

Parameter Estimation in a Pulsatile Hormone Secretion Model

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Abstract

This paper presents an algorithm to estimate parameters of a mathematical model of a bipartite endocrine axis. Secretion of one of the involved hormones is stimulated by the concentration of another one, called release hormone, with the latter secreted in a pulsatile manner. The hormone mechanism in question appears often in animal and human endocrine systems, *i.e.* in the regulation of testosterone in the human male. The model has been introduced elsewhere and enables the application of the theory of pulse-modulated feedback control systems to analysis of pulsatile endocrine regulation. The state-of-the art methods for hormone secretion analysis could not be applied here due to different modeling approach. Based on the mathematical machinery of constrained nonlinear least squares minimization, a parameter estimation algorithm is proposed and shown to perform well on actual biological data yielding accurate fitting of luteinizing hormone concentration profiles. The performance of the algorithm is compared with that of state-of-the art techniques and appears to be good especially in case of undersampled data.

1 Background

Hormones play an important role in living organisms acting as chemical messengers from one cell, or group of cells, to another. Hormones are the signaling elements of endocrine systems that regulate many aspects in the human body, *i.e.* metabolism and growth as well as the sexual function and the reproductive processes.

Hormone production is called secretion and performed by endocrine glands directly into the blood stream in continuous (basal) or pulsatile (non-basal) manner. The latter secretion mechanism was discovered in the second half of the 20th century, [1]. Previously, it was believed that only basal secretion produces hormones. As stressed in [2], pulsatility is now recognized as a fundamental property of the majority of hormone secretion patterns. The term pulsatile generally refers to a sudden burst occurring in the face of a relatively steady baseline process.

The amplitude, frequency and the signal form of hormone pulses can be regulated and impart physiological effects quite similarly to the mechanism of pulse-modulated feedback that is commonly found in control and communication, [3].

One common endocrine system with pulsatile hormone secretion that has been intensively studied is the testosterone regulation system in the human male. Besides of testosterone (Te), it basically includes two other hormones, namely luteinizing hormone (LH) and gonadotropin-releasing hormone (GnRH), which structure yields a simpler study case compared to other endocrine systems. Furthermore, the GnRH-LH-Te axis is essential in medicine with respect to *e.g.* treatments of prostate cancer and reproductive failure as well as development of contraceptives for men. Changes in the dynamics of this endocrine system are also related to aging and obesity, [4].

Within the human male, Te is produced in testes, while the other two hormones are secreted inside the brain. LH is produced in hypophysis and GnRH is secreted in hypothalamus. The pulsatile dynamics of GnRH secretion stimulates the secretion of LH. Further, the secretion of LH stimulates the production of Te. The concentration of Te inhibits the secretion of GnRH and LH, as explained in [4], and implies a negative feedback around the hormone axis. The closed endocrine loop exhibits sustained oscillations that correspond to self-regulation of the biological system.

The hormone concentration change rate is affected by the elimination rate and secretion rate. While hormone elimination rate is defined by the concentration of the hormone itself, the secretion rate is related to concentrations of other hormones. In the GnRH-LH-Te axis, the mathematical modeling of the secretion of LH stimulated by pulses of GnRH is typically done through the deconvolution process. The state-of-the-art software AutoDecon for quantification of pulsatile hormone secretion events [5] produces close estimates for the concentration and basal secretion of LH by applying deconvolution and pulse detection algorithms. However, the resulting characterization does not give much insight into the feedback regulation governing the closed endocrine system since the concentration data are treated as time series.

The latest trend that one can discern in biomathematics is the use of control engineering ideas for formalizing feedback patterns of hormone secretion, [6]. However, the impact of mathematical and particularly control theoretical methods on elucidating mechanisms of endocrine pulsatile regulation is still surprisingly insignificant. One plausible explanation is that control-oriented mathematical models of pulsatile regulation were lacking until recently.

An approximate mathematical formulation of pulsatile regulation in the axis GnRH-LH-Te using pulse modulated systems has been analyzed in [3]. This simple model is shown to be capable of complex dynamic behaviors including sustained periodic solutions with one or two pulses of GnRH in each period. Lack of stable periodic solutions is otherwise a main shortcoming of existing low-order hormone regulation models, *cf.* [7]. Previous deconvolution studies on high resolution LH data [8] have also demonstrated that each visible LH concentration pulse corresponds to a number of secretion events, usually one of much higher amplitude than other. Yet, bifurcations in the pulse modulated model of [3] provide the only mathematical explanation for how secondary secretion events arise.

In the present article, an algorithm to estimate parameters of a mathematical model of a bipartite endocrine axis is presented. Secretion of one of the involved hormones is stimulated by the concentration of another one, called release hormone, with the latter secreted in a pulsatile manner. Following [3], release hormone secretion events are specified by a train of weighted Dirac delta-functions produced by the pulse-modulated controller. It should be emphasized that Dirac delta-functions are used as part of mathematical machinery habitual to the area of pulse-modulated control, agree with the discontinuous nature of pulsatile regulation, and do not correspond to any modeled biological quantity.

The article is organized as follows. First the underlying model of bipartite endocrine axis with pulsatile secretion is briefly described along with two state-of-the-art hormone secretion estimation methods. Then an algorithm to estimate the parameters of this model is suggested, followed by its evaluation on simulated data to demonstrate the efficacy of the proposed approach. Further, the algorithm is tested on real hormone data representing LH concentration profiles with the results compared to those of the state-of-the-art approaches.

2 Mathematical modeling of pulsatile endocrine regulation

A simple model of basal testosterone regulation in the human male has been suggested by [9] and given rise to a class of mathematical constructs known as Smith models. Such a model can

be written as

$$\begin{aligned}\dot{R} &= f(T) - B_1(R), \\ \dot{L} &= G_1(R) - B_2(L), \\ \dot{T} &= G_2(L) - B_3(T),\end{aligned}$$

where $R(t)$, $L(t)$, and $T(t)$ represent the concentration of GnRH, LH, and Te, respectively. The elimination rates of the hormones are given by the functions $B_i, i = 1, 2, 3$, while the secretion rates are described by the functions f, G_1 , and G_2 . Typically, most of the above functions admit linear approximations, *i.e.*

$$\begin{aligned}B_i(x) &= b_i x, \quad b_i > 0 \quad i = 1, 2, 3, \\ G_i(x) &= g_i x, \quad g_i > 0 \quad i = 1, 2.\end{aligned}$$

The secretion rate of releasing hormone $f(T)$ is usually assumed to be nonlinear. Notice that the presence of a nonlinearity is essential in order to obtain sustained oscillations in the closed-loop endocrine system.

In the human, only hormone concentration but not secretion can be measured. Besides, concentration of GnRH cannot either be measured in the human due to ethical issues and has to be inferred.

2.1 Pulsatile Smith model

To model pulsatile regulation, [3] suggests to turn $f(T)$ of the Smith model into a pulse-amplitude-frequency modulation operator:

$$\frac{dx}{dt} = Ax + B\xi(t), \quad y = T(t), \tag{1}$$

where

$$A = \begin{bmatrix} -b_1 & 0 & 0 \\ g_1 & -b_2 & 0 \\ 0 & g_2 & -b_3 \end{bmatrix}, \quad B = \begin{bmatrix} 1 \\ 0 \\ 0 \end{bmatrix}, \quad x(t) = \begin{bmatrix} R(t) \\ L(t) \\ T(t) \end{bmatrix}$$

b_1 , b_2 , g_1 , and g_2 are positive parameters and

$$\xi(t) = \sum_{n=0}^{\infty} \lambda_n \delta(t - t_n)$$

with $\delta(t)$ denoting the Dirac delta-function. The weights λ_n and the firing times t_n are evaluated through nonlinear functions of T implementing the amplitude and frequency pulse modulation. The delta-functions in the equation above emphasize the discontinuous nature of pulsatile regulation and mark the positions of GnRH pulses, while the weights in front of them represent the amount of secreted GnRH at those instances. The main advantage of this description is that it lends itself to mathematical analysis of the closed loop dynamics.

2.2 Parametric and non-parametric pulsatile secretion analysis

The hormone concentration $C(t)$ with the initial condition $C(0)$ is evaluated as the convolution of the secretion rate $S(t)$ and the impulse response of the system $E(t)$ modeling the hormone elimination rate:

$$C(t) = \int_0^t S(\tau)E(t - \tau)d\tau + C(0)E(t). \quad (2)$$

The problem of estimating $S(t)$ from measurements of $C(t)$ is known as deconvolution. There are many linear deconvolution techniques available to estimate $S(t)$ from measurements of $C(t)$, *e.g.* Least Squares (LS) deconvolution, Maximum A Posteriori (MAP) deconvolution, and Wiener deconvolution, see [8] for a comparison among these. Deconvolution is generally an ill-conditioned operation and has to be augmented with some degree of *a priori* information about $S(t)$ to result in a practically useful algorithm. Deconvolution without any prior assumptions about the estimated input is called blind. If the class of inputs is somehow restricted, *e.g.* by specifying a functional basis for it, then one speaks of semi-blind deconvolution. The case when a mathematical model for $S(t)$ is specified and its parameters are estimated from measurements of $C(t)$ is usually termed as model-based deconvolution. Strictly speaking, the latter type of deconvolution is a special case of system identification since an explicit model for the system input is assumed.

