Analytical Construct of Reversible Desensitization of Pituitary-Testicular Signaling: Illustrative Application in Aging

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N.B. Text Figure 4 is in color.

Definitions:

Hysteresis. Dependence of a dose-response function on the time evolution of effector concentrations, such that after (compared with before) a hysteresis-inflection point the magnitude of the response decreases or increases at any given effector concentration.

Onset (initial) dose-response parameter. Any one of potency, sensitivity or efficacy dose-response parameters estimated using paired effector-response data beginning at the onset of a response and continuing until the time of hysteretic inflection.

Recovery (delayed) dose-response parameter. Analogous to onset parameter, except that estimation is based upon the time window beginning with the hysteretic inflection and continuing until the next response onset.
Abstract

Luteinizing hormone (LH) administered in pharmacologic amounts downregulates Leydig-cell steroidogenesis. Whether reversible downregulation of physiological gonadotropin drive operates *in vivo* is unknown. Most analytical models of dose-response functions constructed to date are biased by assuming that no downregulation exists. The present study employs a new analytical platform to quantify potential (but not required) pulsatile cycles of LH-testosterone (T) dose-response stimulation, desensitization and recovery (pulse-by-pulse hysteresis) in 26 healthy men sampled every 10 min for 24 hr. A sensitivity-downregulation hysteresis construct predicted marked hysteresis with a median time delay to LH dose-response inflection within individual T pulses of 23 min and with median T-pulse onset and recovery LH sensitivities of 1.1 and 0.10 (slope units) [P < 0.001]. A potency-downregulation model yielded median estimates of one-half-maximally stimulatory LH concentrations (EC$_{50}$’s, IU/L) of 0.66 and 7.5 (onset and recovery, respectively) [P < 0.001]. An efficacy-downregulation formulation of hysteresis forecast median LH efficacies (ng/dL/min) of 20 (onset of T-secretory burst) and 8.3 (offset of T burst) [P = 0.002]. Segmentation of the LH-T data by age suggested greater sensitivity, higher EC$_{50}$ (increased LH potency), and markedly (2.7-fold) attenuated LH efficacy in older individuals. Each of the three hysteresis models yielded a marked (P < 0.005) reduction in estimated model residual error compared with no hysteresis. In summary, model-based analyses allowing for (but not requiring) reversible pituitary-gonadal effector-response downregulation are consistent with a hypothesis of recurrent, brief cycles of LH-dependent stimulation, desensitization and recovery of pulsatile T secretion *in vivo*, and an age-associated
reduction in LH efficacy. Prospective studies would be required to prove this aging effect.

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Introduction

Biological systems commonly exhibit hysteresis, defined broadly by dependence of a response on its previous history, thus implying that nonidentical initial (onset) and delayed (recovery) pathways operate sequentially (1). Classical instances arise in muscle, lung and gall-bladder contraction and in hormonal signaling (5; 8; 14; 36). Prominent endocrine examples include sex-steroid, glucose, insulin, glucagon and parathormone stimulus-response adaptations (7; 43; 53). Unfortunately to date, hysteretic dose-response fluctuations have usually been ignored in analytical models or imputed to random effects (58). A random-effects model was introduced recently for the pituitary-gonadal axis and justified on statistical grounds (6; 29); however, if de facto hysteretic processes operate over time, a purely random-effects dose-response model would be overly simplistic. Accordingly, more precise understanding of endocrine-signaling systems would ultimately require allowance for and quantification of possible hysteresis, if present.

The present work investigates application of a new analytical construct of allowable dose-response hysteretic adaptations under physiological conditions in vivo. Model constructs permitted analytical estimation of possible (but not obligatory) nonidentical initial and recovery dose-response parameters, viz. potency, efficacy or sensitivity, as recently suggested for corticotropin-cortisol regulation in humans (26). The analysis targets the gonadal axis, wherein studies have revealed unequivocal pharmacological downregulation of gonadotropin/hCG drive of steroidogenesis (9; 19; 20; 34; 51; 52; 60; 61). The issues are whether allowable cycles of physiological downregulation can be modeled in vivo in unstressed uninfused individuals, and whether hysteretic adaptations
are influenced by physiological context, such as age.

**Methods**

**Overview**

Three distinct hysteretic constructs (potency, sensitivity or efficacy) were evaluated using archival data in 13 young (ages 18-30 yr) and 13 older men (ages 60-78 yr). Healthy inclusion criteria were described earlier (32; 56), along with diet, activity and weight status. BMI ranges (kg/m²) were similar at 20-26 (young) and 22-28 (older).

Upon informed consent and with Institutional Review Board approval, 26 healthy subjects underwent blood sampling every 10 min for 24 hr in a clinical research unit to allow later measurements of serum luteinizing hormone (LH) [effector] and testosterone (T) [response] concentrations. These data have been subjected to linear cross-correlation and conventional dose-response analyses previously. They are employed here in an entirely new analytical context.

**Analytical construct**

The goal was to relate time-varying LH concentrations (input, effector) to time-varying T secretion rates (output, response) via a new hysteresis dose-response model in individual young and older men. This requires constructing a 5-parameter nonlinear (logistic) dose-response function with allowance for inhibitory (negative) hysteresis after an estimable time lag. **Figure 1** illustrates the concept. The concept was first outlined in (26) for corticotropin’s feedforward onto cortisol secretion. For the gonadal axis, LH concentration profiles (145 samples in each subject over 24 hr) are reconstructed by a modified deconvolution model to yield fitted (reconvolution) concentration values. LH secretion is represented by a train of variable-amplitude pulses superimposed upon
basal (time-invariant) nonpulsatile secretion (39), whereas LH elimination proceeds via a biexponential function (28). The modified deconvolution procedure incorporates an integral-equation model, rather than the usual difference-based model as discussed fully in the technical Appendix (B. Estimation Algorithm: stages I-III). An advantage of the integral form is that elimination and secretion are viewed as occurring continuously between consecutive samples, which corresponds to biological reality. In addition, the modified methodology presented here includes (a) permissible addback of initially deleted pulses if significant via the Akaike information criterion, and (b) allowance for two possible LH secretory-burst shapes (waveforms), one in the day and the other at night, as suggested recently for ACTH (27). T concentrations are deconvolved analogously to yield sample T secretion rates for subsequent use in constructing dose-response hystereses models. Then, after aligning pulsatile LH concentrations and T secretory responses, the protocol estimates 5-parameter nonlinear LH $\rightarrow$ T feedforward (logistic-like) dose-response functions. The unique aspect is estimation of a classical 4-parameter (basal, potency, sensitivity and efficacy) dose-response model simultaneously with a fifth downregulation parameter, and a hysteresis-inflection time point. The inflection point is the time delay before allowable downregulation of the LH-T dose-response function optimized over the collection of T pulses. Downregulation is implemented as an allowable decrease in potency, sensitivity or efficacy of LH-T action after the estimable inflection point from T secretory-pulse onset.

