

Operating Characteristics of the Male Hypothalamo-Pituitary-Gonadal Axis: Pulsatile Release of Testosterone and Follicle-Stimulating Hormone and Their Temporal Coupling with Luteinizing Hormone*

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ABSTRACT. To appraise the physiological pattern(s) of episodic testosterone and FSH release in man, we withdrew blood samples at 10-min intervals for 24–36 h in a total of 15 normal men. We subjected the resulting FSH (15 men) and testosterone (5 men) time series to 3 statistically based and mathematically independent procedures for detecting hormone pulsatility, *viz.* Cluster analysis, the Detect program, and Fourier transformation. The Cluster technique disclosed discrete testosterone and FSH peaks occurring at mean (\pm SEM) interpulse intervals of 112 ± 14 and 85 ± 3.4 min, respectively. These values were not significantly different from the mean LH interpulse interval of 95 ± 11 min. The average durations of the testosterone and FSH pulsations were 90 ± 11 and 59 ± 3 min, respectively. The mean testosterone pulse amplitude reached a maximal value of 910 ± 92 ng/dL (31.5 ± 3.2 nmol/L), which represented a mean increase of 242 ± 26 ng/dL (8.4 ± 0.9 nmol/L) above the preceding nadir. FSH pulses had a maximum of 7.2 ± 0.3 IU/L, and an incremental amplitude of 1.3 ± 0.1 IU/L. An independent pulse detection procedure, Detect, yielded a testosterone pulse frequency of 12.3 ± 0.8 pulses/day [$P = \text{NS vs. Cluster program}$ (13 ± 1.9 pulses/day)]. The Cluster and Detect estimates of FSH pulse frequency were also similar, *viz.* 16 ± 1.9 and 16 ± 0.6 pulses/day. Further analysis by Fourier transformation revealed significant circadian periodicities for serum testosterone, FSH,

and LH, which had mean nyctohemeral amplitudes of 185 ng/dL (6.4 nmol/L), 0.38 IU/L, and 1.3 IU/L, respectively.

Cross-correlation analyses disclosed significantly positive uncorrected cross-correlations between LH and testosterone that were maximal at a testosterone lag of 60 min (range, 50–70 min). To eliminate high intrinsic autocorrelations within the testosterone and LH time series, stepwise autoregressive fitting was employed. The resulting partial cross-correlation matrices indicated that LH concentrations at any given instant were significantly positively correlated to testosterone concentrations lagged by 10 and 20 min. Similarly, contemporaneous LH and FSH concentrations were significantly positively correlated ($r = 0.40-0.89$; $P < 0.001$). Moreover, autoregressive modeling disclosed significantly positive partial cross-correlations between LH and FSH at a FSH lag of 10 min.

In summary, we have identified significant pulsatile as well as circadian (24-h) patterns of testosterone and FSH release in normal men. Moreover, cross-correlation and stepwise autoregressive modeling of simultaneous LH and testosterone or simultaneous LH and FSH time series revealed close temporal coupling between LH and testosterone concentrations, such that testosterone and FSH increases occur coincidentally with LH increases or lag LH increases by only 10–20 min. (*J Clin Endocrinol Metab* 65: 929, 1987)

ALTHOUGH the episodic release of LH has been characterized extensively in man, that of FSH and/or testosterone has been more difficult to define to date (1, 2). Since distinctly intermittent release of testoster-

one can be demonstrated in a variety of nonhuman species (3–5), we reexamined the nature of pulsatile testosterone release in normal men. Our reinvestigation of this issue and our examination of FSH pulsatility were further prompted by several major recent developments in the field of endocrine pulse analysis: 1) the demonstration that both intensive and extended sampling paradigms may be required to identify the majority of hormone pulses in the circulation (1); 2) the recent availability of statistically validated and methodologically independent procedures for objectively delineating significant hormone pulses (6, 7); and 3) an interest in possible concordance between two endocrine series (*e.g.* LH and testosterone, or LH and FSH).

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In this work, blood samples were withdrawn at 10-min intervals for 24–36 h in 15 men to provide intensive and extended testosterone, FSH, and LH concentration time series for analyses. The simultaneous serum testosterone, FSH, and LH profiles were then subjected to independent, statistically based pulse detection methods for identifying both discrete hormone peaks and underlying periodic (e.g. circadian) rhythms. In addition, cross-correlation analyses and stepwise autoregressive modeling were used to determine possible temporal coupling between LH and testosterone and/or LH and FSH in normal men.

Materials and Methods

Experimental protocol

Fifteen normal men (age range, 22–27 yr; median age, 23) were studied after provision of written informed consent, approved by the Human Investigation Committee of the University of Virginia School of Medicine. Each man had a normal medical history, physical examination (including testicular volumes), and serum biochemical tests. The latter included immunoactive LH, FSH, prolactin, TSH, T_4 , total and unbound testosterone, estradiol, and measurements of hepatic, renal, and hematological function. Eight men submitted complete semen analyses, which were normal. The men were admitted the evening before the study, when an iv heparin well was inserted in a forearm vein. Beginning at 0800 h the next morning, blood was withdrawn at 10-min intervals for 36 h (5 men) or for 24 h (10 men). Serum samples were frozen for the subsequent RIA of testosterone and gonadotropin concentrations. The men were permitted to ambulate and eat regular meals during the study.

Serum testosterone concentrations were measured in 5 men in duplicate by solid phase RIA using reagents obtained from Diagnostic Products, Inc. (Los Angeles, CA; lot 21101). The individual median intraassay coefficients of variation (assessed in each man from 217 sample replicates) ranged from 5.2–8.7% (group median value, 6.3%). Serum immunoactive FSH and LH concentrations were assayed in all 15 men, exactly as described previously (8), with kits from Clinetics Corp. (Tustin, CA) with intraassay coefficients of variation of 2.3–5.5% (median, 3.3%) for FSH and 4.6–6.2% (median, 5.5%) for LH, respectively. In each of the immunoassays, all samples from an individual man were analyzed in the same assay to eliminate interassay variance.

Discrete peak detection

The Cluster analysis program is designed to search for all significant increases and decreases within data (LH, FSH, or testosterone) series in relation to measurement error within the experimental samples of each subject (6). Measurement imprecision (within-sample variance) was modeled as a power function of sample dose (concentration). Other program parameters were constrained after optimization, as defined in *Results (Detection of discrete ultradian testosterone and FSH peaks)*. A significant cluster of increased or decreased hormone concen-

trations is judged from the power-function estimate of sample variance using pooled t statistics applied to the moving test nadir and peak clusters that begin with the onset of the experimental series and traverse all points. The locations and widths of all individual peaks (significant increases followed by decreases) are identified, the total number of peaks is counted, and the mean interpeak interval is calculated in minutes. The following additional pulse parameters are also determined: mean peak duration in minutes, maximal peak height (highest absolute value attained within the peak), fractional peak height (percent increase above prepeak nadir), incremental peak amplitude (algebraic difference between maximal peak height and prepeak nadir), and area under the peak (proportional to the product of the mean peak value minus the lower of the pre- and postpeak nadirs times the peak width). Interpulse valleys are identified as regions embracing nadirs without intervening upstrokes.

As an independently formulated peak detection algorithm, Detect was used with tolerances set automatically and constrained to a 1% false positive rate (7). The parameter for the variance model was a_2 equals the square of the median intraassay coefficient of variation determined from all experimental samples in each man.

