Modeling of Hormone Secretion-Generating Mechanisms With Splines: A Pseudo-Likelihood Approach

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SUMMARY. A flexible and robust approach is proposed for the investigation of underlying hormone secretion-generating mechanism. Characterizing hormone time series is a difficult task as most hormones are secreted in a pulsatile manner and pulses are often masked by the slow decay. We model hormone concentration as a filtered counting process where the intensity function of the counting process is modeled nonparametrically using periodic splines. The intensity function and parameters are estimated using a combination of weighted least squares and pseudo-likelihood based on the first two moments. Our method uses concentration measurements directly which avoids the difficult task of estimating pulse numbers and locations. Both simulations and applications suggest that our method performs well for estimating the intensity function of the pulse-generating counting processes.

KEY WORDS: circadian rhythm; counting process; depression; endocrinology; filtered process; hormone secretion; periodic spline; pseudo-likelihood; weighted least squares.

1. Introduction

Hormones control basic physiological processes such as growth, metabolism, reproduction and stress-related adaption. Synthesis and secretion of hormones are the most highly regulated aspect of endocrine control. Such control is mediated by circadian rhythms and positive/negative feedback circuits, and is susceptible to pathological disturbances in various disease states (Plotsky and Vale, 1985; Veldhuis, Carlson and Johnson, 1987; Sartorio et al., 2000; Wang, Ke and Brown, 2003).

A pulsatile pattern of secretion is seen for virtually all hormones, with variations in pulse characteristics that reflect specific physiologic states (Veldhuis et al., 1987). Well documented researches suggest that various diseases are related to hormone dysregulation. For example, about 30% of major depression patients demonstrate hypercortisolemia and 66% of melancholic depressed subjects show non-suppression of cortisol to dexamethasone (Carroll et al., 1981; Linkowski et al., 1985; Pfohl et al., 1985; Rubin et al., 1987). In recent years, there is increasing evidence that the secretion patterns, rather than the absolute concentration level, are important in triggering the response of a target tissue. Therefore, it is necessary to characterize the latent secretion patterns and to investigate the disease effects on these patterns.

Young, Carlson and Brown (2001) conducted an experiment to study disease effects on secretion patterns of hormones ACTH and cortisol in depressed women. They performed ten-minute sampling for ACTH and cortisol from 9:00 AM for 24 hours in 25 premenopausal depressed women and 25 controls matched by age and menstrual cycle day. Figure 1 shows the ACTH and cortisol time series of two typical pairs of subjects.

[Figure 1 about here.]

Except the point process approach in Anderson and O'Sullivan (1993), all existing statis-

tical methods for the investigation of hormone secretion pattern use a two-stage analysis. At the first stage, single hormone time series from each subject is analyzed for pulse locations, masses and shape parameters including decay rate, and basal secretion. At the second stage, estimates at the first stage are treated as observations and covariate effects on pulse patterns are analyzed using observations across subjects Young et al. (2001). The first stage analysis is often referred to as the pulse detection for which many methods have been developed in the literature (Veldhuis et al., 1987; O'Sullivan and O'Sullivan, 1988; Guo, Wang and Brown, 1999; Johnson, 2003; Yang, Liu and Wang, 2006). The second stage analysis usually involves applications of standard statistical methods. To study pulse frequencies at the second stage, Anderson and O'Sullivan (1993), Keenan, Evans and Veldhuis (2003), Keenan et al. (2003) and Keenan and Veldhuis (2003) assumed a stationary renewal process for pulse events while O'Sullivan and O'Sullivan (1988) and Keenan and Veldhuis (1997) assumed a non-homogeneous Poisson process. Variations in the first stage analysis are usually ignored. The two-stage analysis relies on good estimates at the first stage which is reasonable for relatively clean signals (e.g. Luteinizing hormone). However, for hormones such as ACTH and cortisol, signals are noisier (Figure 1). In these cases, the first stage analysis is technically challenging and sometimes none of the current methods provides good estimates for some subjects. On the other hand, the dynamics are often similar across subjects, possibly driven by the circadian rhythm (Figure 1). This kind of information has not been fully explored.

The goal of this paper is to develop a flexible statistical method for the investigation of secretion-generating mechanisms and disease effects on these mechanisms, without the need to identify pulse locations. Several authors noticed that the frequency of pulses varies during the day. They postulated that pulsatile secretions are regulated by the circadian rhythm (Veldhuis et al., 1989; Winer, Shaw and Baumann, 1990; Wang and Brown, 1996; Keenan and Veldhuis, 1997). However, due to the complexity of the problem, it is only recently that

attempts have been made to quantify secretion-generating mechanisms by tractable mathematical forms (Keenan and Veldhuis, 1997). As Keenan and Veldhuis (1997), we assume that pulses are generated from a counting process modulated by an underlying deterministic nyctohemeral "oscillator" such as the suprachiasmatic circadian clock. However, instead of using a parametric model, we will model the underlying intensity functions nonparametrically using a periodic spline. Keenan and Veldhuis (1997) used the likelihood based on the outcome of pulse detection at the first-stage analysis to estimate all parameters. Our estimation procedure, similar to that in Anderson and O'Sullivan (1993), does not require pulse detection. Therefore, compared to the two-stage analysis, our method has the advantage that the inferences are unconditional on the estimated pulse locations. Anderson and O'Sullivan (1993) assumed that pulses are generated by a renewal process which led to a stationary process for the hormone concentration. Therefore, their method cannot be used to investigate dynamics during the day such as the circadian rhythm. The stationary assumption is quite restrictive in practice. We estimate the nonparametric intensity function using penalized weighted least squares and parameters using profiled pseudo-likelihood. Requiring the first two moments only, our estimation method is robust to distributional assumptions.