**Figure 1** schematizes the pulsatile downregulation hysteresis concept. In particular, *LH concentrations* (reconvolved) are related to *T secretion rates* (deconvolved) via five
simultaneously estimable dose-response parameters and a time-delayed hysteresis inflection point. Random effects on T secretory-burst amplitude and the standard deviation (SD) of residual model error are calculated concurrently, as described previously (23; 24).

The existence of parameter shifts was tested by the signed-ranks test of hysteretic onset and recovery parameters. Justification for each model was evaluated by likelihood-ratio testing using a chi-square statistic, wherein comparisons were made of each hysteresis model against a no-hysteresis construct on a subject-by-subject (N = 26) basis using twice the (negative) log-likelihood function differences at two degrees of freedom. The final step involved assessment of statistical contrasts by age and also by type of downregulation model using a Wilcoxon rank-sum (two-sample) test [young vs older comparison] and 2-way ANCOVA of log-transformed model-error SD values [effect of age and model type], respectively. Multiple means were compared via post hoc Tukey’s honestly significantly different test (45; 46). Data are presented in the figures as box-and-whisker plots (median, interquartile range, 90% confidence interval and extreme range), and given in the text as the median, range.

Results

The modified integrative-deconvolution methodology (Technical Appendix) has never been applied to LH-concentration time series. Hence, significant results are presented in Figure 2. Statistical comparisons disclosed prominent effects of age (N = 13 older vs N = 13 young men). Confirmatory of single-waveform analyses (30), older compared with young men had more frequent LH secretory bursts per 24 hr [P = 0.008], and smaller LH secretory bursts (lower mass of LH released per pulse, MPP) [P =
New findings were that the daytime LH secretory-burst mode (time delay from objective secretory-burst onset to maximum) was 45% shorter ($P = 0.005$), whereas basal (nonpulsatile) LH secretion was 58% higher ($P = 0.035$), in older than young men. The nighttime modes of LH secretory bursts also differed by age ($P = 0.043$), viz. young 16 (3 - 18.4) min and older 12 (3 - 17.7) min.

**Figure 3** shows estimates of initial and recovery (hysteretic) dose-response parameter values for models of sensitivity (Panel A), potency (Panel B) and efficacy (Panel C) hystereses. P values reflect paired nonparametric comparisons in the group of 26 subjects of onset vs recovery LH-T dose-response parameter estimates. All three models predicted strong downregulation [$P < 0.005$]. In the sensitivity model (Panel A), 25 of 26 men exhibited a hysteresis-like downregulating shift. Median onset and recovery sensitivities were 1.1 and 0.10 slope units, defining an 11-fold shift [$P < 0.001$]. In the potency model (Panel B), LH-T potency shifts were inferable in all 26 men. Median onset and offset EC$_{50}$ values (IU/L) for the 26 subjects were 0.66 and 7.5 [$P < 0.001$ paired comparison]. Panel C presents estimates of LH-T efficacy (asymptotically maximal T secretion, ng/dL/min). The median onset efficacy value [$N = 26$ individuals] was 20 and offset value 8.3 ng/dL/min ($P = 0.002$). Downregulation of efficacy was inferable in all subjects.

**Table 1** shows ANCOVA results of model-error (SD) comparisons using the no-hysteresis model SD as the covariate and segmentation by age (2 factors) and model type (3 factors). Each hysteretic model reduced mean model residual error significantly, viz. all $P < 0.005$. Among the three hystereses models, the potency-model yielded a lower residual model error than the efficacy construct ($P = 0.011$). Main effects of age
and model type were both significant at P < 0.001, but there was no significant interaction (P = 0.32). Residual model-error (SD) was lower by age only in the sensitivity model (P = 0.025). Compared with no hysteresis, model justification was confirmed by likelihood-ratio testing using a chi-square comparison of twice the difference of (negative) log-likelihood functions: Table 1.

Illustrative individual outcomes for the 3 hysteresis models in a young and older man are given in Figure 4 (color). The three columns (left, middle, right) depict sensitivity, efficacy and potency model estimates obtained in a young (Panel A) and an older male (Panel B). The top row depicts the deconvolved T-secretion estimate and hysteresis-predicted T-secretion fit. The middle row displays maximum-likelihood estimates (MLE) of logistic dose-response parameters. The solid line denotes the estimated initial (onset) mean dose-response curve, and the dashed line the delayed (recovery) mean dose-response curve (after the hysteretic time delay from the T pulse onset). Each set of dose-response curves (multiple interrupted lines) reflects multiple pulse-by-pulse dose-responses hysteretic cycles, which differ only by allowable random effects in efficacy. The bottom row presents the LH concentration (input) signal, with and without the allowable LH-T alignment shift.

For the separate cohorts of young (N = 13) and older (N = 13) men, median sensitivity shifts were 8.6-fold larger in older (1.8 slope units) than young (0.21 slope units) volunteers (P < 0.001) indicating greater downregulation: Table 2. Box-and-whisker plots in Figure 5 show that absolute values of both onset (initial) and offset (recovery-phase) exponential sensitivity estimates were higher in older than young individuals (P < 0.001 and P = 0.004, respectively). Efficacy shifts did not differ by age (P = 0.34).
These data (median, range) are summarized by age and model in Table 3. Analysis of earlier data wherein ketoconazole was used to block partially T synthesis (59) abolished hysteresis (N = 6 men, plots not shown).

The definition of potency here [Appendix] also is an exponential dose-response term, which yields an EC$_{50}$ (estimated LH concentration stimulating one-half maximal T secretion) when the potency value is divided by the sensitivity coefficient (23; 27). Because potency in the potency-hysteresis model and sensitivity in the sensitivity-hysteresis model are both estimated before (onset) and after (recovery) LH-associated downregulation, two EC$_{50}$ values are calculable in each subject. There is only a single EC$_{50}$ estimate in the efficacy-shift model. For the potency and sensitivity-shift models in the combined cohorts (N = 26), onset and recovery EC$_{50}$'s were 0.66 and 7.5 IU/L and 2.7 and 15 IU/L, respectively [both P < 0.001]. Median EC$_{50}$ shifts (IU/L) [difference between onset and recovery values] in the potency-hysteresis model were similar in young and older men (P = 0.42 age effect): Table 2 and 3. In contradistinction, the downregulating shift in LH EC$_{50}$ was 3.9-fold smaller in older (4.5 IU/L) than young (13 IU/L) men in the sensitivity-hysteresis model (P = 0.015). At the same time, individual onset and recovery EC$_{50}$’s in the sensitivity model were reduced (apparent potencies were enhanced) in aging men (P = 0.001 for onset, and P = 0.011 for recovery): Figure 6. Assessment of efficacy by age stratum predicted 2.7-fold higher LH efficacy in the sensitivity-shift model in young than older men; viz., 43 (20 - 247) and 16 (5.9 - 96) ng/dL/min (P = 0.004): Figure 7.

