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***AutoDecon*, a Deconvolution Algorithm for Identification and Characterization of Luteinizing Hormone Secretory Bursts: Description and Validation Using Synthetic Data**

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Abstract

Hormone signaling is often pulsatile, and multi-parameter deconvolution procedures have long been utilized to identify and characterize secretory events. However, the existing programs have serious limitations, including the subjective nature of initial peak selection, lack of statistical verification of presumed bursts, and user-unfriendliness of the application. Here, we describe a novel deconvolution program, *AutoDecon*, which addresses these concerns. We validate *AutoDecon* for application to serum luteinizing hormone (LH) concentration time series using synthetic data mimicking real data from normal women and then comparing the performance of *AutoDecon* to the performance of the widely-employed hormone pulsatility analysis program *Cluster*. The sensitivity of *AutoDecon* is higher than *Cluster*: ~96% vs. ~80% ($p = 0.001$). However, *Cluster* had a lower false-positive detection rate than *AutoDecon*: 6% vs 1%, $p = 0.001$. Further analysis demonstrated that the pulsatility parameters recovered by *AutoDecon* were indistinguishable from those characterizing the synthetic data and sampling at 5- or 10-minute intervals was optimal for maximizing the sensitivity rates for LH. Accordingly, *AutoDecon* presents a viable non-subjective alternative to previous pulse detection algorithms for the analysis of LH data. It is applicable to other pulsatile hormone-concentration time series and many other pulsatile phenomena. The software is free and downloadable at <http://mljohnson.pharm.virginia.edu/home.html>.

Keywords

Hormone oscillations; pulsatility; luteinizing hormone; deconvolution analysis; software

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INTRODUCTION

It has long been established that signaling within many endocrine systems is pulsatile. As a result, considerable effort has been dedicated to the development of quantitative methods to objectively identify and characterize such pulsatile signals [1,2]. As an example, it has been long recognized that the pulsatile release pattern of gonadotropin releasing hormone (GnRH) is crucial to the effectiveness of this hormone [3]. The luteinizing hormone (LH) dynamics are directed by the GnRH, which is synthesized and then released by hypothalamic neurons in a pulsatile manner and stimulates the pulsatile release of the gonadotropins LH and follicle stimulating hormone (FSH) from cells within the anterior pituitary gland [3]. From a physiologic perspective, the seminal work of Knobil and his colleagues in the Rhesus monkey documented that GnRH had to be administered in a pulsatile fashion to effect the secretion of LH and FSH by gonadotropes within the pituitary [3]. Direct measurement of GnRH within the hypothalamic-hypophyseal portal circulation simultaneously with measurement of LH in the pituitary effluent of the rat [4,5] and sheep [5,6] subsequently provided direct confirmation of the one-to-one coupling of GnRH and LH secretion. More recently, studies by Marshall and colleagues have demonstrated a role for GnRH pulse frequency in controlling gene expression of both LH and FSH [7]. Taken as a group, these studies clearly demonstrate that the pulsatile nature of GnRH and gonadotropin release is vital to reproduction and not simply an epiphenomenon.

Given the close coupling between bursts of GnRH and those of the gonadotropins [4–9]—and the fact that hypothalamic GnRH cannot for all practical purposes be measured in the human—the identification and characterization of such LH and FSH pulses has enjoyed considerable investigative interest; i.e., it has been argued that if the frequency of gonadotropin burst activity can be elucidated, then inferences about the frequency of GnRH burst activity can be made in the absence of direct measurement of this hypothalamic hormone.

Early computer assisted attempts to identify hormone pulses focused on the identification and subsequent characterization of “perturbations” within hormone concentration time series [1, 10–16]. Some of these early and influential pulse analysis methods such as *Cluster* [10], *Detect* [11], *Santen and Bardin* [12], *Ultra* [13], *Pulsar* [14], *Cycle Detector* [15], and the *Santen and Bardin* “2-by-2” method [16], are reviewed elsewhere [2]. None provided detailed information about the specifics of the secretory events and their subsequent elimination – two important phenomena which together direct the perturbations observed in the data. More recently, deconvolution methods have been used to identify and characterize hormone secretory burst activity [17–18]. Although these techniques represent a major advance in our ability to model pulsatile hormonal data, they still contain significant limitations, including the subjective nature of the choice of candidate secretory bursts, lack of robust statistical verification of resolved secretory bursts, and the user-unfriendly interface of the programs.

To address these concerns, we have developed a novel fully automated deconvolution procedure *AutoDecon* [19,20]. In the current study we describe *AutoDecon* and validate its performance for application to serum LH concentration time series. Coupled GnRH and LH data from sheep [8,9] were utilized here as an example of the performance algorithm. The validation was performed on synthetic data created by mimicking real data obtained from normal women under four conditions: during the (1) early follicular (EF), (2) late follicular (LF), and (3) mid-luteal (ML) phases of the menstrual cycle and (4) in women who had completed the menopausal transition (post-menopausal; PM). These four groups were selected to provide a considerable range of values within the physiologic range for the various LH secretory burst characteristics. We also sought to determine whether for this specific hormone, sampling at 5- or 10-minute intervals would prove optimal for maximizing true positive detection rates as compared to 15- and 20-minute sampling. The synthetic data was also used

to compare the performance of *AutoDecon* to the performance of the currently widely-employed hormone pulsatility analysis program *Cluster* [10].

METHODS

Building upon our original multi-parameter deconvolution approach, *Deconv* [17], *AutoDecon* automates the following mathematical deconvolution procedure.