There are many algorithms for hormone secretion analysis based on the deconvolution methods, *e.g.* WEN Deconvolution[8], WINSTODEC[10], AutoDecon[5]. Some more general software estimation of compartmental models such as NONMEM [11] and SAAM II [12] can as well be adopted for that purpose. In this paper, a method for estimation of parameters in (1)

is sought, especially for the case of undersampled hormone data.

Next, a brief description of WEN deconvolution and AutoDecon is presented. These two algorithms are selected as reference for the evaluation of the approach suggested in this paper.

2.2.1 WEN Deconvolution

The main problem with the linear deconvolution methods is their high noise sensitivity often resulting in biologically meaningless negative secretion estimates. A nonlinear technique developed in [8] and called WEN (White Exponential Noise) deconvolution guarantees nonnegative secretion estimates being obtained from sampled data of hormone concentration. However, *a priori* knowledge of the half-life time of the hormone in question is needed. Under variation of the half-life time values, the secretion estimate exhibits inconsistency.

Apparently, it is difficult to obtain public domain software implementing modern regularized deconvolution and WEN deconvolution presents a viable alternative to it due to the ease of programming. Since the algorithm does not assume any other property of the input signal $S(t)$ than positivity, it is instructive to compare its performance on simulated and experimental data with that of model-based deconvolution methods explicitly stipulating the dynamic secretion pattern.

2.2.2 AutoDecon

Assuming the signal form of hormone secretion enables the estimation of the hormone half-life time from concentration data. The state-of-the-art software applying such approach to evaluating pulsatile secretion dynamics is AutoDecon described in [5]. AutoDecon is further development of the widely used Cluster algorithm presented in [13] and estimates hormone half-life time, basal secretion, and initial hormone concentration. AutoDecon is downloadable for public free of charge.

The algorithm is based on the convolution model, *i.e.* (2). For a single compartment model, the elimination rate is defined as

$$E(t) = e^{-k_1 t}, \quad k_1 = \frac{2}{h_{L_1}}$$

where h_{L_1} is the hormone half life time. For an endocrine model with two compartments, it is

stipulated that

$$E(t) = (1 - f_2)e^{-k_1t} + f_2e^{-k_2t}, \quad k_2 = \frac{2}{h_{L_2}} \quad (3)$$

where h_{L_1} and h_{L_2} represent the elimination half-lives and f_2 is the magnitude fraction part of the second compartment. The hormone secretion function is then defined with following equation

$$S(t) = S_0 + \sum_k e^{\log H_k - \frac{1}{2}(\frac{t-\tau_k}{S_D})^2}.$$

The secretion rate is thus assumed to have a Gaussian form that would occur at irregularly time τ_k with different heights H_k and S_D representing the width of secretion. The basal secretion is denoted by S_0 . The choice of secretion function appears to be a matter of on-going discussion in endocrinology, as [2] would rather take Gamma distribution form to represent the pulsatile secretion. Clearly, from concentration data alone, the signal form of pulsatile secretion cannot be discerned.

The estimation algorithm of AutoDecon is composed of three modules, namely the fitting, insertion, and triage. The fitting process is based on weighted nonlinear least-squares with Nelder-Mead Simplex algorithm. The insertion module would add a presumed secretion event at the location based on a calculated value called probable position index. The triage module performs a test to specify whether the inserted secretion should be kept or removed. The whole algorithm works through a certain combination of these three modules.

Performing quite well at explaining hormone data, AutoDecon does not produce a mathematical description of endocrine systems with pulsatile regulation that lends itself to closed-loop analysis. This shortcoming is rectified in the present paper using pulsatile model (1) and a parameter estimation algorithm tailored to its specific features. Since AutoDecon, similar to the technique proposed in this paper, employs model-based deconvolution, it is selected as reference to illustrate the differences in estimates arising due to the dissimilarities in the secretion models and estimation algorithms.

2.3 Bipartite pulsatile hormone secretion model

Widely known as a powerful tool for hormone secretion analysis, the deconvolution-based methods mentioned above do not produce parameter estimates of the Smith model. Obtaining the values of these parameters would open up for deeper understanding of the control feedback in endocrine systems. For instance, all the nonlinear feedback phenomena described in [3] and

arising due to the pulsatile hormone secretion are parameter-dependent and need corresponding parameter estimation techniques to be applied in practice.

As mentioned above, there is no general agreement on the true form of hormone pulsatile secretion. Release hormones (RH) have short half-life times and it is therefore reasonable, at low sampling frequencies, to model the secretion of such a hormone as an instantaneous event, *e.g.* by means of a Dirac delta-function. This modeling approach is also consistent with the mathematical tools of pulse-modulated control systems.

Below, a linear mathematical model describing the relationship between the pulsatile secretion of a RH and the concentration of the hormone it releases, denoted as $H(t)$, will be derived. With the concentration of RH denoted as $R(t)$, model (1) written for the bipartite endocrine system in question gives

$$A = \begin{bmatrix} -b_1 & 0 \\ g_1 & -b_2 \end{bmatrix}, \quad B = \begin{bmatrix} 1 \\ 0 \end{bmatrix}, \quad y(t) = H(t), \quad x(t) = \begin{bmatrix} R(t) \\ H(t) \end{bmatrix}.$$

The closed loop hormone regulation system is supposed to exhibit sustained nonlinear oscillations of a certain period in order to model self-regulation. From [3], it is known that multiple pulses of RH within the oscillation period can occur. Notably, release of RH does not always result in a significant increase of the concentration of H, but can also manifest itself as reduced decay rate of $H(t)$.

Assume that k pulses of RH occur at the firing times $0, t_1, \dots, t_{k-1}$ within the least period of the closed system solution τ :

$$\Theta(t) = \lambda_0 \delta(t) + \lambda_1 \delta(t - t_1) + \dots + \lambda_{k-1} \delta(t - t_{k-1}).$$

Then, the concentration of RH evolves as

$$\begin{aligned} R(t) &= (R(0) + \lambda_0) e^{-b_1 t}, & 0 \leq t < t_1, \\ R(t) &= \eta_1(b_1) e^{-b_1 t}, \eta_1(x) = R(0) + \lambda_0 + \lambda_1 e^{x t_1}, & t_1 \leq t < t_2, \\ &\vdots \\ R(t) &= \eta_{k-1}(b_1) e^{-b_1 t}, \eta_{k-1}(x) = R(0) + \lambda_0 + \lambda_1 e^{x t_1} + \dots + \lambda_{k-1} e^{x t_{k-1}}, & t_{k-1} \leq t < \tau, \end{aligned}$$

and the measured hormone concentration is given by

$$\begin{aligned}
H(t) &= H(0)e^{-b_2t} + \frac{(R(0) + \lambda_0)g_1}{b_2 - b_1}(e^{-b_1t} - e^{-b_2t}), & 0 \leq t < t_1, \\
H(t) &= H(0)e^{-b_2t} + \frac{g_1}{b_2 - b_1}(\eta_1(b_1)e^{-b_1t} - \eta_1(b_2)e^{-b_2t}), & t_1 \leq t < t_2, \\
&\vdots \\
H(t) &= H(0)e^{-b_2t} + \frac{g_1}{b_2 - b_1}(\eta_{k-1}(b_1)e^{-b_1t} - \eta_{k-1}(b_2)e^{-b_2t}), & t_{k-1} \leq t < \tau.
\end{aligned}$$

The number of RH pulses within the least oscillation period depends on the structure of the endocrine system and the values of its parameters. In mathematical analysis of the GnRH-LH-Te axis, periodical solutions with one and two pulses of GnRH have been discerned. In what follows, the case with two pulses of RH-H in the least period is therefore considered.

$$\Theta(t) = \lambda_0\delta(t) + \lambda_1\delta(t - t_1).$$

The case of one RH pulse in the least period follows by letting $\lambda_0 = \lambda_1$ and $t_1 = \tau/2$ which is frequency doubling.