Statistical analysis

Comparisons of peak properties were made by nonparametric methods (Wilcoxon ranked sign test), and departures from normality were assessed by the Wilks-Shapiro or Anderson-Darling statistic (9). Where departures from normality were demonstrated, median rather than mean values are given.

Fourier analysis of the testosterone FSH and LH time series: investigation of circadian periodicities

The original hormone-concentration time series comprising N values in replicate was analyzed as a Fourier expansion. The Fourier transform of the concentration data yields a function that traverses all data points exactly, as a linear composite of distinct cosine and sine functions with $(N - 1)/2$ periodicities (10). Since each coefficient of this linear combination of sine and cosine functions is orthogonal, the SD of the individual coefficients can be determined by nonlinear least squares estimation models (11). In this work, we used this analytical tool to assess the magnitude of significant circadian (24-h) periodicities in the testosterone, FSH, and LH series. Periodicities were considered significant whenever their amplitudes exceeded zero by 1.96 SD.

Auto- and cross-correlation analyses of testosterone and LH series

To search for significant correlations within and between hormone time series, data were also subjected to auto- and cross-correlation analyses. The auto- or cross-correlation coefficient (r_k) measures the correlation between two values a distance (lag) k time units apart within one series or between two distinct series (12). The corresponding SE of the correlation coefficient for a series of N samples at lag k can be estimated as $(N - k)^{-1/2}$ (12). Significant auto- and cross-correlations can

be inferred when r_k values exceed zero by more than twice their (appropriately lagged) SEs. This methodology was employed to search for correlations within and between the testosterone, FSH, and/or LH series. In addition, because of sustained high auto- and cross-correlations within and between the testosterone, FSH, and/or LH series, further analysis of the partial auto- and cross-correlation coefficients was undertaken using stepwise autoregressive fitting. The partial auto- or cross-correlation coefficient at lag k measures the excess correlation at lag k , which is not accounted for by an autoregressive model of order $(k - 1)$ (11).

Results

Detection of discrete ultradian testosterone and FSH peaks

Using Cluster analysis, delimited increases and decreases (peaks) in hormone concentrations can be identified at a given maximal false positive rate on signal-free noise, *e.g.* $P \leq 0.05$. The observed pulse frequency can be considered further in relation to the following model (reviewed in Ref. 2):

observed pulse frequency α true positives

+ false positives - false negatives

In the above model, observed pulse frequency is proportional to the true positive (but unknown) pulse frequency and is artificially increased by false positive (α) errors and undesirably decreased by false negative (β) errors (2). We used this model to optimize the detection of true positive testosterone and FSH peaks, by initially holding the maximal false positive rate (determined on signal-free noise) constant at 5% or less while testing a range of cluster configurations. Assuming no interaction between signal and noise, maximal observed pulse frequency should then reflect the lowest false negative error and, hence, most closely approximate the desired true positive estimate. To this end, we evaluated various cluster sizes and relative upstroke/downstroke thresholds by testing 16 permutations of cluster configurations with t statistics chosen to constrain the maximal false positive rate to 5% or less. This optimization effort yielded maximal testosterone and FSH (or LH) peak detection at cluster configurations of 1×2 and 2×2 (test points in nadir and peak), respectively. Thus, for further analysis, we used 1) a cluster configuration of 1×2 with t statistics of 2.98 and 2.0 for significant ($P < 0.05$) testosterone increases and decreases, respectively, and 2) a cluster configuration of 2×2 with t statistics of 2.65 and 2.50 for significant ($P < 0.05$) FSH (or LH) increases and decreases, respectively.

Testosterone pulsatility. Using Cluster analysis to mark each bounded region of an increase followed by a decrease in testosterone concentrations as a peak, we estimated

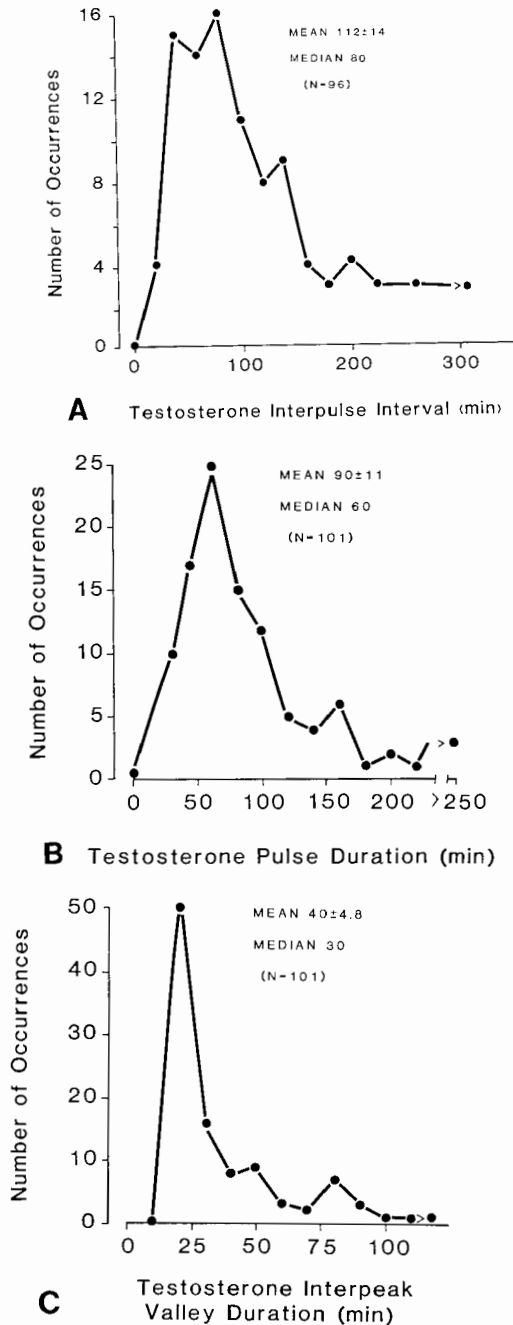


FIG. 1. Frequency histograms of testosterone interpulse intervals (A), testosterone peak durations (B), and testosterone interpeak valley durations (C). In each panel, the frequency distribution of the indicated testosterone pulse parameter was significantly non-Gaussian. The histograms include all data collected from five normal men. Values in parentheses denote the total number of observations.

the frequency, amplitude, and interpulse properties of testosterone pulses in the five normal men sampled at 10-min intervals for 36 h. We found a mean \pm SEM (median) number of significant testosterone peaks of 20 ± 3 (16)/36 h. Correspondingly, the interpeak interval (duration, in minutes, elapsing between consecutive

TABLE 1. Characteristics of spontaneous testosterone peaks in normal men

Subject no.	Peak duration (min)	Maximal peak (ng/dL) ^a	Fractional amplitude (%)	Incremental amplitude (ng/dL) ^a	Area (ng/dL · min) ^a
1	96	565	34	141	7,520
2	112	989	39	260	14,300
3	62	890	50	286	10,800
4	111	1,094	33	261	23,900
5	67	1,013	38	263	13,300
Mean	90	910	39	242	14,000
±SEM	11	92	3	26	2,700
Median	96	989	38	261	13,300

Results are from five normal men sampled at 10-min intervals for 36 h.

^aTo convert nanograms per dL to nanomoles per L, multiply by 0.03467.