Estimated time delays (min) from onset of a T secretory burst to downregulation (hysteretic-inflection delays) were similar in all 3 models and invariant of age (ANCOVA
P ≥ 0.21). The median (absolute range) across the 26 subjects and 3 models was 23 min (10-43 min). Model error (SD) was lower in the older cohort.

**Discussion**

The present analyses demonstrate that hysteresis models of allowable reversible quenching of pulsatile gonadotropin (LH)-testicular (T) stimulation can be constructed and applied to physiological data obtained *in vivo*. According to such constructs, paired LH-T time series in healthy men exhibit evidence of dose-response downregulation within individual T pulses. Statistical model justification was highly significant (P < 10^{-4}) compared with a no-hysteresis construct. In addition, the sensitivity-downregulation construct unveiled distinct age-associated adaptations in the male gonadal axis. Foremost predictions were lower LH efficacy (asymptotically maximal T secretion due to LH action) [P = 0.004], and greater absolute downregulation of T-response sensitivity to LH (sensitivity-shift size, P < 0.001), in older than young men. If verified in larger and prospective cohorts, these outcomes would point to dynamic pituitary-gonadal effector-response coupling *in vivo*, which may be influenced by one or more factors associated with healthy aging.

The accompanying studies differ from earlier model analyses in several important ways. First, the methodology extends the recent concept of allowable (estimable) dose-response hysteresis (26) to a major regulatory interface in the male gonadal axis. Second, feedforward LH → T dose-response functions are estimated conditional upon deconvolution-based reconstruction of LH concentrations and T secretion rates, as respective input (effector) and output (response) signals (6). Third, the deconvolution methodology is integrative and permits two waveforms (shapes) of LH and T secretory
bursts. The latter allowance is based upon an idea presented recently for the ACTH-cortisol axis (27). And, fourth, according to such analyses, older age is associated with diminished LH efficacy, and apparently heightened T sensitivity and LH potency. The last two preliminary findings could be consistent with the broader concept of target-organ denervation hypersensitivity, which is recognized in neuronal, vascular, renal, muscular and other adaptive physiological systems (15; 22; 22; 38; 41; 44; 49; 50). A relative LH-deficiency state was inferred here in older men by their lower mass of LH secreted per burst (Figure 2, P = 0.007). In this regard, increased Leydig-cell responsivity (decreased EC$_{50}$ of exogenous LH stimulation) has been observed in vitro after short-term in vivo LH deficiency in the adult male rat (17) and in the presence of adenosine in Leydig-tumor cells (11). Selective enhancement of target-tissue responses is also evident in glucose-insulin (48), calcium-PTH (53) and other endocrine interfaces (15; 44; 49; 60).

Whether the primary mechanism mediating reduced LH efficacy/lower T-secretory responses to inferably maximal concentrations of LH in vivo in aging volunteers is decreased LH biopotency or diminished Leydig-cell responsivity is not known definitively. Indeed, older men have normal or decreased in vitro LH bioactivity (13; 40). Attenuated LH efficacy would be consistent with diminished T responses to pharmacological hCG stimulation (54; 55), and with inferences in the aged male rat (18; 42). However, infused hCG is distinctly nonphysiological in the male, due both to lutropic dose and binding kinetics (12; 34). In the present uninjected physiological state, the sensitivity-hysteresis model predicted a 2.7-fold reduction in median LH efficacy under endogenous LH-pulse drive, viz. 43 (young) vs 16 (older) ng T secreted per min
per unit distribution volume (dL) \( [P = 0.004] \). Earlier methods of dose-response reconstruction inferred lower absolute LH efficacies in men \( (e.g., \ 3.8 - 5.8 \ \text{ng/dL/min}) \) \( (32; \ 33) \). Based upon available direct measures of T kinetics \( (57) \), the nominal T distribution volume is 25 L \( (250 \ \text{dL}) \) per square meter of surface area. If so, calculated initial (onset) LH efficacy \( (\text{mg of T secreted per day per } 1.73 \ \text{m}^2) \) by age stratum would be 26 in young and 9.9 in older men. These asymptotic estimates are consistent with the several-fold augmentative effect of pharmacological doses of hCG on unstimulated daily T secretion rates of 4-8 mg T in healthy young individuals \( (40) \). However, no studies have estimated directly LH-T dose-response properties or maximal T secretion rates using randomly ordered pulses of recombinant human LH in men.

The median time delay of 23 min from T secretory-burst onset to hysteretic downregulation would be consistent with rapid \( (\text{within } 20-30 \ \text{min}) \) pharmacological desensitization observed in several other endocrine systems \( \textit{in vivo} \) and \( \textit{in vitro} \) \( (2; \ 3; \ 16; \ 21; \ 35) \). Here, reversible cycles of desensitization were consistently inferable \( (\text{viz. in } 25 \ \text{of } 26 \ \text{subjects}) \) under physiological conditions for the endogenous LH-T system. Consistently inferable downregulation in all three model forms would be consonant with the capability of submaximally stimulatory LH concentrations to induce Leydig-cell response refraction in some \( \textit{in vitro} \) studies \( (19; \ 20) \).

In summary, the aptness of the three complementary analytical models of reversible hysteresis in framing the dynamics of pituitary gonadotropin-gonadal sex-steroid coupling suggests that this type of quantitative methodology should find application in other physiological contexts, in which brief, recurrent and reversible agonist-response adaptations are postulated.
Perspectives and Significance

Demonstrating unperturbed effector-response cycles under physiological conditions *in vivo* is inherently difficult. Conventional approaches include administering a pharmacological effector at two separate times so as to compare initial and delayed responses, and/or mathematically simulating potential mechanisms of tachyphylaxis, hypersensitivity or tolerance (37; 47). Neither of these strategies necessarily recapitulates underlying physiological effector-response adaptations. If time-dependent response adaptations are assumed to reflect altered dose-response dynamics, then the presently proposed analytical-estimation model may be appropriate. The objective is to obtain noninvasive, uninjected, nonpharmacological estimates of recurrently adaptive effector-response properties. Because both response desensitization and response hypersensitization accompany G-protein-coupled signaling (4; 10), quantification of successive effector-response cycles in such systems may be especially informative to physiologists. Accordingly, the concept of rapid *in vivo* hysteresis should provide a basis for comparable analyses in many other regulatory and integrative physiological systems.
Technical Appendix

A: Three Estimation Models

In many biological systems, \textit{in vivo} elimination is not well represented by a single exponential decay. Rather there are often \textit{two components}: a fast term reflecting advection, diffusion and mixing in the blood, and a slow component embodying irreversible metabolism or clearance (58). The combined kinetics can be described by two coupled differential equations.

