

## CLINICAL STUDY

# An improved computational method to assess pituitary responsiveness to secretagogue stimuli

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## Abstract

**Objective:** The quantitative assessment of gland responsiveness to exogenous stimuli is typically carried out using the peak value of the hormone concentrations in plasma, the area under its curve (AUC), or through deconvolution analysis. However, none of these methods is satisfactory, due to either sensitivity to measurement errors or various sources of bias. The objective was to introduce and validate an easy-to-compute responsiveness index, robust in the face of measurement errors and interindividual variability of kinetics parameters.

**Design:** The new method has been tested on responsiveness tests for the six pituitary hormones (using GH-releasing hormone, thyrotrophin-releasing hormone, gonadotrophin-releasing hormone and corticotrophin-releasing hormone as secretagogues), for a total of 174 tests. Hormone concentrations were assayed in six to eight samples between –30 min and 120 min from the stimulus.

**Methods:** An easy-to-compute direct formula has been worked out to assess the 'stimulated AUC', that is the part of the AUC of the response curve depending on the stimulus, as opposed to pre- and post-stimulus spontaneous secretion. The weights of the formula have been reported for the six pituitary hormones and some popular sampling protocols.

**Results and Conclusions:** The new index is less sensitive to measurement error than the peak value. Moreover, it provides results that cannot be obtained from a simple scaling of either the peak value or the standard AUC. Future studies are needed to show whether the reduced sensitivity to measurement error and the proportionality to the amount of released hormone render the stimulated AUC indeed a valid alternative to the peak value for the diagnosis of the different pathophysiological states, such as, for instance, GH deficits.

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## Introduction

In order to assess the capability of the pituitary gland to secrete a given hormone, one possibility is to administer a secretagogue (e.g. gonadotropin-releasing hormone (GnRH), thyrotrophin-releasing hormone (TRH), growth hormone-releasing hormone (GHRH), corticotrophin-releasing hormone (CRH), etc.) and measure the resulting hormone concentrations in blood. Given that hypopituitarism may result from a variable mixture of hypothalamic, infundibular or anterior pituitary dysfunction, an important alternative to releasing-hormone tests is the administration of secretagogues that examine the total interaction of the hypothalamic–pituitary axis. In fact, some pathological conditions affecting the pituitary are revealed

by low values of hormone concentration after stimulation (1). For this purpose, five to nine blood samples are withdrawn within 1–3 h after the stimulus. Once the samples have been assayed the problem arises of processing the response curve to obtain a quantitative index of responsiveness.

So far, the most popular index has been the magnitude of the peak value of the plasma hormone concentration measured after a provocative stimulus. For instance, in the large majority of European Community countries and North America, although with different cut-off values, the national health services provide, free of charge, replacement treatment for growth hormone (GH) deficiency in short children only when the peak GH value is below a given threshold in two independent responsiveness tests,

performed with different secretagogues (2, 3). In addition, the diagnosis of GH deficiency in adults relies on the magnitude of the peak value of the GH response to proper provocative tests (4).

However, the shortcomings of the use of the peak value are apparent: the assessment relies on a single measurement and therefore is not robust in the face of measurement errors; moreover, the value of the peak depends on the choice of the sampling instants, which adds to the uncertainty of such an index.

A reasonable alternative appears to be to use the area under the curve (AUC) computed, for instance, through the trapezoidal rule (5). Although the AUC relies on multiple measurements, it is not a precise index of responsiveness for two reasons: first, due to the clearance, it also incorporates the effects of pre-stimulus secretion, i.e. spontaneous secretion that occurred before stimulation and second, unless the sampling protocol embraces the complete response, it neglects the tail of the response falling beyond the last sample. Although the former problem may be somehow obviated by discounting for the baseline, there is no easy remedy for the latter problem. In fact, lengthening the observation window not only would increase costs and patients' discomfort, but would also increase the probability of capturing post-stimulus spontaneous pulses not directly elicited by the stimulus.

