Spectrum of the Pulsatile Characteristics of LH Release in Normal Men

JOHANNES D. VELDHUIS,* ALAN D. ROGOL,† WILLIAM S. EVANS,* ALI IRANMANESH,‡ GERMAN LIZARRALDE,‡ AND MICHAEL L. JOHNSON†

*From the Divisions of Endocrinology and Biomathematics, the *Departments of Internal Medicine, and †Pediatrics and Pharmacology, the University of Virginia School of Medicine, Charlottesville, and the ‡Department of Internal Medicine, Veterans Administration Hospital, Salem, Virginia*

To assess the spectrum of LH pulse characteristics in normal men, blood samples from 36 individuals were drawn at 20-minute intervals for 8 hours. The subsequent immunoactive LH concentrations were analyzed by computer algorithms to delineate the frequency and amplitude characteristics of pulsatile LH secretion. The absolute range for LH pulse frequency estimated by a modified threshold method was 1–6 pulses/8 hr, with a mean (± SEM) of 3.36 ± 0.17 (median - 3) pulses/8 hr. The distribution differed significantly from a Gaussian pattern. The mean LH pulse amplitude expressed as a percent increase above nadir was 92.1 ± 6.1 (median-91.5%). When LH pulse amplitude was defined as an increment (mIU/ml) above nadir, the mean value was 5.13 ± 0.4 (median - 4.8) mIU/ml. These two expressions of amplitude were positively correlated ($P < 0.01$), while the incremental (mIU/ml) pulse amplitude correlated inversely with pulse frequency ($P < 0.01$). To examine the influence of more intensified rates of venous sampling on the spectrum of LH pulse properties, blood was sampled at 4-minute intervals for 8 hours in a subgroup of 13 men. Under these conditions, estimated LH pulse frequency was significantly higher, with a mean of 10.31 ± 1.87 (median - 9) pulses/8 hr compared with 20-minute sampling in the same individuals ($P < 0.001$). Although the estimates of LH pulse frequency at 4-minute and 20-minute sampling intervals were significantly correlated ($P < 0.01$), the dispersion of the LH pulse frequency estimates was considerably larger at more rapid rates of sampling. There was an absolute range of 2–20 pulses/8 hr for the 4-minute sampling, and 1–6 pulses/8 hr for the 20-minute sampling in the same individuals. This increase in LH pulse frequency and the broader dispersion of the range of frequencies estimated at 4-minute compared with 20-minute sampling intervals were confirmed using either another pulse detection algorithm, or separate criteria designed to adjust false-positive error rates in relation to sampling intensity. It was concluded that eugonadal men exhibit a broad spectrum of pulsatile LH characteristics, and the range of LH pulse attributes is even greater at more intensive rates of venous sampling. The results of this study in normal men demonstrate that a wider dispersion of physiologic LH pulse characteristics must be recognized in man. Such information is particularly important whenever possible departures from normal patterns of episodic gonadotropin secretion are sought in various clinical disorders. Moreover, these observations indicate that a wide range of LH pulse signal characteristics will maintain testosterone production effectively in the normal male.

Key words: LH, pulses, men, gonadotropin, hormone, normal range.

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The episodic pattern of gonadotropin release provides a pulsatile signal that is necessary for normal physiologic function of the hypothalamic-pituitary-gonadal axis in experimental animals and man (Nankin and Troen, 1971; Midgely and Jaffe, 1971; Knobil, 1980). Although intermittent patterns of LH release in normal men have been recognized for more than a

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Reprint requests: Johannes D. Veldhuis, M.D., Division of Endocrinology, Box 202, Department of Internal Medicine, University of Virginia School of Medicine, Charlottesville, Virginia 22908.

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decade, the full normal range of LH pulse characteristics has not been defined. The principal limitations encountered to date include not only the small number of individuals studied in any one report (usually five or six subjects), but also uncertainty regarding the most appropriate frequency for blood sampling as well as imperfect assessment of the degree of false-positive and false-negative errors associated with pulse enumeration (Veldhuis et al, 1984a). Despite these difficulties, a more comprehensive appraisal of the full normal range of LH pulse characteristics among healthy individuals clearly is needed to adequately assess altered patterns of gonadotropin secretion in clinical states of infertility or hypogonadism.

In the present work, we have addressed these problems by examining the spectrum of pulsatile characteristics of LH release in 36 normal men, and by using more refined pulse analysis methods in an effort to estimate the apparent "true-positive" LH pulse frequency. Moreover, in a subgroup of 13 individuals, we have evaluated the range of LH pulse properties discerned at more intensive (4-minute) rates of venous sampling. To our knowledge, these represent the most extensive normative male populations studied to date.

Subjects and Methods

Subjects

Thirty-six normal men aged 21 to 30 participated in this study after providing written informed consent. The study was approved by the Human Investigation Committee of the University of Virginia. Each man had a normal fertility history and semen analysis, and a normal physical examination, including normal external genitalia and testicular volumes. In addition, on the day of study, serum concentrations of the following hormones measured by specific radioimmunoassay were within the normal range: total and free testosterone (50–250 pg/ml), estradiol (10–50 pg/ml), free T_4 (0.6–2.5 ng/dl), prolactin (2–18 ng/ml), LH (2–15 mIU/ml), and FSH (2–18 mIU/ml) (Evans et al, 1980; Veldhuis et al, 1984a).

Procedures

The men were admitted to the Clinical Research Center. An indwelling heparin-lock needle was inserted into a forearm vein at least 1 hour before blood sampling. Blood was removed at 4-minute intervals for 8 hours in 13 men, and at 20-minute intervals for 8 hours in 23 other men. The two groups of men could not be distinguished by demographic or biochemical criteria. The volunteers remained fasting and supine for the sampling sessions, which began at 8:00 A.M.

