A Flexible Model For Human Circadian Rhythms

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Summary

Many hormones and other physiological processes vary in a circadian pattern. Although a sine/cosine function can be used to model these patterns, this functional form is not appropriate when there is asymmetry between the peak and nadir phases. In this paper we describe a semi-parametric periodic spline function that can be fit to circadian rhythms. The model includes both phase and amplitude so that the time and the magnitude of the peak or nadir can be estimated. We also describe tests of fit for components in the model. Data from an experiment to study immunological responses in humans are used to demonstrate the methods.

Key words: Bayesian confidence intervals, chronobiology, GCV, L-spline, periodic smoothing spline, self modeling.

1 Introduction

Many hormones and other physiological measurements in the human vary in a circadian pattern. For example, it is well-known that body temperature is lowest during sleep in the early morning and rises after awakening. Since the 24-hour periodicity is entrained, possibly due to the day/night or sleep/awake cycles that are characteristics of the environment, the cycle length is fixed. However, the pattern over time need not be symmetric; that is, the peak and nadir may not be separated by 12 hours and/or the amplitude and width of the peak may differ from those of the nadir. For these reasons a single sine/cosine wave with a 24-hour period does not necessarily fit data from these series adequately. Although adding harmonics can improve the fit, the resulting function is more difficult to interpret. Examination of observed series of data for several characteristics from a study of normal subjects (Kronfol, Nair, Zhang, Hill and Brown (1995)) also indicated that the assumption that the function has no local minima or maxima, other than the global minimum and maximum, may not hold for all series. Therefore, we chose to consider models for circadian rhythms that are more general than the sine/cosine functional form.

We first considered a nonparametric smooth curve based on fitting a periodic spline function. SEMOR (self modeling) using a first-order spline to model a common curve for these kinds of data was introduced by Lawton and Sylvestre (1971) and Lawton, Sylvestre and Maggio (1972). Several authors investigated other choices for the common curve: a smoothing spline (Guardabasso, Rodbard and Munson (1987), Guardabasso, Munson and Rodbard (1988)), a kernel function (Härdle and Marron (1990)) and a free-knot spline (Lindstrom (1992)). See also Kneip and Gasser (1988).

Our proposed method is to model the common curve by a periodic spline (spline on the circle). A generalized cross-validation method is used to select the smoothing parameter at each iteration. Our proposed method is similar to that of Guardabasso et al. (1987) and Guardabasso et al. (1988), although not enough technical details were given in these papers to fully compare our method with theirs. Our choices of common curve, smoothing parameter selection method, estimation procedure and test procedure may be more appropriate for human circadian data.

In Section 2 we describe the data for which the models are developed. The model and estimation procedure are presented in Section 3. We then develop a test based on bootstrap procedure and construct Bayesian confidence intervals for the common curve (Section 4). Analyses of the data sets in Section 2 are presented in Section 5.

2 Human Circadian Rhythms

Blood samples were collected every two hours for 24 hours from a group of healthy normal volunteers and analyzed for parameters that measure immune functions and hormones of the HPA axis (Kronfol et al. (1995)). In addition, core body temperature was measured at the time of each blood drawing. The experiment was approved by the Human Subjects Review Committee at the University of Michigan Medical Center.

Figures 1 and 2 present the observed values for adrenocortocotropic hormone (`ACTH`) on a log scale and core body temperature (`cbt`), respectively, for nine healthy subjects measured at every 2 hours from 8:00 am one day to 6:00 am the next morning. Two `ACTH` observations for subject 7 and one `ACTH` for subject 9 are near zero and are excluded from the analysis. Some `cbt` values are missing for several subjects.

Put Figure 1 here

$\boxed{\text{Put Figure 2 here}}$

The data are noisy and it is difficult to identify patterns among subjects. The investigator hypothesizes that there is a common curve for all individuals. However, the time axis may be shifted and the magnitude of the values may differ between subjects; i.e., there may be phase and amplitude differences between subjects.

Initially, a parametric sine/cosine function was fitted to the data to estimate the common curve (Scales, Vander, Brown and Kluger 1988, Kronfol et al. 1995). The curve is symmetric and not sufficiently flexible. Therefore, we chose to develop a semi-parametric spline function to improve estimation.

3 The Model and Estimation

Assume a general functional form for the data:

$$y_{ij} = \mu_i + \alpha_i f(t_{ij} - \tau_i) + \epsilon_{ij}, \quad i = 1, \cdots, m; \quad j = 1, \cdots, n_i, \tag{1}$$

where

m is the total number of subjects,

n_i is the total number of observations of subject i,

y_{ij} is the response of ith individual at the jth time point t_{ij},

μ_i is the 24-h mean of the ith individual,

$\alpha_i > 0$ is the amplitude (maximum deviation from the mean) of the ith individual,

$0 \leq \tau_i < 1$ is the phase (horizontal shift) of the ith individual. For simplicity, we transform the 24-h period into [0,1].