A. Review of the deconvolution method

Equation 1 presents the mathematical form of the convolution integral used in the original deconvolution approach

$$C(t) = \int_0^t S(z)E(t-z)dz + C(0)E(t) \quad (1)$$

where $C(t)$ is the concentration of hormone in the serum at any time, t ; $E(t-z)$ is the hormone elimination function at time $t-z$ after the secretion event and can be described reasonably as either a one- or two-component model; and $S(z)$ is the secretory rate as a function of time. In the original formulation the secretion rate is modeled by Eq. 2,

$$S(t) = S_0 + \sum_k e^{\left[\log H_k - \frac{1}{2} \left(\frac{t - PP_k}{SecretionSD} \right)^2 \right]}, \quad (2)$$

where the secretion rate is assumed to be the sum of Gaussian shaped events which occur at times PP_k have differing heights (amplitudes), H_k , and the same standard deviation, $SecretionSD$. Note that the amplitudes of secretion events are expressed as the base ten logarithm of the height, $\log H_k$, in order to constrain the heights to physiologically relevant positive values. The positive constant S_0 is the basal secretion.

The choice of a mathematical model with Gaussian shaped secretion events, as in Eq. 2, is an approximation based upon the 10-minute time resolution of the human LH data. For example, the 30 second sampled GnRH time series in sheep shown in Fig. 2 of Moenter et al [8] demonstrates that the GnRH secretion events are slightly skewed with a full-width at half-height of approximately 3.5 minutes. Thus, a reasonable approximation of the pulse shape of these GnRH data is a Gaussian with a standard deviation of approximately 1.5 minutes. However, when these data are binned into 10-minute sampling intervals as is the case with our human LH time series, the GnRH events appear as a single elevated datum point with the adjoining data points essentially falling to the baseline. Consequently, any mathematical form for the secretion events which effectively descends back to baseline on either side of a single elevated value is a sound approximation for data with a 10-minute sampling interval.

The hormone elimination function, $E(t-z)$, is assumed to follow a single-compartment pharmacokinetic model (Eq. 3)

$$E(t-z) = e^{-\frac{\ln 2(t-z)}{HL}} \quad (3)$$

where HL is the one-component elimination half-life, or a two-compartment (Eq. 4) model,

$$E(t-z) = (1 - f_2)e^{-\frac{\ln 2(t-z)}{HL_1}} + f_2e^{-\frac{\ln 2(t-z)}{HL_2}} \quad (4)$$

where HL_1 and HL_2 are the elimination half-lives and f_2 is the amplitude fraction of the second component.

$C(0)$ in Eq. 1 is the concentration of the hormone immediately before the first datum point, i.e. at time equal to zero. This model is rigorously correct for Eq. 3, the single-compartment pharmacokinetic elimination model. However, for the two-compartment elimination model, Eq. 4, it is only correct under the assumption that all of the concentration at time $t = 0$ is the result of an instantaneous secretion event which occurs at this initial moment. This assumption is required because little information pertaining to the secretion and elimination prior to the first datum point is contained within the data.

B. The *AutoDecon* Algorithm

AutoDecon utilizes a combination of three modules: a parameter *fitting* module, an *insertion* module, and a *triage* module.

B.1. The *Fitting* Module—The *fitting* module in *AutoDecon* optimizes selected secretory parameters (basal secretion, peak positions and amplitudes, half-life, and/or *SecretionSD*). To this end, it performs weighted nonlinear least-squares parameter estimations by the Nelder-Mead Simplex algorithm [21–23]. It fits Eqs. 1–4 to the data by determining the parameters of the secretion function, Eq. 2, and the elimination function, Eq. 3 or 4, so that they have the highest probability of being correct. This module is essentially the original *Deconv* algorithm [17] with the exception that the Nelder-Mead Simplex parameter estimation algorithm is used instead of the damped Gauss-Newton algorithm which was employed by *Deconv*.

Our implementation of the Nelder-Mead Simplex algorithm is based upon the *Amoeba* routine [22] which was modified such that convergence is assumed when both the variance-of-fit and the individual parameter values do not change by more than 2×10^{-5} or when 15,000 iterations have occurred. The default control parameters for the *Amoeba* routine [22] were utilized: i.e., $\rho = 1$, $\chi = 2$, $\gamma = 1/2$, $\sigma = 1/2$.

The Nelder-Mead algorithm is commonly employed in science and engineering. This algorithm is typically slower to converge than some other parameter estimation algorithms such as a damped Gauss-Newton [17] and the Levenberg-Marquardt algorithms [24]. However, in our experience the Nelder-Mead simplex method appears to be less sensitive to the choice of initial parameter estimates. Furthermore, the Nelder-Mead algorithm does not require derivatives, thus making it easier to implement. It also allows for minimization criteria other than least-squares, such as the least sum of absolute values. It should be noted that the Nelder-Mead simplex algorithm has not been mathematically proven to converge in all cases [25,26].

B.2. The *Insertion* Module—The *insertion* module inserts a presumed secretion event at the time of the maximal value of the following Probable Position Index, PPI:

$$PPI(z) = \begin{cases} -\frac{\partial \text{Variance-of-Fit}}{\partial H_z} & \text{if } \frac{\partial \text{Variance-of-Fit}}{\partial H_z} < 0 \\ 0 & \text{if } \frac{\partial \text{Variance-of-Fit}}{\partial H_z} \geq 0 \end{cases} \quad (5)$$

The parameter H_z in Eq. 5 is the amplitude of a presumed secretion event at time z . The index function $PPI(z)$ will have a maximum at the time where the insertion of a secretion peak will result in the largest decrease in the variance-of-fit. It is important to note that the partial derivative of the variance-of-fit that appears in Eq. 5 can be evaluated directly. Specifically, the definition of variance-of-fit is

$$\text{Variance - of - Fit} = \sum_i \left(\frac{Y_i - C(t_i; H_z)}{SEM_i} \right)^2 \quad (6)$$

where the changed notation (the modification of $C(t)$ to $C(t;H)$) reflects the addition to Eq. 2 of a term corresponding to a new pulse of height H_z at time z . Therefore, the partial derivative in Eq. 5 can be calculated as shown in Eq. 7 where the summation is over all data points.