TABLE 2. Significant correlation between 36-h mean serum testosterone concentrations and/or individual characteristics of testosterone peaks in five normal men

Correlated parameters	r value
Pulse frequency · interpulse interval	-0.995
Pulse frequency · peak duration	-0.935
36-h mean testosterone · maximal peak amplitude	+0.998
36-h mean testosterone · valley mean	+0.990
36-h mean testosterone · nadir mean	+0.989
36-h mean testosterone · incremental amplitude	+0.885

Linear correlations between distinct testosterone peak properties and/or 36-h mean serum testosterone concentrations in five normal men sampled at 10-min intervals for 36 h.

maximal values attained within adjacent testosterone peaks) averaged 112 ± 14 (132) min. The resultant histogram of the interpulse intervals for significant testosterone peaks is shown in Fig. 1A. This distribution of testosterone interpulse intervals was significantly non-Gaussian ($P < 0.01$).

The other principal characteristics of testosterone peaks in the five men studied are summarized in Table 1. The median duration of a peak was 96 min, its maximal value was 989 ng/dL (34.3 nmol/L), and its fractional amplitude was a 38% increase above the preceding nadir. The testosterone peak durations were also non-Gaussian in distribution, as illustrated in Fig. 1B. The testosterone peaks encompassed a median increment of 261 ng/dL (9.0 nmol/L) above nadir values. The calculated median testosterone peak area was 13,300 ng/dL · min (461 nmol/L · min). In addition, linear regression analyses revealed that 36-h mean serum testosterone concentrations were significantly positively correlated with certain characteristics of the testosterone peaks and valleys, as summarized in Table 2.

The intervals during which no significant increases or decreases occurred in serum testosterone concentrations were identified as interpeak valleys. The mean \pm SEM (median) duration of such interpulse valleys was 40 ± 5

(38) min. The distribution of the interpulse valley durations was also significantly non-Gaussian (Fig. 1C). During these apparently quiescent intervals, the mean testosterone concentration was 730 ± 74 ng/dL (25.3 ± 2.6 nmol/L). The mean nadir (minimum) testosterone concentration contained within the valleys was 654 ± 70 ng/dL (22.7 ± 2.4 nmol/L).

An independent discrete peak detection procedure, Detect, identified 19.4 ± 1.2 testosterone peaks/36 h. This value agrees with the Cluster estimate of 20 ± 3 testosterone pulses/36 h.

As indicated quantitatively above, significant spontaneous serum testosterone excursions occurred in the normal men. Such episodic testosterone profiles for 2 men are illustrated in Fig. 2. In contrast, as shown in the lower panel of Fig. 2, 216 samples assayed from a single pool of male serum in replicate (noise estimate) yielded a testosterone profile devoid of spontaneous pulsatility.

FSH pulsatility. Cluster analysis disclosed a total of 224 FSH peaks in the 15 men studied, with a mean \pm SEM (median) number of significant FSH peaks of 16 ± 0.6 (16)/24 h. Correspondingly, the interpeak interval (duration, in minutes, between maximal values attained in adjacent FSH peaks) averaged 85 ± 3 (88) min. The frequency histogram of FSH interpulse intervals associated with all 224 FSH peaks is shown in Fig. 3A. This distribution of FSH interpulse intervals was significantly non-Gaussian ($P < 0.01$).

Additional physiological characteristics of the FSH peaks are summarized in Table 3. The median duration of an FSH peak was 62 min (Fig. 3B), its maximal height was 7.3 IU/L (Fig. 3C), its fractional height was a 23% increase above the preceding nadir, and its incremental amplitude was 1.3 IU/L (Fig. 3D) above nadir.

The median FSH peak area was 52 IU/L · min (Table 3). In addition, intervals during which no significant increases or decreases occurred in serum FSH concentrations (interpeak valleys) had a mean \pm SEM (median) duration of 26 ± 1 (25) min. The distribution of inter-

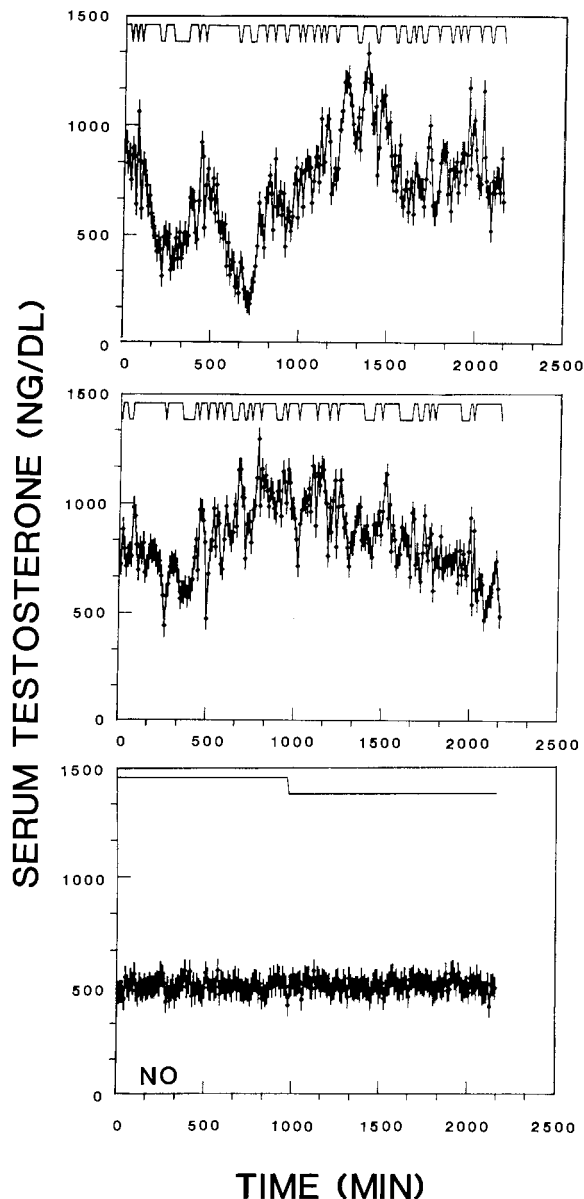


FIG. 2. Profiles of episodic testosterone secretion in two normal men (upper and middle panels). Blood samples were withdrawn at 10-min intervals for 36 h. In the lower panel, 216 sample replicates from a single pool of male serum (designated NO for noise) are depicted for comparison. Serum testosterone concentrations (and the range of the sample duplicates) are given on the vertical axis as nanograms per dL, and time is given on the horizontal axis in minutes. Above each testosterone pulse profile, the schematized deflections depict the computerized (Cluster analysis) designation of statistically significant increases and decreases in the data, and thereby corresponding pulses. To convert testosterone concentrations from nanograms per dL to nanomoles per L, multiply the former values by 0.03467.

pulse valley durations was also significantly non-Gaussian (not shown). During these quiescent intervals, the mean FSH concentration was 6.0 ± 0.3 IU/L. The mean nadir (minimum) FSH concentration contained within the valleys was 5.6 ± 0.3 IU/L.

As an independent discrete peak detection procedure, the Detect program was also used. Although the range of estimates was larger for the Detect method, this algorithm identified 16 ± 1.9 FSH peaks/24 h, which agrees with the mean Cluster estimate of 16 ± 0.6 pulses/24 h ($P = \text{NS}$; Fig. 4).

As indicated by the above quantitative analyses, statistically significant spontaneous FSH pulsations occur in the circulation of normal men. Temporal profiles of episodic FSH release for two men are illustrated in Fig. 5.