We introduce the model and estimation method in Sections 2 and 3 respectively. We validate the proposed method via simulations in Section 4. We fit the data from Young et al. (2001) in Section 5 and conclude with some remarks in Section 6.

2. The Model

Experiments are usually conducted in such a way that blood samples are withdrawn from multiple subjects (patients and controls) at regular time intervals for a period of time, say every 10 minutes for 24 hours. For simplicity, we transform the observation time interval of 24 hours to the interval $[0, 1]$. Let t_{ij} be the jth observation time of the ith subject and $y(t_{ij})$

denote the observed hormone concentration. We assume that (O'Sullivan and O'Sullivan, 1988)

$$y(t_{ij}) = \gamma_i + \alpha_i \int_{-\infty}^{\infty} p(\boldsymbol{\beta}, t_{ij} - x) dN_i(x) + \epsilon_{ij}, \quad i = 1, \cdots, m, \quad j = 1, \cdots, n_i, \tag{1}$$

where γ_i is the baseline secretion, α_i is the pulse amplitude, p is a pulse shape function with parameters $\boldsymbol{\beta}$, $N_i(t)$ is a counting process representing the number of pulses to occur up to time t, and ϵ_{ij} is the random error. We will assume that γ_i's are iid random variables with mean γ and variance σ_γ^2, α_i's are iid random variables with mean α and variance σ_α^2, and ϵ_{ij}'s are iid random variables with mean zero and variance σ^2. Furthermore, we assume that γ_i, α_i, ϵ_{ij} and $N_i(t)$ are mutually independent.

Compared to the model (2.4) in O'Sullivan and O'Sullivan (1988), γ_i is added to allow a non-zero baseline, the integral is taken on the whole real line, and model (1) is assumed for a population of subjects rather than a single subject. Note that pulses outside the actual observation period, especially those close to the boundaries, affect observations within the period. O'Sullivan and O'Sullivan (1988) used a finite interval containing the observation period to account for all non-negligible pulses. Our method does not require the specification of such a finite interval.

Model (1) expresses the hormone concentration at time t as sum of contributions from baseline and individual pulses. The pulse shape function p describes how a single pulse is accumulated and then released. We assume that (O'Sullivan and O'Sullivan, 1988)

$$p(\boldsymbol{\beta}, s) = \begin{cases} \exp(\beta_1 s), & s \leq 0, \\ \exp(-\beta_2 s), & s > 0, \end{cases} \tag{2}$$

where β_1 the infusion rate and β_2 the decay rate. Let $\boldsymbol{\beta} = (\beta_1, \beta_2)^T$ which will be referred to as the pulse shape parameters.

Neuroendocrine ensembles communicate with their remote and proximal target cells via

an intermittent pattern of hormone pulses. Biomedical researchers are interested in the mechanisms by which neuroendocrine signals are generated and how these mechanisms are affected by demographic, environmental and psychological variables such as depression. The secretion pattern often exhibits random behavior which cannot be explained by a deterministic model. We assume that the seemingly erratic, random bursts of secretory activity by the glands are regulated by a underlying circadian oscillator (Keenan and Veldhuis, 1997). Specifically, we assume that $N_i(t)$'s are iid counting processes with a positive *periodic* intensity function $h(t)$. We assume that all subjects have the same phase. It is a reasonable assumption for the data described in Section 1 since the experiment was well controlled (Young et al., 2001). We have also checked this assumption indirectly by fitting models in Wang et al. (2003) where estimates of subject-specific phases are very close. The main interest of this paper is to estimate the intensity function for a population, test if it follows the circadian rhythm, and investigate differences between populations. We use the logarithm transformation, $\eta(t) = \log(h(t))$, to relax the positive constraint. We assume that $\eta \in W_2(per)$ (Wahba, 1990), where

$$W_2(per) = \{f : f \text{ and } f' \text{ are abs. cont., } f(0) = f(1),\ f'(0) = f'(1)\ , \int_0^1 (f''(t))^2 dt < \infty\}. \quad (3)$$

We note that the assumption of a constant amplitude α_i for subject i is quite restrictive. A more flexible model will allow a different amplitude for each pulse (Anderson and O'Sullivan, 1993). We have tried a more general model with a random amplitude for each pulse as in Anderson and O'Sullivan (1993). We found that, due to the complexity of the model, the pseudo-likelihood approach does not provide a good estimate for the variance of amplitude within a subject. This is perhaps why Anderson and O'Sullivan (1993) fixed the variance parameter as zero (i.e. assumed a constant amplitude for each subject) in their simulations and applications. In this paper we are mainly interested in population parameters such as the intensity function. In our simulations, we generated observations with a random

amplitude for each pulse and random pulse shape parameters for each subject. We found that the pseudo-likelihood approach provides good estimates for the intensity function and other parameters even when the variation of amplitudes within a subject is large and this variation is ignored.

3. Estimation

The parameters of interest are $\boldsymbol{\theta} = (\gamma, \alpha, \boldsymbol{\beta}^T, \sigma_\gamma^2, \sigma_a^2, \sigma^2)^T$ and the log intensity function η. Note that we do not assume specific distributions for the baselines γ_i, the amplitudes α_i and random errors ϵ_{ij}. There is no closed form representation of the likelihood function even when these random variables are assumed Gaussian. Due to the fact that the number of pulses and pulse locations are unknown and the observations are further masked by the slow decay to the baseline, it is very difficult to calculate the exact likelihood.