We assume that ϵ_{ij}'s are iid with $\epsilon_{ij} \sim \mathrm{N}(0, \sigma^2)$.

The function f in (1) is the common curve. For our analysis, we need f to be a periodic function with period equal to 1. For identifiability, we also need the integral of f to be equal to zero and $\sup_{t \in [0,1]} |f(t)| = 1$. More precisely, let

$$\begin{aligned} W_2^0(per) \;=\; \{ \quad & f : f \text{ and } f' \text{ absolutely continuous}, \;\; \int_0^1 (f^{(2)}(t))^2 dt < \infty, \\ & \int_0^1 f(t)dt = 0, \;\; f(0) = f(1), \;\; f'(0) = f'(1)\}. \end{aligned} \tag{2}$$

Our model space for f is $\{f \in W_2^0(per) : \; \sup_{t \in [0,1]} |f(t)| = 1\}$.

A natural extension of the parametric sine/cosine model (Scales et al. (1988)) is to add some harmonics to the common curve. It then is difficult to calculate the amplitude and phase. The model (1) has the advantage that the common curve is flexible. Wahba (1990) proves that with equally spaced data, such a spline is equivalent to downweighting the higher frequency sine/cosine terms, which prevents overfitting. These weights depend on a smoothing parameter, which can be selected by a data-based method such as generalized cross-validation or unbiased risk method (see Wahba (1990)). The model (1) also has the advantage that differences between subjects can be easily compared using tests of parameters.

To estimate the parameters and f, we follow a two-step procedure. If we know the common curve f, for each i we can estimate the parameters μ_i, α_i and τ_i by solving the nonlinear least-square problem:

$$\min \frac{1}{n_i}\sum_{j=1}^{n_i}(y_{ij}-\mu_i-\alpha_i f(t_{ij}-\tau_i))^2. \tag{3}$$

Let $\boldsymbol{\mu}^T=(\mu_1,\cdots,\mu_m)$, $\boldsymbol{\alpha}^T=(\alpha_1,\cdots,\alpha_m)$ and $\boldsymbol{\tau}^T=(\tau_1,\cdots,\tau_m)$. Given current estimates for the parameters $\boldsymbol{\mu}$, $\boldsymbol{\alpha}$ and $\boldsymbol{\tau}$, we can estimate f by solving the penalized least-square problem:

$$\min_{f\in W_2^0(per)}\left\{\frac{1}{m}\sum_{i=1}^{m}\frac{1}{n_i}\sum_{j=1}^{n_i}(y_{ij}-\mu_i-\alpha_i f(t_{ij}-\tau_i))^2+\lambda\int_0^1(f^{(2)}(t))^2dt\right\}, \tag{4}$$

and by normalizing f:

$$f(t)\leftarrow f(t)/\sup_{t\in[0,1]}f(t). \tag{5}$$

$W_2^0(per)$ is a reproducing kernel Hilbert space with reproducing kernel $R(s,t) = -B_4([s-t])/24$, where $[s-t]$ is the fractional part of $s-t$ and $B_4(t) = (t-0.5)^4 - (t-0.5)^2/2 + 7/240$. Consider $\tilde{y}_{ij} = y_{ij} - \mu_i$ as observations. It is easy to see that the linear functionals $L_{ij}f = \alpha_i f(t_{ij} - \tau_i)$ are bounded. From Thereom 1.3.1 of Wahba (1990), the solution to (4) has the form $f(t) = \boldsymbol{\xi}^T(t)\boldsymbol{c}$, where $\boldsymbol{\xi}(t)$ is a vector with elements $\xi_{ij}(t) = L_{ij}R(t,\cdot) = \alpha_i R(t, t_{ij} - \tau_i)$. When λ is fixed, $\hat{\boldsymbol{f}}_\lambda = (\hat{f}_\lambda(t_{11} - \tau_1), \cdots, \hat{f}_\lambda(t_{mn_m} - \tau_m))^T$ is a linear function of $\tilde{\boldsymbol{y}} = (\tilde{y}_{11}, \cdots, \tilde{y}_{mn_m})^T$: $\hat{\boldsymbol{f}}_\lambda = A(\lambda)\tilde{\boldsymbol{y}}$, where $A(\lambda)$ is the so called "hat", or influence, matrix. The quantity $trA(\hat{\lambda})$ is used as the degrees of freedom of the nonparametric function. λ in (4) is a smoothing parameter. It controls the trade-off between the goodness of fit and the roughness of the estimate. It is very important to choose the smoothing parameter based on data. We use the generalized cross-validation (GCV) method to choose the smoothing parameter. That is, we estimate the smoothing parameter λ by minimizing the GCV score

$$V(\lambda) = \frac{1/n||(I - A(\lambda))\tilde{\boldsymbol{y}}||^2}{(1 - trA(\lambda)/n)^2}. \tag{6}$$

Algorithm:

1. START: Get an initial estimate f^0 of f. Normalize f^0. Then for each

$i = 1, \cdots, m$, solve (3) to get initial estimates μ_i^0, α_i^0 and τ_i^0 of μ_i, α_i and τ_i.

