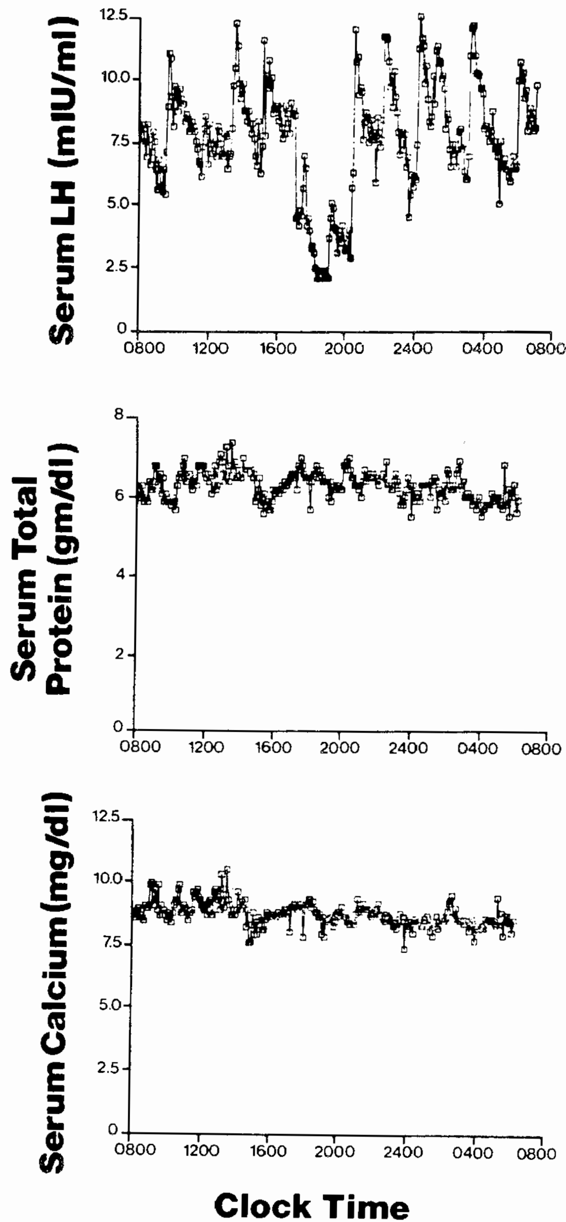

FIG. 3. Sera from one man were analyzed for serum immunoactive LH (*upper panel*), total protein (*middle panel*), or calcium (*lower panel*) in samples obtained at 5-min intervals for 24 h. In contrast to the prominent fluctuations in serum LH concentrations, there were no significant fluctuations in the serum concentrations of protein and calcium. These results indicate that the immunoactive LH pulsations observed are not attributable to major variations in sample processing and/or hydration.