\textbf{Differential Eq}: \( X(t) = X^{(1)}(t) + X^{(2)}(t) \), and \( X^{(1)}(0) = aX(0), \ X^{(2)}(0) = (1-a)X(0) \) \hspace{1cm} (1)

\( \frac{dX^{(1)}(t)}{dt} = -\alpha^{(1)}X^{(1)}(t) + aZ(t), \ \frac{dX^{(2)}(t)}{dt} = -\alpha^{(2)}X^{(2)}(t) + (1-a)Z(t) \)

with the corresponding integral-equation representation being:

\textbf{Integral Eq}: \( X(t) = (ae^{-\alpha^{(1)}t} + (1-a)e^{-\alpha^{(2)}t})X(0) + \int_{0}^{t}(ae^{-\alpha^{(1)}(t-s)} + (1-a)e^{-\alpha^{(2)}(t-s)})Z(s) \mathrm{d}s \) \hspace{1cm} (2)

where \( X(t) \) denotes the concentration at time \( t \), with \( X^{(1)}(t) \) for the fast and \( X^{(2)}(t) \) for the slow component. The fast and slow rates of elimination are \( (\alpha^{(1)}, \alpha^{(2)}) \), and the fast and slow fractions \( a \) and \( (1-a) \). The symbol \( Z \) denotes the secretion rate. Because data are not observed continuously, but at some sampling rate \( (\Delta t) \), observations are made at times \( (t_1, t_2, \ldots, t_n) \), \( t_{i+1} - t_i = \Delta t \). The two models are discretized (made evaluable at the sampling times) as follows:

\textbf{Difference Eq}: \( X(t_{i+1}) = (a\bar{\alpha}^{(1)} + (1-a)\bar{\alpha}^{(2)})X(t_i) + \Delta t \ Z(t_i) \) \hspace{1cm} (3)

\textbf{Discrete-time Integral Eq}:

\( X(t_{i+1}) = (a\bar{\alpha}^{(1)} + (1-a)\bar{\alpha}^{(2)})X(t_i) + \int_{t_i}^{t_{i+1}}(ae^{-\alpha^{(1)}(t_i-s)} + (1-a)e^{-\alpha^{(2)}(t_i-s)})Z(s) \mathrm{d}s \) \hspace{1cm} (4)
where $\tilde{\alpha}^{(1)} = \exp(-\alpha^{(1)} \Delta t)$ and $\tilde{\alpha}^{(2)} = \exp(-\alpha^{(2)} \Delta t)$. To date, virtually all endocrine modeling has utilized the difference equation (3) rather than the integral equation (4) in estimation. The integral-equation model subsumes the difference-equation model, and hence is more comprehensive.

In the difference-equation approximation, the fractions $(1 - \alpha^{(1)} \Delta t)$ and $(1 - \alpha^{(2)} \Delta t)$ are often used in place of $\tilde{\alpha}^{(1)}$ and $\tilde{\alpha}^{(2)}$, representing approximations to derivatives at zero; such use is appropriate when $\Delta t$ is small. An advantage of using $\tilde{\alpha}^{(1)}$ and $\tilde{\alpha}^{(2)}$ is that the difference and differential equation models are constrained to have the same half-lives of elimination (or so close as possible for $\Delta t$ sampling).

When $\Delta t$ is small compared with the half-lives and/or with the rapidity of change in the secretion rate ($Z$), there is not much difference between the difference and integral models. However, when this is not the case, inaccurate estimates can occur. Specifically, equation (3), compared with integral equation (4), does not account for time-varying secretion ($Z$) and elimination that are in fact taking place within the time interval $[t_i, t_{i+1}]$ defining consecutive observation times. It is as if elimination were occurring only at time $t_i$ and secretion were constant at the initial value $Z(t_i)$ over the interval. When the sampling interval is similar to or longer than the half-life of elimination, inaccurate estimates occur, since time-varying secretion and elimination within the interval are ignored.

The difference equation model (3) can be derived from the discrete-time integral equation model (4) by assuming a constant secretion inside the integral over the interval at the initial value $Z(t_i)$ and ignoring elimination (i.e., $\alpha$ is taken as 0 in the exponent).
An intermediate model (5), which lies between (models (3) and (4), can be constructed by assuming constant secretion inside the integral at the initial value $Z(t_i)$, but allowing constant-rate elimination over the interval:

**Intermediate Eq Model:**

$$X(t_{i+1}) = (a\,\bar{\alpha}^{(3)} + (1-a)\,\bar{\alpha}^{(2)})X(t_i) + a((1-\bar{\alpha}^{(1)})/\alpha^{(1)}) + (1-a)((1-\bar{\alpha}^{(2)})/\alpha) \right] Z(t_i) \quad (5)$$

One might propose using the integral equation model (4) exclusively. The difficulty is that the numerical integrations that are involved in model (4) make the computations beyond the reach of the resources of most investigators, especially when one factors in the selection of pulse times. An alternative is to frame the basic procedure as a sequence of three stages, as done here. The first stage employs the difference-equation (3) model; the parameters are estimated; and the AIC-penalized maximum likelihood pulse times chosen. The second stage utilizes intermediate equation (5) to create more accurate slow half-life estimates and serve as a transition to the third stage. The third stage uses the integral-equation (4) model, which is the most accurate for estimating a variable secretion-rate function, $Z$.

**B. Estimation**

Since the secretion rate function $Z$ is predicated on release occurring at certain (unobserved) pulse-times in a given hormone concentration profile, a previously published pulse-detection algorithm was applied (24). The algorithm is a nonlinear diffusion equation, which selectively smoothes the profile using a diffusion coefficient that is inversely related to the degree of positivity of the local gradient in the concentration profile. One begins with all local minima; those followed by a rapid or marked increase are smoothed minimally, whereas those of slow or little increase are
smoothed more. The smoothed profile is not used in parameter estimation, just in
collection of possible pulse times. As the algorithm proceeds, a sequence of
diminishing numbers of pulse times is constructed, denoting increasing susceptibility of
the pulse time to smoothing: \( P = \{ P^{(1)}, P^{(2)}, \ldots, P^{(M-1)}, P^{(M)} \} \). Here, \( P^{(1)} \) is the most robust
pulse time and \( P^{(M)} \) the least robust (most diminutive and easily smoothed).

Putative pulse sets are constructed by including pulse times with indices up to
\( m \), \( P_{m} = (P^{(1)}, \ldots, P^{(m-1)}, P^{(m)}) \), \( 1 \leq m \leq M \). There are \( M \) such sets, wherein pulse times in
any given set \( P_{m} \) are not arranged in increasing order of sampling time, but in order of
susceptibility to smoothing (removal). Thus, denote the pulse times of \( P_{m} \), rearranged in
increasing order of time, as \( T_{m} = (T^{(1)}, \ldots, T^{(m-1)}, T^{(m)}) \). Parameter estimation then
proceeds by penalized maximum-likelihood estimation (MLE) conditional on each pulse-
time set, where penalization is on the number of pulse times \( m \). Both the Akaiki (AIC)
and Bayesian (BIC) information criteria can be used as model comparators.