2. (h+1)ST ITERATION: For fixed μ_i^h, α_i^h and τ_i^h, solve (4) with λ selected by GCV to get $f^{(h+1)}$ as an improved approximation of f. Normalize f^{h+1}. Then for each $i = 1, \cdots, m$, solve (3) to get improved estimates μ_i^{h+1}, α_i^{h+1} and τ_i^{h+1}.

3. STOPPING CRITERIA: Stop iterating when for a prespecified $\delta > 0$,

$$|RSS(\boldsymbol{\mu}^h, \boldsymbol{\alpha}^h, \boldsymbol{\tau}^h, f^h) - RSS(\boldsymbol{\mu}^{h+1}, \boldsymbol{\alpha}^{h+1}, \boldsymbol{\tau}^{h+1}, f^{h+1})| < \delta, \tag{7}$$

where RSS is the residual sum of squares

$$RSS(\boldsymbol{\mu}, \boldsymbol{\alpha}, \boldsymbol{\tau}, f) = \frac{1}{m} \sum_{i=1}^{m} \frac{1}{n_i} \sum_{j=1}^{n_i} (y_{ij} - \mu_i - \alpha_i f(t_{ij} - \tau_i))^2. \tag{8}$$

As with most iterative methods, a good initial estimate is important. The algorithm may not converge or may converge to a local minimum when the initial estimate is far from the optimum. We used the solution to (4) with $\mu_i = \sum_{j=1}^{n_i} y_{ij}/n_i$, $\alpha_i = 1$, $\tau_i = 0$ as the initial estimate f^0 to analyze `ACTH`, `cbt` and many other hormones. They all converged within 7 iterations and gave reasonable estimates. We also tried to use some other reasonable initial estimates (such as, $\mu_i = \sum_{j=1}^{n_i} y_{ij}/n_i$, $\alpha_i = \max_j |y_{ij} - \mu_i|$, $\tau_i = 0$) and to start with the non-linear least-square problem with an initial estimate f^0 (such as, the sin/cosine function). They all converge to similar estimates. Several methods can be used to solve (3). Since the sample sizes are small for each subject, we use a grid search to solve (3). RKPACK (Gu (1989)) is used to solve (4). Since the algorithm fits a smoothing spline with different data at each iteration, we estimate the smoothing parameter at each iteration (see Gu (1992a)).

4 Hypothesis Tests and Bayesian Confidence Intervals

It is of interest to test whether a circadian rhythm exists:

$$H_0: \ \boldsymbol{\alpha} = 0, \qquad H_1: \ \boldsymbol{\alpha} \neq 0, \tag{9}$$

and furthermore, if all individuals have a similar phase:

$$H_0: \ \tau_i = \tau_j \text{ for all } i \text{ and } j, \qquad H_1: \ \tau_i \neq \tau_j \text{ for some } i \text{ and } j. \tag{10}$$

Using the "extra sum of squares" principle, we define the statistics

$$F = \frac{(RSS(\boldsymbol{\mu}_{H_0}, \boldsymbol{\alpha}_{H_0}, \boldsymbol{\tau}_{H_0}, f_{H_0}) - RSS(\boldsymbol{\mu}_{H_1}, \boldsymbol{\alpha}_{H_1}, \boldsymbol{\tau}_{H_1}, f_{H_1}))/(df_{H_0} - df_{H_1})}{RSS(\boldsymbol{\mu}_{H_1}, \boldsymbol{\alpha}_{H_1}, \boldsymbol{\tau}_{H_1}, f_{H_1})/df_{H_1}}. \tag{11}$$

The degrees of freedom for residuals df_{H_0} and df_{H_1} can be calculated as the total number of observations minus the total number of parameters and the degrees of freedom for the smoothing spline. For example, to test (9), $df_{H_0} = \sum_{i=1}^m n_i - m$ and $df_{H_1} = \sum_{i=1}^m n_i - 3m - trA(\hat{\lambda}) + 1$. Since the numerator and denominator may not be independent in (11), F may not have a F distribution. We use a bootstrap procedure to calculate the null distribution of F. That is, we fit the data under H_0. We then generate bootstrap samples from this fit. These bootstrap samples are fitted under both H_0 and H_1 to get bootstrap estimates F^* of F under H_0. The empirical distribution of these F^*'s are used as the null distribution.