$$\frac{\partial \text{Variance - of - fit}}{\partial H_z} = \sum_i \left[\frac{2}{SEM_i^2} (Y_i - C(t_i;H_z)) \frac{\partial C(t_i;H_z)}{\partial H_z} \right] \quad (7)$$

In Eq. 7, Y_i corresponds to the experimentally observed hormone-concentration and SEM_i to the estimated uncertainty (i.e., the measurement error) at the i^{th} datum point. The standard error of the mean of a small number of replicate measurements does not provide a reliable measure of the estimated uncertainty (i.e., the measurement error) of the i^{th} datum point. Therefore, when the number of replicates is less than ~20, the SEM_i should be estimated as shown in Eq. 8,

$$SEM_i = \sqrt{\left(\frac{\text{assay sensitivity}}{2}\right)^2 + \left(\text{concentration}_i \frac{CV}{100}\right)^2} \quad (8)$$

where *assay sensitivity* is twice the standard deviation of a large number of replicates of a control sample which has a known hormone concentration of zero and *CV* is the intra-assay coefficient of variation of the assay at the optimal range of the assay. The *assay sensitivity* is commonly called the *Minimal Detectable Concentration*.

B.3. The Triage Module—The *trriage* module performs a statistical test to decide if a presumed secretion event should be removed. This test requires two weighted nonlinear least-squares parameter estimations (an application of the *fitting* module), one with the presumed peak present and one with the presumed peak removed. The ratio of the variance-of-fit resulting from these two parameter estimations is given by an F statistic which is a function of the probability, P , that the presumed secretion event does not exist, as in Eq. 9.

$$\frac{\text{Variance - of - Fit}_{\text{removed}}}{\text{Variance - of - Fit}_{\text{present}}} = 1 + \frac{2}{ndf} F \text{ Statistic } (2, ndf, 1 - P) \quad (9)$$

This is the F-test for an additional term [27] where the additional term is the presumed secretion event. The 2's in Eq. 9 reflect the fact that an additional secretion event increases the number of parameters being estimated by 2 (the location and the logarithmic amplitude of the new secretion event). The number of degrees of freedom, *ndf*, is the number of data points minus the total number of parameters being estimated when the secretion event is present. Typically, a probability level of $P = 0.05$ is used and the (user-defined) choice of the P -value will determine the performance characteristics of the *AutoDecon* algorithm.

Each cycle of the *trriage* module performs this test for every secretion event in an order determined by the size of the amplitude of the event, from the smallest to largest. If a secretion event is found to be non-significant it is removed and the *trriage* module is restarted (i.e., a new cycle is initiated). Thus, the *trriage* module continues until there are no non-significant secretion events to be removed. If m is the current number of secretion events, each cycle of the *trriage* module performs at most $m + 1$ weighted nonlinear least-squares parameter estimations (each time invoking the *fitting* module).

B.4. Combination of the Insertion, Triage, and Fitting Modules—The *AutoDecon* algorithm combines the three modules (i.e. the *fitting* module, the *insertion* module, and the *trriage* module) in the following order:

1. By default it is assumed that no presumptive secretion peaks exist. However, the algorithm can be initialized with any number of user defined presumptive secretion peaks.
2. The *fitting* module estimates the basal secretion (S_0 in Eq. 2) assuming that no secretion events are present. Steps 2–5 are performed with user-supplied physiologically reasonable initial values for the elimination half-life (*HL*), *SecretionSD*, and $C(0)$ while secretion event positions and amplitudes are simultaneously optimized by the *fitting* module.
3. The *triage* module tests the significance of all of the current and presumptive secretion events and removes all non-significant secretion events.
4. The *insertion* module adds a presumptive secretion event and then the *fitting* module adjusts the positions and amplitudes of all the current and presumptive secretion events present.
5. The *triage* module tests the significance of the current and presumptive secretion events and removes any non-significant events.
6. Steps 4 and 5 are repeated until the number of secretion events does not increase.
7. The *fitting* module estimates *HL*, *SecretionSD*, and $C(0)$. For this, and for all subsequent steps, the *fitting* module simultaneously optimizes all model parameters (event positions, logarithmic heights, *SecretionSD*, and $C(0)$).
8. The *triage* module then tests the significance of the current and presumptive secretion events and removes the non-significant events.
9. The *insertion* module adds a presumptive secretion event, as in step 4.
10. The *triage* module then tests the significance of all of the current presumptive secretion events and removes the non-significant ones while simultaneously estimating all model parameters.
11. Steps 9 and 10 are repeated until the number of presumptive secretion events does not increase.
12. Finally the residuals (i.e., the differences between the data points and the fitted curve) are examined for trends which might indicate fitting problems. Specifically, if the first datum point is a negative outlier (i.e., the datum point is significantly lower than expected; see the next section “*Outliers*”) or the last datum point is a positive outlier (i.e., the datum point is significantly higher than anticipated) then it is possible that the algorithm failed to identify a partial secretion event at either the start or end of the time series, respectively. If only a portion of the secretion event is present within the data time series then the *AutoDecon* algorithm might not resolve this partial event and thus an outlier may be present. When this is observed the offending datum point is temporarily removed and the entire *AutoDecon* algorithm is repeated with the current values as the initialization.

The net result of this twelve-step algorithm (i.e. *AutoDecon*) is a non-subjective procedure that simultaneously locates and estimates the properties of the secretion events while determining the basal secretion and elimination half-life.