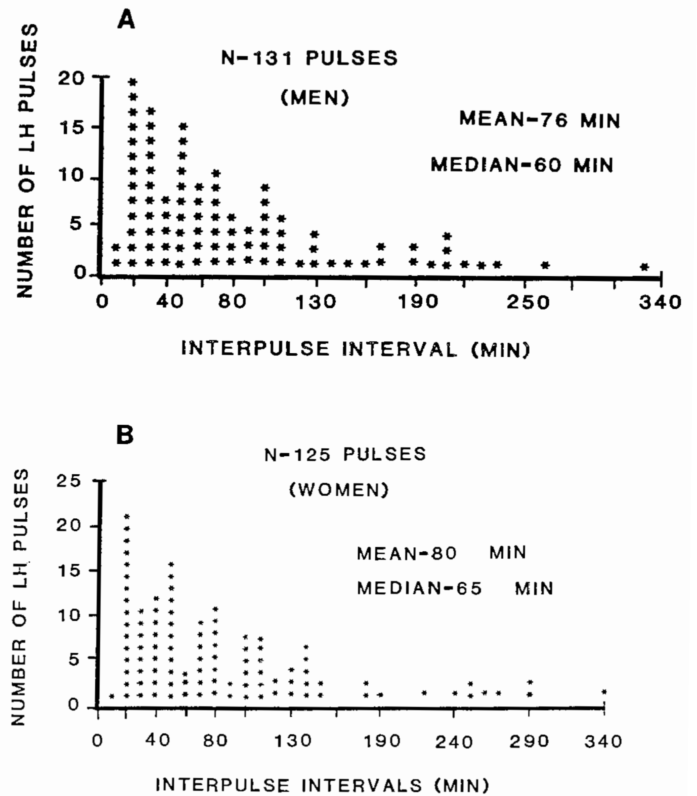

FIG. 4. Frequency histograms of individual interpulse intervals for normal men (A) and women (B) sampled at 5-min intervals for 24 h. These data represent 131 values in men and 125 values in women.

30% to ±81% (median, ±53%) in the eight men and from ±18% to ±19% (median, ±45%) in the eight women.

The counting error inherent in the estimate of observed LH pulse frequency was substantially reduced by sampling every 5 min compared with that less with sampling at less frequent intervals. Moreover, as the duration of sampling was increased from 6 to 24 h, the precision of the estimate for hourly pulse frequency increased significantly. In particular, as shown in Fig. 6, there was a progressive decline in the counting error for more frequent sampling (5 *vs.* 20 min) as well as for more extended sampling (24 *vs* 6 h) in both men and women. For example, the counting errors (expressed as the coefficient of variation for the observed LH pulse frequency estimate) were as large as ±82% and ±85%, respectively, in men and women sampled at 20-min intervals for 6 h. However, the counting error declined to ±24% for both men and women sampled at 5-min intervals for a full 24 h.

In contrast to significant differences in LH pulse frequency in relation to sampling intensity, LH pulse amplitude was not so greatly influenced by sampling intensity. As shown in Table 2, the mean ± SEM LH pulse amplitude, given as a percent increase, did not change significantly at more rapid rates of sampling. Similar observations applied when LH pulse amplitude was expressed as an increment (milliinternational units per ml).

To illustrate the influence of more intensified rates of venous sampling on the actual configurations of LH pulses, the pulse profiles for 5- and 20-min LH series over 24 h are compared graphically in Fig. 7A in three men and in Fig. 7B in three women, whose LH pulse patterns spanned the ranges observed in this study.