Denote μ_i^*'s, α_i^*'s and τ_i^*'s as the estimates after the algorithm converged. Let $\tilde{\tilde{y}}_{ij} = y_{ij} - \mu_i^*/\alpha_i^*$, $\tilde{\tilde{t}}_{ij} = t_{ij} - \tau_i^*$ and $\tilde{\tilde{\epsilon}}_i = \epsilon_i/\alpha_i^*$. Then we have $\tilde{\tilde{y}}_{ij} = f(\tilde{\tilde{t}}_{ij}) + \tilde{\tilde{\epsilon}}_{ij}$, where $\tilde{\tilde{\epsilon}}_{ij}$'s are independent and $\tilde{\tilde{\epsilon}}_{ij} \sim \mathrm{N}(0, \sigma^2/(\alpha_i^*)^2)$. We reestimate f by solving the penalized weighted least-square problem:

$$\min_{f \in W_2^0(per)} \left\{ \frac{1}{m} \sum_{i=1}^m \frac{1}{n_i} \sum_{j=1}^{n_i} (\alpha_i^*)^2 (\tilde{\tilde{y}}_{ij} - f(\tilde{\tilde{t}}_{ij}))^2 + \lambda \int_0^1 (f^{(2)}(t))^2 dt \right\}. \qquad (12)$$

We than can construct the well-known Bayesian confidence intervals for the solution of (12). These Bayesian confidence intervals allow us to judge the shape of the common curve f. See Wahba (1983), Gu (1990) and Gu (1992b) for details about how to solve (12) and how to construct Bayesian confidence intervals.

We can also use L-splines (Kimeldorf and Wahba 1971, Ramsay and Dalzell 1991, Ansley, Kohn and Wong 1993) to check if a simple sin/cosine parametric model is appropriate. Let L be the linear differential operator: $L = D^2 + (2\pi)^2$, where $D^2 f$ is the second derivitive of f. Let $\mathcal{H}_1 = \ker L = \text{span}\{\sin 2\pi t, \cos 2\pi t\}$. Define a linear operator $B: W_0^2(per) \to R^2$ such that $Bf = (\int_0^1 f(t) \sin 2\pi t dt, \int_0^1 f(t) \cos 2\pi t dt)^T$. Let $\mathcal{H}_2 = \ker B$. Define inner products on $\mathcal{H}_1$, $\mathcal{H}_2$ and $\mathcal{H}$ as $\langle f, g\rangle_1 = (Bf)^T(Bg)$, $\langle f, g\rangle_2 = \int_0^1 (Lf)(Lg)dt$, and $\langle f, g\rangle = \langle f, g\rangle_1 + \langle f, g\rangle_2$. Then it is easy to check that $W_0^2(per) = \mathcal{H}_1 \oplus \mathcal{H}_2$ and the reproducing kernel of $\mathcal{H}_2$ for L is

$$R_2(s,t) = \sum_{\nu=2}^{\infty} \frac{2}{(2\pi)^4(1-\nu^2)^2} \cos 2\pi\nu(s-t). \tag{13}$$

We have now decomposed $W_0^2(per)$ into a space corresponding to the sin/cosine parametric model ($\mathcal{H}_1$) and its residue ($\mathcal{H}_2$). Let P_i be the projection operator from $W_0^2(per)$ onto $\mathcal{H}_i$, $i = 1, 2$. Then $\int_0^1 (Lf)^2 dt = \|P_2 f\|_2^2$ measures deviation of f from the sin/cosine parametric model. Putting a penalty on $P_2 f$, we reestimate f by solving the penalized weighted least-square problem:

$$\min_{f \in W_2^0(per)} \left\{ \frac{1}{m} \sum_{i=1}^{m} \frac{1}{n_i} \sum_{j=1}^{n_i} (\alpha_i^*)^2 (\tilde{\tilde{y}}_{ij} - f(\tilde{\tilde{t}}_{ij}))^2 + \lambda \int_0^1 (Lf)^2 dt \right\}. \tag{14}$$

We can check $P_2\hat{f}$ together with its Bayesian confidence intervals to see if $P_2 f = 0$. A formal test of $P_2 f = 0$ can also be employed (see Cox, Koh, Wahba and Yandell (1988)).

5 Example of Circadian Rhythm

Figures 1 and 2 present the plots of `ACTH` and core body temperature `cbt` for the nine healthy subjects in the study described in Section 2. Define acrophase r as the location of the maximum response. Estimates of the 24-h mean μ, amplitude α and acrophase r for ACTH and temperature of all individuals and their means are listed in Tables 1 and 2, respectively.

$\boxed{\text{Put Table 1 here}}$

$\boxed{\text{Put Table 2 here}}$

The standard deviations of the estimates of acrophase are large since there are at most 12 observations for each individual.

The common curves with their 90% Bayesian confidence intervals are plotted in Figure 3. Each curve contains two peaks. To see if the small peaks are real, we search for the spline with smallest smoothing parameters that have only one peak and one nadir. These curves (dotted lines) are also plotted in Figure 3. For `ACTH`, some parts of the one-peak curve are outside the Bayesian confidence intervals, providing evidence that the second peak is real. For `cbt`, the one-peak curve is inside the Bayesian confidence intervals. Because of the small sample size, we may not have enough power to detect a second peak for `cbt`.