Sampling Processing and RIA

Samples were allowed to clot at room temperature, and the serum was stored at -20 C before assay. All samples from an individual were analyzed in the same assay run to eliminate interassay variability. LH concentrations were determined in triplicate using a modification of the method of Odell et al (1967). The reagents were those described previously (Evans et al, 1980).

To define the intra-assay variability precisely at multiple points along the RIA displacement curve, four or more pools of male serum in each run were assayed as nontuplets (groups of nine replicates). These serum pools yielded 565 individual LH values, each representing a mean of triplicate determinations encompassing the LH concentration range of 2.5–15 mIU/ml. The corresponding mean intra-assay coefficients of variation ranged between 5.2 and 12.1%, with a variability of 5.2, 6.5, 8.0, and 12.1% for LH concentrations of 10 mIU/ml, 6 mIU/ml, 4 mIU/ml, and 2.5 mIU/ml, respectively.

Quantitative Analysis of Pulsatile Hormone Secretion

Pulsatile patterns of LH release were analyzed by a recent modification of the original method of Santen and Bardin (Santen and Bardin, 1973). Instead of applying a fixed 20% threshold, we modified this algorithm to permit detection of pulses that exceeded our relevant individual within-assay coefficients of variation by 3.5 times (or, in some designated figures, by another multiple) (Veldhuis et al, 1984a, 1984b). The relevant within-assay coefficient of variation for any subject was computed from 30 to 90 assay replicates of a pool of serum derived from that individual's samples and run in the same assay. In some indicated cases, data also were analyzed by another, independent algorithm (Pulsar), in which one or more LH values are required to increase above a smoothed baseline in order to constitute a significant pulse (Merriam and Wachter, 1982).

To evaluate the performance of our pulse enumeration method, serial LH values from the multiple serum pools (control series) also were analyzed for the presence of false-positive LH pulses. Assay-associated, false-positive pulses provided one measure of the Type I statistical error in the analysis. Pulse detection was applied to two kinds of LH series: those derived from serum pools, and simulated LH series. The simulated LH series comprised a total of 2880 values that were created using the random-number generator at the University of Virginia IBM Cyber 720 system. As described earlier, the distribution of the standard deviates in the simulated series conformed either to the distribution observed for our 565 LH replicate values (by randomly selecting members from our RIA histogram distribution), or to a Gaussian distribution. The variance for each series was constrained further to a given "intraseries" coefficient of variation designated by the investigator (Veldhuis et al, 1985).

Simulation of Less Frequent Sampling Intervals

Data derived from 4-minute sampling intervals also were analyzed by omitting successive samples to examine constituent series collected at less frequent intervals. For

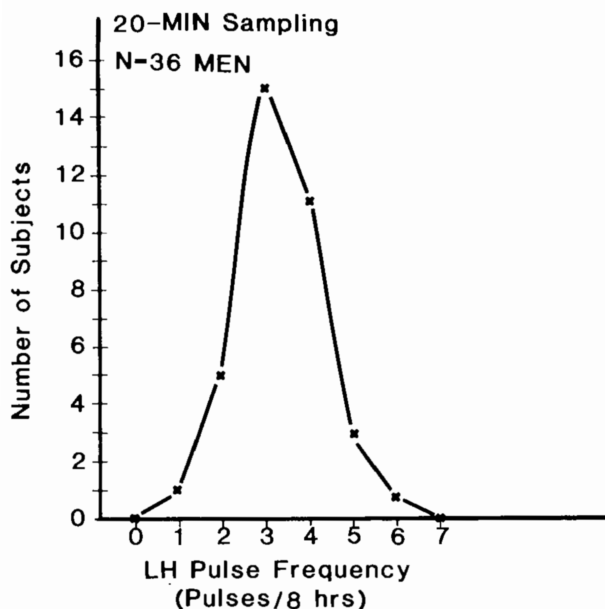

Fig. 1. Distribution of LH pulse frequencies among 36 normal men sampled at 20-minute intervals for 8 hours. Pulse frequency is given as the number of LH pulses observed per 8 hours using an adjusted threshold criterion of 3.5 times the individual intra-assay coefficients of variation.

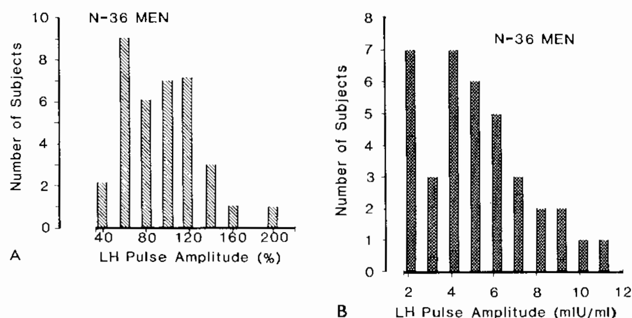

Fig. 2. Distribution of LH pulse amplitudes among 36 eugonadal men. Pulse amplitude was defined as a percent increase above nadir (A) or as an absolute (mIU/ml) increment above nadir (B).

example, deleting every other point in the serial 4-minute values provided a series of samples actually collected at 8-minute intervals.

Statistical Analysis

Data were subjected to analysis of variance for group effects, and paired Student's *t* testing for within-subject comparisons (Winer, 1971). Normality was tested by the Shapiro-Wilks statistic. Correlations were sought by nonparametric methods.

Other Analytic Methods

Plots of sampling intensity vs. apparent LH pulse frequency were analyzed by nonlinear least-squares curve fitting. This analysis permitted us to search for a stable, or plateau, estimate of LH pulse frequency at more rapid rates of venous sampling. The program performs a weighted, least-squares fit of the data points by a modified Gauss-Newton iteration, and assumes a nonlinear, asymmetric, highly correlated variance space for calculations of 67% confidence intervals for the precision of fit (Johnson, 1983).