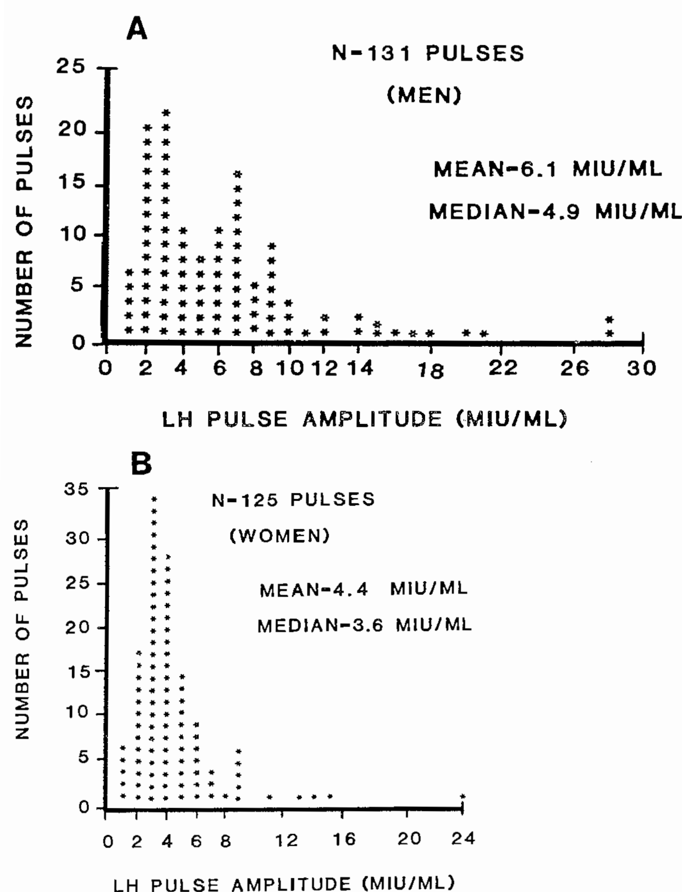

FIG. 5. Frequency histograms of the amplitudes (milliinternational units per ml) of individual LH pulses in normal men (A) and women (B) sampled at 5-min intervals for 24 h. Data are represented as defined in Fig. 4.

Discussion

Although many investigators have evaluated possible alterations in pulsatile gonadotropin release in patients with various pathophysiological conditions, major discrepancies have emerged in the delineation of physiological LH pulse properties in these studies (1–6, 16–21). These discrepancies have become compelling in view of current therapeutic efforts to mimic more precisely the normal attributes of the physiological LH pulse signal. Accordingly, in the present work, we approached these critical problems by using both intensive and prolonged sampling paradigms in normal men and women.

Our results document a major dependence of the LH pulse frequency estimate on both sampling intensity and sampling duration. Thus, sampling every 21.6 min for 24 h would be required to detect as much as 50% of the total number of LH pulses in healthy young men, while sampling every 3.1 min would be needed to detect 90%. Corresponding analyses in women in the early follicular phase indicated that sampling would be required every 18.9 min or every 2.0 min to identify, respectively, 50% or 90% of the LH pulses present. These observations indicate that the vast preponderance of previous sampling paradigms have been inadequate to capture the majority of LH pulses present in the human circulation.

Based on the apparent curvilinear relationship between sampling intensity and LH pulse frequency for 24 h of observation, we could extrapolate estimated LH pulse frequency (or periodicity) toward infinitely rapid sampling rates. The present results document that stable or plateau estimates of physiological LH pulse frequency in men and women are possible. In men, the plateau estimate of LH pulse periodicity averaged 73.8 ± 6.5 min/pulse, which is significantly shorter than that reported using conventional sampling regimens in man (1–6), *e.g.* 143 ± 7 min in 36 normal men sampled at 20-min intervals for 8 h (27) or 147 ± 8 min in 8 normal men evaluated at 20-min intervals for 24 h (this study). Similarly, in normal early follicular phase women, the mean LH pulse periodicity of 70 ± 10 min was shorter than that found at less frequent sampling rates. These extrapolated LH pulse periodicities agreed well with our direct measurements of interpulse intervals at the 5-min sampling rate in the same subjects. Similar estimates were obtained using another independent pulse detection algorithm, in which clusters of points are analyzed by pooled t statistics (our unpublished observations) using cluster analysis (28). Thus, our observations provide internally consistent estimates of physiological LH pulse frequency in normal man and demonstrate for the first time that such estimates stabilize with a sufficiently intensive and prolonged sampling regimen.

The inference that both intensive and prolonged sampling sessions are required to estimate LH pulse frequency accurately could not be attributed to selection of arbitrary pulse detection threshold criteria. Thus, a wide range of threshold criteria (from 20–100%) documented progressive increases in LH pulse frequency at more rapid sampling rates. In each case, plateau estimates of LH pulse frequency were approached at sampling rates of 5–10 min extended over a full 24 h. As anticipated, the use of more stringent threshold criteria resulted in lower plateau estimates. This presumably reflects an underestimate of true LH pulse frequency at higher thresholds, when more false negative errors are likely to occur (16, 19, 20). Conversely, at low pulse detection thresholds, the estimates of LH pulse frequency would presumably be artificially inflated by higher false positive error rates (22). To minimize the latter extreme, we used a suitably adjusted multiple of each individual intraassay coefficient of variation as the threshold for pulse detection (19, 22). This threshold was designed to limit the estimated false positive rate to approximately 1.5% in each individual, independent of his or her particular intrassay coefficient of variation (22).