Put Figure 3 here

To check if a sin/cosine parametric model is appropriate for `ACTH`, we solve (14) and plot $\hat{f}$ and its components in Figure 4. Zero is outside of some parts of the Bayesian confidence intervals for $P_2 f$, indicating a sin/cosine parametric model is not appropriate.

Put Figure 4 here

We can also see that after a horizontal shift, the two common curves of `ACTH` and `cbt` may be negatively associated. The investigator would be interested in whether there is a causal relationship between the two curves. However, the identification of such an association is not proof of causality; both may be separately responding to internal and/or external circadian cues, such as day/night or sleep/awake periods. Numerous studies have established strong links between the central nervous system and the immune system (Ader, Felten and Cohen 1991). Mediators of such interactions have not always been identified with certainty, but the HPA axis (Bateman, Singh, Kral and Solomon 1989) and the sympathetic nervous system (Williams, Peterson, Shea, Schmedte, Bauer and Felten 1981) have often been assumed to play key roles. We are currently working on extending our method to investigate the relationship between two rhythms in order to study the relationship between the rhythms of hormones and those of immune measures. With modifications of the algorithm, it is possible to propose a formal test for the equality of two curves.

Next, we use the bootstrap hypothesis test procedure to test

(a) Is there a circadian rhythm for `ACTH`?

(b) Do all individuals have the same phase for `ACTH`?

(c) Is there a circadian rhythm for `cbt`?

(d) Do all individuals have the same phase for `cbt`?

Histograms of the bootstrap statistics F^* are plotted in Figure 5. The test statistics F are marked with vertical bars. We find that for test (a) $p < 0.01$; for test (b) $0.07 < p < 0.08$; for test (c) $p < 0.01$; and for test (d) $0.10 < p < 0.11$. We also superimpose the corresponding F densities with approximate degrees of freedom. The bootstrap distributions are far from the F distributions and p-values based on these F distributions are smaller than the bootstrap estimates.

Put Figure 5 here

Fits for each individual are plotted in Figure 1 and Figure 2. The fitted curves model the data very well, even for `cbt` of some individuals with many missing values. Residual plots (not shown here) reveal no violation of the assumptions.

6 Discussion and Conclusions

In this paper we have described a method for modelling data with an underlying circadian rhythm using splines. The advantage of using a spline is the ability to model the shape of the curve without predetermining its parametric form. Therefore, the spline provides greater flexibility for the functional forms of the models than using a sine/cosine function.

In order to use the spline it is necessary to define the function such that it cycles at the proper interval and that the derivative is continuous and bounded throughout. That is, when exactly 24 hours of data are collected, there is continuity between the model for the data at the beginning of the study and that at the end of the 24 hours.

By bootstrapping it is possible to generate confidence intervals for the curve and to test hypotheses, such as whether there is one peak or more in the series.

These methods are not limited to circadian rhythms, but may be applied to any time-linked function. One interesting application is to the rich field of chronobiology pioneered by F. Halberg (see Cugini, Leone, Palma, Battisti, Wilson, Kawasaki, Tamura, Halberg and Cornelissen (1990) and references there). The Cosinor analysis used in chronobiology assumes the sin/cosine parametric model, which may be inappropriate for some cases as illustrated in `ACTH` example. Our method provide a test for Cosinor model and an alternative when Cosinor model is not appropriate.

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Table 1: Estimates of μ_i, α_i, r_i and their bootstrap standard deviations for log(ACTH). $\hat{s}(\hat{r})$ are in minutes.

	subject									
	1	2	3	4	5	6	7	8	9	mean
$\hat{\mu}$	2.69	2.08	3.02	2.50	2.26	2.75	1.62	2.31	2.30	2.39
$\hat{s}(\hat{\mu})$	0.12	0.12	0.12	0.12	0.12	0.12	0.15	0.13	0.13	0.13
$\hat{\alpha}$	0.49	0.73	1.22	0.92	0.98	0.67	1.90	1.65	0.92	1.05
$\hat{s}(\hat{\alpha})$	0.31	0.27	0.33	0.30	0.33	0.28	0.43	0.36	0.36	0.33
$\hat{r}$	6:47	7:45	5:50	6:19	4:52	7:16	5:50	9:11	5:50	6:38
$\hat{s}(\hat{r})$	143	99	75	86	88	159	81	62	92	98

Table 2: Estimates of μ_i, α_i, r_i and their bootstrap standard deviations for `cbt`. $\hat{s}(\hat{r})$ are in minutes.

	subject									
	1	2	3	4	5	6	7	8	9	mean
$\hat{\mu}$	36.86	36.61	37.48	35.60	36.44	36.18	37.07	37.12	36.98	36.70
$\hat{s}(\hat{\mu})$	0.18	0.13	0.08	0.07	0.10	0.07	0.08	0.07	0.08	0.09
$\hat{\alpha}$	0.80	0.49	0.37	0.12	0.49	0.43	0.18	0.31	0.86	0.45
$\hat{s}(\hat{\alpha})$	0.49	0.20	0.18	0.18	0.23	0.18	0.17	0.17	0.22	0.22
$\hat{r}$	9:11	17:21	16:23	4:52	20:43	17:50	18:19	20:43	21:12	16:17
$\hat{s}(\hat{r})$	121	118	109	199	139	110	203	153	84	137

Captions of figures

Figure 1: Plots of `ACTH` on log scale for 9 healthy subjects. Dots are observations. Lines are fits by our algorithm.