Let $\mathbf{t}_i = (t_{i1}, t_{i2}, \cdots, t_{in_i})^T$, $\mathbf{y}_i = (y_{i1}, y_{i2}, \cdots, y_{in_i})^T$, $\xi(t) = \mathrm{E}(y(t))$, $\boldsymbol{\xi}_i = \mathrm{E}(\mathbf{y}_i)$, $W_i = \mathrm{cov}(\mathbf{y}_i)$ and $\mathbf{y} = (\mathbf{y}_1^T, \cdots, \mathbf{y}_m^T)^T$. We have (Web Appendix A)

$$\begin{aligned}
\xi(t) &= \gamma + \alpha \int_{-\infty}^{\infty} p(\boldsymbol{\beta}, t-x) h(x) dx, \\
\mathrm{Cov}(y(s), y(t)) &= \sigma_\gamma^2 + (\alpha^2 + \sigma_\alpha^2) \int_{-\infty}^{\infty} p(\boldsymbol{\beta}, s-x) p(\boldsymbol{\beta}, t-x) h(x) dx \\
&\quad + \sigma_\alpha^2 \left(\int_{-\infty}^{\infty} p(\boldsymbol{\beta}, s-x) h(x) dx \right) \left(\int_{-\infty}^{\infty} p(\boldsymbol{\beta}, t-x) h(x) dx \right) + \sigma^2 I(s=t),
\end{aligned}$$

where $I(s=t) = 1$ when $s = t$ and 0 otherwise. For the double exponential pulse shape function (2), we have the following explicit formulae for the mean and covariance (Web Appendix B)

$$\begin{aligned}
\xi(t) &= \gamma + \frac{\alpha}{1 - \exp(-\beta_2)} \int_0^1 \exp(-\beta_2 x) h(t-x) dx \\
&\quad + \frac{\alpha}{1 - \exp(-\beta_1)} \int_0^1 \exp(-\beta_1 x) h(t+x) dx, \\
\mathrm{Cov}(y(s), y(t)) &= \sigma_\gamma^2 + \frac{\exp(-\beta_2(t-s))}{1 - \exp(-2\beta_2)} (\alpha^2 + \sigma_\alpha^2) \int_0^1 \exp(-2\beta_2 x) h(s-x) dx \\
&\quad + \exp(-\beta_2(t-s)) (\alpha^2 + \sigma_\alpha^2) \int_0^{t-s} \exp(-(\beta_1 - \beta_2) x) h(s+x) dx
\end{aligned}$$

$$+\frac{\exp(-\beta_1(t-s))}{1-\exp(-2\beta_1)}(\alpha^2+\sigma_\alpha^2)\int_0^1 \exp(-2\beta_1 x)h(t+x)dx$$
$$+\frac{\sigma_\alpha^2}{\alpha^2}(\xi(s)-\gamma)(\xi(t)-\gamma)+I(s=t)\sigma^2, \quad s\le t. \tag{4}$$

Define the following pseudo-likelihood of $\mathbf{y}$ based on its first two moments (Carroll and Ruppert, 1982; Davidian and Carroll, 1987):

$$l(\boldsymbol{\theta},\eta|\mathbf{y}) \propto -\frac{1}{2}\sum_{i=1}^{m}\log|W_i| - \frac{1}{2}\sum_{i=1}^{m}(\mathbf{y}_i-\boldsymbol{\xi}_i)^T W_i^{-1}(\mathbf{y}_i-\boldsymbol{\xi}_i). \tag{5}$$

For heteroscedastic linear models, Carroll and Ruppert (1982) proposed a robust and efficient estimation procedure using pseudo-likelihood (PL) and weighted least squares (WLS). Their procedure consisted of two parts: (i) estimate mean parameters (γ, α, $\boldsymbol{\beta}$ and η) by WLS with weights (W_i's) fixed at their estimates, and (ii) estimate covariance parameters (σ_γ^2, σ_α^2 and σ^2) by PL with mean parameters fixed at their estimates. Part (i) of Carroll and Ruppert's procedure cannot be applied directly because the mean parameters are not identifiable when the weights W_i's are fixed. For example, the mean will remain the same when we multiply a constant to the parameter α and divide h by the same constant. We note that model (1) itself is identifiable and nonidentifiability came up only if we were to apply Carroll and Ruppert's procedure directly. In the following, we propose a modification of their robust estimation procedure. We estimate η by WLS and all other parameters by a profiled PL.