Results

Pulse Frequency

When the LH series from these 36 men was analyzed for pulse frequency using the modified threshold method (3.5 times the intra-assay coefficient of variation), a wide range in estimates of pulse frequency was observed (Fig. 1). The absolute range for the estimate of LH pulse frequency was 1 to 6 pulses/8 hours, with a mean ± SEM of 3.36 ± 0.17 and a median of 3 pulses/8 hr. This distribution differed significantly from a Gaussian pattern ($P = 0.02$, for a Shapiro-Wilks statistic of 0.918). In the same individuals, the corresponding LH pulse amplitudes expressed as the percent (Fig. 2A) or incremental (mIU/ml) increase (Fig. 2B) also exhibited a wide range of non-Gaussian distributed values ($P < 0.01$). The mean LH pulse amplitude expressed as a percent increase above nadir was 92.1 ± 6.1, with a median of 91.5%, and the incremental (mIU/ml) LH pulse amplitude averaged 5.13 ± 0.4, with a median of 4.8 mIU/ml. Since the mean intra-assay coefficient of variation was approximately 9% in these men, the fractional (percent) LH pulse amplitude exceeded 32% (3.5 times the intra-assay coefficient of variation), while the incremental LH pulse amplitude (mIU/ml) exceeded 2 mIU/ml.

In 13 men, blood samples were also drawn at 4-minute intervals over 8 hours, and the constituent 4-minute and 20-minute series was compared within the same individual. As shown in Fig. 3A, using the adjusted threshold algorithm, there were significantly more pulses detected in normal men at 4-minute than at 20-minute sampling intervals. In particular, at 4-minute sampling intervals, the mean and median LH pulse frequencies were 10.31 ± 1.87 and 9 pulses/8 hr, respectively, compared with corresponding values in the same men sampled at 20-minute intervals of 3.46 ± 0.37 (mean ± SEM) and 3 (median) pulses/8 hr ($P < 0.001$). The range of LH pulse frequency estimates also was considerably larger at more rapid rates of sampling, that is 2–20

pulses/8 hr for 4-minute sampling versus 1–6 pulses/8 hr for 20-minute sampling. The increase in LH pulse frequency estimates and the increasing dispersion of the range of estimates obtained at 4-minute intervals compared with 20-minute sampling intervals also was observed with a second independent pulse detection algorithm (Pulsar). As shown in Fig. 3B, the Pulsar program detected 1.23 ± 0.32 pulses/8 hr at 20-minute sampling intervals and, in the same men, 4.92 ± 0.92 pulses/8 hr with 4-minute sampling ($P < 0.01$). Using this method of analysis, 11 of 13 individuals exhibited an increased number of apparent pulses when sampled at 4-minute compared with 20-minute intervals ($P < 0.01$). The absolute number of pulses delineated by the Pulsar program was less than that for the adjusted threshold method, presumably reflecting, in part, the high thresholds above smoothed baseline required by the Pulsar method in its present form (Veldhuis et al, 1984a). Results from the Pulsar method, however, correlated highly with the adjusted threshold algorithm at both 4-minute ($r = +0.932$, $P < 0.001$) and at 20-minute ($r = +0.675$, $P < 0.05$) sampling intervals. In addition, the results from sampling at 4-minute and 20-minute intervals were themselves correlated significantly within this group of men ($r = +0.753$, $P < 0.01$).

Pulse Amplitude

Pulse amplitudes expressed as increment and percent increases were highly correlated among the 36 men ($r = +0.433$, $P < 0.01$). Incremental amplitude was significantly inversely correlated with LH pulse frequency ($r = -0.580$, $P < 0.001$) in this group. On the other hand, percent amplitude was not significantly correlated with LH pulse frequency ($r = -0.12$).

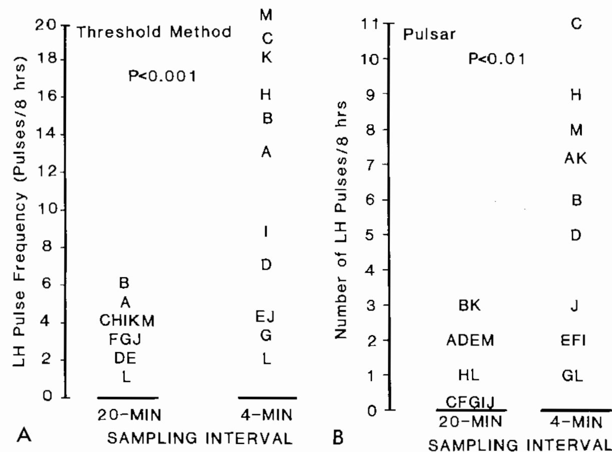

Fig. 3. Comparison between LH pulse frequency estimates derived from 4-minute or 20-minute sampling, using the adjusted threshold criteria (A) defined in the legend of Fig. 1, or a second independent algorithm (Pulsar, B). Letters "A–M" denote the individual pulse frequencies for the 13 men studied.

