Appendix: Variable-Waveform Deconvolution Analysis

From a technical perspective, there are 2 infusion types involving each group (Pl or E2) of postmenopausal women, here denoted as $k = 1, 2$. Each subject, $j = 1, 2, \cdots$, was sampled every 10 min for 4 h under both conditions. At a given time, $t$, the GH secretion rate (unobserved) and the GH concentration (measured) in subject $j$ for condition $k$ are designated by $Z_{j}^{(k)}(t)$ and $X_{j}^{(k)}(t)$, respectively. Nonpulsatile (basal) GH secretion is given by $\gamma^{(k)}$, which was estimated from the lowest GH concentration attained before any stimulus. Burst-like GH secretion at time $T$ is described by two terms: (a) the waveform, a normalized rate of secretion over time, $\psi(\cdot)$; and (b) the mass (amount) released per unit distribution volume in the burst, $M$ (40,43). The waveform (burst shape) is defined by the generalized Gamma probability density:

$$\psi^{(k)}(s) \propto s^{\beta_1^{(k)} - 1} e^{-(s/\beta_2^{(k)})^{\beta_3^{(k)}}}, \quad s \geq 0, \quad k = 1, 2, 3, 4. \quad (1)$$

The three beta parameters of the Gamma distribution permit variable asymmetry or Gaussian-like symmetry of secretory-burst shape (41).

The present analytical formulation is distinctive by way of reconstructing 2 common waveform functions for each cohort (Pl, N = 11; E2, N = 10 women), one for each of the 2 interventions, $k$; and analogously for the mass of secretory bursts $M^{(k)}$, with allowance in the response of any given subject, $j$, for a random variation, $A_{j}^{(k)}$, about the cohort mean. The total (basal and pulsatile) GH secretion rate ($\mu g/L/min$) at time $t$ in subject $j$ under condition $k$ is described by:

$$Z_{j}^{(k)}(t) = \gamma^{(k)} + (M^{(k)} + A_{j}^{(k)}) \psi^{(k)}(t-T), \quad t \geq 0 \quad (2)$$
and the predicted GH concentration at any given time, t, is given by:

\[
X_j^{(k)}(t) = (ae^{-\alpha_1 t} + (1 - a)e^{-\alpha_2 t}) X_j^{(k)}(0) + \int_0^t (ae^{-\alpha_1 (t-r)} + (1 - a)e^{-\alpha_2 (t-r)}) Z_j^{(k)}(r) \, dr
\]

\[
\approx \gamma^{(k)} \times \left( \frac{a}{\alpha_1} (1 - e^{-\alpha_1 t}) + \frac{1-a}{\alpha_2} (1 - e^{-\alpha_2 t}) \right) + \int_0^t (ae^{-\alpha_1 (t-r)} + (1 - a)e^{-\alpha_2 (t-r)}) \times (M^{(k)} + A_j^{(k)}) \psi^{(k)}(r-T) \, dr .
\] (3)

“basal secretion” + “pulsatile secretion”

where \(a\) is the proportion of rapid to total elimination, \(\alpha_1\) and \(\alpha_2\) are rate constants of rapid and slow elimination, and \(X(0)\) is the starting hormone concentration in subject \(j\) under intervention \(k\) (43). Here, \(\alpha_1\) is fixed at the shortest half-life estimable for 10-min sampling, 6.93 min, \(a\) at 0.37, and \(\alpha_2\) at the reported value of 20.8 min for endogenous GH (63).