For any fixed $\boldsymbol{\theta}$, we estimate η as the solution to the following penalized WLS

$$\min_{\eta\in W_2(per)}\left\{\sum_{i=1}^{m}(\mathbf{y}_i-\boldsymbol{\xi}_i)^T\tilde{W}_i^{-1}(\mathbf{y}_i-\boldsymbol{\xi}_i)+N\lambda\int_0^1(\eta''(t))^2dt\right\}, \tag{6}$$

where $N=\sum_{i=1}^m n_i$, $\tilde{W}_i$ is W_i evaluated at the fixed $\boldsymbol{\theta}$ and a previous estimate of η, $\int_0^1(\eta''(t))^2dt$ is a penalty on η, and λ is a smoothing parameter balancing the goodness-of-fit and the smoothness of the function η. Let the solution to (6) be $\hat{\eta}_{\boldsymbol{\theta}}$, where the dependence on $\boldsymbol{\theta}$ is expressed explicitly. We then estimate $\boldsymbol{\theta}$ as the maximizer, $\hat{\boldsymbol{\theta}}$, of the profiled PL $l(\boldsymbol{\theta},\hat{\eta}_{\boldsymbol{\theta}}|\mathbf{y})$. The estimate of η is then $\hat{\eta}_{\hat{\boldsymbol{\theta}}}$. The quasi-Newton method is used to find $\hat{\boldsymbol{\theta}}$. Note

that $\hat{\eta}_{\boldsymbol{\theta}}$ changes with $\boldsymbol{\theta}$ and, therefore, is involved in the computation of the approximate gradient when optimizing the profiled PL. The simpler approach that iterates between WLS and PL (i.e. updates η using the penalized WLS with a fixed $\boldsymbol{\theta}$ and updates $\boldsymbol{\theta}$ using PL with a fixed η) sometimes fails to converge for our problem. We note that in both approaches, whether using the profiled PL or the PL with a fixed η, $\boldsymbol{\theta}$ is identifiable since both $\boldsymbol{\xi}_i$ and W_i involve $\boldsymbol{\theta}$. Constraints on parameters such as non-negativity of variances are enforced.

Due to the logarithm transformation, $\xi(t)$ depends on η non-linearly. Readers are referred to Ke and Wang (2004b) for the existence of a unique solution to (6). We solve (6) using the extended Gauss-Newton method in Ke and Wang (2004b) for nonlinear smoothing spline models. Briefly, at each iteration, we approximate the non-linear functional by its first-order Taylor expansion at the current estimate of η. It reduces (6) to a standard penalized WLS problem for linear smoothing spline models, the solution of which then becomes the next current estimate of η. We then use the new η for the next-round Taylor expansion and for updating $\tilde{W}_i$. For a given initial value of η, the procedure is iterated until convergence to obtain $\hat{\eta}_{\boldsymbol{\theta}}$. Our experience suggests that around five iterations are usually sufficient. Since each iteration involves solving a standard penalized WLS problem with a fixed weight matrix, we select the smoothing parameter at each iteration using either the generalized cross-validation (GCV) or the generalized maximum likelihood (GML) criterion for the standard smoothing spline models (Wahba, 1990; Wang, 1998). This iterative approach for selecting the smoothing parameter is similar in spirit to that in Severini and Wong (1992) for semiparametric regression involving profiled likelihoods and local polynomial models for the nonparametric function. It is also similar in spirit to that in Gu (1992) and Wahba et al. (1995) for spline smoothing with data from exponential families. Simulations indicate that this iterative approach performs well, and the GCV and GML methods have similar performances. See Web Appendix C for details.

Integrals in (4) cannot be computed analytically. In addition, the spline solution in the minimization problem (6) requires double integrals involving a reproducing kernel. Some tedious algebra shows that all integrals in (4) and (6) can be decomposed into summations of integrals of the form $\int_{t_{(j_1)}}^{t_{(j_1+1)}} \mathrm{INT}_1(x)dx$ or $\int_{t_{(j_1)}}^{t_{(j_1+1)}} \int_{t_{(j_2)}}^{t_{(j_2+1)}} \mathrm{INT}_2(x,y)dxdy$ for some integrands INT_1 and INT_2. These single and double integrals on short intervals can then be well approximated by a three-point Gaussian quadrature and a nine-point Gaussian quadrature respectively. See Web Appendix C for details about the reproducing kernel and the integral approximations. The R function `ssr` in the ASSIST package (Ke and Wang, 2004a) is used to fit the standard smoothing spline model, and the R function `optim` is used to find the maximizer of the profiled PL under constraints. The R codes are available from the authors.

4. Simulation

We consider m subjects with equally spaced observation time points at $t_{ij} = (j - 0.5)/150$, $i = 1, \cdots, m$ and $j = 1, \cdots, 150$. For subject i, we first generate the number of pulses, K_i, and their locations, x_{ik}, $k = 1, \cdots, K_i$, according to a non-homogeneous Poisson process with intensity function $h(t) = 35 + 25\sin(2\pi t)$. We then generate the amplitude $\alpha_i \overset{iid}{\sim}$ logNormal$(7, \sigma_\alpha^2)$, infusion rate $\beta_{1i} \sim \mathrm{N}(200, 10)$, decay rate $\beta_{2i} \sim \mathrm{N}(40, 1)$, baseline $\gamma_i \sim$ logNormal$(2, 1)$ and random errors $\epsilon_{ij} \overset{iid}{\sim} \mathrm{N}(0, 0.25)$. Finally, observations

$$y(t_{ij}) = \gamma_i + \sum_{k=1}^{K_i} \alpha_{ik} p(\boldsymbol{\beta}_i, t_{ij} - x_{ik}) + \epsilon_{ij}, \quad i = 1, \cdots, m, \quad j = 1, \cdots, 150, \tag{7}$$

where $\alpha_{ik} \overset{iid}{\sim}$ logNormal$(\alpha_i, 1)$, $k = 1, \cdots, K_i$. Note that amplitudes are different for each pulse and pulse shape parameters are different for each subject. Therefore, model (7) is more general than model (1). We ignore these variations and fit model (1) to the simulated data. The purpose is to find out how our method performs under this more realistic situation.