Consider a given fixed pulse set \( T_{m} = (T^{(1)}, \ldots, T^{(m-1)}, T^{(m)}) \), and describe the waveform
of mass release (realization of secretion rate over time) via a 3-parameter generalized
Gamma density (24). For thyroid-stimulating hormone and corticotropin, there is often a
day-night difference in the waveform, one for day (D) and one for night (N) (25; 31). If
daylight (to be estimated) is defined by the interval: \([\phi_{1}, \phi_{2}]\), then the waveform at pulse
time \( T^{(k)} \), depending upon the day or the night, is

\[
\Psi(s) = \begin{cases} 
  s^{\beta_{1}(1)\beta_{2}(3)-1} e^{-s/\beta_{2}(1)} \beta_{1}(3), & s \geq 0, \\
  s^{\beta_{1}(1)\beta_{2}(3)-1} e^{-s/\beta_{2}(1)} \beta_{1}(3), & s \geq T^{(k)}, \\
  s^{\beta_{1}(1)\beta_{2}(3)-1} e^{-s/\beta_{2}(1)} \beta_{1}(3), & s \geq 0, \\
  s^{\beta_{1}(1)\beta_{2}(3)-1} e^{-s/\beta_{2}(1)} \beta_{1}(3), & T^{(k)} < \phi_{1} \text{ or } \phi_{2} < T^{(k)},
\end{cases}
\]

(6)
The secretion rate is given as the sum of a nonpulsatile (basal) secretion rate, $\beta_0$, and a train of secretory bursts (pulses). Each secretory-burst mass is described as a weak linear function of the preceding interpulse interval $(T^{(k)} - T^{(k-1)})$ plus a random effect $(A^{(k)})$. The latter allows for variations in burst size, which are not explicitly modeled by the linear function (e.g., variability due to nonuniform access of secretory granules to capillaries from burst to burst). Thus, overall secretion is:

$$Z(t) = \beta_0 + \sum_{T^{(k)} \geq t} (\eta_0 + \eta_1 \times (T^{(k)} - T^{(k-1)}) + A^{(k)}) \psi(t - T^{(k)})$$

(7)

Let $\theta$ denote the secretion and elimination parameters for the model, wherein fast and slow rates of elimination $\alpha^{(1)}$ and $\alpha^{(2)}$ are to be estimated, and their fractions ($a$ and $1-a$) taken as literature-based population values. The $m$ random effects (one for each burst) are assumed to be IID normal with mean zero and variance $\sigma_A^2$. For a given pulse set $T_m = (T^{(1)}, ..., T^{(m-1)}, T^{(m)})$, a parameter choice $\theta$, and the secretion rate function, $Z$, any one (or all) of the models (3)-(5) can be applied, resulting in the (true) concentrations with measurement error:

$$Y_i = X(t_i) + \epsilon(i), \quad i=1, ..., n$$

(8)

where the errors are assumed to be IID normal with mean zero and variance $\sigma_\epsilon^2$.

Because the random effects (A’s) in the secretion rate (Z) enter linearly, the resulting likelihood for the observed concentrations (Y’s) is Gaussian. An AIC (or BIC) penalty term is appended, penalizing the number of pulse times $m$, resulting in a penalized log-likelihood function that is maximized over $\theta$ and $m$ as:
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\[ p_l(\theta \mid Y_i, i = 1, \ldots, n; P_m) \]  \hspace{1cm} (9)

The optimization is constrained, since any estimate \( \hat{\theta} \) must result in a non-negative \( Z \);
i.e., the conditional expectation of the secretion rate evaluated at \( \hat{\theta} \) must be non-negative:
\[ E_{\hat{\theta}}[Z(t_i), i = 1, \ldots, n \mid Y_i, i = 1, \ldots, n] \geq 0. \]  This requires calculating the
c Conditional expectations of the A's, conditioned on the Y's:
\[ (\hat{A}^{(1)}, \hat{A}^{(2)}, \ldots, \hat{A}^{(m)}) = E_{\hat{\theta}}[A^{(k)}, k = 1, \ldots, m \mid Y_i, i = 1, \ldots, n]. \]  Values are estimated from the
definition of Z (Eq (7)) and nonnegativity is assessed. The results of the penalized
maximum-likelihood estimation (PMLE) are \( \hat{\theta}, \hat{m} \) and the AIC-optimal pulse-time set
\[ P_{\hat{m}} = (P^{(1)}, \ldots, P^{(\hat{m} - 1)}, P^{(\hat{m})}). \]

In previous analyses (39), allowable pulse-time sets included only the M-specific
collection of decreasing sets. The possibility arises that one or more of the \( \hat{m} \) pulse
times removed could enhance the fit if added to the resulting AIC-optimal set, \( P_{\hat{m}} \). Such
sets were excluded, because smoothing yielded the specific decreasing collection \( P_j, j=1,\ldots,M. \)  To address this possibility, one may evaluate the effect of add-back of
previously removed pulse times; viz., by creating new pulse-time sets
\( \{P_{\hat{m}}, P^{(k)}\}, k=\hat{m} + 1,\ldots,M. \)  For each of the M-\( \hat{m} \) such sets, one calculates the penalized
MLE:
\[ p_l(\theta \mid Y_i, i = 1, \ldots, n; \{P_m, P^{(k)}\}) \], which is compared with that achieved by \( P_{\hat{m}} \) via AIC.
The best of such qualifying single pulse times is added, if one exists. The procedure is
then repeated for the remaining M-\( \hat{m} - 1 \) pulse times, until none of the potential add-back
pulse times is preferred allowing for the AIC penalty. The aggregate of these
procedures constitutes the estimation algorithm.
**Estimation Algorithm:** Observed Data: $Y_i = X(t_i) + \epsilon(i), \ i = 1,...,n$

**Stage I.** Starting parameter $\theta_s$ and putative pulse sets $P_m, m = 1,...,M$

Difference-Eq Model: $X(t_{i+1}) = (a\alpha(1) + (1-a)\alpha(2))X(t_i) + \Delta t Z(t_i)$

Penalized log-likelihood function: $pl(\theta| Y_i, i = 1,...,n; P_m)$

Result: PMLE $\hat{\theta}^{(\text{stage I})}$ and AIC-optimal pulse set $\widetilde{P}_{\text{M+1}} = \{P_{\text{M}}, P^{(k_1)}, P^{(k_2)},..., P^{(k_s)}\}$, including add-back of $s$ pulse times

**Stage II.** Start with results from Stage I, $\hat{\theta}^{(\text{stage I})}$, $\widetilde{P}_{\text{M+1}}$; Intermediate Model replaces the Difference-Eq Model in penalized log-likelihood function:

Model:

$X(t_{i+1}) = (a\alpha(1) + (1-a)\alpha(2))X(t_i) + [a((1-a)\alpha(1)/\alpha(1)) + (1-a)((1-a)\alpha(2)/\alpha)]Z(t_i)$

Penalized log-likelihood function: $pl(\theta| Y_i, i = 1,...,n; \widetilde{P}_{\text{M+1}})$