B.5. Outliers—The detection of outliers is a complex statistical issue with a profuse number of divergent criteria depending upon the specific application. An outlier in the present work is defined as a datum point at which the absolute value of the Z score of the particular residual, Z_k , is greater than 4. These Z scores are calculated as the particular, k^{th} , residual divided by the

root mean squared average value of all of the other residuals as shown in Eq. 10 where N is the total number of residuals.

$$Z_k = \frac{R_k}{\sqrt{\frac{\sum_{i \neq k} R_i^2}{N-1}}}, \quad i \neq k \quad (10)$$

B.6. Initial Parameter Values—The numerical procedures outlined here are based upon a series of weighted nonlinear least-squares parameter estimations. All weighted non-linear least-squares fitting procedures, and specifically *AutoDecon*, require initial approximations of the parameter values that are to be estimated. The *AutoDecon* algorithm requires only four initial parameters. The initial concentration ($C(0)$ in Eq. 1) is typically set to zero. The basal secretion rate (S_0 in Eq. 2) is usually initialized to zero. The standard deviation of the Gaussian shaped secretion events (*SecretionSD* in Eq. 2) is generally initialized as $\frac{1}{2}$ of the data sampling interval. Thus, for 10-minute sampled data the *SecretionSD* will be initialized to 5 minutes. Typical literature values are used for the initial elimination parameters (HL in Eq. 3 and HL_1 , HL_2 , f_2 in Eq. 4). For human LH a single component elimination half-life of 60–80 minutes is normally utilized.

Once the algorithm has been executed, the initial parameter values are the numerical results from the previous step. The *insertion* module inserts presumptive secretion events at a time corresponding to the current maximum of the Probable Position Index (PPI in Eq. 5). The initial area, i.e. mass, of this next presumptive secretion event is evaluated as the difference between the experimentally observed concentration and the current calculated concentration at the time of the presumptive event time.

C. Cluster Algorithm

Cluster, developed by Veldhuis and Johnson [10], identifies hormone nadirs by locating all the statistically significant decreases in the data which are then followed by statistically significant increases. Any region in the data containing a decrease followed by an increase is termed a “nadir” and the regions between nadirs are identified as concentration “peaks” or “pulses”. It must be emphasized, however, that neither *Cluster*, nor any of the other standard pulse detection algorithms, separates a hormone “pulse” into its component parts: i.e., the secretory and elimination components.

D. Validation of *AutoDecon*

D.1. Collection of Serum LH Concentration Time Series from Normal Women—

Twenty-four healthy young (mean age 27 years) premenopausal women and eight healthy older (mean age 59 years) postmenopausal women were studied at the University of Virginia Clinical Research Center. All women had normal thyroid function tests, serum prolactin and serum testosterone concentrations. The premenopausal women were studied in the early follicular (EF) phase (days 2–5 of menses; $n = 8$), the late follicular phase (1–4 days prior to ovulation; $n = 8$), and the mid-luteal phase (days 5–8 after ovulation; $n = 8$) of the menstrual cycle. In the premenopausal group, ovulation was presumed to have occurred during the study cycle when the development of a normal preovulatory follicle was followed by its disappearance, as documented with transvaginal ovarian ultrasonography. All of the older women had experienced menopause greater than one year prior to the study and were admitted only after discontinuing any hormone replacement at least six weeks earlier. All women reviewed and signed consent forms approved by the University of Virginia Human Investigation Committee.

D.2. Protocol—One hour after placement of an indwelling heparin cannula into a forearm vein, blood samples were obtained beginning at 0800 hours and subsequently at 10 minute intervals for 24-hours ($n = 145$ points/subject). On the day of sampling, subjects were provided breakfast, lunch and dinner. Water was allowed *ad libitum*. Daytime naps were prohibited while subjects were encouraged to sleep at night.

D.3. Assays—Serum LH was measured using an immunoradiometric assay (IRMA; Nichols Institute Diagnostics, San Juan Capistrano, CA) [28]. The sensitivity of the IRMA is 0.1 mIU/mL and the intra- and inter-assay coefficients of variation are 4.4% and 7.1%, respectively.

D.4. Creation of Synthetic Serum LH Concentration Time Series—Groups of synthetic serum LH concentration time series were generated and utilized for the testing of the *AutoDecon* algorithm. These synthetic time series were created to mimic the experimentally observed serum LH concentration time series. They were produced so that the locations and sizes of the synthetic secretion events as well as the half-lives, and the *SecretionSD* were known. Thus, when these synthetic time series were analyzed with *AutoDecon* and *Cluster*, comparisons between the correct answers and those produced by the algorithms could be made. The key to the utility of this approach is the accuracy with which the synthetic LH hormone-concentration time series mimic the experimentally observed LH hormone concentration time series.

Typical parameters for early follicular, late follicular, mid-luteal, and post-menopausal LH secretion are shown in Table 1. The common method for constructing synthetic concentration time series involves simulating a series of secretion events based upon the average values as summarized in Table 1 [29,30]. This approach, however, contains the flaw that it incorrectly assumes the parameters shown in Table 1 are not dependent upon one another (i.e. it assumes that they are orthogonal to each other). For example, the size of a secretion event might be related to the size of the previous secretion event, the time elapsed since the previous event, the elimination half-life, or even may contain a circadian rhythm. In addition, the common methods [29,30] assume that the distributions of interpulse intervals and peak areas follow a normal distribution while these are empirically closer to a log normal distribution. Therefore, the approach currently utilized for the simulation of the synthetic series includes a description of the co-variance (i.e. the interrelationships that exist between the values shown in Table 1) and assumes log normal distributions.