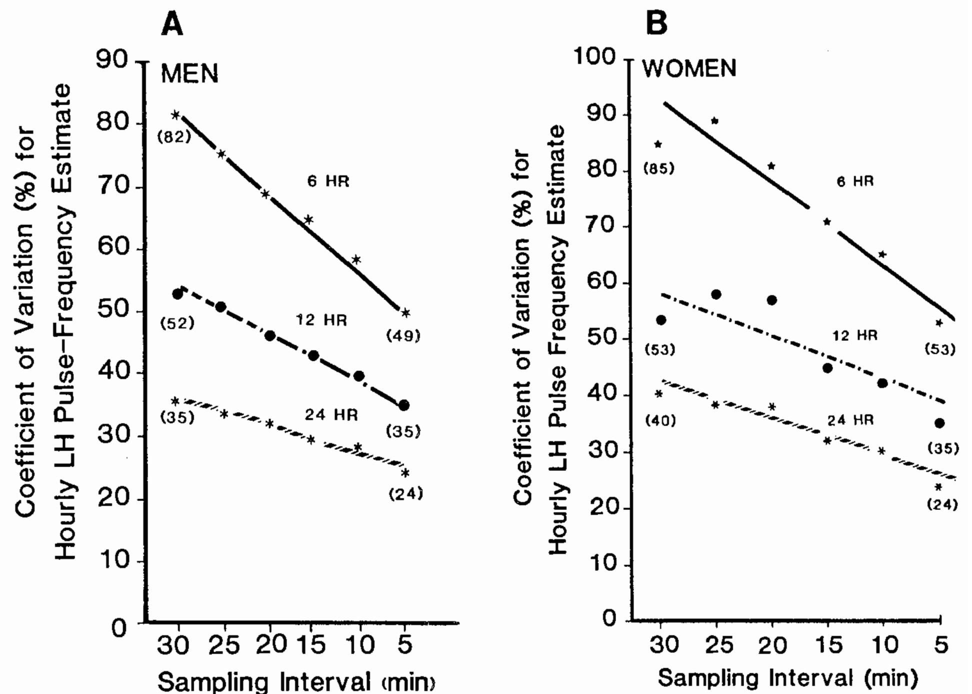

FIG. 6. Relationship between sampling intensity (sampling interval given in minutes) and the coefficient of variation for the LH pulse frequency estimate. For the indicated durations of sampling (6, 12 and 24 h), the coefficients of variation for the hourly LH pulse frequency estimate were calculated at each of several sampling intervals, ranging from 5–30 min. Values in *parentheses* denote the coefficient of variation at the extreme ends of the individual curves.

TABLE 2. Variations in LH pulse amplitude in relation to sampling intensity

Sampling interval (min)[a]	LH pulse amplitude (%)[b]	
	Men	Women
5	73.5 ± 14	83 ± 12.9
10	93.6 ± 11	87 ± 16.4
15	93.9 ± 9.4	100 ± 22.7
20	95.8 ± 10	98 ± 24.3
25	93.1 ± 9.4	102 ± 28.1
30	91.4 ± 7.3	99 ± 20.9
35	95.4 ± 8.6	99 ± 20.4
45	96.0 ± 17	84 ± 16.6
60	99.9 ± 9.6	94 ± 15.8

[a] Interval at which serial blood samples were withdrawn for 24 h.
[b] Mean ± SEM (n = 8). No significant differences were found by analysis of variance.