Result: PMLE $\hat{\theta}^{(\text{stage II})}$; pulse times in $\widetilde{P}_{\text{M+1}}$ allowed to shift (slightly)

**Stage III.** Start with results from Stage II, $\hat{\theta}^{(\text{stage II})}$, $\widetilde{P}_{\text{M+1}}$; Integral-Eq Model replaces the Intermediate-Eq Model in penalized log-likelihood function:

Model:

$X(t_{i+1}) = (a\alpha(1) + (1-a)\alpha(2))X(t_i) + \int_{t_i}^{t_{i+1}} (ae^{-\alpha(1)(t_{i+1}-s)} + (1-a)e^{-\alpha(2)(t_{i+1}-s)})\times Z(s)ds$

Penalized log-likelihood function: $pl(\theta| Y_i, i = 1,...,n; \widetilde{P}_{\text{M+1}})$

Result: PMLE $\hat{\theta}$ ($= \hat{\theta}^{(\text{stage II})}$); pulse times in $\widetilde{P}_{\text{M+1}}$ allowed to shift (slightly)

End of Algorithm
When the pulse times $\hat{P}_{m+i+4}$ and the final parameter estimates $\hat{\theta}$ are obtained, the secretion-rate function $Z$ can be calculated (at the observed sample times) as conditional expectations evaluated at $\hat{\theta}$:

$$\hat{Z}_i(i = 1, \ldots, n) = E_{\hat{\theta}}[Z(t_i), i = 1, \ldots, n | Y_{r,j}, i = 1, \ldots, n],$$

(10)

One can now calculate the model fits, or the predicted concentrations. Fits are obtained by a convolution (expression (4)) using the estimated secretion rate (expression (7)) and estimated biexponential kinetics ($\hat{\alpha}^{(1)}, \hat{\alpha}^{(2)}$), two components of $\hat{\theta}$. The result is the predicted (reconvolved) concentrations:

$$\hat{Y}_i, i = 1, \ldots, n;$$

(11)

C. Application to LH and T Analyses

To illustrate the sequential 3-stage Estimation Algorithm, Appendix Figure 1 depicts the 3 main equations used in the program. Appendix Figure 2 applies these analytical steps using an illustrative LH times series obtained in a young male subject. Each panel displays measured LH concentrations (solid line) and the deconvolution-predicted fit (dashed line), and estimates of the pulse times (x-axis ticks), daytime-waveform interval (demarcated by diamonds), and half-lives, as follows: (Top) Stage I (Difference-Eq Model); (Middle), Stage II (Intermediate-Eq Model); and (Bottom), Stage III (Integral-Eq Model). Appendix Figure 3 presents corresponding stepwise deconvolution data for testosterone (T) obtained in an older male. These 2 profiles typify the spectrum of profiles observed here. Appendix Figure 4 shows more detailed results from the integral-deconvolution stage in the same young subject (LH, Left) and older subject (T, Right). The information given includes: (Top Row) measured concentrations, fits and
pulse times; (Second Row) estimated secretion rates, pulse times and daytime interval; and (Third row) night (N) waveform (solid line) and daytime waveform (dashed line).

D. LH Feedforward on T: Dose-Response with Hysteresis

Methods described in Appendices A-C above yield (a) estimated (integral-model) reconvolved LH concentrations: \( \hat{y}_{L,i}, i=1,\ldots,n \), expression (11), which will be the basis for the LH feedforward signal on testosterone (T) secretion; and (b) estimated T secretion rates: \( \hat{z}_{T,i}, i=1,\ldots,n \), expression (10), which will be the output of the dose-response function. To estimate the dose-response function, there are 2 further considerations: (1) a varying time delay between the onset of an LH pulse and the observed T secretory response; and, (2) potentially variable loss of responsiveness of T to the LH feedforward signal. If one wishes to accurately recover the dose-response structure, both of these considerations must be addressed.

Utilizing the fitted (reconvolved) LH concentrations: \( \hat{y}_{L,i}, i=1,\ldots,n \), the LH feedforward signal \( F_L(t) \) was constructed piecewise, from one T pulse-onset time to the next. This allows one to account for varying time lags between the onsets of LH and T pulses. For each T pulse time \( T_{T,k} \), the LH pulse \( T_{L,j} \) nearest within the allowable time, \([-60,10]\) min, was identified. If an LH pulse exists, it is shifted to the T onset point so that the two onset points are aligned. If no such pulse exists within the time interval, then a time lag of 40 min was applied between the LH and T pulse-onset times (32). The results of this phase of the dose-response analysis are shown in text Figure 3 (bottom row), viz., the fitted LH concentrations (solid line) and time-delayed LH feedforward signal (dashed line).
Estimation proceeds by using estimated T secretion rates $\hat{Z}_{T,i}(i = 1,...,n)$, assumed to be $Z_T(t_i) + \text{error}$. Random effects in efficacy ($A$'s) are included to accommodate pulse-by-pulse size variability. To allow for possible loss of responsiveness to LH drive, a time-delayed shift in the dose-response function is permitted. The shift is assumed to occur at time $M_{LonT}$ (to be estimated) following the T-secretion pulse onset. From pulse onset until time $M_{LonT}$, the LH-T relationship is defined via one dose-response curve, and thereafter (until the next T pulse onset) via a shifted curve. Hence, a hysteresis-like phenomenon occurs, in which the recovery phase of the dose response shifts within a T pulse after an estimable delay, followed by resetting to the initial curve at the next secretory pulse: **text Figure 1**. Dose-response hysteresis types can be evaluated via three models.

**Model 1**: Half-Maximally Effective Stimulus Concentration (LH Potency):

$$
\hat{Z}_T(t_i) = \begin{cases} 
\eta_0 + \frac{\eta_3 + A_{LonT}^{(k)}}{1 + \exp\{-\left(\eta_1^{EP} + \eta_2^{UP} \times F_L(t_i)\right)\}}, & T^{(k)}_T \leq t_i < T^{(k)}_T + M_{LonT} \\
\eta_0 + \frac{\eta_3 + A_{LonT}^{(k)}}{1 + \exp\{-\left(\eta_1^{DOWN} + \eta_2^{UP} \times F_L(t_i)\right)\}}, & T^{(k)}_T + M_{LonT} \leq t_i < T^{(k+1)}_T 
\end{cases} + \varepsilon, i = 1,...,n
$$

(12)

**Model 2**: Dose-Response Slope (Testis Sensitivity):

$$
\hat{Z}_T(t_i) = \begin{cases} 
\eta_0 + \frac{\eta_3 + A_{LonT}^{(k)}}{1 + \exp\{-\left(\eta_1^{EP} + \eta_2^{UP} \times F_L(t_i)\right)\}}, & T^{(k)}_T \leq t_i < T^{(k)}_T + M_{LonT} \\
\eta_0 + \frac{\eta_3 + A_{LonT}^{(k)}}{1 + \exp\{-\left(\eta_1^{DOWN} + \eta_2^{UP} \times F_L(t_i)\right)\}}, & T^{(k)}_T + M_{LonT} \leq t_i < T^{(k+1)}_T 
\end{cases} + \varepsilon, i = 1,...,n
$$