The first step in the simulation process was to analyze a group of experimentally observed LH concentration time series, with several different algorithms (i.e., *AutoDecon*, *Pulse2* and *Pulse4* as in [1,2,10,17,18–20,29]), in order to create a consensus analysis for each of the actual hormone-concentration time series. The resultant analyses were then used to create the values presented in Table 1, as well as the simulations outlined below. Next, a log normal distribution was constructed for the elimination half-life, S_0 (the basal secretion), and the *SecretionSD*. It was assumed that each observed interval between secretion events and each secretion event area might be dependent upon 1) the previous secretion event interval, 2) the previous event area, 3) the basal secretion, 4) the *SecretionSD*, 5) the half-life, and 6) a circadian or other 24-hour rhythm. Thus, a multiple linear regression was utilized to correct the log normal distribution of the event intervals and event areas for the effects of these six potential confounders. The circadian rhythm was formulated as the linear sum of a 24-hour sine wave and a 24-hour cosine wave. Our earlier approach [29] assumed that the distributions were normal instead of the log-normal distributions utilized here.

For each of the simulated hormone concentration time series unique values of the elimination half-life, S_0 , and the *SecretionSD* were randomly selected using a random number generator and the observed log normal distributions. Next, the time of the first secretion event was set to

minus 1440 minutes before the first desired time point. The simulation of each time series was initiated 1440 minutes prior to the first desired time point and continued on for 720 minutes past the last desired time value in order to eliminate the possibility of “edge effects” and correctly simulate any partial secretion events at the start and end of the time series. The mass of the first secretion event was simulated from the observed log normal distribution of event masses. The increase in the hormone concentration due to a Gaussian shaped secretion event is related to the area of the secretion event, i.e. it is proportional to the product of the secretion event height and the event width. Thus, the event area (i.e. the mass or amount of hormone) was used for these simulations. As soon as the first interval and mass were defined, the subsequent intervals and masses were evaluated by randomly selecting from the corresponding log-normal distributions. The process of randomly selecting a value from an observed log normal distribution is complex. The multiple linear regressions corrected log normal distributions of interpulse intervals and burst masses were created from the distributions observed within the experimental data. To select a value from this corrected distribution, a log normally distributed pseudo-random number with the mean and standard deviation of the corrected distribution was generated. Next, this was modified by the potential confounders as obtained from the multiple linear regressions. This is the logarithm of the desired value, i.e., the next interpulse interval or the next burst mass. The net result is to generate pairs of simulated secretion intervals and masses that include potential confounding effects of 1) the previous secretion event interval, 2) the previous event area, 3) the basal secretion, 4) the *SecretionSD*, 5) the half-life, and 6) a 24-hour rhythm. If these confounders are not significant and the original distributions are normal (i.e. not log normal) then the simulation procedure is the same as the previously published simulation methods.

One hundred synthetic data sets (i.e., hormone concentration time series) were generated for each of the subgroups (i.e., early follicular, mid-luteal, late follicular, and the post-menopausal groups). Normally distributed pseudo-random experimental observational error based upon the assay minimal detectable concentration and coefficient of variation was added to the simulated data (see Table I and Eq. 8).

The experimental data from which the 5-minute simulated data sets were constructed was sampled at 10-minute intervals. Thus, the synthetic 5-minute profiles represent an interpolation based upon the assumption that the 10-minute sampled results (i.e. numbers of secretion events, etc.) are representative of what would be observed if the real data had been sampled at 5-minute intervals.

E. The *in vivo* Sheep Data

The coupled GnRH and LH time series in sheep were either provided by Sue Moenter and Fred Karsch [8] or digitized from the published figures [9] by the Pic-to-data software (<http://mljohnson.pharm.virginia.edu/pictodata-home.html>).

F. Statistical Analysis

Analysis of variance was used to compare the number of peaks known to be present in the synthetic LH concentration time series against the number of peaks determined from each corresponding data set by analysis with *AutoDecon* and also by *Cluster*. *Post hoc* testing was done to determine whether there was a significant difference in the number determined by either method as compared to the known number of peaks in the synthetic data. All tests were performed using Systat 11. Performance characteristics [29,30] were determined for each analytical method and for each synthetic LH concentration time series by comparing the known locations for each simulated secretion event, i.e. peak, in the synthetic data and allowing a window of plus or minus 10 minutes for identifying the coincident peaks. True-Positives are apparent secretion events found by the algorithm that closely correspond in time to actual

secretion events. False-Positives (Type I errors) are apparent secretion events found by the algorithm that do not closely correspond in time to actual secretion events. False-Negatives (Type II errors) are the simulated secretion events that are not located by the algorithm. Sensitivity is the percentage of the actual simulated secretion events which are located by the algorithm. The sensitivity and false-positive rates were compared using a two-factor Analysis of Variance with replication. The factors in the analysis were analytical method (*AutoDecon*, *Cluster*) and reproductive cycle status (EF, LF, ML, PM).

G. Concordant Secretion Events

A Monte-Carlo approach (31) was utilized to determine whether the concordance of peak positions was statistically significant or whether this was simply a consequence of a random position of unrelated secretion events. Specifically, we evaluate the probability that j coincidences (i.e., concordances) will occur based upon a random positioning of the distinct events within two time series with n and m distinct events. These probabilities are dependent upon the size of the specific time window employed for the definition of coincidence. We generated 100,000 pairs of time series with the n and m distinct randomly timed events, respectively. The probability distribution for the expected number of concordances was tabulated from the apparent coincident events within these pairs of time series.

RESULTS

An Example Analysis of *in vivo* LH in Sheep

Figure 1 presents an example of the use of the *AutoDecon* algorithm in which we analyzed the ovariectomized ewe data presented in the upper panel of Fig. 1 from Goodman et al [9]. These data were simultaneously and continuously sampled at 10-minute intervals, LH from the jugular vein and GnRH from the portal plasma. The vertical lines in the upper panel of Fig. 1 correspond to the experimentally observed LH time series with the estimated uncertainties evaluated from the reported assay characteristics [9] according to Eq. 8. The symbols spanning the top of the upper panel denote the locations of the 16 secretion events located by the *Cluster* algorithm [10]. The solid connecting line in the upper panel is the calculated curve based upon the 16.5 minute elimination half-life and the 19 secretion events shown in the LH Secretion panel of the present Fig. 1 that the *AutoDecon* algorithm reported for this data. The second panel from the top in Fig. 1 is the measurement uncertainty weighted differences between the data points and the calculated curve in the upper panel. The lower panel presents the corresponding experimentally observed GnRH pattern. The box in the lower panel marks the period when the ewe received a naloxone treatment.