Based on the above given equation, concentration of RH with two fired pulses can be described by the following functions

$$\begin{aligned}
R(t) &= (R(0) + \lambda_0)e^{-b_1t}, & 0 \leq t < t_1, & \quad (4) \\
R(t) &= \eta(b_1)e^{-b_1t}, \eta(x) = R(0) + \lambda_0 + \lambda_1e^{xt_1}, & t_1 \leq t < \tau.
\end{aligned}$$

For the measured concentration, it applies

$$\begin{aligned}
H(t) &= H(0)e^{-b_2t} + \frac{(R(0) + \lambda_0)g_1}{b_2 - b_1}(e^{-b_1t} - e^{-b_2t}), & 0 \leq t < t_1, & \quad (5) \\
H(t) &= H(0)e^{-b_2t} + \frac{g_1}{b_2 - b_1}(\eta(b_1)e^{-b_1t} - \eta(b_2)e^{-b_2t}), & t_1 \leq t < \tau.
\end{aligned}$$

In what follows, an important temporal characteristic of model (4,5) is utilized for estimation of system parameters. It is introduced as the time instant at which the measured hormone concentration achieves maximum for the first time during the oscillation period, *i.e.*

$$t_{\max} = \arg \max_t H(t), \quad 0 \leq t < t_1.$$

Since the scope of this article is only the pulsatile secretion, the initial condition $H(0)$ is taken

out from the model.

$$\begin{aligned} H(t) &= \frac{(R(0) + \lambda_0)g_1}{b_2 - b_1}(e^{-b_1 t} - e^{-b_2 t}), & 0 \leq t < t_1, \\ H(t) &= \frac{g_1}{b_2 - b_1}(\eta(b_1)e^{-b_1 t} - \eta(b_2)e^{-b_2 t}), & t_1 \leq t < \tau. \end{aligned} \quad (6)$$

From (6), this characteristic is uniquely defined by the values of b_1 and b_2

$$t_{\max} = \frac{\ln(b_1) - \ln(b_2)}{b_1 - b_2}.$$

From the biology of the system, it follows that $b_1 > b_2$. Let now $b_1 = b$ and $b_2 = \kappa b$ where $0 < \kappa < 1$. Therefore

$$t_{\max} = \frac{\ln \kappa}{b(\kappa - 1)}.$$

For further use, one can observe that t_{\max} is a monotonically decreasing and bounded function of κ . Indeed,

$$\frac{d}{d\kappa} \frac{\ln \kappa}{\kappa - 1} = \frac{1}{\kappa - 1} \left(\frac{1}{\kappa} - \frac{\ln \kappa}{\kappa - 1} \right) < 0.$$

The first factor of the right-hand side of the equation above is negative and the second factor is positive by evoking the standard logarithmic inequality

$$\frac{x}{1+x} \leq \ln(1+x), \quad x > -1$$

and taking into account the range of κ . The same inequality yields also a useful upper bound

$$t_{\max} = \frac{\ln \kappa}{b(\kappa - 1)} < \frac{1}{b\kappa} = \frac{1}{b_2}. \quad (7)$$

Denote $H_{\max} = H(t_{\max})$. In terms of model parameters

$$H_{\max} = \frac{\lambda_0 g_1^0}{b_2^0 - b_1^0} (e^{-b_1^0 t_{\max}} - e^{-b_2^0 t_{\max}}) = \frac{\lambda_0 g_1}{\kappa b} e^{-b t_{\max}} = \frac{\lambda_0 g_1}{\kappa b} e^{-\frac{\ln \kappa}{\kappa - 1}}.$$

Now, due to the inequality above, the following lower bound can be found

$$H_{\max} > \frac{\lambda_0 g_1}{\kappa b} e^{-\frac{1}{\kappa}}. \quad (8)$$

3 Model parameters

There are seven parameters to be estimated in the mathematical model of the measured output H expressed by (4) and (5), namely g_1 , b_1 , b_2 , λ_0 , λ_1 , t_1 , and $R(0)$. The initial condition $H(0)$, as it has been mentioned above, is taken out from the model for two reasons. On the one hand, it is supposed to be known since measurement data of H are available. On the other hand, the focus of this paper is on the pulsatile secretion of H and, therefore, the basal level of H would be out of the scope. The proposed algorithm would estimate the parameters based on preprocessed data, that is extracted individual major pulse.

Nonlinear least squares estimation was used in [3] to fit a mathematical model of LH secretion stimulated by two consequent GnRH pulses corresponding to a 2-cycle of the closed-loop pulsatile regulation system. Estimates obtained from real data representing four pulses of LH sampled at 10 min and taken from the same human male appear to lay outside the intervals of biologically reasonable values as provided in [14]. Therefore, further research has to be performed, both to find estimates that also are biologically sensible and to explore more data.

3.1 Identifiability

Before performing parameter estimation, it has to be confirmed that the proposed model is identifiable. Compared to a standard system identification setup with an output signal registered for a given input, the parameter estimation in the case at hand has to be performed from a pulse response of the dynamic system. The celebrated Ho-Kalman realization algorithm [15] provides the theoretical grounds for such an estimation. Indeed, under zero initial conditions, for the impulse response of the minimal realization of the transfer function $W(s) = C(sI - A)^{-1}B$ where s is the Laplace variable, one has

$$y(t) = C \exp(At)B, t = [0, \infty).$$

Therefore, the Markov coefficients of the system

$$h_i = CA^iB, i = 0, 1, \dots$$

can be obtained by differentiation of the output at one point

$$h_0 = y(t)|_{t=0_+}, h_1 = \frac{dy}{dt}|_{t=0_+}, h_2 = \frac{d^2y}{dt^2}|_{t=0_+}, \dots$$

and the matrices A, B, C obtained via the Ho-Kalman algorithm.

There are two issues pertaining to the identifiability of model (4,5). The first issue is related to the initial condition of RH concentration $R(0)$. As can be seen from the model equations, this value always appears in a sum with the magnitude of first delta function λ_0 . Hence, it is impossible to distinguish between these parameters, which might be an artifact resulting from consideration of only the extracted pulse. They are further considered as one parameter defined as λ_0 .

Furthermore, λ_0 and λ_1 always appear multiplied by g_1 . The only possible solution is to estimate not the actual values of λ_0 and λ_1 , but rather the ratio between them. Following [3], it is further assumed that $\lambda_0 = 1$.

3.2 Parameter estimation algorithm

A data set of measured hormone concentration typically consists of samples that are taken every 3 – 10 minutes for 20 – 23 hours during one day of observation. In case of pulsatile secretion, such a data set would exhibit several major concentration pulses. Figure 1 shows three pulses of LH extracted from measured data. The data has been preprocessed to remove the basal level. The pulses have similar signal form and are all coherent with the assumption of two secretion events of GnRH within one period of a model solution. The curves also confirm that the secondary GnRH releases occur around the same time instance. It can be conjectured that depending on the amplitude of the secondary GnRH pulse, the concentration of LH can either rise (Data set 3), stay constant (Data set 1) or decay at decreased rate (Data set 2).

By the argument given in the previous subsection, the number of estimated unknown parameters is reduced from seven to five: g_1, b_1, b_2, λ_1 , and t_1 . Figure 2 shows how the estimated parameters influence the pulse form. The ratio between λ_0 and λ_1 would only represent the weights ratio between the Dirac delta-functions. Notice that delta-functions have infinite amplitudes and only used to mark the time instances of GnRH secretion and communicate the amount of secreted hormone through the weight. Estimates of these values are obtained via optimization performed basing on the mathematical model and measured data. Typically, hor-

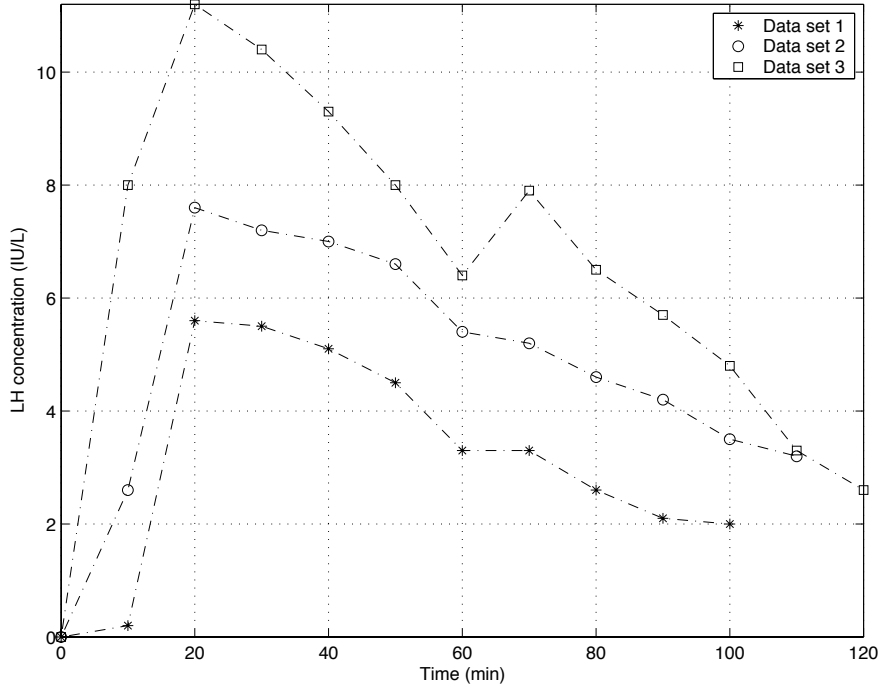


Figure 1: Three pulsatile profiles of LH concentration measured in a human male. Notice that all the pulses exhibit first maximum and secondary GnRH release event approximately at the same time.

more data are undersampled and it is hard to capture the kinetics of most RHs. For instance, a GnRH pulse would decay in around 1-3 minutes, according to biological analysis in [16]. To obtain more reliable parameter estimates from undersampled measurements, the data are processed in several steps.