Analysis of circadian testosterone and FSH excursions

Testosterone periodicities. As shown in Table 4, the five men studied for testosterone pulsatility had highly significant 24-h (circadian) periodicities in testosterone concentrations. The same five men also had significant circadian LH periodicities. The mean amplitude of the 24-h testosterone rhythms was 185 ng/dl (6.4 nmol/L). The mean testosterone acrophase (maximal value) occurred at 0630 h. In addition, each of the five individual testosterone periodograms (Fourier transforms) contained significant testosterone cycles at periodicities of 23, 38, 48, 61, 79, 98, 113, 180, 360, and 717 min/cycle. Certain of these periodicities may represent harmonics, while the mean value of 113 min/cycle is very similar to the mean periodicity (interpeak interval) of 112 ± 14 min detected by Cluster analysis (above). As discussed below, the periodicity of 717 min corresponds closely to a cycle length suggested by partial autocorrelation analysis.

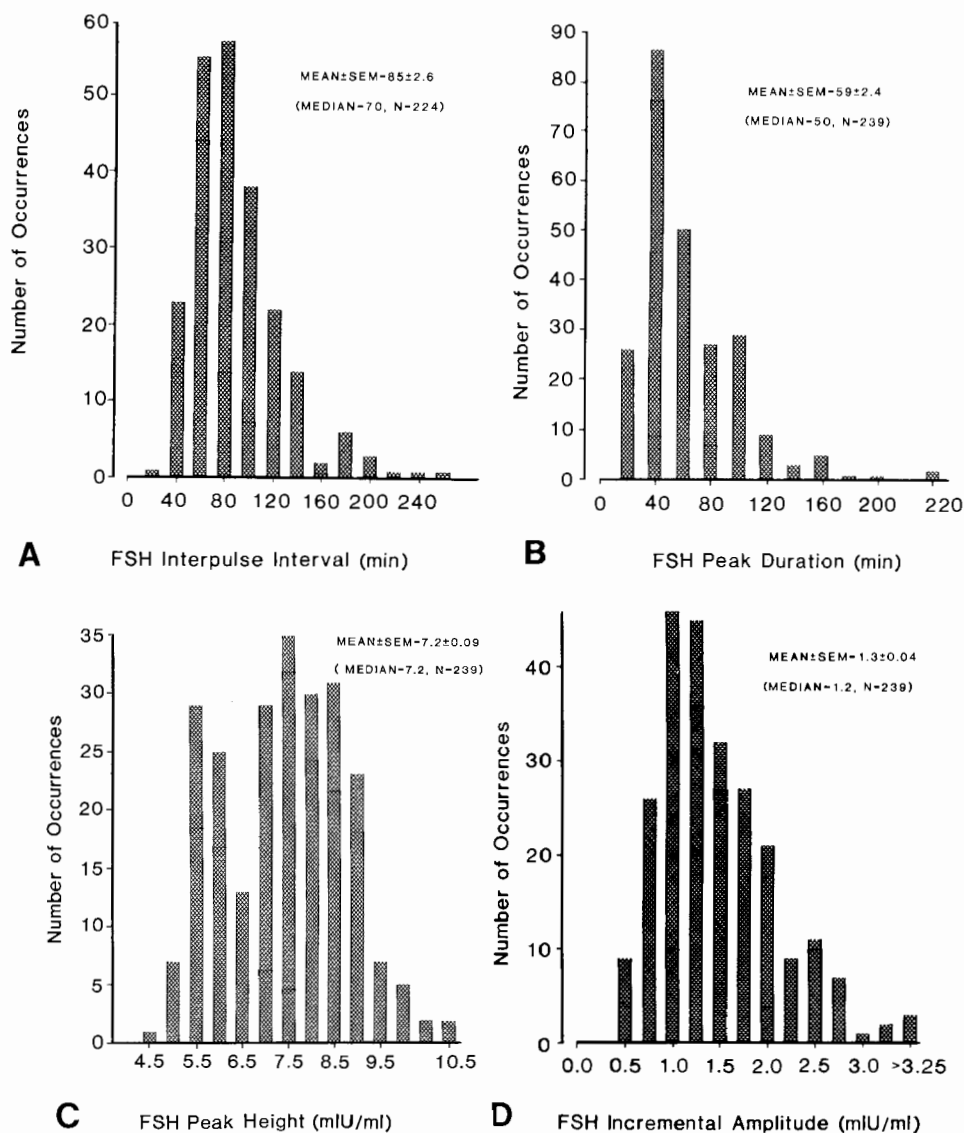
FSH periodicities. In the five men sampled every 10 min for 36 h, we found major significant intrinsic periodicities in their FSH time series at cycle lengths of 85, 107, and 307 min/cycle (Fig. 6). The 85-min cycle length is similar to the mean periodicity (interpeak interval) of 85 ± 3 min detected in the same men by Cluster analysis (above).

Fourier analysis of the 24-h FSH profiles in 15 men revealed significant circadian periodicities, with a mean amplitude of 0.38 ± 0.04 IU/L ($P < 0.001$). The corresponding mean amplitude of the circadian periodicities for LH in these 15 men was 0.88 ± 0.14 IU/L ($P < 0.001$). The mean acrophases for FSH and LH occurred at 0708 and 0736 h, respectively ($P = \text{NS}$).

Autocorrelation analyses of the testosterone and FSH time series and cross-correlations with LH

Testosterone autocorrelations. To search for possible relationships between consecutive and/or variously delayed testosterone concentrations within individuals, autocorrelation analysis was applied. As shown in Fig. 7

FIG. 3. Frequency histograms of FSH interpulse intervals (A), FSH peak durations (B), FSH maximal values (peak height), and incremental peak amplitudes (C and D), as defined by optimized Cluster analysis. In each case, the frequency distribution of the indicated FSH pulse parameter was significantly non-Gaussian. The histograms include all data collected from 15 normal men who underwent blood sampling at 10-min intervals for 24 h. Values in *parentheses* denote the grand median values and the total number of observations. Note that group ($n = 15$ men) median values are given in Table 3. Concentrations in milliinternational units per mL are numerically equivalent to those in international units per L.



for two men, testosterone concentrations were highly autocorrelated over lag intervals of 0 to -18 time units (0 to -18×10 min or -180 min). This observation might be expected in view of serial correlation introduced by metabolic clearance of testosterone. Accordingly, we also examined the partial autocorrelation coefficients at various lags k , which represent excess correlation beyond that contributed by prior correlations at lags $(k - 1)$. In this analysis, all five men had significantly positive partial correlations at lags $k = -1$ and -2 time units (corresponding to 10 and 20 min, respectively) and significantly negative partial autocorrelations at a median lag $k = -34$ (range $k = -16$ to $k = -60$). Such negative partial autocorrelations indicate that at a median lag of 34 time units (or 34×10 min = 340 min = 5.7 h), testosterone concentrations were significantly negatively autocorrelated, *i.e.* high values of testosterone 5.7 h earlier were followed by low subsequent values, and *vice*

versa. This apparent half-cycle length corresponds clearly to the significant Fourier periodicity of 717 min (half-period = 6.0 h), which had an amplitude of 52 ng/dL (1.8 nmol/L). In contrast, the testosterone series comprising replicates of pooled male serum (noise estimate) had no significant autocorrelations (Fig. 7; *bottom panel*).