The *AutoDecon* algorithm identified the eighth LH secretion event which occurred at 450 minutes to be a pair of secretion events that are separated in time by 15 minutes. This is a consequence of the corresponding peak within the LH time series being slightly broader than the other events within the time series. The *Cluster* algorithm did not identify this event as a doublet. This doublet is not obvious within the GnRH time series. However, given the 10-minute sampling of the GnRH data it is impossible to ascertain if this is actually one or two peaks.

From the lower panel of Fig. 1 it is apparent that there are at least 18 GnRH events within this data set. The *Cluster* algorithm failed to locate two of the corresponding LH events. The symbols across the top of the upper panel indicate the locations of the events found by *Cluster*. The *AutoDecon* algorithm located all 18 of these events but split the 8th event into two almost superimposed secretion events. There is also a very small event in the GnRH pattern at approximately 170 minutes which might correspond to a 19th event. Neither analysis algorithm located a corresponding event within the LH time series.

Both algorithms performed very well at locating the temporal positions of the secretion events within the LH time series which corresponded to concentration events within the corresponding GnRH time series to within the 10-minute data sampling window. Specifically, if no temporal relationship existed between the *Cluster* identified LH events and the GnRH events then the probability is less than 0.05 of 8 or more concordant events to within ± 10 minutes. The corresponding probability for all 16 events being concordant is less than 10^{-4} . The probability for the 18 concordant events found by *AutoDecon* is also less than 10^{-4} .

The *AutoDecon* algorithm also provides estimates of the sizes of the secretion events within the LH time series. The mechanism by which GnRH concentration stimulates the pulsatile secretion of LH is undoubtedly a complex multiple step process. However, the cross correlation between the areas of the 18 GnRH concentration events (excluding the one at 170 minutes) and the areas of the corresponding LH secretions events identified by *AutoDecon* is 0.72 when the doublet at 450 minutes is included and 0.70 when the doublet is excluded. Both of these are significant at the 0.01 level.

Figure 1 illustrates some of the difficulties of using actual experimental data to test various analysis algorithms. The time resolution of the data makes it impossible to ascertain whether the doublet at 450 minutes reported by the *AutoDecon* is actually two nearly superimposed secretion events or an incorrectly identified doublet. Thus, the corresponding *false-positive* rate for *AutoDecon* for this data set is either 0.0 or 5.3%, respectively. Similarly, is the 19th maximum within the GnRH time series which occurs at 170 minutes an actual GnRH event? If it is real then the Sensitivity of the *Cluster* algorithm for these data is 84% while the *Sensitivity* would be 89% if it were not an actual event. When simulated data is employed to test the algorithms, the locations and sizes of the secretion events are known *a priori*. Clearly, using the experimental GnRH time series as an indicator of the exact locations of the expected LH secretion events requires the investigator to make arbitrary decision about which peaks within the GnRH time series are to be considered as actual events.

Additionally, the elimination half-life for this sheep LH data determined by *AutoDecon* is approximately 16.5 minutes while it is 60–90 minutes for humans (see Table I). This corresponds to less than two data points per half-life in sheep and more than six data points per half-life for humans. Thus, the performance characteristics based upon the sheep data will not necessarily represent those expected with human data.

It is also impossible to manipulate these data to address questions about different portions of the human menstrual cycle. Similarly it is impossible to change the experimental sampling intervals, noise levels, pulse frequencies, etc.

Validation With Synthetic Data

Our approach to validating the *AutoDecon* pulse detection algorithms involves the creation of synthetic data sets where the number, position and characteristics of the pulses are “known” *a priori*. In Fig. 2, representative 24-hour serum LH concentration time series obtained from normal pre- and post-menopausal women are shown in the left panel (32). Depicted in the right panel are representative synthetic LH concentration time series that were modeled based on the experimental data. To assess the ability of *AutoDecon* to identify and characterize LH secretory bursts within the synthetic data, 100 simulated data sets mimicking hormone-concentration time series from EF, LF, ML and PM women were analyzed and the results compared to those obtained with *Cluster*.

Identification of LH Secretory Bursts/Pulses by *AutoDecon* and *Cluster*

The mean ($\pm$ SEM) numbers of LH secretory bursts known to be present in the synthetic data series, together with those recovered by analysis with *AutoDecon* and *Cluster* are shown in Table 2. Of importance is that for all groups (EF, LF, ML and PM), the number of bursts identified with *AutoDecon* corresponded closely with the numbers known to characterize the synthetic data series. However, *Cluster* identified approximately 15–25% fewer ($p < 0.001$) pulses than were known to be present in the synthetic data.

To assess whether the specific pulses identified with *AutoDecon* and *Cluster* were identical to those created in the synthetic data series, the percent sensitivity and false-positive rates of detection were appraised and are shown in Fig. 3. The sensitivity detection rate (%) with *AutoDecon* was higher than that for *Cluster* for the EF (97.0 vs. 81.6; $P < 0.001$), LF (97.8 vs. 82.9; $P < 0.001$), ML (99.3 vs. 74.5; $P < 0.001$), and PM (93.8 vs. 80.7; $P < 0.001$) simulations (see Table 3). In contrast, analysis with *Cluster* resulted in lower false-positive rates than did *AutoDecon* for the EF (6.6 vs. 0.2; $P < 0.001$), LF (6.5 vs. 0.3; $P < 0.001$), ML (9.5 vs. 3.1; $P < 0.001$) and PM (6.3 vs. 0.3; $P < 0.001$) simulations.