Figure 2: Plots of `cbt` for 9 individuals. Dots are observations. Lines are fits by our algorithm.

Figure 3: Plots of the common curves. Dashed lines are their 90% Bayesian confidence intervals. Dotted lines are one-peak common curves with increased smoothing parameters.

Figure 4: Plots of the common curve of `ACTH` and its components estimated by L-spline. From left to right are $P_1\hat{f}$, $P_2\hat{f}$ and $\hat{f}$. Dashed lines are their 90% Bayesian confidence intervals.

Figure 5: Histograms of F^*'s scaled as a probability density. Horizontal bars are test statistics F's. Dashed curves are density functions of F distributions with approximate degrees of freedom.

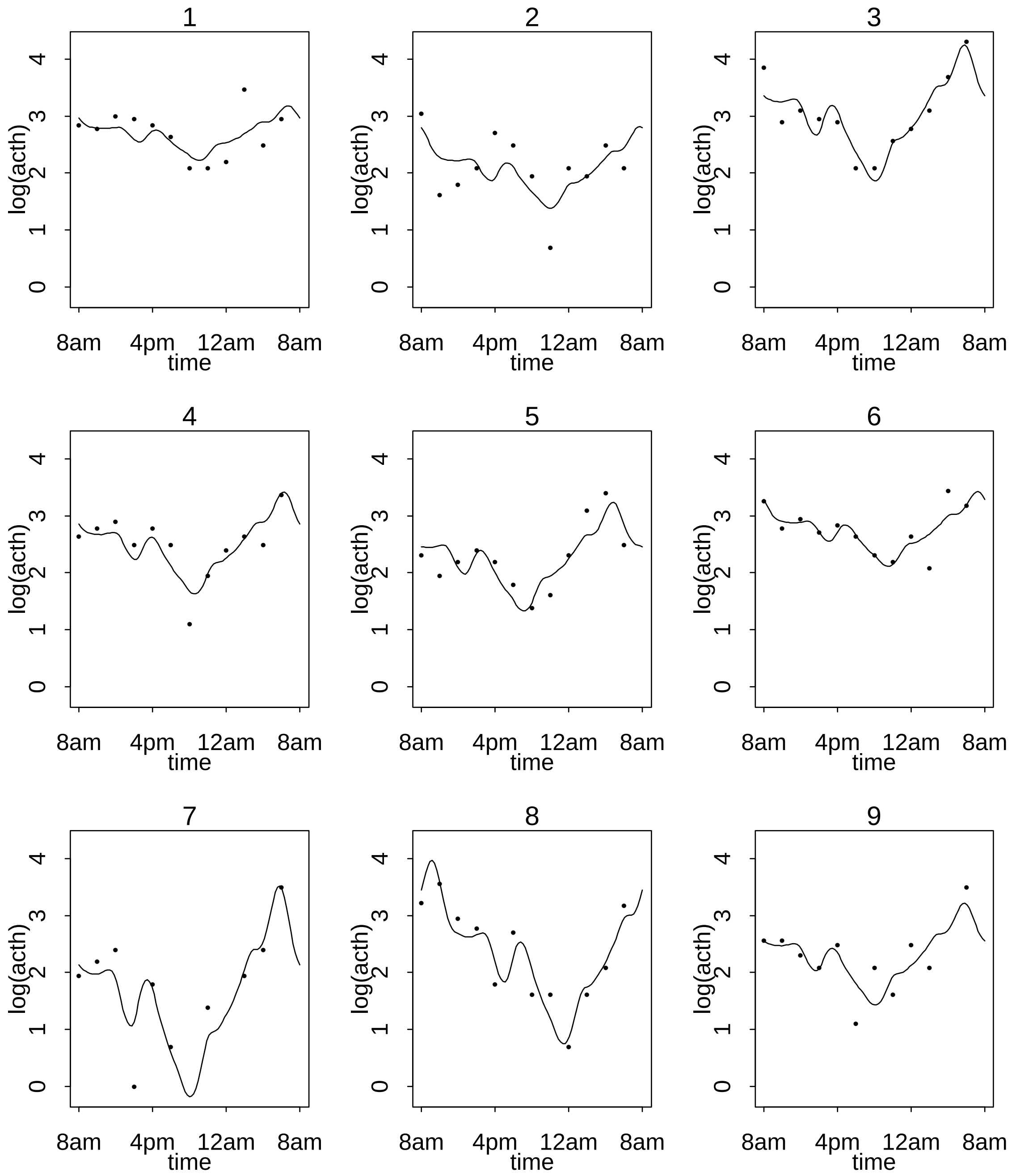

Figure 1: Plots of ACTH on log scale for 9 healthy subjects. Dots are observations. Lines are fits by our algorithm.