Stability of Pulse-Frequency Estimates Within a Sampling Session

To assess the stability of the pulse frequency and amplitude estimates obtained at 20-minute sampling

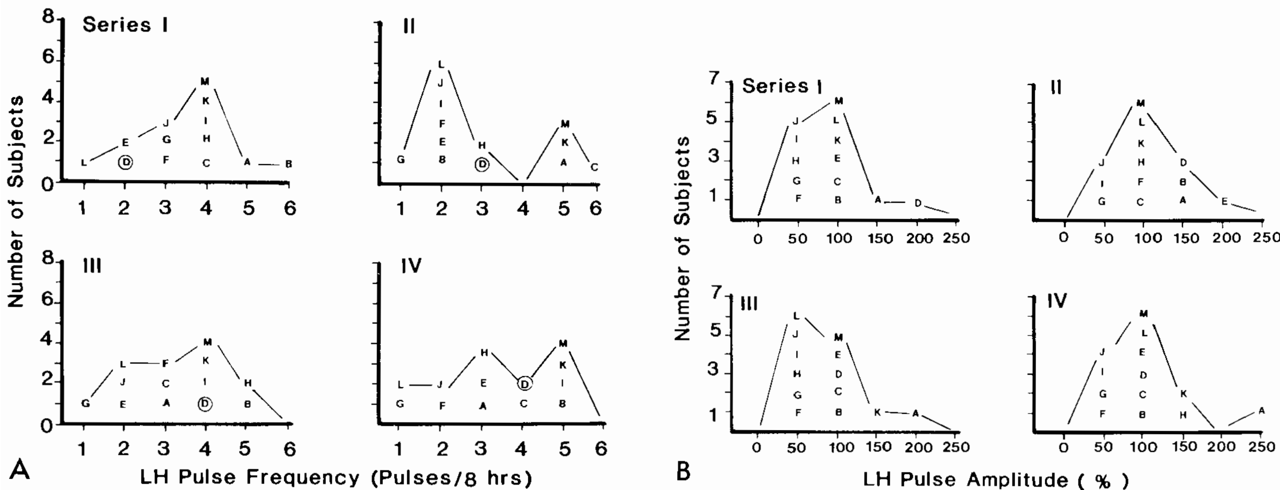

Fig. 4. Stability of the LH pulse frequency (A) and pulse amplitude (B) estimates in each of 13 normal men who were studied by venous sampling at 4-minute intervals for 8 hours. For each individual, the four constitutent 20-minute series beginning at +4, +8, +12, and +16 min are presented as series I, II, III, and IV. The letters correspond to those for the parent series (beginning at t = 0 minutes) shown in Fig. 3A.

intervals, each of the 13 4-minute LH series was analyzed for its various 20-minute constituents. These constituents are related to each other by a 4-minute time shift. These analyses revealed that even a 4-minute time shift in 20-minute sampling series could lead to different estimates of LH pulse frequency among individual men, although the mean estimates for the group did not change significantly. In particular, the mean (± SEM) LH pulse frequency estimates for each of the individual 20-minute series in the group of 13 men were: 3.36 ± 0.27 (20-minute series starting at 4-minutes); 3.08 ± 0.4 (20-minute series starting at 8 minutes); 3.23 ± 0.34 (starting at 12 minutes); and 3.31 ± 0.41 (starting at 16 minutes) pulses/8 hr (P = NS) compared with 3.46 ± 0.37 pulses/8 hr (original series starting at t = 0). In contrast, as shown in Fig. 4A, the individual estimates of LH pulse frequency did differ significantly in some men even in association with this slight temporal shift. For example, in subject B, the pulse frequency estimates ranged from 2 to 6 pulses/8 hr depending on when sampling was initiated. In subject D, the range was from 2 to 4 pulses/8 hr. Thus, considerable individual variation is concealed by group mean estimates.

LH pulse amplitude also varied substantially within individuals when the 20-minute sampling series was analyzed in relation to 4-minute time shifts, which created four separate 20-minute LH series for each individual, differing by 4, 8, 12, or 16 minutes from the first series (no time shift) (Fig. 4B). Although significant (two-fold) differences in LH pulse amplitudes within individuals were observed in relation to a temporal shift of the sampling, the mean LH pulse amplitudes for the group as a whole did not differ significantly. These pulse amplitudes were 97.7 ±