We consider a factorial design with two levels of m, $m = 30$ and 100, and three levels of σ_α^2, $\sigma_\alpha^2 = 1, 4$, and 10. We repeat 100 times for each simulation setting. The infusion rate

is very fast comparing to the sampling rate. Therefore, it is usually difficult to get a good estimate of the infusion rate, especially when the number of subjects is not large. For $m = 30$, we assume that the infusion rate is known. This is not uncommon in practice. For example, Anderson and O'Sullivan (1993) assumed that the infusion rate, the baseline and the error variance are all known. For $m = 100$, we use initial values $\boldsymbol{\theta}_0 = (1, 4, (50, 20)^T, 0, 0, 0)^T$ and $h_0 = 10$. The same initial values are used for $m = 30$ except that β_1 is fixed at the truth. The algorithm is rather robust to the choice of the initial values. We estimate the smoothing parameter λ in (6) using both GCV and GML methods. Results are similar. The presented results are based on the GML criterion.

Tables 1 and 2 summarize the estimation results for $m = 30$ and $m = 100$ respectively. For a non-homogeneous Poisson process on $[0, 1]$, the expected number of events is $\mathrm{E}N(1) = \int_0^1 h(t)dt$. We also list estimates of the expected number of pulses in Tables 1 and 2. For the intensity estimates, we compute the integrated mean squared errors as

$$IMSE = \frac{1}{R}\sum_{r=1}^{R}\int_0^1 \left(\exp(\hat{\eta}^r_{\hat{\boldsymbol{\theta}}}) - h(x)\right)^2 dx,$$

where $R = 100$ is the number of simulation repetitions and $\hat{\eta}^r_{\hat{\boldsymbol{\theta}}}$ is the estimated log-intensity from the rth repetition. Figures 2 and 3 show fits corresponding to the 5th, 25th, 50th, 75th and 95th percentiles of the IMSE.

For comparison, we also estimate the intensity function using the kernel density. According to the property of a non-homogeneous Poisson process, conditional on the total number of pulses, the pulse locations are iid with density $h(x)/\int_0^1 h(x)dx$. Treating the generated pulse locations from all subjects as observations, we compute the kernel density estimate with optimal window width chosen by the direct plug-in method. We estimate $\int_0^1 h(x)dx$ by the average number of pulses per subject. Then an estimate of h is obtained by multiplying the two estimates. We note that this approach uses unobservable pulse numbers and

locations as observations. Therefore, the comparison puts our method in a disadvantageous position.

From the simulation results, the proposed estimation method performs reasonably well. There are some upward biases in the estimates of $\boldsymbol{\theta}$. The estimate of the intensity function is on average unbiased for both sample sizes, so is the estimated pulse number. Our intensity estimates corresponding to the 5th, 25th and 50th percentiles of the IMSE are comparable to their kernel counterparts. Larger sample size boosts the performance of our procedure significantly.

[Table 1 about here.]

[Table 2 about here.]

[Figure 2 about here.]

[Figure 3 about here.]

5. Application

We now apply our method to the data described in Section 1. Young et al. (2001) used a two-stage analysis to investigate secretion patterns for depressed and normal women. For each subject, they first applied the method in Guo et al. (1999) to detect pulse locations and estimate all parameters. For the first stage analysis, they also tried the Pulsefit (Kushler and Brown, 1991) and deconvolution (Veldhuis and Johnson, 1992) methods which led to unreasonable estimates of half-lives due to slow decay. They then applied t-test to the estimates of secretion patterns including the number of pulses, amplitude, half-life and baseline to investigate differences between depressed women and their normal controls. Young et al. (2001) concluded that baselines for both ACTH and cortisol are elevated, and there are no significant differences in pulsatile components.

Our overall objective remains the same: to investigate differences in secretion patterns of ACTH and cortisol between depressed and normal women. Our approach is different: we concentrate on the underlying secretion-generating mechanisms.

We fit model (1) to the patient group and normal group separately. It is difficult to get a precise estimate of β_1 due to the fast infusion rate. We compute the estimate of β_1 using a grid search. Specifically, we compute estimates of all parameters with β_1 fixed at one of the 15 equally spaced points in the interval $[100, 700]$. Then we estimate β_1 as the one that gives the largest PL. It turns out for both hormones in both groups, $\beta_1 = 700$ is the optimal choice. Estimates of other parameters are rather close when $\beta_1 > 700$.

We compute the hormone half-life as $\tau = 1440 \log(2)/\beta_2$ minutes. Estimates of parameters for ACTH and cortisol are listed in Table 3. Estimates of the intensity function are shown in Figure 4. We use the bootstrap procedure to construct 95% confidence intervals for all parameters and the intensity function (Wang and Wahba, 1995). Bootstrap samples of size 100 are drawn from the fitted model (1) with $N_i(t)$ being a non-homogeneous Poisson Process. These 95% bootstrap confidence intervals are also shown in Table 3 and Figure 4.

[Table 3 about here.]

[Figure 4 about here.]