- Step 0: Extract a data set representing one pulse of H from measured data;
- Step 1: Evaluate maxima and minima within the data set;
- Step 2: Calculate initial values of \hat{g}_1^0 , \hat{b}_1^0 , \hat{b}_2^0 , $\hat{\lambda}_1^0$ and \hat{t}_1^0 for estimation;
- Step 3: Estimate \hat{g}_1 , \hat{b}_1 , and \hat{b}_2 from the measurements within the interval $t < \hat{t}_1$;
- Step 4: Estimate $\hat{\lambda}_1$ and \hat{t}_1 from the measurements within the interval $t \geq \hat{t}_1$;
- Step 5: Estimate \hat{g}_1 , \hat{b}_1 , \hat{b}_2 , $\hat{\lambda}_1$ and \hat{t}_1 from all points of the data set using the estimates obtained at Step 3 and Step 4 as initial conditions.

The operations executed at each step of the estimation algorithm are explained in what follows.

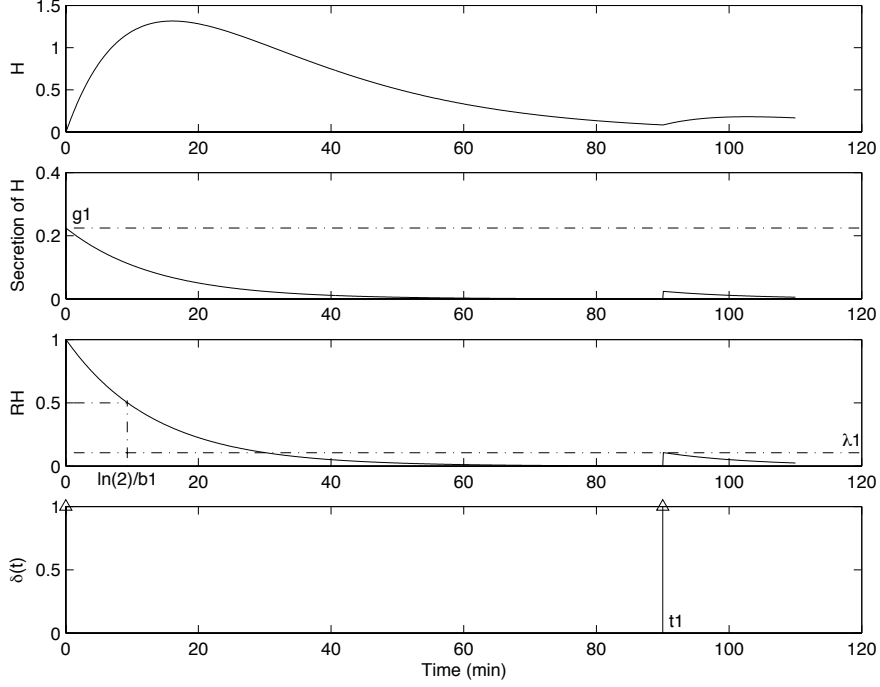


Figure 2: Estimated parameters of model dynamics. Amplitudes of Dirac delta-functions are infinite.

3.2.1 Pulse extraction from measured data

Provided with hormone concentration data comprising several pulses, it is necessary to extract each pulse from the data set prior to performing parameter estimation. Then the basal level of hormone concentration at onset of each pulse is omitted to assure that the changes in concentration are due to pulsatile secretion. This pulse extraction provides the measured data set $H_m(k)$ with n sample points and the sampling interval h where the first measurement is taken at $k = 0$, and the last measurement at $k = n - 1$. The value of each measurement values $H_m(k)$ are subtracted by the initial value $H_m(0)$. It should be noted that these measurements are sampled instances of a continuous output. In what follows, the sampling instance is denoted with k and t represents the continuous time variable, *i.e.* $t = kh$, $k = 0, 1, \dots, n - 1$.

3.2.2 Maxima, minima and initial estimation of t_1

From a measured data set, the information about local maxima and local minima of the hormone concentration H is collected. A simple approach for it is described in Appendix A. Initialization of parameter estimates is performed by inspecting the extreme values and their temporal locations. The global maximum represents the highest concentration level of H which is caused by release of the first RH pulse. The local minimum most probably marks the location of the

second RH pulse firing. Finally, the other local maximum shows the highest concentration of H after the second RH pulse has been fired.

The peak concentration value H_{\max} is given by

$$H_{\max} = H_m(k_{\max}) = \sup_k H_m(k), \quad 0 \leq k < n$$

$$\hat{t}_{\max} \approx k_{\max}h$$

where k_{\max} is the sample number at which the highest concentration of H is achieved. A global maximum always exists in the measured data due to the nature of pulsatile secretion. However, not all data sets would contain local minima and local maxima. In Fig. 1, Data set 1 and Data set 2 exhibit no local minima while Data set 3 does. Therefore, two cases are considered in the next step.

1. *Data with local maxima and local minima:* An example of data with local maxima and local minima is Data set 3 on Fig. 1. For this case, it is easier to estimate the time at which second RH pulse occurs. The local minimum marks the firing time \hat{t}_1 .

$$H(\hat{t}_1) \approx H_m(k_{\min}) = \min_k H_m(k), \quad k_{\max} < k < n$$

$$\hat{t}_1 \approx k_{\min}h.$$

The second maximum H_{\max_2} , which occurs because of the second RH pulse, is then obtained from the information about the local maximum.

$$H_{\max_2} = H_m(k_{\max_2}) = \max_k H_m(k), \quad k_{\min} \leq k < n$$

$$\hat{t}_{\max_2} \approx k_{\max_2}h.$$

2. *Data without local minima:* This case arises when the magnitude of second RH pulse is relatively small and causes rather a decrease in the decay rate of H concentration instead of producing another peak. Examples of this kind of behavior are given by Data set 1 and Data set 2 in Fig. 1.

To produce the initial estimate \hat{t}_1^0 , the ratio between two consecutive data samples $\rho(k)$ is calculated. The lowest ratio value indicates the position of the second RH pulse \hat{t}_1 . Due

to a reduction in the decay rate of H concentration, the difference between concentration level at two consecutive samples is relatively low compared to the level difference at other instances. Because the concentration level varies, evaluating the ratio is more reliable than evaluating the level difference.

$$\rho(k_{\min}) = \min_k \frac{H_m(k)}{H_m(k+1)}, \quad k_{\max} < k < n-1$$

$$\hat{t}_1 \approx k_{\min}h$$

$$H(\hat{t}_1) \approx H_m(k_{\min}).$$

In this case, secondary peak does not exist and, within the interval $k_{\min} \leq k < n$, the maximal value is achieved on the boundary k_{\min} . Therefore, this point is estimated as the firing time of the second RH pulse

$$\hat{t}_{\max_2} \approx \hat{t}_1$$

$$H_{\max_2} = H_m(k_{\min}).$$

With the collected information of \hat{t}_{\max} , H_{\max} , $H(\hat{t}_1^0)$, \hat{t}_{\max_2} , and H_{\max_2} the estimation algorithm could proceed to the next step.

3.2.3 Initial parameter estimates

The introduced above mathematical model has five parameters that need to be identified. Therefore, at least five equations are required to provide the initial values of these parameters \hat{g}_1^0 , \hat{b}_1^0 , \hat{b}_2^0 , $\hat{\lambda}_1^0$ and \hat{t}_1^0 . These initial values constitute a starting point for the optimization algorithm. The following equations to estimate initial parameters are considered.

- The time instant at which highest H concentration is achieved – \hat{t}_{\max} ;
- The value of maximum H concentration – H_{\max} ;
- The value of H concentration at the time of second RH pulse – $H(\hat{t}_1^0)$;
- The time instant of local maximum of H concentration after second RH pulse is secreted – \hat{t}_{\max_2} ; and
- The value of second local maximum of H concentration – H_{\max_2} .

All necessary information to calculate those values is readily obtained from previous sections. Below is a detailed description of each equation that is used to produce initial parameter estimates.