Characterization of LH Secretory Burst Properties by *AutoDecon*

Several properties known to characterize the synthetic LH secretory bursts are shown in Table 4 together with those recovered by analysis with *AutoDecon*. These findings demonstrate that the LH burst mass, half-duration, and estimated half-life which are known to define the synthetic bursts and those recovered by *AutoDecon* were statistically indistinguishable.

Impact of Sampling Frequency on Identification of LH Secretory Bursts

The impact of sampling frequency on the detection of LH secretory burst detection sensitivity rates in the simulated data is shown in Fig. 4. Notably, sampling at 5- and 10-minute intervals resulted in similar rates of secretory burst detection for each of the four groups appraised; i.e., 99 and 97% for the EF (5- and 10-minute sampling intervals, respectively); 98 and 98% for the LF; 99 and 99% for the ML; and 98 and 94% for the PM groups. However, sampling at 15- and 20-minute intervals resulted in sensitivity recovery rates of 87 and 65% for the EF; 83 and 58% for the LF; 89 and 84% for the ML; and 66 and 36% for the PM groups.

DISCUSSION

Previous implementations of the deconvolution procedure applied to hormone-concentration time series [17] have significant limitations, including the subjective process through which prospective secretory bursts are chosen as initial “guesses”, the lack of rigorous statistical methods with which to define what is or is not a secretory burst, and the overall difficulty in using the software—i.e., its inherent unfriendly nature for users. In an effort to utilize the power of deconvolution for hormone secretion analysis and address these concerns, we have recently developed a deconvolution method known as *AutoDecon* [19,20]. This algorithm utilizes the fundamental principles and mathematics which subserve *Deconv*, but the process is fully automated and utilizes a statistically based algorithm to test the significance of secretion events. Thus, *AutoDecon* eliminates the subjective nature of the previous use of *Deconv*.

An approach that has proven useful in validating novel pulse detection algorithms involves the creation of synthetic data sets where the number, position and characteristics of the pulses are “known”. In the current study we chose to model the synthetic files based upon “real” data which were obtained under several physiologic circumstances: different phases of the menstrual cycle in premenopausal women and in the setting of “hypergonadotropic-hypogonadism” encountered in post-menopausal women. One would predict differences in not only secretory burst frequency but in certain burst characteristics and in elimination functions

within these groups. These expected characteristics were incorporated into simulations, and the *AutoDecon* and *Cluster* algorithm were applied to ascertain how well each characteristic was recovered by each algorithm.

Although *Cluster* identifies and appraises hormone pulses and not secretory bursts, we thought it useful to compare directly the ability of *Cluster* and *AutoDecon* to assess the frequency with which LH is released within pulses/bursts. Moreover, given the practical issues associated with undertaking these labor-intensive studies, we evaluated the effect of sampling frequency in order to identify an optimal protocol which provides sufficient information for analysis with *AutoDecon*.

Our results demonstrate that *AutoDecon* performs significantly better than does *Cluster* with regard to the identification of true secretory bursts (sensitivity). This analysis indicates that *AutoDecon* provides an even balance between the sensitivity rate of greater than ~95% and the false-negative rate of less than ~5%. Conversely, *Cluster* has an extremely low false-positive rate of ~1% at the expense of a very poor sensitivity rate of only ~80%. These percentages only apply to how the respective algorithms perform with the LH hormone-concentration time series which the simulated data mimic. Different percentages are expected for different hormones and/or different experimental protocols.

In terms of characterizing LH secretion burst properties such as mass and half-duration, *AutoDecon* did extremely well. Moreover, with regard to an estimation of half-life, *AutoDecon* recovered values indistinguishable from those known to be encoded within the synthetic data series. It is important to emphasize that *Cluster* does not yield information about secretory burst mass or half-duration, nor does it provide estimates of the hormone half-life.

In summary, *AutoDecon* is a user-friendly, multi-parameter deconvolution method which works well in terms of identifying and subsequently characterizing LH secretory bursts. *AutoDecon* appears to perform better than does *Cluster* in terms of recognition of true-secretory bursts, in addition to providing specific information about secretion and elimination which *Cluster* does not. Although *AutoDecon* will need to be validated for use with other endocrine systems, it holds significant promise for the analysis of a wide array of hormonal signals.

The *AutoDecon* software is an integral part of the *Pulse_XP* software which may be downloaded free of charge from <http://mljohnson.pharm.virginia.edu/home.html>.

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Figure 1.

An example of the use of the *AutoDecon* algorithm applied to the simultaneously measured LH and GnRH data is presented in the upper panel of Fig. 1 from Goodman et al [6b] for an ovariectomized ewe. These data were simultaneously sampled at 10-minute intervals; LH from the jugular vein and GnRH from the portal plasma. The upper panel of the present Fig. 1 contains the experimental observed LH time series with the estimated uncertainties evaluated from the reported assay characteristics of 0.11 ng/tube and CV of 8.7% [6b] according to Eq. 8 (vertical lines). The symbols across the top of the upper panel mark the locations of the 16 secretion events identified by the *Cluster* algorithm. The solid connecting line in the upper panel is the calculated curve based upon the 16.5 minute elimination half-life and the 19 secretion events shown in the LH secretion panel which the *AutoDecon* algorithm reported for this data. The second panel from the top is the measurement uncertainty weighted differences between the data points and the calculated curve in the upper panel. The lower panel is the experimentally observed GnRH pattern. The box in the lower panel marks the period when the ewe received a 1 mg/kg-h naloxone treatment.

LH Concentration-Time Series

Figure 2.