Testosterone and LH cross-correlations. The individual temporal profiles of LH, FSH, and testosterone concentrations in one man sampled at 10-min intervals for 36 h are shown in Fig. 8. For illustrative purposes, discrete peaks are presented using a 1% false positive rate by Cluster analysis. The unmodified profiles in all five men were subjected to cross-correlation analysis. As summarized in Fig. 9, all five men had significant cross-correlations between LH and testosterone concentrations. Maximal uncorrected cross-correlations were found at latencies (lags in min) between LH and testosterone of

TABLE 3. Characteristics of spontaneous FSH pulsations identified by Cluster analysis in 15 normal men

Subject no.	Peak frequency (no./24 h)	Interpulse interval (min)	Peak duration (min)	Maximal peak ht (IU/L)	Fractional peak ht (% increase)	Area (IU/L·min)	Increment (IU/L)	Valley duration (min)
A	21	66	44	6.0	23	30	1.1	21
B	20	68	51	5.5	21	30	0.9	20
C	15	94	59	6.0	28	43	1.3	33
D	16	85	62	8.3	30	64	1.9	23
E	13	103	72	6.7	22	53	1.2	29
F	19	67	42	7.9	18	27	1.2	25
G	16	77	57	5.2	24	36	1.0	24
H	16	82	55	9.2	20	52	1.5	25
I	15	91	63	8.1	22	53	1	27
J	13	110	79	8.1	33	93	2.0	24
K	15	91	64	7.3	21	54	1	27
L	14	98	71	7.3	22	63	1.3	27
M	14	94	65	6.5	23	55	1.2	30
N	16	88	56	8.6	26	74	1.7	28
O	16	83	62	7.3	26	52	1.5	20
Mean	16	85	59	7.2	24	52	1.3	26
SE	0.6	3.4	2.6	0.3	1.0	4.6	0.1	0.9
Median	16	88	62	7.3	23	53	1.3	25

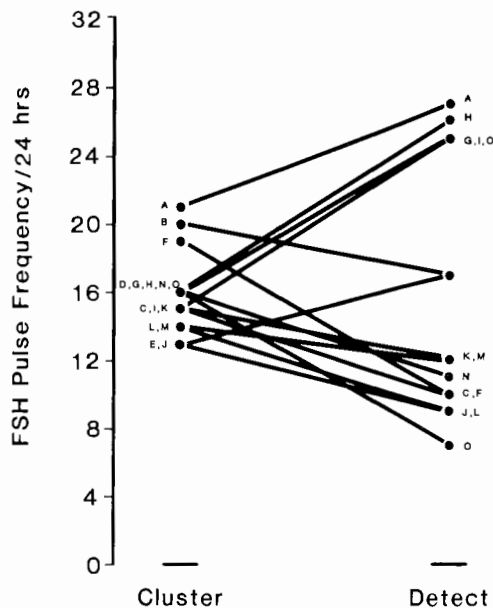


FIG. 4. Comparison between Cluster and Detect estimates of FSH pulse frequency in 15 normal men. Estimates for individual men are denoted by alphabetic characters.

50–70 min (Table 5). In addition, LH alone and testosterone alone manifested significant and sustained positive autocorrelations. Such auto- and cross-correlations for LH and testosterone are illustrated for one man in Fig. 10.

The significant autocorrelations within the testosterone and LH series noted above could give rise to spurious cross-correlations (12). Accordingly, we used stepwise

autoregressive modeling to determine partial cross-correlation coefficients at various lags k , in which the influences of prior correlations at lags $(k - 1)$ are removed. As summarized in Table 6, each man had highly significant positive partial cross-correlation coefficients at LH-testosterone lags of 10 and 20 min. This indicates that testosterone concentrations are significantly positively correlated to LH concentrations 10 and 20 min earlier. Moreover, stepwise autoregressive fitting demonstrated that the delayed association of LH and testosterone (at lags of 60–100 min) could be accounted for nearly completely by the partial correlations identified at lags of 10–20 min.

Using serum collected from two hypogonadotropic men who received a bolus iv injection of pure human LH in an earlier study (13), we measured serial testosterone concentrations before and after the LH infusion. The resultant cross-correlation and autoregression analyses are shown in Table 7. Most notably, autoregressive fitting revealed that temporal coupling between LH and testosterone also involved a 10- to 15-min testosterone lag in these men. Consequently, we can exclude the possibility that temporal linkage between LH and testosterone merely reflects synchronous endogenous oscillations in MCRs.

FSH and LH cross-correlations. To search for possible interrelationships between concurrent and/or variously delayed FSH and LH concentrations, cross-correlation analysis was applied to the 14 men who had uniformly detectable concentrations of LH and FSH. As shown in

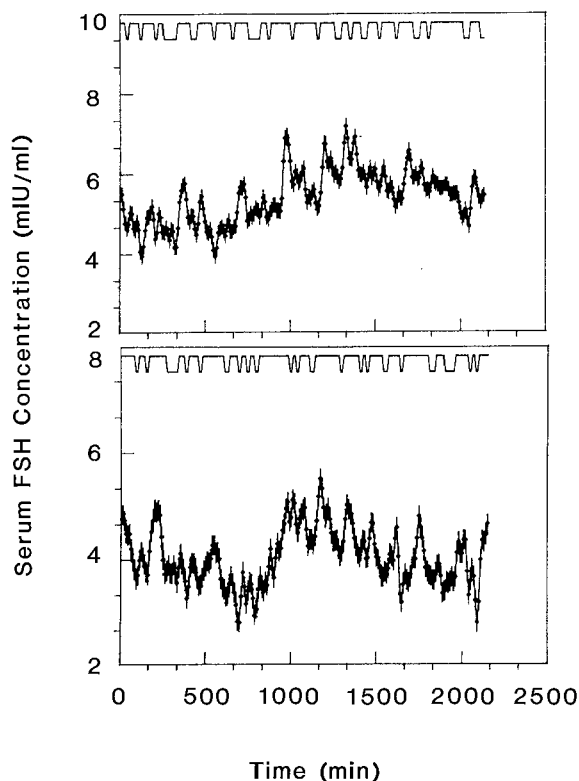


FIG. 5. Profiles of episodic FSH secretion in two normal men. Blood samples were withdrawn at 10-min intervals for 36 h. Serum FSH concentrations and their intrasample SD are given on the vertical axis in milliinternational units per mL (which is equivalent to SI units of international units per L). Time is given on the horizontal axis in minutes. Above each FSH pulse profile, the schematized deflections depict the computerized (Cluster) designation of statistically significant increases and decreases in the data, which serve to identify the locations and durations of significant FSH pulses.

TABLE 4. Amplitudes of significant testosterone and LH circadian periodicities

Subject no.	Testosterone (ng/dL) ^a	LH (IU/L)
1	134	1.8
2	195	0.52
3	269	1.2
4	136	1.7
5	193	1.2
Mean	185	1.28
±SEM	26	0.23

Data are from five men sampled at 10-min intervals for 36 h.

^aTo convert nanograms per dL to nanomoles per L, multiply by 0.03467.