1. *The time instant at which highest H concentration is achieved* – \hat{t}_{\max} . This value can be obtained by taking first derivative of H in (5) for $0 \leq t < t_1$ equal to zero and solving the equation for t

$$\hat{t}_{\max} = \frac{\ln b_1^0 - \ln b_2^0}{b_1^0 - b_2^0}. \quad (9)$$

2. *The value of maximum concentration of H* – H_{\max} . This value is calculated from (5) as

$$H_{\max} = \frac{\lambda_0 g_1^0}{b_2^0 - b_1^0} (e^{-b_1^0 \hat{t}_{\max}} - e^{-b_2^0 \hat{t}_{\max}}). \quad (10)$$

3. *The concentration of H at the time of second RH pulse* – $H(\hat{t}_1^0)$. Since the estimated point of the second RH pulse is obtained, the measured concentration data at that point can be used to obtain more information on the model parameters.

$$H(\hat{t}_1^0) = \frac{\lambda_0 g_1^0}{b_2^0 - b_1^0} (e^{-b_1^0 \hat{t}_1^0} - e^{-b_2^0 \hat{t}_1^0}). \quad (11)$$

4. *The time instant of local maximum of H concentration after second RH pulse is secreted* – \hat{t}_{\max_2} . The instance where second maximum would occur is obtained similarly to H_{\max} and results in the following equation

$$\hat{t}_{\max_2} = \frac{\ln b_1^0 (\lambda_0 + \lambda_1^0 e^{b_1^0 \hat{t}_1^0}) - \ln b_2^0 (\lambda_0 + \lambda_1^0 e^{b_2^0 \hat{t}_1^0})}{b_1^0 - b_2^0}. \quad (12)$$

5. *The value of second (local) maximum of H concentration* – H_{\max_2} . Similar with the first maximum, however for the concentration of H taken within the interval $t_1 \leq t < \tau$.

$$H_{\max_2} = \frac{g_1^0}{b_2^0 - b_1^0} (\eta(b_1^0) e^{-b_1^0 \hat{t}_{\max_2}} - \eta(b_2^0) e^{-b_2^0 \hat{t}_{\max_2}}). \quad (13)$$

Solving equations (9-13) provides fair initial values of \hat{g}_1^0 , \hat{b}_1^0 , \hat{b}_2^0 , $\hat{\lambda}_1^0$ and \hat{t}_1^0 for further parameter estimation.

3.2.4 Parameter estimation using constrained weighted nonlinear least squares

With the knowledge of initial values of the parameters evaluated from inspection of hormone concentration data, more accurate estimates of model parameters can be obtained by taking into account all the samples of the data set. The method which is utilized here is weighted nonlinear least squares with positivity constraints motivated by the biological nature of the system. The algorithm comprises three steps of fitting the solution of (4,5) to data samples. In the first step, estimates of g_1 , b_1 , and b_2 are produced. The next step is performed to estimate λ_1 and t_1 . Then the final estimation step is carried out to yield better parameter estimates all together.

1. *Estimation of g_1 , b_1 , and b_2 .* The first optimization step of parameter estimation is performed using measured data $H_m(k)$ for $0 \leq k < [\frac{t_1}{h}]$, where $[\cdot]$ represents the integer part of a real number. Within this interval, only g_1 , b_1 , and b_2 are affecting the concentration of H, while the model response afterwards is also influenced by the other two parameters. The weighting for this estimation is chosen so that data representing the global maximum have the highest priority. Then the fit within the interval $[\frac{t_{\max}}{h}] < k < [\frac{t_1}{h}]$ will be given higher weights compared to that within the interval $0 \leq k < [\frac{t_{\max}}{h}]$.
2. *Estimation of λ_1 and t_1 .* The other two model parameters are estimated from measured data $H_m(k)$ which are sampled after the estimated secretion instance of second RH pulse $[\frac{t_1}{h}] \leq k < n$ and taking advantage of the estimates of g_1 , b_1 , and b_2 resulting from the previous step. In this way, it is meant to produce estimates of λ_1 and t_1 that would approximate this part of the measured data, without changing the fitting of the preceding samples of the data set. The measured data $H_m(k)$ at sampling instances $k = [\frac{t_1}{h}]$ and $k = [\frac{t_{\max_2}}{h}]$ are given the highest priority when applying the nonlinear least squares. Then the fit in the interval $[\frac{t_{\max_2}}{h}] < k < n$ is given higher weight than that within $[\frac{t_1}{h}] \leq k < [\frac{t_{\max_2}}{h}]$.
3. *Estimation of all parameters.* The last optimization step is performed to estimate the parameters by considering all samples of the data set. Until this point, the influence of parameters g_1 , b_1 , and b_2 on the second part of measured data has not been considered. Based on initial values from the previous two estimation steps, this last step would provide estimation improvement. The weighting for this step follows the values evaluated above.

Further details on the used least squares algorithm are provided in Appendix B.

4 Experiments

Experiments with data are performed to illustrate efficacy of the suggested estimation approach. First, simulated LH data based on the bipartite pulsatile secretion model of Section 2.3 are utilized and then the method is put to test on previously published hormone data.

4.1 Experiments with simulated data

The data are produced by capturing at each sampling instance the output value of the LH regulation model (4,5) with two instances of pulsatile secretion in the least period. For congruence with the real hormone data case discussed in the next section, sampling period is set equal to 10 minutes. Two sets of simulated data are used and the nominal model parameters for them are given in Table 1. Data set 1 demonstrates a situation when the secondary GnRH pulse is large enough to produce an obvious secondary peak in the LH concentration profile. Data set 2 illustrates a weaker secondary secretion event that only reduces the hormone concentration decay rate.

A number of cases is constructed to investigate the performance of the estimation approach in different situations, namely:

- estimation with initialization of parameter estimates close to actual values,
- estimation with initialization of parameter estimates far from actual values,
- estimation under measurement disturbance.

Under these conditions, the parameter estimation approach developed here, the WEN deconvolution approach, and AutoDecon approach are tested. For the WEN deconvolution approach, the hormone kinetics is modeled according to (3).

Initial parameter values. The performance of the WEN deconvolution approach is highly dependent on the accuracy of the assumed values of the LH decay rate, while the parameter estimation approach and AutoDecon can perform very well even in the case of unknown initial parameter values, relying instead on their estimates. The parameter values of k_1 and k_2 in the two-compartment secretion model for the WEN deconvolution approach follow the values of the

parameters b_1 and b_2 of the pulsatile model. Deconvolution with nominal parameter values in Table 1 is used to illustrate the performance with perfect knowledge of the hormone kinetics and while deconvolution with initial values reveals the effects of parameter uncertainty.

As expected, both the parameter estimation approach and the WEN deconvolution with nominal parameter values (WEN deconvolution (N)) explain simulated LH concentration data well, Fig. 3a. Yet, the secretion estimates produced by the WEN deconvolution method are inaccurate. The discrepancy between the actual and estimated secretion depends apparently on the sampling rate and is addressed later. For inaccurate (initial) assumed parameter values, the WEN deconvolution method (WEN deconvolution (I)) yields significant errors also in the estimated LH concentration. Notably, as Fig. 3b indicates, the performance of the WEN deconvolution improves for Data set 2 where the secondary secretion event is less prominent. Apparently, AutoDecon estimates the LH profile very well for the main pulse but neglects the existence of the secondary pulse. Similar to the WEN deconvolution approach, its performance improves for Data set 2 due to the small value of secondary pulsatile secretion. AutoDecon’s secretion profile also differs from the simulated model. This is understandable because in AutoDecon pulsatile secretion model is based on different assumption, *i.e.* it follows normal distribution.

Besides of the nominal value of the parameters, the initial and the final parameter estimates produced by the parameter estimation approach are also shown on Table 1. The accuracy of the parameter estimates is sufficient to make the simulated LH concentration and secretion practically indistinguishable in Fig. 3 from the corresponding estimates. Inspecting the estimated values shows that t_{\max} and H_{\max} satisfy bounds (7) and (8).

Parameter set	g_1	b_1	b_2	λ_1	t_1
Nominal 1	0.2243	0.0748	0.0514	0.1056	90
Initial 1	0.5000	0.0500	0.0220	0.0010	80
Estimated 1	0.2203	0.0693	0.0541	0.1047	90
Nominal 2	0.3200	0.0750	0.0330	0.0500	75
Initial 2	0.5000	0.0500	0.0220	0.0100	90
Estimated 2	0.3130	0.0715	0.0337	0.0515	75

Table 1: Parameter sets for generation of simulated data and their estimates by the proposed method.