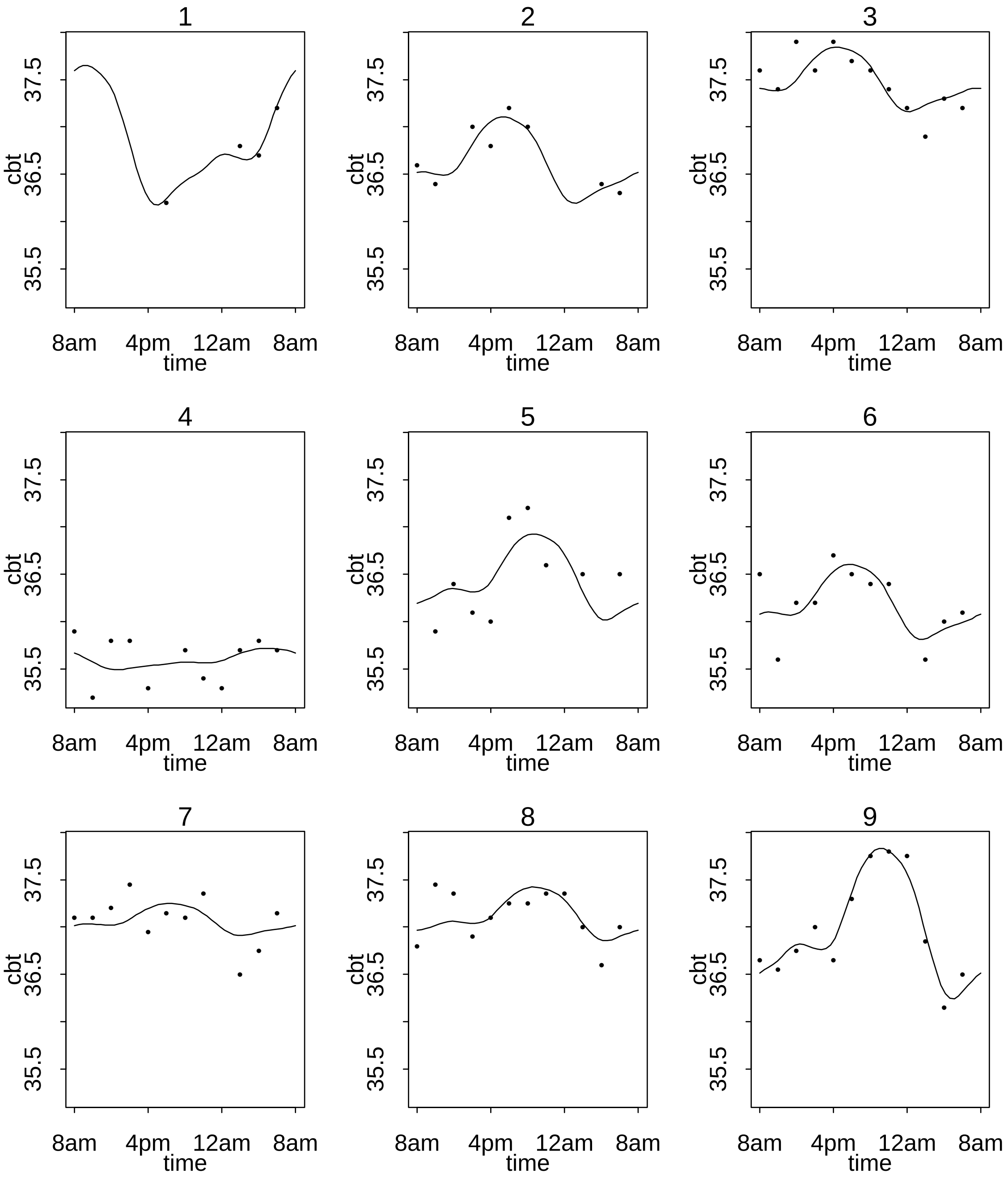

Figure 2: Plots of `cbt` for 9 individuals. Dots are observations. Lines are fits by our algorithm.