Table 8, FSH and LH concentrations were highly cross-correlated in all 14 men at zero lag (simultaneous samples). In addition, at a lag of $k = \pm 1$ (10 min), both LH and FSH showed high degrees of autocorrelation. This observation might be expected in view of the slow metabolic clearance of FSH and LH. Accordingly, autore-

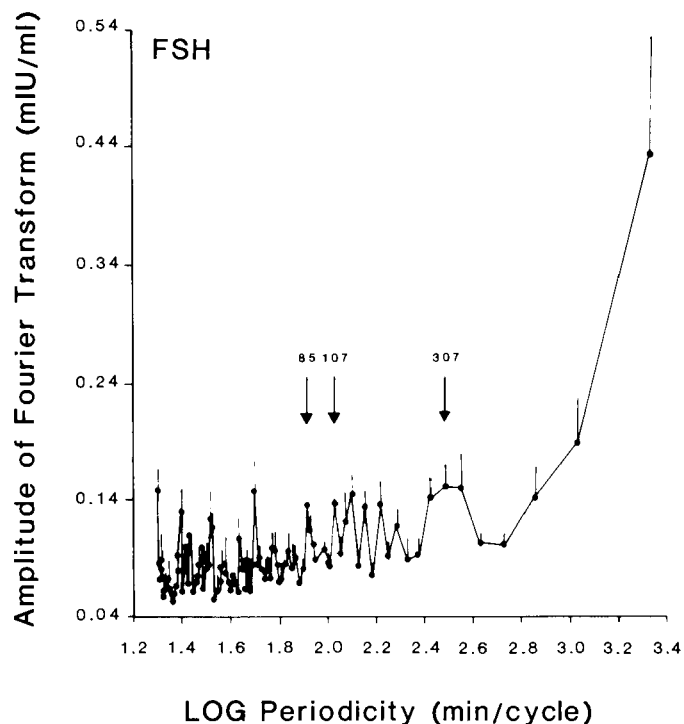


FIG. 6. Periodogram of FSH time series. Mean amplitudes (milliinternational units per mL or international units per L) of Fourier transforms of the 36-h FSH time series in five men are given on the vertical axis, and the logarithm of the periodicity (minutes per cycle) is given on the horizontal axis. The periodicities of the most prominent FSH amplitudes are indicated by numbers and arrows above the periodogram tracing.

gressive modeling was used to remove bias otherwise introduced by significant positive autocorrelations in both series. As summarized in Table 8, 12 of 14 men had significant positive partial correlations between LH and FSH at a lag of $k = 1$ (10 min), when LH was allowed to lead FSH. These positive lagged cross-correlations indicate that FSH concentrations have a significant positive correlation not only with simultaneous LH concentrations, but also with LH concentrations 10 min earlier. In contrast, 2 control FSH and LH series representing 145 replicated samples of pooled male serum (noise pool) had no significant auto- or cross-correlations.

Comparisons of testosterone, FSH, and LH rhythms

Discrete peak detection. When Cluster analysis was applied to the 36-h LH series, a mean LH pulse frequency of 21 ± 2.2 pulses/36 h was found. This estimate is not significantly different from those for testosterone and FSH in the same men, *viz.* 20 ± 2.9 pulses/36 h (testosterone) and 24 ± 2.4 pulses/36 h (FSH). However, mean testosterone (and/or FSH) and LH pulse frequencies or interpulse intervals were not significantly correlated among the 5 men.

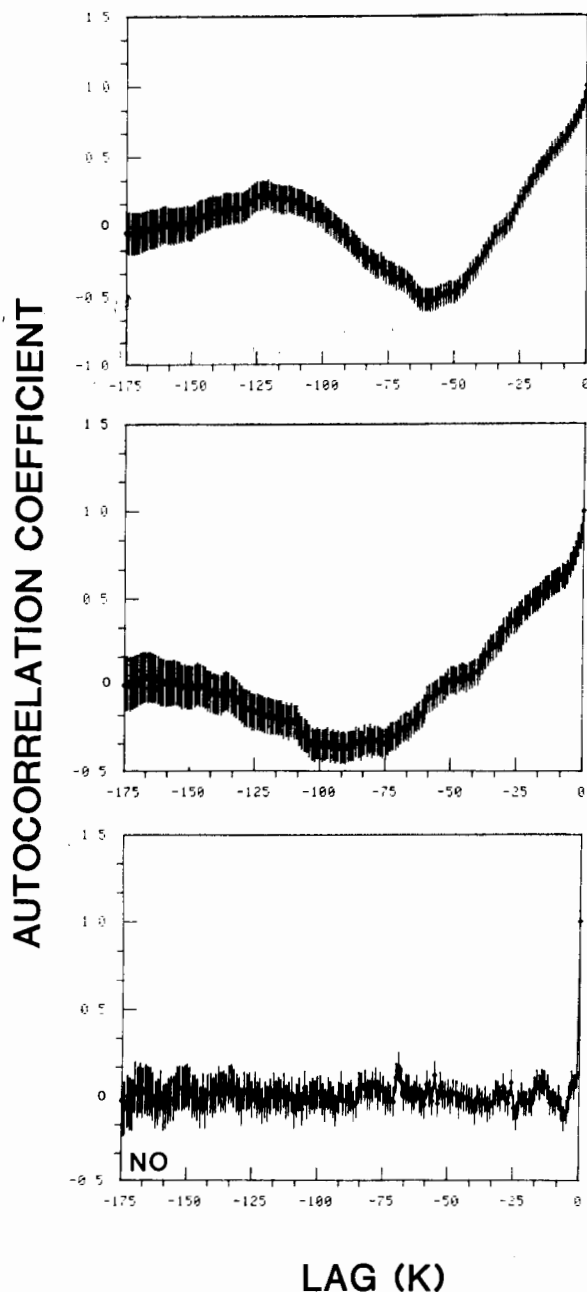


FIG. 7. Autocorrelograms for two men in whom blood was sampled at 10-min intervals for 36 h for the subsequent measurement of serum testosterone. The vertical axis gives the autocorrelation coefficient \pm SD, and the horizontal axis gives the lag (k) at which the autocorrelation coefficient was determined. The lag value k is given in time units, each of which corresponds to 10 min. For comparison with the physiological testosterone time series (upper and middle panels), the autocorrelogram of randomly varying measurements (designated NO for noise) with a median intraseries coefficient of variation of 6.3% is shown in the lower panel. Note that at zero lag, series are perfectly autocorrelated ($r_k = 1.0$). At all other lags, randomly varying series would show autocorrelation coefficients not significantly different from zero.

Circadian periodicities. Like testosterone and FSH, LH also exhibited significant 24-h (circadian) periodicities