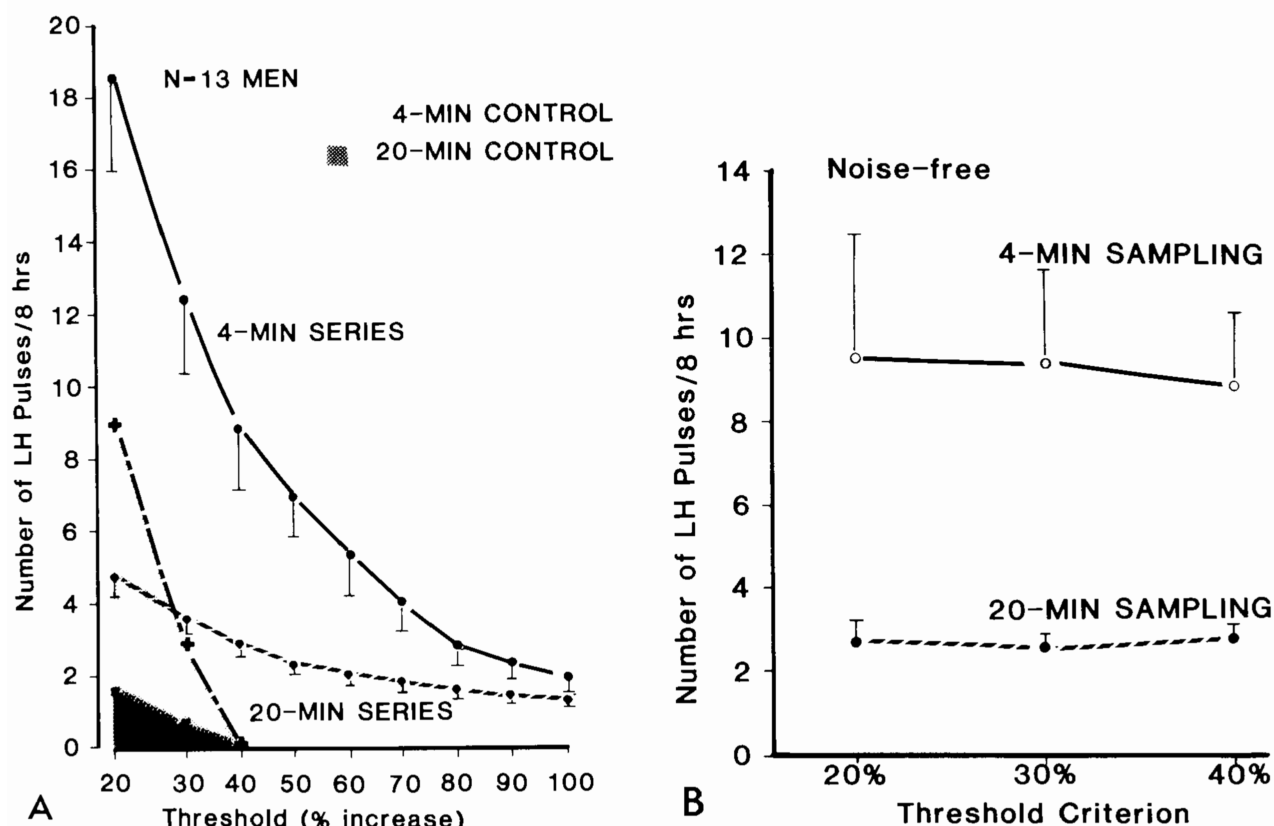

Fig. 5. A shows the influence of increasingly stringent threshold criteria on apparent LH pulse frequency for 13 normal men sampled at 4-minute intervals for 8 hours. For each man, both the 4-minute and the parent 20-minute series were evaluated for the presence of LH pulses using pulse-detection thresholds of 20, 30, and 40...100%. In addition, as shown by the shaded regions, serial replicates of pooled male sera, which contained 120 samples (to match the 4-minute series) or 25 samples (to conform to the 20-minute series), were also analyzed as controls. These control series were taken as a measure of false-positive "pulses." **B** shows that at the 20, 30, and 40% thresholds, the corresponding estimates of false-positive pulses (shaded regions, A) were substrated to yield an approximation of "noise-free" or true-positive LH pulse frequency at the two sampling rates. Data are means ± SEM (n = 13 men).

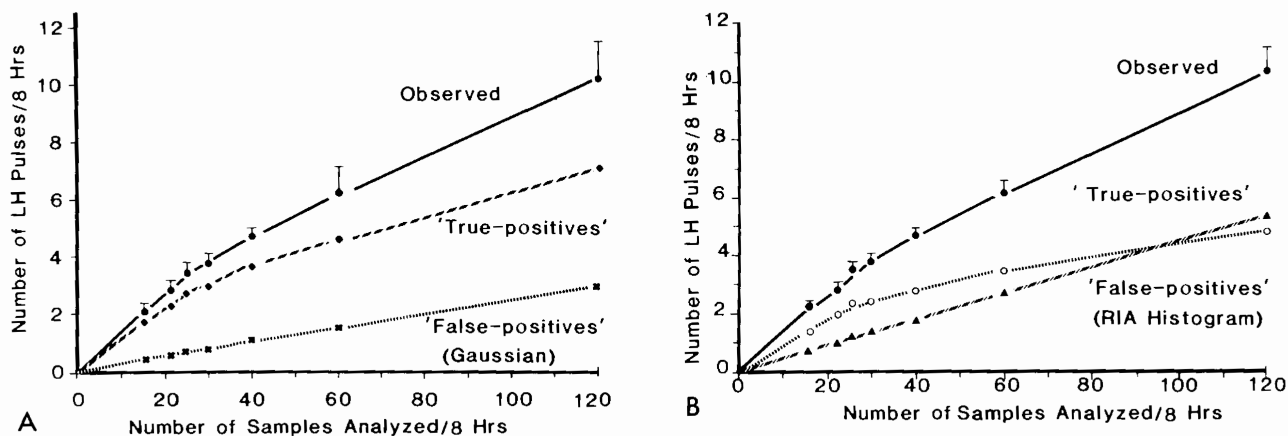

Fig. 6. Relationship between pulse frequency (vertical axis) and sampling intensity (number of samples analyzed/8 hr, horizontal axis) in 13 eugonadal men sampled at 4-minute intervals for 8 hours. The uppermost curve depicts the observed pulse frequency, and the lowermost line, the estimated false-positive rate derived from either a Gaussian distribution of RIA measurement error (A) or our RIA histogram (B). The middle curve was obtained by substraction, and provides an estimate of "true-positive" LH pulse frequency. The asymptote of this curve was extrapolated by nonlinear least-squares curve fitting. Note that the numbers of samples analyzed (eg, 40, 60, 120) correspond inversely with the sampling rate every 12, 8, or 4 minutes, respectively.

12.7, 102 ± 12.9, 89.6 ± 12.1, and 99.6 ± 14.4%, respectively ($P =$ NS, compared with 92.1 ± 6.1% in the parent series starting at $t = 0$).

Assessment of Presumptive True-positive LH Pulses

To test whether the increase in apparent LH pulse frequency observed in the 13 men at 4-minute sampling intervals could be attributed to an increase in false-positive LH pulses enumerated, increasingly stringent threshold criteria for pulse detection were evaluated (Fig. 5A). The original (20%) threshold criterion of Santen and Bardin (1973) yielded significantly more LH pulses than higher thresholds of 30%, 40%, 50...100% applied to either the 4-minute series or the 20-minute series. The greater number of LH pulses estimated at lower thresholds could be attributed in part to false-positive LH pulses based upon our analysis of replicate control series. These control series comprised pooled male sera, which were assayed either as three sets of 120 replicates each (in the case of the 4-minute series) or as six sets of 25 replicates each (in the case of the 20-minute series) to yield sample numbers equivalent to those in the experimental groups. Notably, with increasingly stringent threshold criteria, the number of peaks in the control series declined to a value of ≤ 0.3% at a threshold criterion of 40% (approximately 4.4 times the average intra-assay coefficient of variation for these men). Figure 5A, however, clearly indicates that the 20-minute and 4-minute volunteer series differed significantly even at this high threshold criterion of 40%, yielding LH pulse frequency estimates of 8.85 ± 1.66 and 2.85 ± 0.34 pulses/8 hr, respectively ($P < 0.01$).