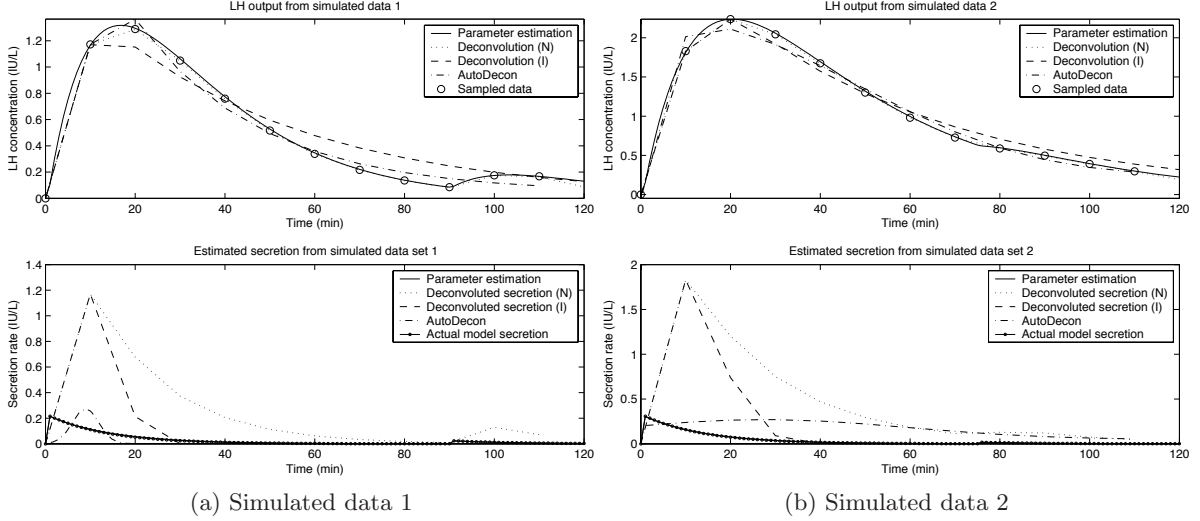


Figure 3: Performance of the tested estimation approaches on simulated data sets, sampling time 10 min.

Sampling rate. As noted before, the secretion estimation provided by the WEN deconvolution approach does not represent the actual secretion rate of the simulated model. The reason to it is the inadequate sampling rate in the data sets. In fact, much higher sampling rates are technically feasible. For instance, in [17], 30 s samples of portal blood from short term ovariectomized ewes have been taken. As concluded by stochastic analysis in [16], an appropriate sampling period of the LH secretion profile should be around 2 – 3 minutes. In [3], from a control engineering perspective, the suitable sampling time is estimated to be in the range 0.6 – 1.9 minutes. Lowering the sampling time to one minute increases the agreement of the estimated secretion with the actual simulated one, see Fig. 4. The figure also depicts the estimation provided by AutoDecon. Typically for deconvolution approaches, AutoDecon performs very well on high resolution sampled data. The secretion profile produced by AutoDecon fits well the secretion model when high resolution data are used. Furthermore, in the same figure, it can be noticed that AutoDecon yields negative estimates in the attempt to follow the simulated LH concentration in the beginning of the data set. The estimated secretion profile also has some negative values, *i.e.* in Fig. 4a within the time interval $t = \{50, 80\}$. As pointed out in Section 2.2.1, the problem of negative secretion estimates in deconvolution has been successfully resolved in [8].

Measurement disturbance. The last simulation test is the performance evaluation with random additive hormone concentration measurement disturbance. For simulation purpose, a

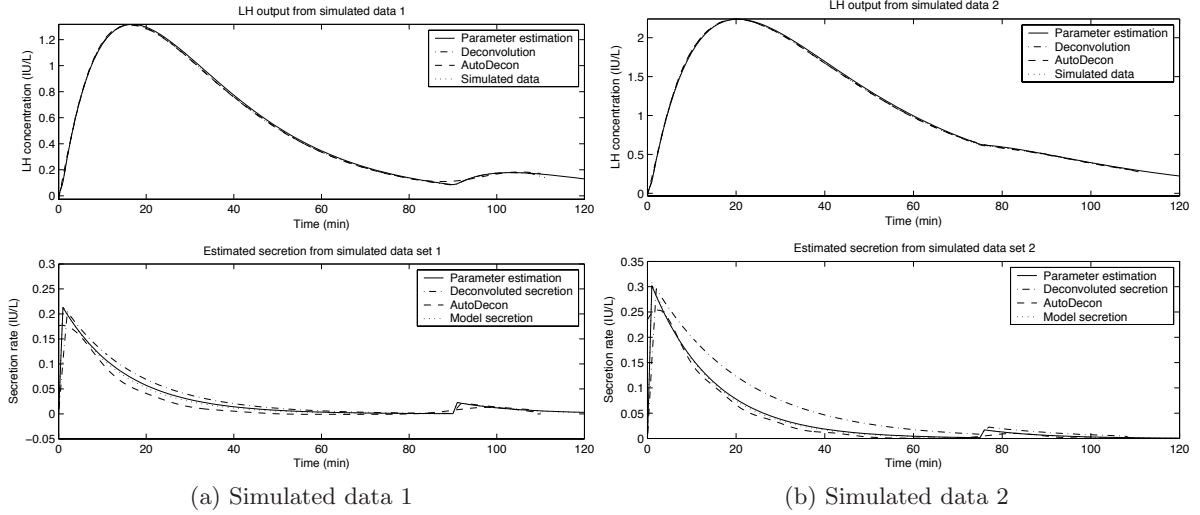


Figure 4: Performance of the tested estimation approaches on simulated data sets, sampling time 1 min.

random signal with zero mean and variance of 10^{-6} is introduced as measurement disturbance. This value is chosen based on the accuracy of radioimmunoassay (RIA) that is used for measuring LH concentration in blood sample. The standard deviation of RIA measurement is known to be on the level of ng/ml, therefore the chosen for simulation value appears to be reasonable. On disturbed data, the tested approaches perform quite well when it comes to the LH concentration estimation, see Fig. 5. The parameter estimation approach is able to estimate the secretion profile correctly and the estimate is not influenced much by the measurement disturbance. This is also confirmed by Monte Carlo simulation where 50 different realizations of measurement disturbance have been used to perturb each of the simulated data sets. The estimation results summarized in Table 2 indicate good robustness properties of the developed technique against additive measurement disturbance.

	g_1	b_1	b_2	λ_1	t_1
Nominal parameter of simulated data 1	0.2243	0.0748	0.0514	0.1056	90
Estimation mean (μ)	0.2203	0.0691	0.0543	0.1060	90.2186
Estimation standard deviation (σ)	0.0009	0.0016	0.0012	0.0021	0.4678
Nominal parameter of simulated data 2	0.3200	0.0750	0.0330	0.0500	75
Estimation mean (μ)	0.3131	0.0715	0.0337	0.0514	75.5961
Estimation standard deviation (σ)	0.0010	0.0005	0.0002	0.0006	1.1153

Table 2: Monte Carlo simulation results.

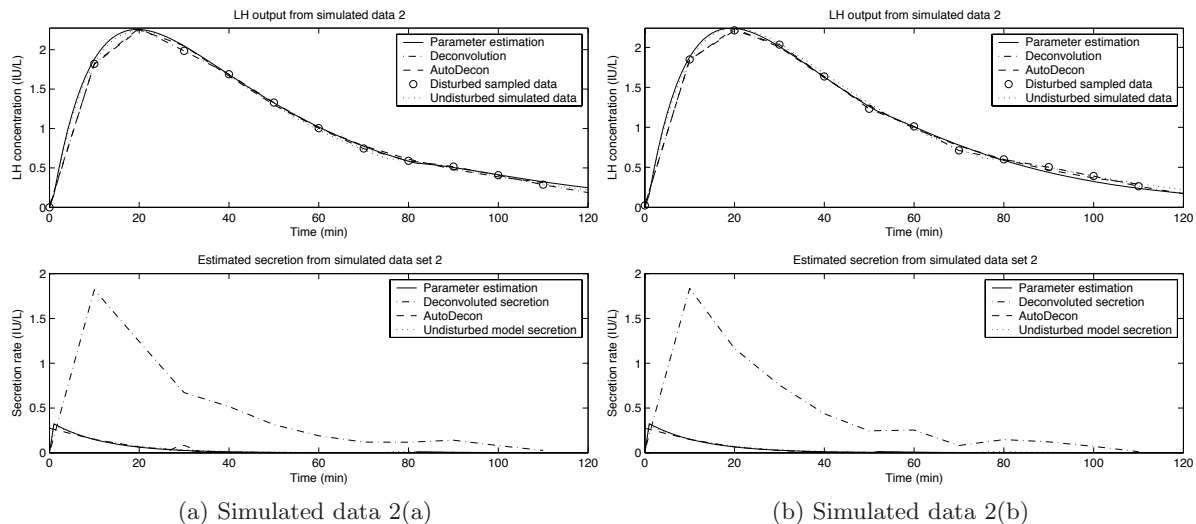


Figure 5: Performance of the tested estimation approaches on simulated data sets with measurement disturbance, sampling time 10 min.

4.2 Experiments with measured data

As it has been shown in the previous section, the proposed estimation approach yields better results on simulated data when compared to the state-of-the-art deconvolution algorithms, especially in the case where high sampling rate is not available. However, this does not prove that the approach also would perform better on actual biological data since the other two approaches are based on different model assumption. Indeed, any mathematical model is just an approximation of reality and cannot capture all the particularities of the actual phenomena.