The high frequency pattern of LH pulsations demonstrable with 5-min sampling rates was not attributable simply to variations introduced by sample processing, since serial measurements of two stable serum constituents (total protein and calcium concentrations) in 288 samples collected over 24 h at 5-min intervals did not fluctuate. This information is particularly important because available methods for estimating pulse frequency have dealt exclusively with false positive errors introduced by within-assay variance (16–22). The present results suggest that preassay variance is minor, at least under the present conditions of sampling.

Our exhaustive sampling protocols also revealed that the range of normal LH pulse frequencies among young men and women is large and is increased at more rapid rates of venous sampling. We suggest that this broader range of LH pulse frequencies reflects an increase in the contribution of lower amplitude pulses found at more rapid sampling rates in some individuals. This implies that deviations from the normal range under different pathological conditions may be even more difficult to document than previously supposed. This problem is further compounded by the relatively high counting error inherent in estimates of LH pulse frequency, even under extended sampling conditions. Based on these results, we suggest that sampling be conducted at a rate of at least every 10 min and extended over 24 h wherever possible. Where blood volumes represent a limiting factor, we would emphasize longer sampling durations at the expense of somewhat less intensive sampling intervals.

In addition to the wide dispersion of LH pulse properties among different subjects, our use of extended intensive sampling protocols revealed marked variability within individuals. Thus, interpulse intervals within individual subjects had respective coefficients of variation ranging from 31–84% (men) and 63–99% (women). Similarly, the coefficients of variation for LH pulse amplitudes (milliinternational units per ml increments above nadir) in individual subjects ranged from 30–81% (men) and from 18–91% (women). This striking nonuniformity of LH pulse properties within individual healthy men and women has not been recognized previously, but has several distinct implications. First, nonuniformity of LH interpulse intervals may be of physiological importance, since even small changes in GnRH pulse frequency can