The difference between estimated LH pulse frequency in the control (pooled serum) replicates and experimental groups in Fig. 5A would presumably provide an estimate of the "true-positive" LH pulse frequency. Accordingly, Fig. 5B summarizes the results of estimating "true-positive" LH pulse frequency at thresholds of 20, 30, and 40%. The estimated "true-positive" LH pulse frequency is calculated as the difference between the observed LH pulse frequency in the 4-minute or 20-minute series and the corresponding estimated false-positive error rate from the control (pooled serum) replicates. These

TABLE 1. False-Positive Rates of LH Peaks as Determined by RIA Histogram or Inferred from a Gaussian Distribution*

Number of Samples/Series (Equivalent Sampling Interval, Min.)	Gaussian Noise	RIA Histogram
16 (32)	0.40 ± 0.03	0.71 ± 0.04
21 (24)	0.53 ± 0.04	0.93 ± 0.05
25 (20)	0.64 ± 0.04	1.11 ± 0.06
30 (16)	0.76 ± 0.05	1.33 ± 0.08
40 (12)	1.01 ± 0.06	1.78 ± 0.09
60 (8)	1.53 ± 0.09	2.67 ± 0.14
120 (4)	3.04 ± 0.19	5.33 ± 0.29

*Means ± SD, number of spurious "pulses" per series, assuming a pulse detection threshold of 32% and an intraseries coefficient of variation of 9%. The sampling interval (min) given in parentheses assumes an 8-hour sampling session.

TABLE 2. Estimates of True-Positive LH Pulse Frequency in Relation to Three Models of False-Positive Error Applied to Rapid (4-Minute) Sampling in Normal Men

	Models of "False-Positive" Error Term		
Parameters	Gaussian	RIA Histogram	Nonlinear Least-Squares Curve Fitting
Pulse frequency	12.8 (8.5–16.9)* pulse/8 hr	7.6 (4.9–10.3) pulse/8 hr	11.1 (5.7–16.8) pulse/8 hr
False-positive rate	2.50†	4.44	3.06

*Parentheses give 95% confidence limits for the estimates derived from 13 men.

†Number of "false-positive" pulses per 100 samples analyzed. The "false-positive" models are defined in the Methods section.

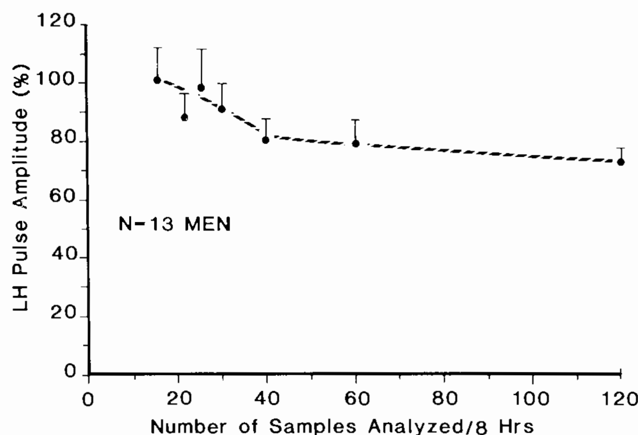

Fig. 7. Lack of major influence of sampling intensity (plotted as in Fig. 6) on LH pulse amplitude (percent) in 13 normal men, each sampled at 4-minute intervals for 8 hours.

estimates of "true-positive" LH pulse frequency were remarkably uniform at either 20, 30, or 40% thresholds. In each case, there were approximately threefold more pulses detected at 4-minute sampling than at 20-minute sampling in this group of 13 normal men ($P < 0.01$).

Influence of Sampling Rate on Apparent True-positive LH Pulse Frequency

The concept of removing presumptive false-positive peaks from the observed series was applied further to samples analyzed across a wide range of sampling rates: 4-, 8-, 12-, 16-, 20-, 24-, and 32-minute sampling intervals. In Fig. 6A, a false-positive rate was computed in which "false-positives" were assumed to conform to a random Gaussian distribution of standard deviates with a 9% intraseries coefficient of variation (Veldhuis et al, 1985). At a pulse detection threshold of 32%, the number of false-positive LH pulses increased linearly with increasing sample number. The number of estimated "true-positive" LH pulses was defined as observed minus false-positive pulses. This assumes a simple algebraic relationship between false-positive, true-positive, and observed LH pulse frequencies. In this model, "true-positive" pulse frequency increased rapidly when the sampling interval was reduced from 32 minutes to 12 minutes, and then rose less rapidly when the sampling interval was decreased further to 4 minutes (Fig. 6A). This tendency for the estimate of "true-positive" LH pulses to approach a plateau value at abbreviated sampling intervals could be extrapolated by nonlinear least-squares curve-fitting to an apparent maximum of 12.8 (8.5–16.9, 95% confidence limits) pulses/8 hr.