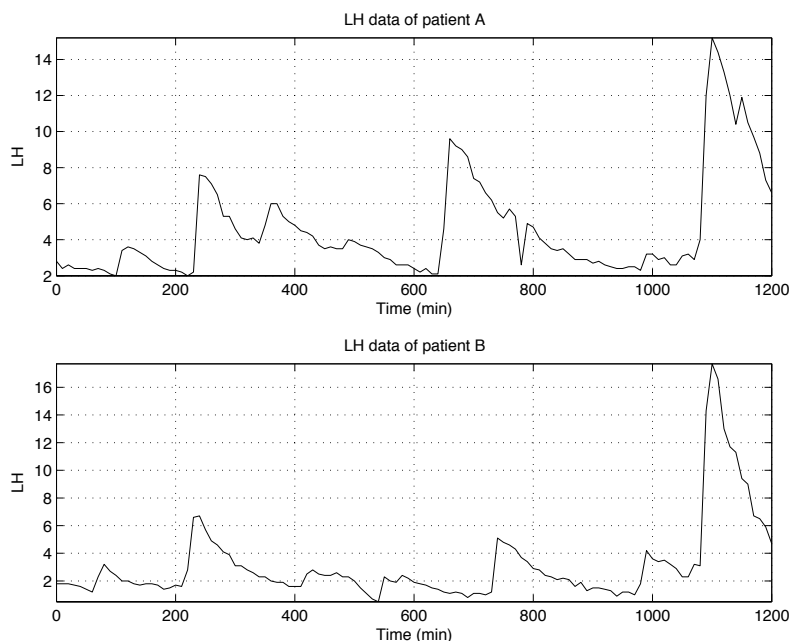


Figure 6: LH concentration data for two healthy men, sampling time 10 min.

To investigate feasibility of the proposed parameter estimation technique on real data, it is applied to data of LH serum concentration provided by Prof. Veldhuis of Mayo Clinic and described in [18]. The evaluation data are collected from two healthy men (Patient A and Patient B) and sampled periodically every 10 minutes starting from 18.00 until 14.00 on the following day, see Fig. 6. From ocular inspection, there are about 6 major pulses within the LH concentration data of patient A, and 5 major pulses in the data from patient B.

Since the focus of this paper is on the pulsatile hormone regulation, the basal level of each pulse is subtracted from the hormone concentration values. Along with the WEN deconvolution approach, comparison is also performed with AutoDecon.

Fig. 7 shows some typical experimental results on real hormone data. It represents the cases both with and without clear secondary maximum on sampled data for both patients. Fig. 7a and Fig. 7d show the case where secondary local maximum does not appear in sampled data, while Fig. 7b and Fig. 7c represent the case where the sampled data exhibits an obvious secondary peak. It can be seen that the proposed parameter estimation approach fits the sampled LH concentration data better compared to the two deconvolution algorithms, especially when the examined data set has a clear secondary peak. Occasionally, the WEN deconvolution also produces results that indicate the release of a secondary GnRH pulse. For example, in Fig. 7a, the GnRH secretion profile estimated by the conventional deconvolution approach clearly exhibits a second pulse. Similarly to the experiments with simulated data, AutoDecon sometimes estimates very small negative value of the basal secretion. This is probably related to the symmetrical (normal distribution shaped) signal form of the secretion.

The fact that the deconvolution approaches do not always recognize secondary GnRH secretion events in LH pulses does not necessarily lead to significant errors in the estimate of the secreted GnRH but rather ignores an interesting dynamic phenomenon whose existence is predicted mathematically in [3]. In fact, a much more serious problem in deconvolution methods is the large discrepancy in the estimated hormone secretion due to uncertainty in the metabolic constants. This shortcoming is intrinsic to the deconvolution approach and cannot be alleviated without assuming a model of the RH secretion profile, as *e.g.* is done in AutoDecon. However, since there is no generally accepted pulsatile secretion model in endocrinology, the estimation results depend on what secretion model is assumed.

Parameter estimates produced by the proposed approach are summarized in Table 3. Once again, the resulting parameter estimates are within the expected boundaries for t_{\max} and H_{\max}

given by (7) and (8). Clearly, variation in the estimates of b_1 and b_2 is high. This problem is expected because of the low sampling rate. However the variation of ratio between two exponential rates κ is reasonable. In [19], the exponential rates for RIA LH profile have been examined yielding the fast decay rate $k_1 = 3.87 \times 10^{-2}$ and the slow one $k_2 = 7.69 \times 10^{-3}$, with the ratio between them equals to 0.19. The mean value of κ from Table 3 is 0.26 which is not very far from the value obtained ratio from [19]. Regarding the estimated values of t_1 , it is interesting to note that the results of regularized deconvolution analysis of LH data sampled with 5 min sampling time in [20] clearly show two instances of pulsatile secretion separated by approximately a one hour long interval. This agrees well the values in Table 3 obtained by the present method from much sparser data.

An inherent drawback of the proposed algorithm is that it is based on a simple linear model and cannot capture nonlinearities of the LH dynamics, as seen on Fig. 7a where the estimation misses the second sampling instance.

Patient	Data Set	\hat{g}_1	\hat{b}_1	\hat{b}_2	$\hat{\lambda}_1$	\hat{t}_1	κ
A	1	0.2223	0.0698	0.0392	0.0674	91	0.56
	2	1.0942	0.1319	0.0233	0.0878	50	0.18
	3	0.2839	0.0565	0.0430	0.0583	51	0.76
	4	0.8597	0.0752	0.0162	0.0813	60	0.22
	5	0.6333	0.1936	0.0297	0.1551	58	0.15
	6	1.7058	0.1070	0.0182	0.2634	60	0.17
B	7	0.5284	0.1644	0.0319	0.1169	50	0.19
	8	0.7623	0.0928	0.0217	0.0415	90	0.23
	9	0.9104	0.1790	0.0165	0.0637	110	0.09
	10	0.6677	0.1554	0.0196	0.2252	39	0.13
	11	2.5608	0.1098	0.0260	0.1164	48	0.24

Table 3: Estimated parameters of the major pulses of measured data given on Fig. 6.

5 Conclusion

In this study, an algorithm for parameter estimation of pulsatile hormone regulation based on the model introduced in [3] is developed and evaluated on simulated and experimental biological data. The suggested technique performs very well on undersampled simulated data. The two deconvolution based algorithms developed from different model assumption, one which is the state-of-the-art software for identification and characterization of hormone data, produce different hormone secretion profile with the nominal one. It is demonstrated on experimental

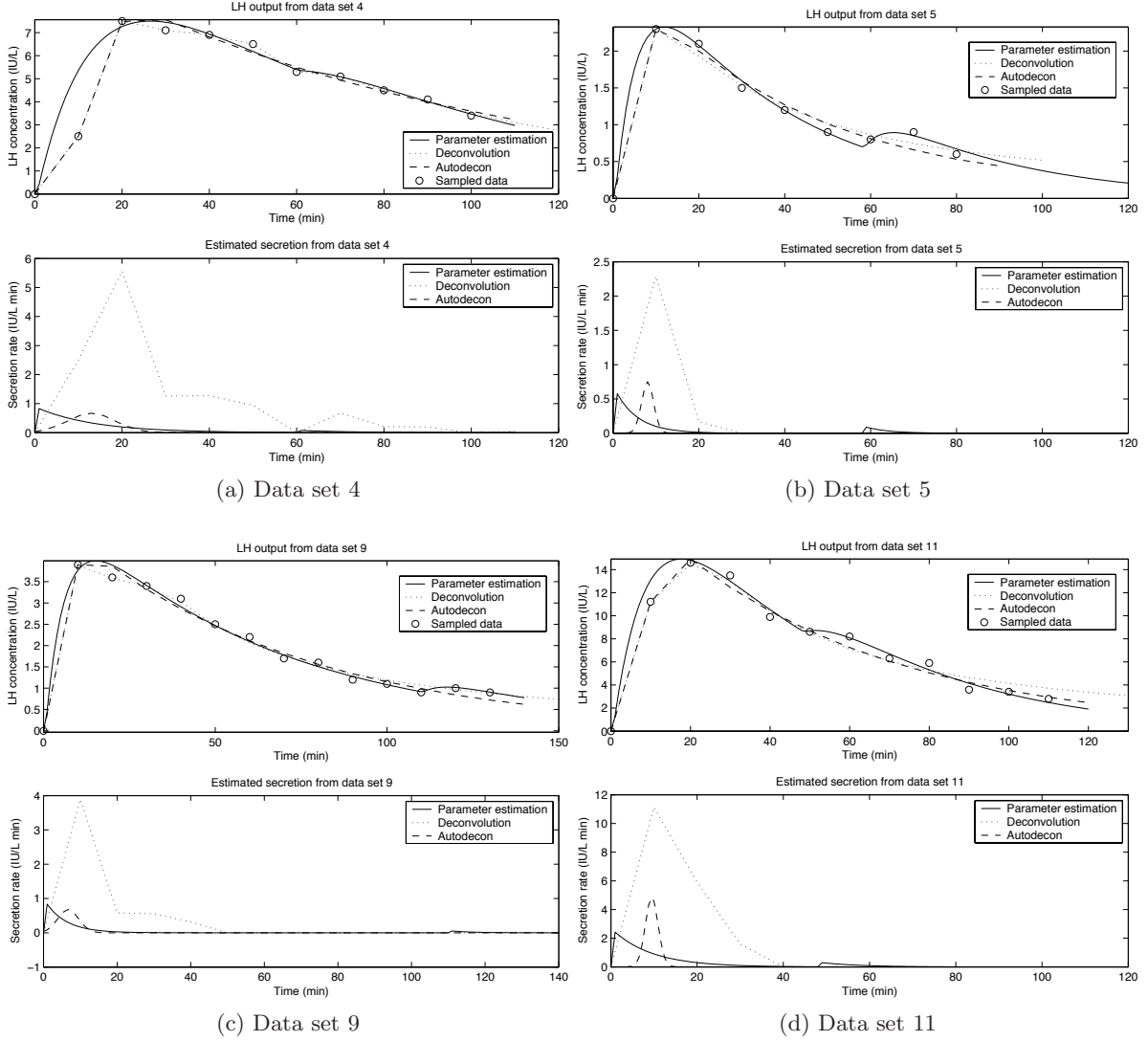


Figure 7: Performance of the tested estimation approaches on selected LH data sets, sampling time 10 min.

