Appendix:

The present research is directed at discerning the feedback effects of T on LH secretion. In conjunction with the use of ketoconazole, 4 doses of T addback were administered. From a modeling perspective, one must decide on the appropriateness and the degree to which parameters might be considered common, for a given subject, among the four series. Moreover, evidence suggests that slow-phase LH half-lives can be altered by reduced T concentration levels. We do fix and hold constant the fast half-life of LH at 18 min. Consequently, it was most appropriate to not assume any *a priori* (but rather to test for any possible) relationship between the parameters for LH secretion, across the 4 T doses.

The models for LH secretion and concentration are as follow. For pulsatile-secreting hormones, the pulse times are fundamental (1). The first step is to create a collection of possible sets, each consisting of putative pulse times. For a given LH concentration time-profile, the local minima are possible onset points of pulses. The LH pulse times were estimated by a recently developed selective-smoothing method (2). The procedure involves the application of a nonlinear diffusion equation to the measured LH concentration time series, wherein the diffusion coefficient is inversely related to the rate of increase in hormone concentrations. A local minimum at which its rate of increase is small is smoothed more than one for which the rate of increase is large. The algorithm produces a collection of decreasing sets of potential pulse-onset times (minima) by removing least-contributory putative pulse times one at a time. For uniform implementation of algorithmic parameters, each LH profile was first stationarized using a (heat equation-based) method to remove low-frequency trends,

and then normalized to [0,1] (3). For a given subject, we denote the 4 T addback doses by k (k = 0, 2.5, 5.0, 7.5 (mg)), and a pulse set by: $P^{k,1}, P^{k,2}, \ldots P^{k,m}$, where the number of pulses, *m*, will depend on *k*. LH secretion rates ($Z_L^{(k)}$) and concentrations ($X_L^{(k)}$), are then assumed to be given by:

$$Z_L^{(k)}(t) = \beta_0^{(k)} + \sum_{P^{(k,l)} \le t} (\eta_0^{(k)} + \eta_1^{(k)} \times (P^{(k,l)} - P^{(k,l-1)}) + A^{(k,l)})\, \psi^{(k)}(t - P^{(k,l)}) \qquad (1)$$

$$X_L^{(k)}(t) = (ae^{-\alpha_1^{(k)} t} + (1-a)e^{-\alpha_2^{(k)} t}) X_L^{(k)}(0) + \int_0^t (ae^{-\alpha_1^{(k)}(t-r)} + (1-a)e^{-\alpha_2^{(k)}(t-r)}) \times Z_L^{(k)}(r)\, dr \qquad (2)$$

where $\psi^{(k)}$, for each of the 4 T doses (k), is the unit area-normalized rate of secretion over time (waveform), described by a 3-parameter generalized Gamma density:

$$\psi^{(k)}(s) \quad \propto \quad s^{\beta_1^{(k)} \beta_3^{(k)} - 1}\, e^{-(s/\beta_2^{(k)})^{\beta_3^{(k)}}} \qquad \text{k=0.0, 2.5, 5.0, 7.5 (mg).}$$

The fast and slow rates of elimination of LH are $\alpha_1^{(k)}$ and $\alpha_2^{(k)}$, and a and $1-a$ the corresponding fractions. The rates of basal LH secretion is $\beta_0^{(k)}$ and the mass accumulation rate is $\eta_1^{(k)}$. Secretory-burst mass is a linear function of the preceding interpulse interval $(P^{(k,l)} - P^{(k,l-1)})$ plus a pulse-by-pulse random effect $A^{k,l}$ that allows for unmodeled variation in successive burst size. Sample hormone concentrations with experimental uncertainty, $\varepsilon_L^{(k)}(i)$, are then defined by:

$$Y_{L,i}^{(k)} = X_L^{(k)}(t_i) + \varepsilon_L(i), \text{ i=1,…,n.}$$

For each putative pulse time set, parameter estimation proceeds by penalized maximum-likelihood estimation (MLE), where the resulting likelihood function is penalized by the number of pulse times in the set, using the Akaike Information Criteria (AIC) penalization. One then obtains the MLE $\hat{\theta}_L^{(k)}$ and the corresponding pulse time

set. Estimates of secretion rates are then obtained as conditional expectations evaluated at the MLE $\hat{\theta}_L^{(k)}$:

$$\hat{Z}_{L,i}^{(k)}(i = 1,..., n) = E_{\hat{\theta}_L^{(k)}}[Z_{L,i}^{(k)}(t_i), i = 1,..., n | Y_{L,i}^{(k)}, i = 1,..., \mathrm{n}],\ k = 0.0, 2.5, 5.0, 7.5\ ,$$

which involves the "reconstruction" of the random effects: $E_{\theta_L^{(k)}}[A^{(k,l)} \mid Y_{L,i}^{(k)}, i = 1,...,n]$.

The random effects and observational errors are assumed to be i.i.d Gaussian and independent of one another

A convolution of secretion rates with the estimated biexponential kinetics, a linear operation, results in predicted (reconvolved) concentrations: $\hat{Y}_{L,i}^{(k)}$, i=1,…,n; and the 4 doses (k) of T.

Consequently, for each subject, one obtains the estimated parameters $\hat{\theta}_L^{(k)}$ and the estimated secretion rate $\hat{Z}_{L,i}^{(k)}(i = 1,...,n)$, for each of the four T doses (0.0, 2.5, 5.0 and 7.5 mg). From these, one then obtains, for each of the four doses, the values used in further analysis: (average) LH secretory burst mass, depicted in Figures 2 and 3; and, total 12-hr basal secretion and total 12-hr pulsatile secretion, displayed in Figure 6.

Finally, using the interpulse intervals from the resulting AIC pulse time set, one can then estimate pulse frequency and pulse regularity, using a previously published Weibull model (renewal process), with parameters λ (frequency) and γ (regularity). The Poisson model corresponds to $\gamma = 0$. The γ parameter allows for pulse time patterns more regular than those occurring under a Poisson model. In a Weibull renewal process, the conditional probability density (in s) for next pulse time $P^{(k,l)}$, given $P^{(k,l-1)}$, is:

$$f(s \mid P^{(k,l-1)}) = \gamma \times (\lambda^{(k)})^{\gamma^{(k)}} (s - P^{(k,l-1)})^{\gamma^{(k)}-1} e^{-(\lambda^{(k)})^{\gamma^{(k)}} (s-P^{(k,l-1)})^{\gamma^{(k)}}}$$

The likelihood function under this model then produces, for each of the four T doses (k) for each subject, pulse frequency estimates: $\hat{\lambda}^{(k)}$ and pulse regularity estimates: $\hat{\gamma}^{(k)}$. The estimated pulse frequencies are displayed in Figures 4 and 5. There were no statistical differences in the regularity parameter due to T dose, and hence those estimates are not displayed.

References

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