The tendency for the presumptive "true-positive" LH pulse frequency to plateau at rapid sampling rates was more evident when the number of false-positive peaks was estimated from the actual distribution of false-positive LH peaks observed in a histogram developed from 565 replicates in our RIA ("RIA Histogram," Fig. 6B). Based upon the LH series from this RIA histogram, the estimated false-positive detection rates were uniformly higher than those inferred from a Gaussian distribution (Table 1), averaging 4.4% rather than 2.5%. Using the false-positive estimate from our actual RIA histogram, the relationship between sampling intensity and LH pulse frequency appeared to plateau at an estimated pulse frequency of 7.6 (4.9–10.3) pulses/8 hr. This value does not differ significantly from that observed after subtraction of demonstrable assay-associated false-positive LH pulses accompanying the 4-minute sampling data, which was 8.85 ± 1.66 pulses/8 hr (Fig. 5B). A third method of estimating the asymptote of the relationship "number of LH pulses observed/8 hr vs. number of samples analyzed," which simply determines the best fit for a curvilinear plus linear model (Johnson, 1983), yielded an intermediate estimate, see Table 2 ("Nonlinear least-squares curve fitting"). We would emphasize that these estimates of false-positive rates apply to intra-assay error, and do not include other possible sources of error prior to sample assay. As such, these estimates provide only approximations of true-positive results.

In contrast to the striking effect of sampling intensity on estimated LH pulse frequency, LH pulse amplitude was influenced sparingly by the rate of sampling (Fig. 7). There was a slight trend toward decreased pulse amplitude at more rapid rates of sampling, with a mean LH pulse amplitude of 101.3 ±

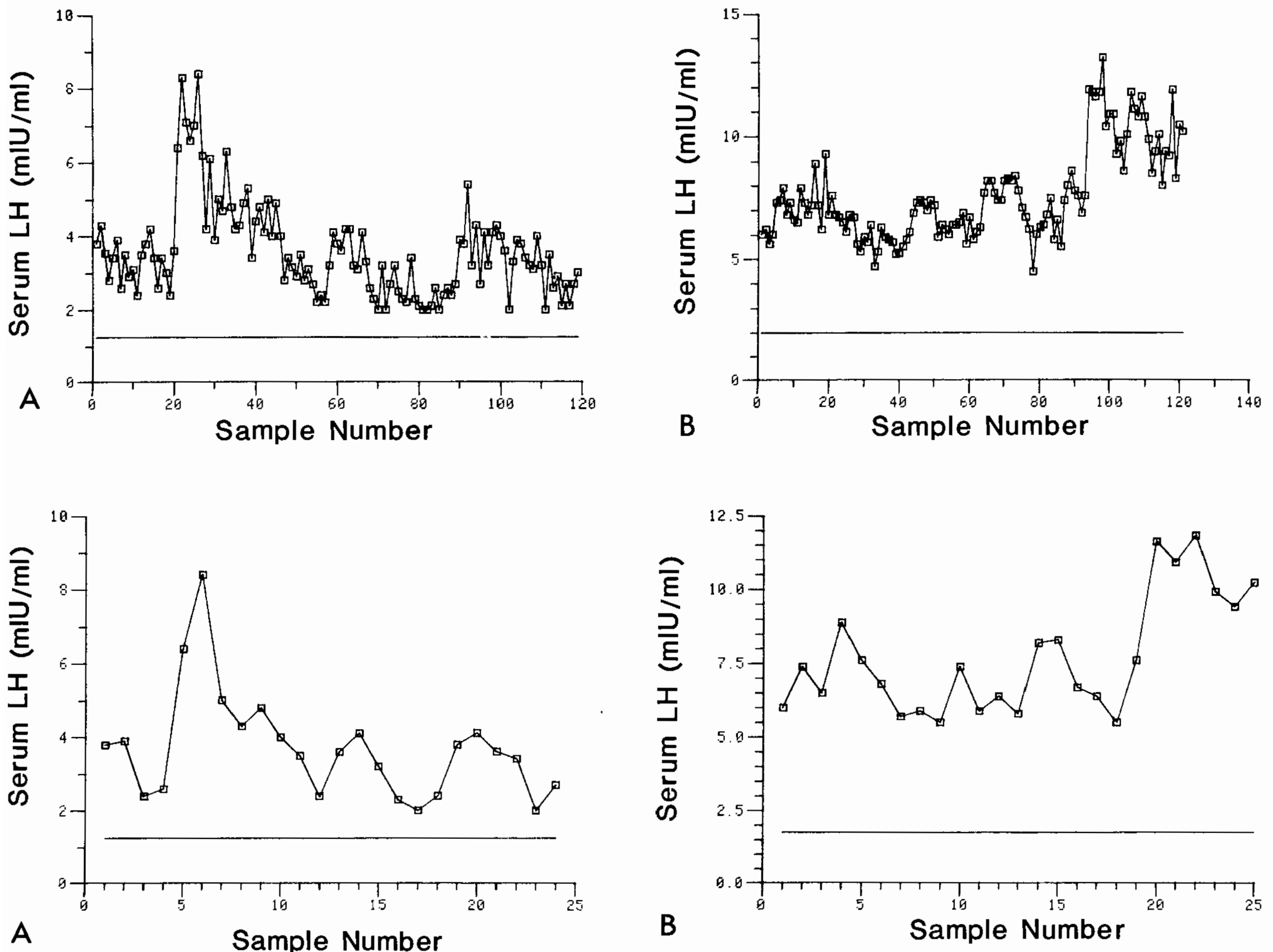

Fig. 8. Illustration of LH pulse profiles in two normal men (A, B) sampled at 4-minute intervals for 8 hours. The vertical axis gives serum LH concentrations (mIU/ml), and the horizontal axis consecutive sample numbers for blood withdrawn every 4 minutes over an 8-hour session. For each individual, the upper panel gives the original 4-minute LH series, and the lower panel a constituent 20-minute series derived by omitting four of five consecutive values from the parent series.