Representative 24-hour serum luteinizing hormone (LH) concentration profiles obtained from premenopausal (upper panels) and post-menopausal (bottom panel) subjects that were used as the basis for the simulations. For each subject, serial serum LH concentrations (mIU/mL) were measured at 10-min intervals over 24h (32). Intrasample SDs are shown as vertical lines through each data value. Different scales are used for the vertical axes because of the wide range of serum LH concentrations.

Figure 3.

Mean sensitivity (%; upper panel) and false-positive (%; lower panel) rates of secretory burst detection using *AutoDecon* (□) or *Cluster* (■). The synthetic data sets from which these bursts were recovered were modeled from human data obtained in premenopausal women during the early follicular (EF), late follicular (LF) and mid-luteal (ML) phases of the menstrual cycle and post-menopausal (PM) women. * $P < 0.001$, *AutoDecon* vs. *Cluster*.

Figure 4.

Effect of sampling intensity on the ability of *AutoDecon* to detect LH secretory bursts within synthetic data series. In the case of all four groups, the results obtained when samples were collected at 5- and 10-minute intervals were quite similar (i.e., 98–99 % and 94–99% for the 5- and 10-minute sampling intervals, respectively) to the known parameters. In contrast, sampling at 15- and 20-minute intervals was associated with the recovery of fewer secretory events (i.e., 66–89 % and 36–84 % for the 15- and 20-minute sampling intervals, respectively).

Table 1

Values of the parameters used in the creation of synthetic luteinizing hormone concentration-time series.

	Early Follicular	Late Follicular	Mid-Luteal	Post-Menopausal
Number of data points	145	145	145	145
Time between data points	10 min.	10 min.	10 min.	10 min.
Number of replicates	2	2	2	2
Interpulse interval	89.3 $\pm$ 1.079	79.8 $\pm$ 1.07	99.2 $\pm$ 1.19	73.7 $\pm$ 1.07
Log Area	0.346 $\pm$ 1.14	0.573 $\pm$ 1.12	0.215 $\pm$ 1.39	1.23 $\pm$ 1.05
SecretionSD	2.32 min.	2.498 min.	3.68 min.	3.51 min.
Single component Half-Life	69.6 min.	80.2 min.	61.4 min.	88.7 min.
MDC (I.e. sensitivity)	0.337	0.726	0.205	1.715
Noise CV	9.09%	4.94%	7.69%	1.985%

Table 2

Mean ($\pm$ SEM) number of peaks known to be present in the synthetic luteinizing hormone concentration time series and the number recovered by analysis with *AutoDecon* and *Cluster*. For each group studied (i.e., early follicular, late follicular, mid-luteal and post-menopausal), values identified with different superscripts differ significantly ($p < 0.001$) by analysis of variance with Duncan's Multiple Range test.

	Synthetic	AutoDecon	Cluster
Early Follicular	17.6 $\pm$ 0.76 ^a	18.2 $\pm$ 0.79 ^a	13.2 $\pm$ 0.18 ^b
Late Follicular	15.9 $\pm$ 0.26 ^a	16.7 $\pm$ 0.26 ^a	13.3 $\pm$ 0.19 ^b
Mid-Luteal	12.0 $\pm$ 0.35 ^a	13.1 $\pm$ 0.32 ^a	9.3 $\pm$ 0.17 ^b
Post-Menopausal	18.4 $\pm$ 0.29 ^a	18.5 $\pm$ 0.41 ^a	14.9 $\pm$ 0.20 ^b

Table 3
Performance characteristics of *AutoDecon* and *Cluster* based upon synthetic data which mimics normal human subjects at different phases of the menstrual cycle.

	AutoDecon				Cluster			
	% Sensitivity	% TP	% FP	% FN	% Sensitivity	% TP	% FP	% FN
Early Follicular	97.0	93.4	6.6	3.0	81.6	99.8	0.2	18.4
Late Follicular	97.8	93.5	6.5	2.2	82.9	99.7	0.3	17.1
Mid- Luteal	99.3	90.5	9.5	0.7	74.5	96.9	3.1	25.5
Post- Menopausal	93.8	93.7	6.3	6.2	80.7	99.7	0.3	19.3

Table 4

Mean ($\pm$ SEM) LH burst mass, burst half-duration and luteinizing hormone (LH) half-life characterizing the synthetic data series and recovered by application of *AutoDecon* to the synthetic data series. In each case, and for all groups (i.e. early follicular, late follicular, mid-luteal and post-menopausal) *AutoDecon* returned values that are statistically indistinguishable from the values known to characterize the synthetic data series ($p > 0.05$).

	LH Burst Mass (IU/L)		LH Burst Half-Duration (min)		LH Half-Life (min)	
	Synthetic	AutoDecon	Synthetic	AutoDecon	Synthetic	AutoDecon
Early Follicular	2.2 $\pm$ 0.59	2.1 $\pm$ 0.56	5.9 $\pm$ 1.3	6.5 $\pm$ 7.5	74.9 $\pm$ 2.5	71.7 $\pm$ 2.6
Late Follicular	3.7 $\pm$ 0.18	3.6 $\pm$ 0.15	6.3 $\pm$ 0.14	6.6 $\pm$ 0.16	90.2 $\pm$ 4.0	81.3 $\pm$ 2.7
Mid- Luteal	1.4 $\pm$ 0.04	1.4 $\pm$ 0.04	9.3 $\pm$ 0.28	9.2 $\pm$ 0.31	65.2 $\pm$ 1.6	64.3 $\pm$ 1.6
Post- Menopausal	7.9 $\pm$ 0.13	8.5 $\pm$ 0.20	8.2 $\pm$ 0.12	8.5 $\pm$ 2.0	94.4 $\pm$ 1.7	80.2 $\pm$ 2.9