Results show that on average, patients tend to have elevated baseline, pulse intensity and number, longer half-life, and smaller pulse amplitude for both hormones. While the between-subject variations of amplitude and baseline for ACTH are close between patients and controls, patients on average exhibit a lot larger variation in their corresponding cortisol measurements. However, none of the differences are statistically significant at the 0.05 level. Using a different approach, we reach similar conclusions as those in Young et al. (2001). Furthermore, our new method reveals insights into the underlying secretion-generating mech-

anisms which cannot be addressed by those methods in Young et al. (2001). Figure 4 shows that the intensity functions are not constant during the day, suggesting pulsatile secretions are regulated by a underlying circadian oscillator. The same conclusion has been reached for Luteinizing hormone secretion in horses (Keenan and Veldhuis, 1997). More pulse events occur in the early morning. For both ACTH and cortisol, depressed women have more pulse events throughout the day. The difference between intensity functions for depressed women and their normal controls is not statistically significant for either hormones. Regulation of the circadian oscillator on the intensity function at the secretion level partly explains the circadian rhythm observed at the concentration level (Young et al., 2001; Wang et al., 2003).

Our method does not require pulse detection in the first stage analysis, while variations in the first stage analysis were ignored in Young et al. (2001). For example, Young et al. (2001) found patients have significantly elevated ACTH baseline (p-value=0.045). Omission of the variation in the first stage analysis may be part of the reason for the more significant p-value.

6. Discussion

We present a novel approach for modeling the hormone-generating mechanism. It models the hormone pulses as generated from a counting process where the intensity function is estimated non-parametrically using periodic splines. It allows us to explore regulation by the circadian rhythm and disease effects on the regulation. Compared with current methods, our approach is robust and avoids the difficulty of identifying pulse locations. The method in this paper is intended for the situation when the experiment is well controlled such that all subjects have roughly the same phase and the population parameters are the main interests. It does not explore subject-specific and pulse-specific variations (Yang et al., 2006). When subjects have different phases, the method in Keenan and Veldhuis (1997) should be used

where a first stage analysis for pulse detection is needed. Methods to explore both subject-specific and pulse-specific variations have yet to be developed.

For simplicity of implementation and to save computational time, we used an iterative approach to select the smoothing parameter. Drawbacks of this iterative approach are that the convergence is not guaranteed and the goal function is not explicitly defined. Further research on properties of this iterative approach or new direct methods is needed. For example, as in Gu and Ma (2005), it may be better to estimate the smoothing parameter jointly with $\boldsymbol{\theta}$.

The model (1) is a filtered counting process model with mixed effects. Many physical phenomena such as radioactive emission measurements and opto-electronic signals can be modeled as a filtered counting process (Snyder and Miller, 1991). Our proposed estimation procedure can be modified for these applications.

7. Supplementary Materials

Web Appendices referenced in Section 3 are available under the Paper Information link at the Biometrics website http://www.tibs.org/biometrics.

Acknowledgement

This research was supported by NIH Grant R01 GM58533. The authors would like to thank Elizabeth, A. Young, MD, and Professor Morton B. Brown from the University of Michigan for allowing us to use the hormone data. The authors thank the editor, associate editor and two referees for constructive comments that substantially improved an earlier draft.

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[p]

Table 1

Simulation results when $m = 30$.

		$\sigma_\alpha^2 = 1$	$\sigma_\alpha^2 = 4$	$\sigma_\alpha^2 = 10$
	TRUE	Mean(SD)	Mean(SD)	Mean(SD)
γ	2	2.43(0.28)	2.41(0.34)	2.45(0.39)
β_2	40	42.52(2.99)	42.39(3.04)	42.63(3.87)
α	7	7.20(0.58)	7.30(0.77)	7.14(0.97)
σ^2	0.25	0.27(0.11)	0.26(0.04)	0.32(0.16)
σ_α^2	1 | 4 | 10	1.11(0.92)	4.39(1.98)	10.60(7.12)
σ_γ^2	1	1.25(0.81)	1.10(0.84)	1.55(1.23)
E$N(1)$	35	34.66(5.47)	34.33(5.79)	35.04(6.52)

[p]

Table 2

Simulation results when $m = 100$.

		$\sigma_\alpha^2 = 1$	$\sigma_\alpha^2 = 4$	$\sigma_\alpha^2 = 10$
	TRUE	Mean(SD)	Mean(SD)	Mean(SD)
γ	2	2.15(0.16)	2.24(0.22)	2.20(0.27)
β_1	200	198.91(9.74)	198.33(10.25)	199.62(14.21)
β_2	40	40.61(1.78)	40.77(2.15)	40.02(2.50)
α	7	7.17(0.31)	7.29(0.41)	7.11(0.52)
σ^2	0.25	0.25(0.03)	0.25(0.03)	0.25(0.04)
σ_α^2	1 | 4 | 10	1.09(0.49)	4.39(1.04)	10.64(3.68)
σ_γ^2	1	0.97(0.43)	1.06(0.55)	1.16(0.92)
E$N(1)$	35	33.96(2.52)	33.30(3.03)	35.23(3.77)

[p]

Table 3

Parameter estimates with 95% bootstrap confidence intervals.