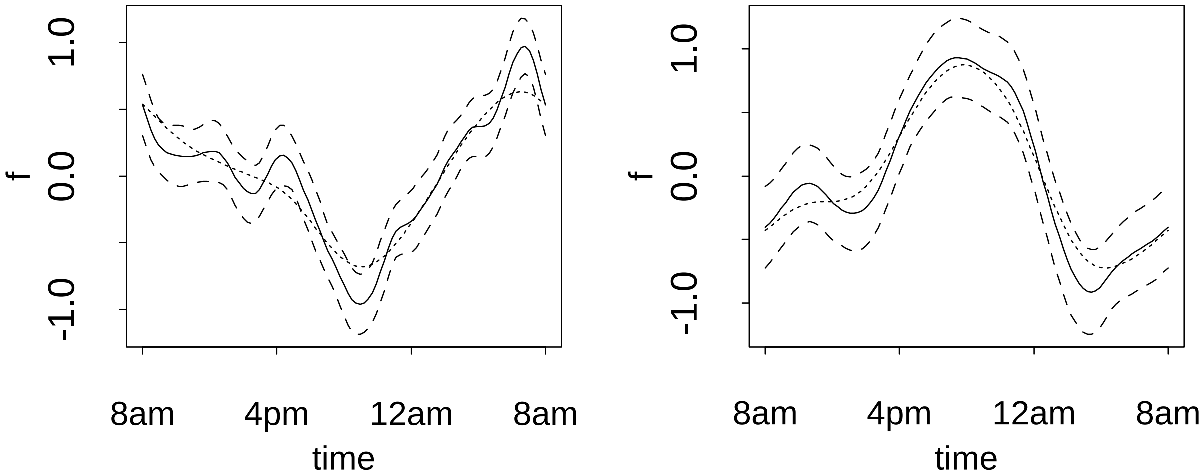

Figure 3: Plots of the common curves. Dashed lines are their 90% Bayesian confidence intervals. Dotted lines are one-peak common curves with increased smoothing parameters.

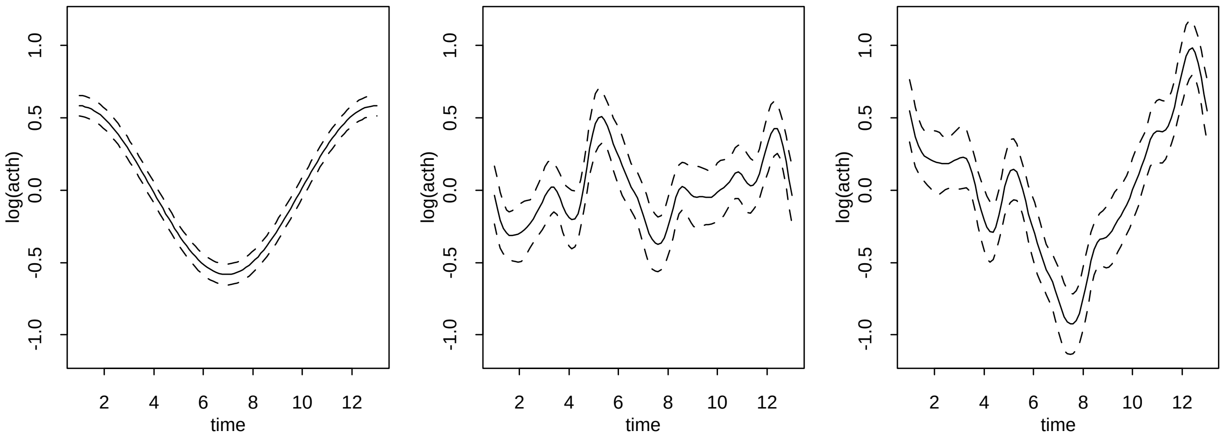

Figure 4: Plots of the common curve of `ACTH` and its components estimated by L-spline. From left to right are $P_1\hat{f}$, $P_2\hat{f}$ and $\hat{f}$. Dashed lines are their 90% Bayesian confidence intervals.

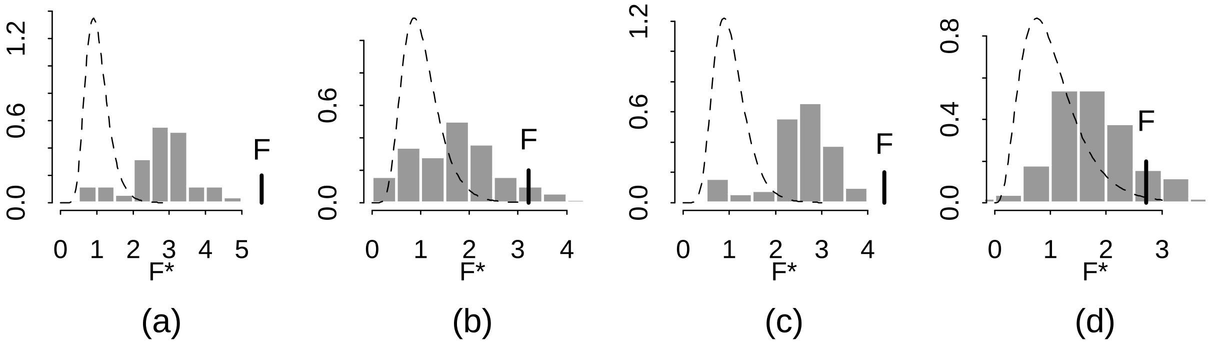

Figure 5: Histograms of F^*'s scaled as a probability density. Horizontal bars are test statistics F's. Dashed curves are density functions of F distributions with approximate degrees of freedom.