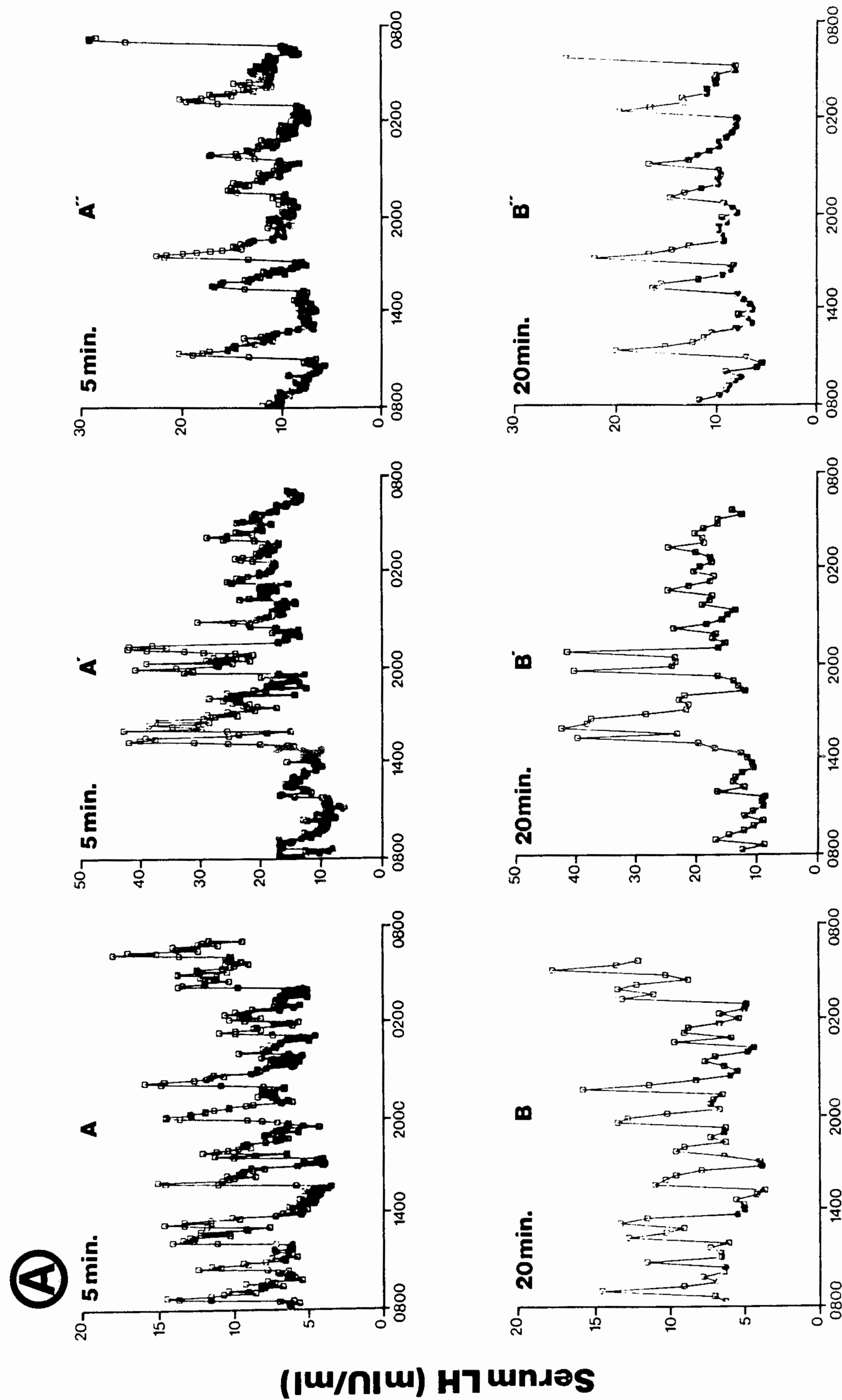

FIG. 7A

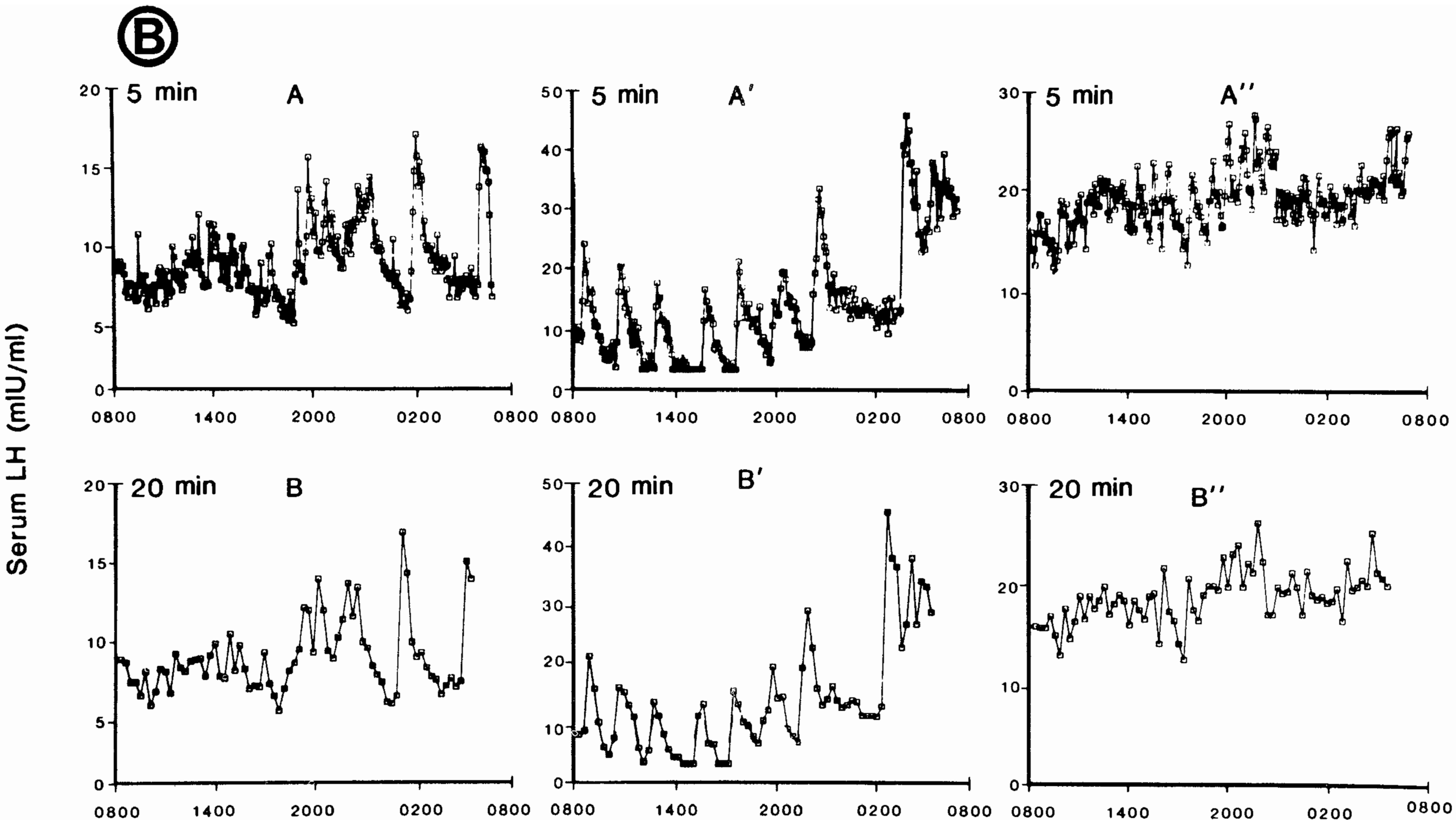

FIG. 7. Physiological patterns of pulsatile LH release in men and women are depicted for three individuals sampled at 5-min intervals (*upper panel*; A, A′, and A′′) compared with the constituent 20-min series in the same individuals (*lower panel*; B, B′, and B′′). In these studies, normal men and women were sampled at 5-min intervals for 24 h. The parent 5-min series provided constituent 20-min LH series for comparison. The three profiles shown were selected to illustrate the range of LH pulse frequency estimates observed in this group of individuals. A, Men; B, women.

result in profound changes in gonadotropin and steroid hormone secretion (29). For example, in hypothalamus-pituitary-disconnected rams, increasing exogenous GnRH pulse frequency from 2 to 1 h not only elicited enhanced testosterone production, but also resulted in activation of steroid negative feedback (29). Moreover, in men with selective serum FSH elevation and idiopathic oligospermia, increasing exogenous GnRH pulse frequency from 2 to 0.5 h reduced mean FSH levels to normal without significantly altering serum LH, testosterone, or estradiol concentrations (30). Secondly, exogenous GnRH infusion protocols have largely adhered to fixed dose and/or fixed pulse frequency schedules, which would not mimic the inherently nonuniform nature of the endogenous LH pulse signal in the normal human. Accordingly, fixed dose and/or fixed frequency GnRH treatment regimens may not be fully appropriate to model physiological patterns of pituitary hormone release, which have distinctly nonuniform properties.

Our documentation of detailed LH pulse configurations in normal men and women under conditions of intensive venous sampling for prolonged intervals provides the most precise delineation of the contour of physiological LH pulses presently available. On the average, the LH pulses in men and women contained, respectively, 7.6- ± 0.6 and 5.3 ± 0.3 points/pulse. The identification of such LH pulses provides a higher degree of certitude than single point analyses (16). In addition, such information should be of considerable importance to investigators attempting to approximate physiological LH pulse contours with more refined pulse detection models. Moreover, a more precise knowledge of LH pulse configurations is likely to assist in estimating false negative error rates, which to date have been difficult to ascertain in view of uncertainties about the intrinsic configuration (and range of configurations) of physiological LH pulses in men and women.

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