(13)

**Model 3**: Asymptotic Maximum (LH Efficacy):

C:\temp\32527_1_art_file_828379_lbjqkt.doc
Desensitization in male gonadal axis

\[
\hat{Z}_T(t_i) = \begin{cases} 
\eta_0 + \frac{\eta_3^{(k)} + A_{LonT}^{(k)}}{1 + \exp\left\{-\left(\eta_1 + \eta_2 \times F_i(t_i)\right)\right\}}, T^{(k)}_T \leq t_i < T^{(k)}_T + M_{LonT} \\
\eta_0 + \frac{\eta_3^{DOWN} + A_{LonT}^{(k)}}{1 + \exp\left\{-\left(\eta_1 + \eta_2 \times F_i(t_i)\right)\right\}}, T^{(k)}_T + M_{LonT} \leq t_i < T^{(k+i)}_T
\end{cases} + \epsilon_i, j = 1, \ldots, n 
\] (14)

Each model is fit separately, allowing subsequent comparisons of model residual error (see Statistics, Methods). In addition, generalized likelihood-ratio testing was used to evaluate each hysteresis model compared with the no-hysteresis construct.
Acknowledgments

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41. **Martin F, Andreu E, Rovira JM, Pertusa JA, Raurell M, Ripoll C, Sanchez-Andres JV, Montanya E and Soria B.** Mechanisms of glucose hypersensitivity in


Legends

Figure 1. Schema of dose-response hysteresis concept. Hormone (effector) concentrations within a pulse [top left] drive target-gland secretion rates [top right]. The relationship between input (concentration) and output (secretion) is estimated as a dose-response logistic function [below]. The onset (solid arrows) and recovery (open arrows) phases of the dose-response interface are estimated simultaneously with a hysteresis inflection point (delay time) [Appendix].

Figure 2. Salient differences in LH secretion by age using the dual-waveform integrative deconvolution model. Data are box-and-whisker plots from 13 young and 13 older men. Median, interquartile (25% and 75%) and interdecile (10% and 90%) confidence intervals with individual extreme values are shown.

Figure 3. Individual onset (initial) and recovery (delayed) dose-response parameters in 26 individuals. The 3 panels reflect results from sensitivity, potency and efficacy downregulation models.

Figure 4. This figure is color. Illustrative dose-response estimates for LH concentration-dependent drive of T secretion in one young (Panel A) and one older (Panel B) man. The top row gives deconvolution-calculated T secretion (continuous lines) and dose-response hysteresis predicted T secretion (interrupted lines) rates [vertical bars on x axis denote T pulse-onset times]; the middle row presents mean dose-response (solid curve for initial and interrupted curve for delayed) estimates with allowable random effects on efficacy (dotted lines) and allowable hysteretic shifts in any one of potency (left), sensitivity (middle) and efficacy (right); and the bottom row shows the time-shifted reconvolved LH-concentration profile (interrupted curve with vertical
bars on x axis to denote LH-pulse locations) and unshifted LH-concentration profile (continuous line with diamonds for unshifted LH secretory-pulse locations).

**Figure 5.** Age enhances Leydig-cell T-secretory sensitivity assessed before (onset) [left] and after (recovery) [middle] analytically estimated hysteretic downregulation of LH-stimulated T secretion in 26 (13 older and 13 young) healthy men. Age augments the difference between onset and recovery sensitivities of LH-T drive [right], denoting greater desensitization. Box-and-whisker plots (see Figure 2) on a natural logarithmic scale.

**Figure 6.** Potentiation by age of submaximal LH drive of T secretion in healthy men. Data are LH EC$_{50}$ values estimated in the sensitivity-hysteresis (downregulation) model [Figure 1]. Lower values denote greater LH potency. Box-and-whisker plots on a natural logarithmic scale.

**Figure 7.** Attenuation by age of estimated LH efficacy (asymptotically maximal LH-stimulated T secretion rates) in the sensitivity-downregulation model. See Figure 4 for data format.

**Appendix Figure Legends**

**Figure 1.** Three-step deconvolution procedure-based equation system [Appendix A and B]. See full discussion in Appendix.

**Figure 2.** Three-step deconvolution analysis applied to 24-hr LH concentration-time series, as discussed in Appendix, where each panel is reviewed.

**Figure 3.** Comparable deconvolution plot to that of Figure 2 but for T concentration data.
Desensitization in male gonadal axis

Figure 4. Illustrative detailed deconvolution of the LH (*left*) and T (*right*) concentration profiles shown in Appendix Figures 2 and 3. Interrupted green lines (*top*) are reconvolution curves (fits of the data). Continuous lines are measured hormone concentrations (*top*) and calculated secretion rates (*middle*). Rhomboids on the x axes denote day-night waveform demarcations (*top*: concentrations; *middle*: secretion rates). Day and night secretory-burst waveforms (Gamma-probability densities) are plotted (*bottom*).
Table 1. Residual Model Error (SD) by Age and Model Type

<table>
<thead>
<tr>
<th>Model</th>
<th>Young (N = 13)</th>
<th>Older (N = 13)</th>
<th>Unpaired P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No hysteresis</td>
<td>7.2 ± 0.54</td>
<td>6.2 ± 0.97</td>
<td>0.89</td>
</tr>
<tr>
<td>Sensitivity model(^A)</td>
<td>5.6 ± 0.47</td>
<td>3.6 ± 0.33</td>
<td>0.025</td>
</tr>
<tr>
<td>Potency model(^A*)</td>
<td>4.6 ± 0.50</td>
<td>3.3 ± 0.31</td>
<td>0.53</td>
</tr>
<tr>
<td>Efficacy model(^B)</td>
<td>5.4 ± 0.44</td>
<td>4.1 ± 0.43</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Data are the mean ± SEM for SD of model error. Analysis was by two-way ANCOVA with Tukey's test.

\(^{A}\) P < 0.001 vs no hysteresis.

\(^{B}\) P = 0.003 vs no hysteresis.

\(^{*}\) P = 0.011 vs efficacy model.

By generalized likelihood-ratio testing of each hysteresis-model type against no hysteresis, the new models reduced the absolute value of the log-likelihood function by P < 0.01 in 24 of 26 individuals (potency model) or 25 of 26 individuals (other two models) yielding overall P < 10\(^{-4}\) for each type.
**Table 2. Hysteretic *Shifts* (Differences) in Individual Sensitivity, Efficacy and Potency Models of LH → T Drive by Age**

<table>
<thead>
<tr>
<th>Model</th>
<th>Young (N = 13)</th>
<th>Older (N = 13)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity shift in sensitivity model (slope units)</td>
<td>0.21 (0 - 3.0)</td>
<td>1.8 (0.61 - 3.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>EC$_{50}$ shift in sensitivity model (IU/L)</td>
<td>13 (0 - 153)</td>
<td>4.5 (0.48 - 472)</td>
<td>0.015</td>
</tr>
<tr>
<td>EC$_{50}$ shift in potency model (IU/L)</td>
<td>5.8 (1.4 - 209)</td>
<td>7.9 (2.2 - 237)</td>
<td>0.42</td>
</tr>
<tr>
<td>Efficacy shift in efficacy model (ng/dL/min)*</td>
<td>8.6 (4.8 - 86)</td>
<td>7.9 (1.1 - 263)</td>
<td>0.34</td>
</tr>
</tbody>
</table>

*Shifts* are defined as arithmetic differences in parameter values before and after putative downregulation.