	ACTH		Cortisol	
	Control	Patient	Control	Patient
γ(ug/dl)	1.76(1.39,2.72)	2.01(1.66,2.71)	2.58(2.52,3.12)	2.98(2.41,4.07)
τ(mins)	27.92(21.47,30.46)	28.85(21.79,32.13)	28.82(23.02,30.11)	31.70(24.71, 35.19)
α(ug/dl)	4.51(3.29,5.81)	3.28(2.44,4.10)	6.51(5.63,7.50)	5.74(4.86,6.75)
σ^2	0.13(0.05,0.19)	0.14(0.08,0.20)	0.12(0,0.21)	0.16(0,0.34)
σ_α^2	1.35(0,3.98)	1.51(0.10,3.40)	0.44(0,2.16)	1.61(0,3.73)
σ_γ^2	1.32(0.28,4.41)	0.80(0.21,1.70)	0.33(0,0.66)	1.91(0.29,4.11)
E$N(1)$	14.79(10.33,25.05)	21.78(13.83,39.85)	25.39(10.70,35.01)	33.64(24.42,46.74)

[p]

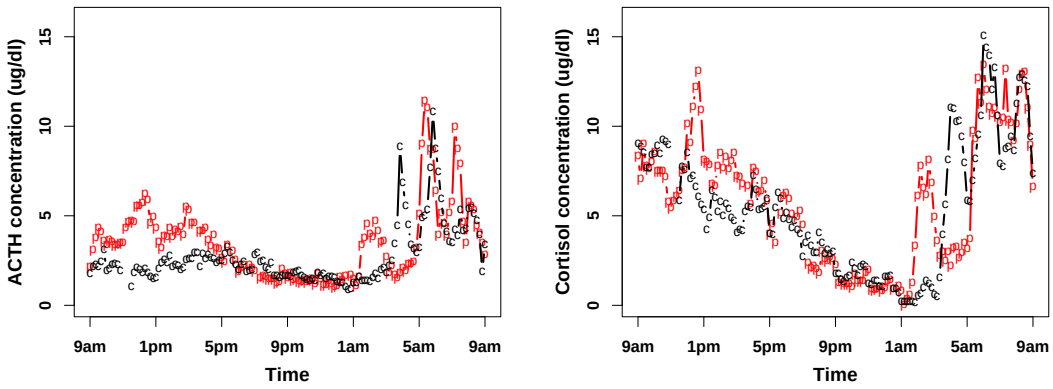

Figure 1. Plots of hormones ACTH (left) and cortisol (right) concentration from two matched pairs of subjects. Labels "p" and "c" represent patient and control respectively.

[p]

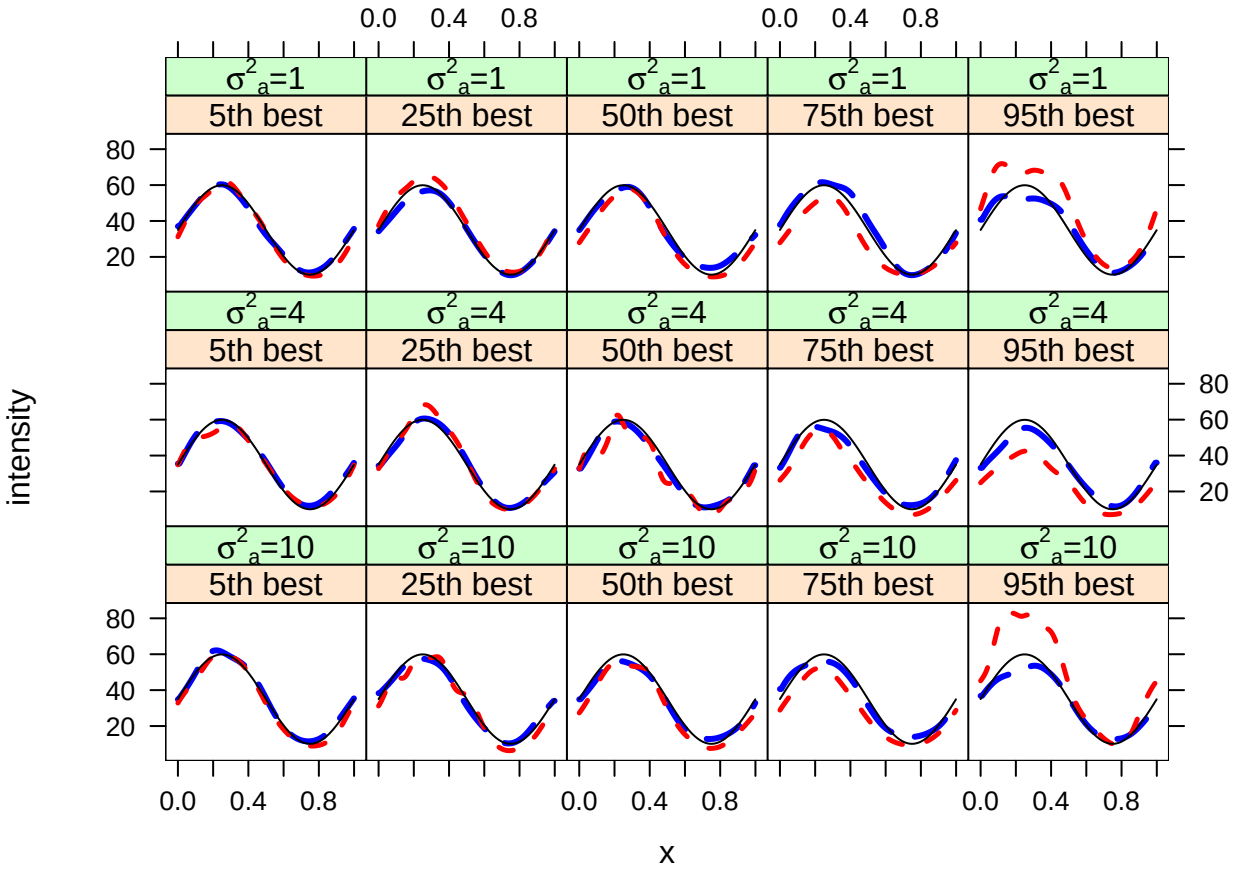

Figure 2. Plots of the true intensity function (black solid), kernel estimates (blue long-dashed) and estimates from our method (red short-dashed) when $m = 30$.