12% and 75.8± 6.2% at 32-minute and 4-minute sampling intervals, respectively ($P = 0.047$).

Patterns of LH Pulses

The 4-minute and 20-minute sampling series from two subjects are depicted in Fig. 8 to illustrate the pattern(s) of the LH pulse signal observed in normal men.

Discussion

Although many reports have evaluated relative changes in LH secretion under various pathophysiologic conditions (Nankin and Troen, 1971; Midgley and Jaffe, 1971; Santen and Bardin, 1973; Knobil, 1980; Evans et al, 1980; Veldhuis et al, 1984b), there is virtually no precise information regarding the full spectrum of normal patterns of pulsatile LH release in men. Available reports, including our earlier analyses (Nankin and Troen, 1971; Midgley and Jaffe, 1971; Santen and Bardin, 1973; Knobil, 1980; Evans et al, 1980; Veldhuis et al, 1984b), have been severely restricted by the small numbers (five or six) of individuals studied. In the present study of 36 healthy, adult males, we observed a remarkably wide range of LH pulse properties.

Using adjusted threshold criteria, the absolute range of LH pulse frequency estimates was six-fold when blood was sampled at 20-minute intervals. LH pulse amplitudes also exhibited a wide dispersion of values, expressed as a percent or increment (mIU/ml) above nadir. The latter characteristic of LH pulse amplitude was correlated in a significant inverse

fashion with estimated pulse frequency. This observation suggests that, at higher rates of pulsatile LH release, the incremental increase in the LH concentration is attenuated. This result agrees with studies assessing LH pulse amplitude in relation to accelerated pulse frequencies induced by exogenously infused gonadotropin-releasing hormone or by endogenous stimuli in experimental animals (Knobil, 1980; Wildt et al, 1981; Karsch et al, 1983).

To appraise LH pulse properties at more rapid sampling rates, 13 men were studied by blood withdrawal at 4-minute intervals. A significant increase was found in the mean and the range of LH pulse frequencies. The implication that more rapid rates of venous sampling accentuate the dispersion of LH pulse frequency estimates was confirmed by a second independent computer algorithm (the Pulsar program). Although the Pulsar estimates were considerably lower than those using adjusted threshold criteria, the results of the two distinct methods were highly correlated.

The LH series collected at 4-minute intervals also permitted us to test the stability of the LH pulse estimates. For example, when the 20-minute constituents of the original 4-minute series were analyzed beginning at +4 minutes, +8 minutes, +12 minutes, or at +16 minutes instead of at time 0, we were able to study four additional, separate LH series in each individual. Notably, the mean estimate of LH pulse frequency for the whole group was highly stable. In any individual, however, the estimate of LH pulse frequency could be altered up to three-fold by a small time shift, while pulse amplitude was minimally affected. Such observations suggest a significant dependence of the LH pulse frequency estimate on sampling duration.

To assess the range of "true-positive" LH pulse frequencies in normal men more precisely, we have used two formulations of the false-positive error rate associated with pulse analysis. The false-positive error rate was estimated from either a Gaussian distribution or our observed (slightly skewed) RIA distribution (Veldhuis et al, 1985). Presumptive false-positive errors were subtracted from observed LH pulse frequencies as one indirect measure of the number of apparent "true-positive" LH pulses. The subsequent estimates of "true-positive" LH pulses increased markedly as the sampling interval was abbreviated from every 32 minutes to every 12 minutes, and approached a plateau value at the shortest sampling intervals used (8 and 4 minutes). This tendency for the estimated number of "true-positive" LH pulses to plateau at the most rapid rates of sampling had not been recognized in earlier studies, presumably because the number of subjects was small, or because data were analyzed without rate-dependent adjustments for false-positive errors. Even so, we would emphasize that our RIA-associated false-positive estimates do not necessarily encompass other sources of error extrinsic to actual assay performance.

We also applied nonlinear least-squares analysis to define the curvilinear relationship between sampling intensity and apparent "true-positive" LH pulse frequency (observed minus false positives). These analyses assume an algebraic relationship between false-positive, true-positive, and observed LH pulse frequencies, and suggest that a mean of between 7.6 and 12.8 pulses/8 hr (depending on the false-positive model used) would occur in the circulation of normal men if an infinite number of samples were analyzed over an 8-hour interval. We stress that such values must be regarded as the best available estimates, rather than absolute values, because there is no independent way of determining the exact rate of false-negative error (ie, how many true LH pulses are missed), or of determining false-positive errors extrinsically introduced to the assay. Moreover, the development of alternative pulse detection methods depending upon multiple point analyses may ultimately permit more refined estimates of LH pulse frequency.

We have observed that there is a remarkable dispersion of LH pulse frequency estimates in normal men. The range of values observed for LH pulse frequency or amplitude is significantly amplified when more intensive rates of venous sampling are utilized. Such schedules of more intensive venous sampling permit the detection of significantly larger numbers of presumptively "true-positive" LH pulses. Notably, the estimate of "true-positive" LH pulse frequency appears to plateau at very rapid rates of venous sampling, while the observed rate of apparent LH "pulses" continues to increase. Our studies indicate that this ostensible increase can be attributed in large part to an increasing contribution of false-positive peaks at more rapid sampling rates.

We conclude that clinical investigators must recognize a wide range of LH pulse characteristics expressed among healthy men, and that significant deviations are introduced if frequency-adjusted, false-positive error rates are not evaluated. These considerations will be particularly important in future attempts to document important departures from

normal patterns of pulsatile gonadotropin secretion in selected pathophysiologic states. Moreover, the wide spectrum of LH pulse frequencies and amplitudes observed in this large group of eugonadal men (having normal serum testosterone concentrations) suggests that a broad range of LH pulse signal characteristics will effectively maintain testosterone production in the human male.

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