Data are the median (range). Comparisons are by the rank-sum test.

*There is no EC$_{50}$ shift in the efficacy model by definition ([Methods](#)).
Table 3. LH-T Hysteresis Feedforward Model Data in Men.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity Model Ln Sensitivity (slope)</th>
<th>Potency Model LH-T Ln EC\textsubscript{50} (IU/L)</th>
<th>Efficacy Model Ln Efficacy (ng/dL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Onset</td>
<td>Recovery</td>
<td>Onset</td>
</tr>
<tr>
<td>Young (N = 13)</td>
<td>-1.2 (-4.0 to 1.1)</td>
<td>-2.5 (-4.6 to -1.8)</td>
<td>3.0 (2.2 to 4.5)</td>
</tr>
<tr>
<td>Older (N = 13)</td>
<td>0.88 (-0.42 to 1.4)</td>
<td>-0.91 (-4.2 to -0.065)</td>
<td>3.2 (2.2 to 5.6)</td>
</tr>
<tr>
<td>Both (N = 26)</td>
<td>0.058 (-4.0 to 1.4)</td>
<td>-2.3 (-4.6 to -0.065)</td>
<td>3.0 (2.2 to 5.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Data are the median (range), given as natural logarithmic values.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1

Schema of Hysteretic Dose-Response Model

**Effectors**
- Concentration

**Response**
- Secretion Rate

Time

Time-Lagged Paired Concentration/Secretion

Inflection Point

- Hysteresis Onset
- Hysteresis Recovery

Effector Concentration

Secretion Response
Figure 2  Age Contrasts in Dual-Waveform LH Analyses

**Frequency**
- P = 0.008

**Daytime Mode**
- P = 0.005

**MPP**
- P = 0.007

**Basal Sec**
- P = 0.035
Figure 3A  Model of LH-T Sensitivity Downregulation

- Young (N = 13)
- Older (N = 13)

- Median slope for young group: 1.1
- Median slope for older group: 0.10

*P < 0.001*
Figure 3B  Potency Model of LH-T EC$_{50}$ Hysteretic Regulation

$P < 0.001$

- Onset
- Recovery

$\text{Ln LH EC}_{50}$ (IU/L)

-$\text{median} = 0.66$

-$\text{median} = 7.5$

$\circ$ Young ($N = 13$)

$\bullet$ Older ($N = 13$)
Figure 3C  Model of LH-T Efficacy Downregulation

- **P = 0.002**

- Young (N = 13)
- Older (N = 13)

- Median = 20
- Median = 8.3

Ln Efficacy (ng/dL/min)

Onset  Recovery
Three Models of LH-T Hystereses in Young Male Sensitivity Efficacy Potency

Figure 4A

Sensitivity

Efficacy

Potency

T Secretion Rate (ng/dL/min)

Time (min)

LH Concentration (IU/L)

LH Concentration (IU/L)

Time (min)
Figure 4B  Three Models of LH-T Hystereses in Older Male
Figure 5  LH-T Slopes in Sensitivity-Hysteresis Model

Onset

Recovery

Shift

\[ P < 0.001 \]

\[ P = 0.004 \]

\[ P < 0.001 \]
Figure 6  LH-T EC₅₀ in Sensitivity-Hysteresis Model

Onset  
P = 0.001

Recovery  
P = 0.011

Shift  
P = 0.015

Ln EC₅₀ (IU/L)
Figure 7  LH-T Efficacy in Sensitivity-Hysteresis Model

![Box plot showing Ln (ng/dL/min) for young and older groups with N = 13 for both. The P value is 0.004.](Veldhuis\SEC\Data\Feedforward Model\Figs for LH_Thysteresis Paper\Fig7.ppt)
Appendix Figure 1 Algorithmic Steps in Integral Deconvolution

<table>
<thead>
<tr>
<th>Observed Data</th>
<th>( Y_i = X(t_i) + \varepsilon(i), \ i = 1, \ldots, n )</th>
</tr>
</thead>
</table>

- **Difference-Equation Model**
  \[
  X(t_{i+1}) = (a \bar{\alpha}^{(1)} + (1-a) \bar{\alpha}^{(2)}) X(t_i) + \Delta t Z(t_i)
  \]

- **Intermediate Model**
  \[
  X(t_{i+1}) = (a \bar{\alpha}^{(1)} + (1-a) \bar{\alpha}^{(2)}) X(t_i) + [a((1-\bar{\alpha}^{(1)})/\alpha^{(1)}) + (1-a)((1-\bar{\alpha}^{(2)})/\alpha)] Z(t_i)
  \]

- **Integral-Equation Model**
  \[
  X(t_{i+1}) = (a \bar{\alpha}^{(1)} + (1-a) \bar{\alpha}^{(2)}) X(t_i) + \int_{t_i}^{t_{i+1}} \left( a e^{-\alpha^{(1)}(t_{i+1}-s)} + (1-a) e^{-\alpha^{(2)}(t_{i+1}-s)} \right) Z(s) \, ds
  \]

- Calculate secretion rates; calculate fitted concentrations
Appendix Figure 2

Three-Stage Deconvolution of LH in Young Man

Differential Eq: 13 pulses
half-lives 18, 57 min

Intermediate Eq/Pulse Addback: 18 pulses
half-lives 18, 50 min

Integral Eq: 18 pulses
half-lives 18, 52 min

LH Concentration (IU/L)

Time (min)

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Appendix Figure 3

Three-Stage Deconvolution of T in Older Man

Differential Eq: 14 pulses
half-lives 1.9, 27 min

Intermediate Eq/Pulse Addback: 17 pulses
half-lives 1.9, 22 min

Integral Eq: 17 pulses
half-lives 1.9, 23 min

Testosterone Concentration (ng/dL)

Time (min)
Appendix Figure 4
Integrative Deconvolution Model: LH and T Secretion

- LH Con (IU/L)
  - 18 pulses
  - Half-lives: 18, 52 min

- T Con (ng/dL)
  - 17 pulses
  - Half-lives: 1.9, 23 min

- LH Sec Rate (IU/L/min)
- T Sec Rate (ng/dL/min)

- Probability
  - Day
  - Night

X:\SEC\Data\Feedforward Model\Figs for AJP LH_Thyteresis Paper\AppendixFig4.ppt