[p]

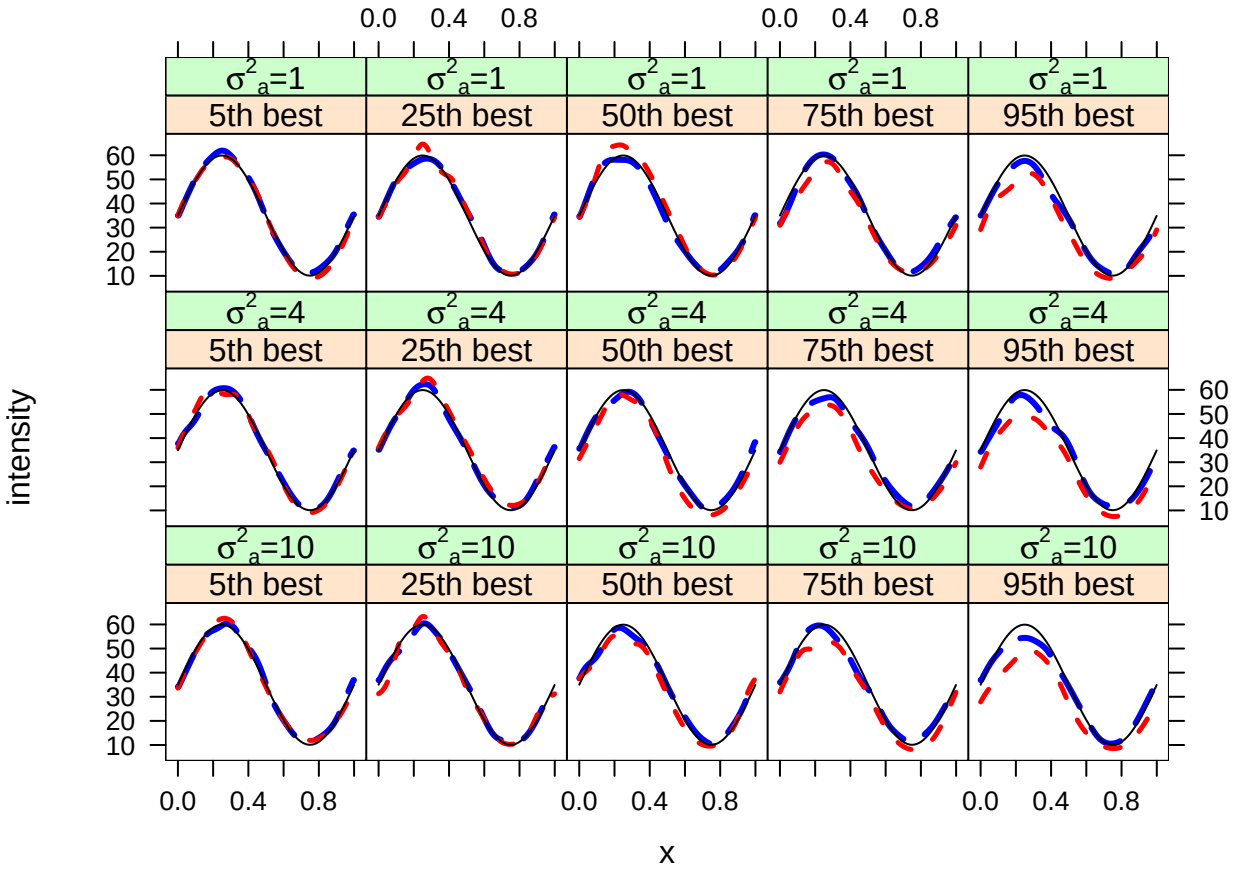

Figure 3. Plots of the true intensity function (black solid), kernel estimates (blue long-dashed) and estimates from our method (red short-dashed) when $m = 100$.

[p]

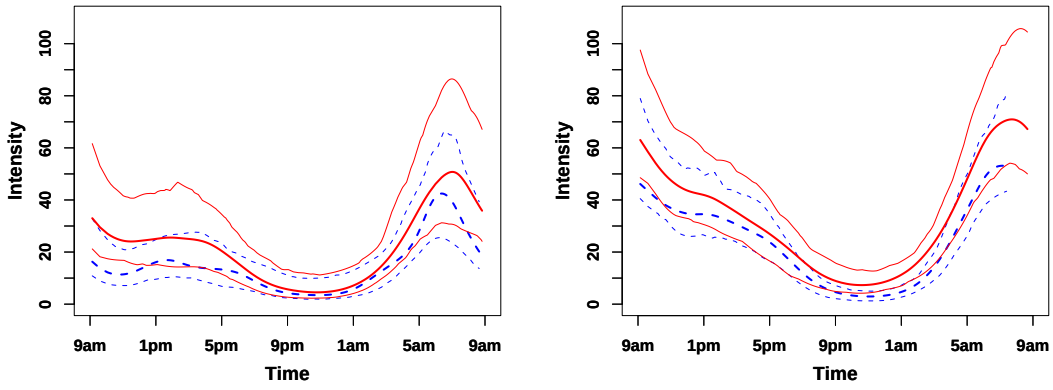

Figure 4. Plots of intensity function estimates with 95% bootstrap confidence intervals for ACTH (left) and cortisol (right). Red solid lines are for patients and blue dashed lines for controls.