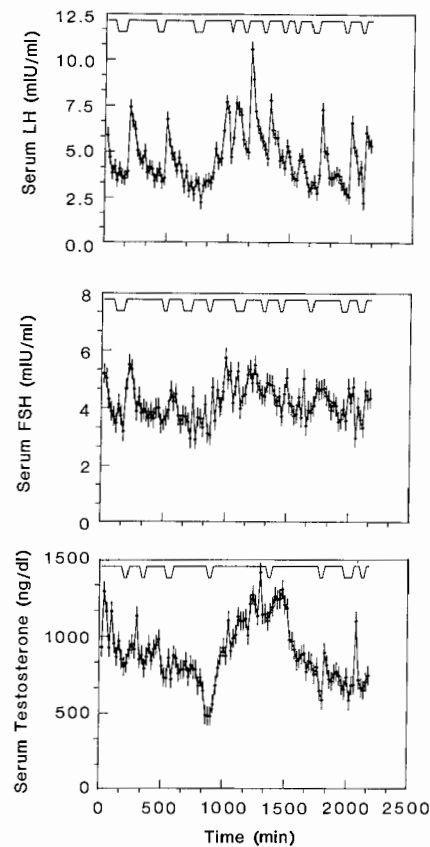


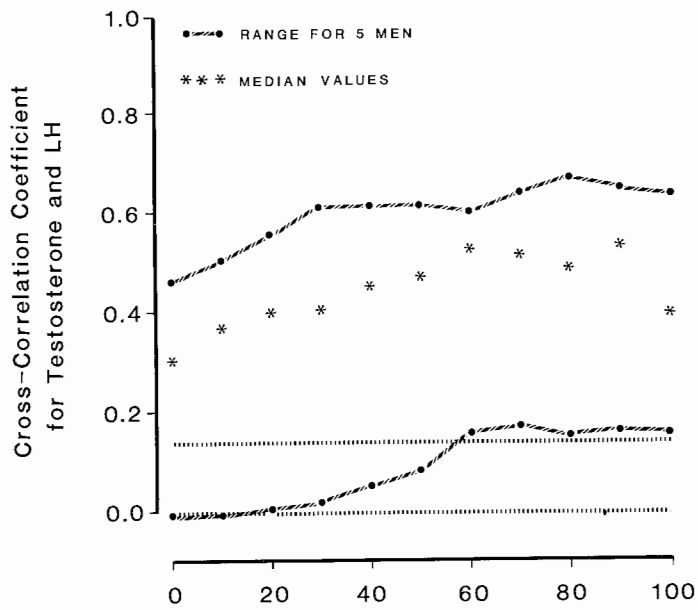
FIG. 8. Simultaneous profiles of episodic LH, FSH, and testosterone release in a normal man. Serum LH (upper panel), FSH (middle panel), and testosterone (lower panel) concentrations were measured in blood samples withdrawn at 10-min intervals for 36 h. The data were analyzed at a maximal false positive rate of 1% and are otherwise presented as defined in Figs. 2 and 5.

in all five men studied for 36 h. The mean amplitude was 1.3 IU/L. Circadian LH amplitudes were not significantly correlated with those for testosterone or FSH in same men.

Discussion

We used several new and independent analytical models to investigate the nature of spontaneous FSH and testosterone release and to seek an association between LH and testosterone and/or FSH secretion in normal men. These methods were applied to a large series of hormone concentrations generated by the withdrawal of blood samples at 10-min intervals for at least 24 h. Under these circumstances, we were able to delineate statistically unambiguous pulses of testosterone and FSH in the circulation of normal men, characterize their physiological properties, and assess their relationship(s) with LH.

The use of two recent discrete peak detection methods that are statistically based, Cluster analysis (5) and the Detect program (6), permitted us to enumerate signifi-



LAG (min) between LH and Testosterone

FIG. 9. Cross-correlations between testosterone and LH time series in five normal men. ●, Ranges of observed cross-correlation coefficients. *, Median cross-correlation coefficients. The interrupted horizontal line designates 2 SE above zero ($P < 0.025$). Thus, cross-correlation values above the horizontal line denotes significantly positive cross-correlation coefficients. The horizontal axis gives the latency or lag (k), which represents the distance in minutes separating the LH and testosterone concentrations when testosterone lags behind LH.

TABLE 5. Lags at which maximal uncorrected cross-correlations occur between testosterone and LH in normal men

Subject no.	Lag (latency in min)	Cross-correlation coefficient ^a
1	70	0.22
2	60	0.48
3	50	0.61
4	70	0.65
5	60	0.18
Median value	60	0.48

^aThe SE of the cross-correlation coefficient in these analyses was 0.0680.

cant and distinct serum testosterone and FSH concentration peaks in normal men. Testosterone and FSH peaks occurred, respectively, at average interpeak intervals of 112 and 85 min. These values were not significantly different from the mean LH interpulse interval of 95 min. Such delimited testosterone and FSH pulses were separated by regions of nonpulsatility (interpeak valleys) of, respectively, 40 and 25 min in mean duration. These observations using cross-validated computerized peak detection algorithms significantly extend earlier assessments of physiological episodic pulsations of testosterone and FSH in normal men (14–16), in which

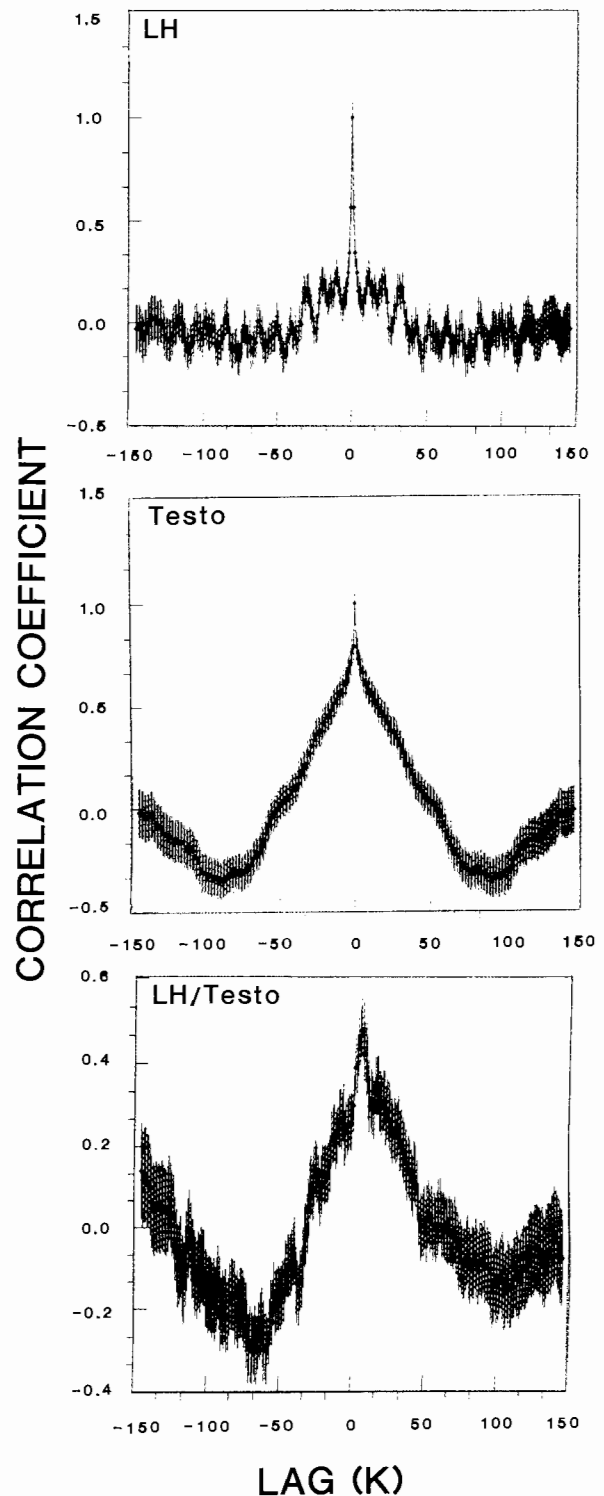


FIG. 10. Auto- and cross-correlation profiles for testosterone (Testo) and LH in a normal man. Autocorrelation profiles are shown for LH (top panel) and testosterone (middle panel). The cross-correlation profile between testosterone and LH is also given (bottom panel). In each panel, the vertical axis depicts the correlation coefficient (\pm SD), and the horizontal axis the lag, which is the time interval (minutes) by which the correlated values are separated.

TABLE 6. Summary of stepwise autoregression fitting of LH and testosterone time series

Lag (min)	Subject no.				
	1	2	3	4	5
10	225 ^a	354 ^a	466 ^a	534 ^a	407 ^a
20	19 ^a	28 ^a	20 ^a	20 ^a	17 ^a
30				12 ^b	11 ^b

Values are the χ^2 test statistic for the partial autoregression coefficient at the indicated lag.

^a $P < 0.01$.

^b $P < 0.05$.