data that the model with two instances of GnRH release within one period of LH pulsatile secretion is feasible. Furthermore, the parameter estimation technique could fit the measured data as good as the state-of-the-art deconvolution approaches without neglecting the existence of secondary GnRH release.

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A Identifying maxima and minima

To locate maxima and minima within the data set, evaluation of the numerical derivative of the system output is performed.

$$D(k) = \frac{H_m(k+1) - H_m(k)}{h}, \text{ for } 0 < k < n - 1.$$

The points at the beginning and the end of the data set are excluded. Then the points of probable maxima or minima are evaluated by finding the sampling instance k_i where the following inequality holds

$$D(k_i)D(k_i - 1) \leq 0.$$

To find out whether the extremum is either maximum or minimum, the following conditions are checked

$$k_i : \begin{cases} \max & \text{if } D(k_i - 1) > 0 \\ \min & \text{if } D(k_i - 1) < 0. \end{cases}$$

B Constrained weighted nonlinear least squares

The estimation approach used within this article is to minimize the least square error between measured data $H_m(k)$ and model output $H(\mathbf{x}, kh)$ with \mathbf{x} representing the parameters vector $\{g_1, b_1, b_2, \lambda_1, t_1\}$, *i.e.*

$$\hat{\mathbf{x}} = \arg \min_{\mathbf{x}} F(\mathbf{x}) = \frac{1}{2} \min_{\mathbf{x}} \sum_{k=0}^{n-1} W(k) \left(H_m(k) - H(\mathbf{x}, kh) \right)^2.$$

The weighting factors of the output error at each sampling instance k are represented by $W(k)$ and their values are assigned based on the rules given in Section 3.2.4.

The minimum value of $F(\mathbf{x})$ is achieved when its gradient equal to zero. The gradient is calculated as

$$\mathbf{F}'(\mathbf{x}) = \begin{bmatrix} \frac{\partial F}{\partial x_1}(\mathbf{x}) \\ \vdots \\ \frac{\partial F}{\partial x_5}(\mathbf{x}) \end{bmatrix}$$

where

$$\begin{aligned} \frac{\partial F}{\partial x_j}(\mathbf{x}) &= \sum_{k=0}^{n-1} W(k) \frac{\partial f_k}{\partial x_j}(\mathbf{x}) f_k(\mathbf{x}) \\ f_k(\mathbf{x}) &= H_m(k) - H(\mathbf{x}, kh). \end{aligned}$$

Function $f_k(\mathbf{x})$ is the discrepancy between measured data and model output. Its gradient $\mathbf{J}(\mathbf{x})$

is described by the following equation

$$\begin{aligned} (\mathbf{J}(\mathbf{x}))_{kj} &= \frac{\partial f_k}{\partial x_j}(\mathbf{x}) \\ &= -\mathbf{H}'_{kj}(\mathbf{x}). \end{aligned}$$

The matrix \mathbf{H}' consists of partial derivatives of hormone H concentration with respect to the model parameters. The values of $H(t)$ given by (5) can be separated in two time intervals to facilitate derivation

$$H(t) = \begin{cases} H_a(t) & \text{for } 0 \leq t < t_1 \\ H_a(t) + H_b(t) & \text{for } t_1 \leq t < T \end{cases}$$

where

$$\begin{aligned} H_a(t) &= \frac{\lambda_0 g_1}{b_2 - b_1} (e^{-b_1 t} - e^{-b_2 t}), \\ H_b(t) &= \frac{\lambda_1 g_1}{b_2 - b_1} (e^{b_1(t_1-t)} - e^{b_2(t_1-t)}). \end{aligned}$$

Now, a step (Heaviside) function $\sigma(t)$ is introduced

$$\sigma(t) = \begin{cases} 0 & \text{for } 0 \leq t < t_1 \\ 1 & \text{for } t_1 \leq t < \tau \end{cases}$$

yielding

$$H_b(t) = \frac{\lambda_1 g_1 \sigma(t)}{b_2 - b_1} (e^{b_1(t_1-t)} - e^{b_2(t_1-t)}).$$

The gradient $\mathbf{H}'(\mathbf{x}, t)$ is calculated by partial derivation as

$$\begin{aligned} \left[\frac{\partial H}{\partial g_1} \quad \frac{\partial H}{\partial b_1} \quad \frac{\partial H}{\partial b_2} \quad \frac{\partial H}{\partial \lambda_1} \quad \frac{\partial H}{\partial t_1} \right] &= \left[\frac{\partial H_a}{\partial g_1} \quad \frac{\partial H_a}{\partial b_1} \quad \frac{\partial H_a}{\partial b_2} \quad \frac{\partial H_a}{\partial \lambda_1} \quad \frac{\partial H_a}{\partial t_1} \right] \\ &+ \left[\frac{\partial H_b}{\partial g_1} \quad \frac{\partial H_b}{\partial b_1} \quad \frac{\partial H_b}{\partial b_2} \quad \frac{\partial H_b}{\partial \lambda_1} \quad \frac{\partial H_b}{\partial t_1} \right] \end{aligned}$$

where

$$\begin{aligned}\frac{\partial H_a}{\partial g_1} &= \frac{\lambda_0}{b_2 - b_1} \left(e^{-b_1 t} - e^{-b_2 t} \right) \\ \frac{\partial H_b}{\partial g_1} &= \frac{\lambda_1 \sigma(t)}{b_2 - b_1} \left(e^{b_1(t_1-t)} - e^{b_2(t_1-t)} \right)\end{aligned}$$

$$\begin{aligned}\frac{\partial H_a}{\partial b_1} &= \frac{\lambda_0 g_1}{(b_2 - b_1)^2} \left((b_1 t - b_2 t + 1) e^{-b_1 t} - e^{-b_2 t} \right) \\ \frac{\partial H_b}{\partial b_1} &= \frac{\lambda_1 g_1 \sigma(t)}{(b_2 - b_1)^2} \left((b_2 - b_1)(t_1 - t) e^{b_1(t_1-t)} + e^{b_1(t_1-t)} - e^{b_2(t_1-t)} \right)\end{aligned}$$

$$\begin{aligned}\frac{\partial H_a}{\partial b_2} &= \frac{\lambda_0 g_1}{(b_2 - b_1)^2} \left(-e^{-b_1 t} + (1 - b_1 t + b_2 t) e^{-b_2 t} \right) \\ \frac{\partial H_b}{\partial b_2} &= \frac{\lambda_1 g_1 \sigma(t)}{(b_2 - b_1)^2} \left((b_1 - b_2)(t_1 - t) e^{b_2(t_1-t)} - e^{b_1(t_1-t)} + e^{b_2(t_1-t)} \right)\end{aligned}$$

$$\begin{aligned}\frac{\partial H_a}{\partial \lambda_1} &= 0 \\ \frac{\partial H_b}{\partial \lambda_1} &= \frac{g_1 \sigma(t)}{b_2 - b_1} \left(e^{b_1(t_1-t)} - e^{b_2(t_1-t)} \right)\end{aligned}$$

$$\begin{aligned}\frac{\partial H_a}{\partial t_1} &= 0 \\ \frac{\partial H_b}{\partial t_1} &= \frac{\lambda_1 g_1 \sigma(t)}{b_2 - b_1} \left(b_1 e^{b_1(t_1-t)} - b_2 e^{b_2(t_1-t)} \right).\end{aligned}$$

The minimization is performed using Matlab function `fmincon`, which is available on Optimization Toolbox for constrained minimization. The implementation of the positivity constraints

$$\begin{aligned}x_i &> 0, i = 1, \dots, 5 \\ \mathbf{x} &= \{\hat{g}_1, \hat{b}_1, \hat{b}_2, \hat{\lambda}_1, \hat{t}_1\}\end{aligned}$$

is to assure that the optimization would produce parameters with biologically reasonable values, *i.e.* positive values.