The model is represented fully by the following set of parameters, \(\theta^{(k)}\), given \(k = 1, 2\):

\[
\theta^{(k)} = (\beta_1^{(k)}, \beta_2^{(k)}, \beta_3^{(k)}, \gamma^{(k)}, M^{(k)}, \sigma_A^{(k)}, \sigma_\varepsilon^{(k)})
\] (4)

Measured GH concentrations, \(Y_{ij}\), are considered a discrete time sampling (indexed by \(i\) of \(n\) data points) of the foregoing continuous processes, with superimposed measurement and observational error, \(\varepsilon_i\):

\[
Y_{ij} = X_j^{(k)}(t_i) + \varepsilon_i, \quad i = 1, \ldots, n.
\]

The discretized secretion rate, \(Z_{ij}^{(k)} = Z_j^{(k)}(t_i), i=1,\ldots,n,\) is estimated by the conditional expectation evaluated at the maximum likelihood estimate, \(\hat{\theta}^{(k)}\):

\[
\hat{Z}_{ij}^{(k)}(i = 1,\ldots,n) = E_{\hat{\theta}^{(k)}}[Z_{ij}^{(k)}, i = 1,\ldots,n \mid Y_{ij}^{(k)}, i = 1,\ldots,n].
\] (5)
The solution involves statistical estimation of individual subject random effects contributing to GH secretory-burst mass: $E_{θ^{(k)}}, \{A_j^{(k)} \mid Y_{j,i}^{(k)}, i = 1,\ldots,n\}$, assuming that the latter and observational errors are independently identically distributed and uncorrelated random Gaussian variables. In contrast, for a given subject, $j$, and intervention, $k$, random effects, $A_j^{(k)}$, may be correlated. Therefore, statistical comparisons are performed both within subject by secretagogue type and between cohorts by PI vs E2 status. The final parameter set is given in Appendix Figure 1.

Variances and covariances of parameters are obtained explicitly from the inverse of the estimated information matrix: $Σ^{(k)} = -(\frac{∂^2 l^{(k)}}{∂θ^{(k)} ∂θ^{(k)}})^{-1}$, evaluated at the maximum likelihood estimate, $\hat{θ}^{(k)}$. Thereby, statistical confidence intervals are calculated directly for basal secretion, $\hat{γ}^{(k)}$, and waveform parameters, $\hat{β}_1^{(k)}, \hat{β}_2^{(k)}$ and $\hat{β}_3^{(k)}$ of $ψ^{(k)}(s) \propto s^{β_1^{(k)}} e^{-(s_l/β_2^{(k)})^β_4^{(k)}} s \geq 0, k = 1,2,3,4$. The statistical mode of the waveform (time delay to attain the maximal GH secretion rate within a burst) is given algebraically as $h(\hat{β}_1^{(k)}, \hat{β}_2^{(k)}, \hat{β}_3^{(k)}) = \hat{β}_2^{(k)} (\hat{β}_1^{(k)} - (1/\hat{β}_3^{(k)}))(1/\hat{β}_3^{(k)})$. Variance of this value is computed by the multivariate delta method as: $\sum_{i,j=1}^3 \sigma_{ij}^{(k)} \frac{∂h}{∂β_i^{(k)}} \frac{∂h}{∂β_j^{(k)}}$ evaluated at $(\hat{β}_1^{(k)}, \hat{β}_2^{(k)}, \hat{β}_3^{(k)})$, where $σ_{ij}^{(k)}$ is the (i,j) element of $Σ^{(k)}$.

A nonlinear-parameter estimation procedure was applied recursively to candidate sets of pulse-onset times, which were determined a priori by an image boundary-detection procedure applied to each 4-h GH time series. Thereby, the entire estimation process is objective and verifiable (40,42,43).
Appendix Figure 1

Parameter set used in the statistical estimation of GH secretory responses to saline and ghrelin in PI and E2-treated women. Symbols are defined in the Appendix.
Total GH secretion rate \( Z_j^{(k)} (t) \)

\( (k) = \text{secretagogue} \)

\( j = \text{subject} \)

\[
= \gamma^{(k)} + \sum_l \left( M^{(k)} + A^{(k)}_{j,l} \right) \psi^{(k)} (t - T_{j,l}^{(k)}) + \varepsilon
\]

- common basal for a given secretagogue
- common waveform for given secretagogue
- random effect on each burst mass

shared\slides\Ghrelin E2 Appendix Fig.ppt