TABLE 7. Relationships between bolus LH injection and subsequent testosterone secretion in two hypogonadotropic men

Time (min)	Cross-Correlation Coefficients	
	Subject A ^a	Subject B ^a
0 ^b	0.37	0.20
10	0.34	0.26
15	0.43	0.29
20	0.55 ^c	0.35
25	0.30	0.42
30	0.31	0.43
35	0.37	0.50 ^c
45	0.13	0.52 ^c
55	0.32	0.23
65	0.16	0.15
75	-0.18	0.05

The baseline and peak LH concentrations were 2.5 and 35 IU/L (subject A) and 1.6 and 27 IU/L (subject B), from which LH half-lives were previously calculated (13), with corresponding basal and peak testosterone concentrations of 180 and 570 ng/dL (subject A) and 160 and 320 ng/dL (subject B). To convert nanograms per dL to nanomoles per L, multiply the testosterone concentration by 0.03467.

^a Autoregressive modeling further demonstrated that the maximal partial autoregressive coefficient occurred at 15 min for subject A ($P < 0.01$; $\chi^2 = 40$) and at 10 min in subject B ($P < 0.01$; $\chi^2 = 53$, with a critical value of 13 for 4 degrees of freedom).

^b LH (35 μ g) was injected at time zero.

^c Maximal value for the cross-correlation coefficient ($P < 0.05$).

sampling duration and intensity were relatively limited and statistically based peak detection algorithms were not used.

By additionally subjecting the testosterone and FSH series to Fourier transformation, we were able to search for circadian and other regularly occurring periodicities that would not be identified by discrete peak detection methods (above). The subsequent periodograms (plot of Fourier transform amplitude *vs.* periodicity) revealed a range of statistically significant testosterone and FSH periodicities in these normal men. Most prominent were significant circadian (24-h) testosterone and FSH rhythms, which had mean amplitudes of 185 ng/dL (6.4 nmol/L) and 0.38 IU/L, respectively. The latter compares with an amplitude of 1.3 IU/L for the nyctohemeral

periodicity of LH in the same men. The circadian periodicities in all men for all three hormones were statistically significant, as assessed by Fourier transformation with nonlinear least squares parameter estimation, in contrast to inferences from some previous reports, which employed less intensive sampling paradigms and did not provide statistical confidence limits for the estimates of circadian amplitudes (reviewed in Ref. 2). Without such error estimates that reflect intraassay precision in the experimental samples, earlier inferences regarding circadian periodicities cannot be interpreted with statistical reliability. Our statistically bounded quantitation of various periodicities revealed not only circadian rhythms, but also multiple ultradian fluctuations in the FSH and testosterone time series. At present, the exact physiological significance of such higher frequency periodicities is not known, although rapidly cycling testosterone rhythms have also been described recently in salivary fluid in men (17).

Our further assessment of autocorrelations within the testosterone and FSH series revealed not only expected positive correlations among immediately consecutive hormone values (as would be predicted from short term serial correlation introduced by metabolic clearance), but also in the case of testosterone unanticipated delayed negative autocorrelations. In particular, at any given time, serum testosterone concentrations were significantly negatively correlated with those in the same individual 5.7 h earlier. We speculate that this cycle might reflect ultradian negative feedback oscillations within the testosterone time series in man, akin to those that have been suggested recently in rats (18).

Comparison of LH, FSH, and testosterone pulse frequencies in the same men yielded similar mean estimates. In addition, all three hormones exhibited significant circadian (24-h) periodicities in each man. Further analyses of possible relationships among FSH, testosterone, and LH concentrations demonstrated a close temporal coupling in all men. In particular, stepwise autoregressive fitting disclosed highly significant positive cross-correlations between simultaneous FSH and LH concentrations and between testosterone and LH concentrations at lags of 10 and 20 min. The latter findings indicate that increases or decreases in testosterone concentrations at any given time can be predicted to a significant degree by corresponding increases or decreases in LH concentrations 10 and 20 min earlier. This close temporal association between LH and testosterone permits us to postulate that Leydig cells are rapidly responsive to LH *in vivo*. The latter inference was supported by our further analyses of testosterone responses to injected LH in two men and is concordant with that put forward in the intact rat (17). Our finding of a very close temporal coupling between LH and testosterone differs from cer-

TABLE 8. Autocorrelation, cross-correlation, and autoregressive modeling of FSH and LH time series in 14 normal men

Subject	Cross-correlation between FSH and LH at lag $k = 0^a$	Autoregressive modeling at lag $k = 1$ (10-min delay)				
		LH autocorrelation	FSH autocorrelation	LH and FSH cross-correlation		
				LH leads FSH	FSH leads LH	
A	0.41 ^b	+ ^a	0 ^c	+ ^c	0 ^c	
B	0.75	+	0	+	0	
C	0.63	+	+	0	0	
D	0.87	+	0	+	0	
F	0.89	+	0	+	0	
G	0.48	+	+	+	0	
H	0.72	+	+	+	+	
I	0.62	+	+	+	+	
J	0.73	+	+	+	0	
K	0.64	+	+	+	0	
L	0.61	+	+	+	0	
M	0.40	+	+	+	+	
N	0.74	+	0	+	0	
O	0.67	+	+	0	+	

Subject E had occasional undetectable (<1.8 IU/L) serum gonadotropin concentrations, which rendered the correlation matrix indeterminate.

^a Simultaneous serum FSH and LH concentrations are considered. Each correlation coefficient was statistically significant ($P < 0.01$).

^b The standard deviations of the cross-correlation coefficient is 0.08 for these series. Hence, cross-correlation values listed in this column are significant ($P < 0.001$).

^c "0" and "+" denote respectively, nonsignificant ($P > 0.05$) and significantly positive ($P < 0.05$) correlation coefficients.

tain earlier reports, in which a more prolonged delay between LH and testosterone peaks (50–90 min uncorrected lag) was suggested (1, 14, 15). These differences may reflect in large part the lack of autoregressive analysis applied to previous observations, since we also found similar uncorrected lags (*viz.* 50- to 70-min lags). However, the latter, when corrected by autoregressive modeling, can be accounted for by underlying cross-correlations unique to even earlier lags (*i.e.* 0- to 20-min latencies between LH and testosterone).

Assuming that FSH and testosterone MCRs do not vary in a periodic fashion, our results offer a clear characterization of the pulsatile nature of FSH and testosterone release. Moreover, recent studies by Winters and Troen (19) of testosterone release into the human spermatic vein revealed distinctly pulsatile patterns, which are consistent with, and even more prominent than, those in the peripheral circulation. Although the exact impact of these pulsatile signals on their respective target tissues is not yet known, recent *in vitro* studies of rat Sertoli cell function in perfused cultures indicate that Sertoli cell secretory patterns vary not only in relation to FSH concentration, but also in response to changes in the frequency and duration of FSH pulses (20).

In summary, we investigated the nature of spontaneous testosterone and FSH rhythms in the circulation of normal men using extended venous sampling in con-

junction with new statistically based analytical methods. Such approaches offer a quantitative appraisal of amplitude and frequency properties of physiologically intermittent testosterone, LH, and FSH secretion. They demonstrate a close temporal coupling between LH and FSH, and LH and testosterone release in these men.

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Erratum

(for Veldhuis, et al: "Operating characteristics of the male hypothalamo-pituitary-gonadal axis..." *J Clin Endo Metab* 65:929, 0987.)

In the above article, the times given for the acrophases for testosterone, LH and FSH were incorrect as written, and should read (clock times):

0429 (+ 80 min) for testosterone;
 0448 (+ 27 min) for LH;
 and 0304 (+ 33 min) for FSH.