Conceptually, the correct index of responsiveness would be the amount of hormone released by the hypophysis during a proper time-interval (e.g. 1 h) immediately after the stimulus. Such an amount is proportional to the AUC of the instantaneous secretion rate (ISR), that is the flux of hormone coming out of the gland. Although the ISR is not directly measurable, several studies have shown that deconvolution analysis can be successfully employed to obtain the ISR from hormone concentrations in plasma either for spontaneous or stimulated secretion (6–13). Estimation of the ISR is an inverse problem, whose solution requires knowledge of the hormone kinetics in the specific subject. In the analysis of clinical responsiveness tests, the individual kinetics parameters are not available (unless a second independent experiment is carried out) and population values have to be used. At present, commercial and easy-to-use programmes for deconvolution analysis are not widespread, so that deconvolution seems to be beyond the reach of clinicians. However, if only the total amount of released hormones (i.e. the AUC of the ISR) is needed, a recent study has shown that such a quantity can be reliably and easily estimated directly from the hormone concentrations in plasma (14). In practice, it suffices to compute the linear combination of the concentration data through proper weights depending on the specific hormone and the sampling protocol adopted. In any case, since the weights are based on the population values of the hormone kinetics, there is an error due to deviations

of the individual parameters from the population ones.

In the present study, a novel index of responsiveness is introduced in order to reduce the effect of inter-individual kinetics parameter variability. More precisely, we consider the 'stimulated AUC' ( $AUC_{stim}$ ), which is the part of the AUC of the plasma concentration depending on the stimulus (other parts are due to pre- and post-stimulus spontaneous secretion). Such stimulated AUC is proportional (through the clearance) to the AUC of the ISR but, differently from the ISR, its estimation should depend less heavily on the precise knowledge of the kinetics parameters, which influence only the discounting of pre-stimulus and post-stimulus spontaneous secretion.

## Materials and methods

One-hundred and thirty-four healthy subjects (85 males and 49 females; age range 14–72 years) were admitted to the study after giving informed consent. The study protocol was approved by the Ethical Committee of Istituto Auxologico Italiano. All subjects were of normal height weight, body mass index ( $kg/m^2$ ), and they were not under medication.

### Tests

1. GnRH test (LHRH-Ferring, Germany, 100  $\mu g$ , i.v.): blood samples were collected in 40 subjects at –30, 0, 15, 30, 45, 60, 90 and 120 min for serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations (U/l).

2. TRH test (Ares Serono, Switzerland, 200  $\mu g$ , i.v.): blood samples were collected at –30, 0, 15, 30, 45, 60, 90 and 120 min for serum TSH (24 subjects) and prolactin (PRL; 20 subjects) concentrations (mU/l and  $\mu g/l$  respectively).

3. GHRH-29 (Geref, Serono, Italy, 1  $\mu g/kg$  body weight, i.v.): blood samples were collected in 30 subjects at 0, 15, 30, 45, 60, 90 and 120 min for serum GH concentrations ( $\mu g/l$ ).

4. Ovine CRH (Novabiochem, Switzerland, 1  $\mu g/kg$  body weight, i.v.): blood samples were collected in 20 subjects at –30, 0, 20, 30, 60 and 120 min for serum ACTH concentrations (ng/l).

All tests started between 0800 and 0830 h after the subjects had fasted overnight; no side-effects were observed during the tests, apart from transient facial flushing after the administration of GHRH and CRH.

### Hormone assays

Serum LH, FSH, thyrotrophin (TSH), PRL, GH and plasma ACTH levels were determined using commercial radioimmunoassay and immunoradiometric kits (LH and FSH: Ciba Corning Diagnostics, Italy; TSH: DPC,

USA; PRL: Chiron, Italy; GH: Sorin, Italy; ACTH: Nichols Institute, San Juan Capistrano, CA, USA). Two GH profiles are reported in Fig. 1. The intra- and interassay coefficients of variation were all less than 6%. All samples were analyzed at the same time.

**Response assessment**

**Peak value** The immediate and most adopted way to assess the gland response to the stimulus is to consider the peak value of the response, namely the highest plasma concentration among those measured after the stimulus. There is no guarantee that the true peak value of the concentration curve will coincide with one of the collected samples (see Fig. 1A). Moreover, being based on a single sample, the peak value is particularly exposed to noise sensitivity (see Fig. 1B for example). In fact, if the peak value was correctly measured, the subject would exhibit a decrease of concentration from the third to the fourth sample which would be faster than allowed by the GH kinetics. Hence, it is much more reasonable to assume that the peak was strongly biased by experimental error.

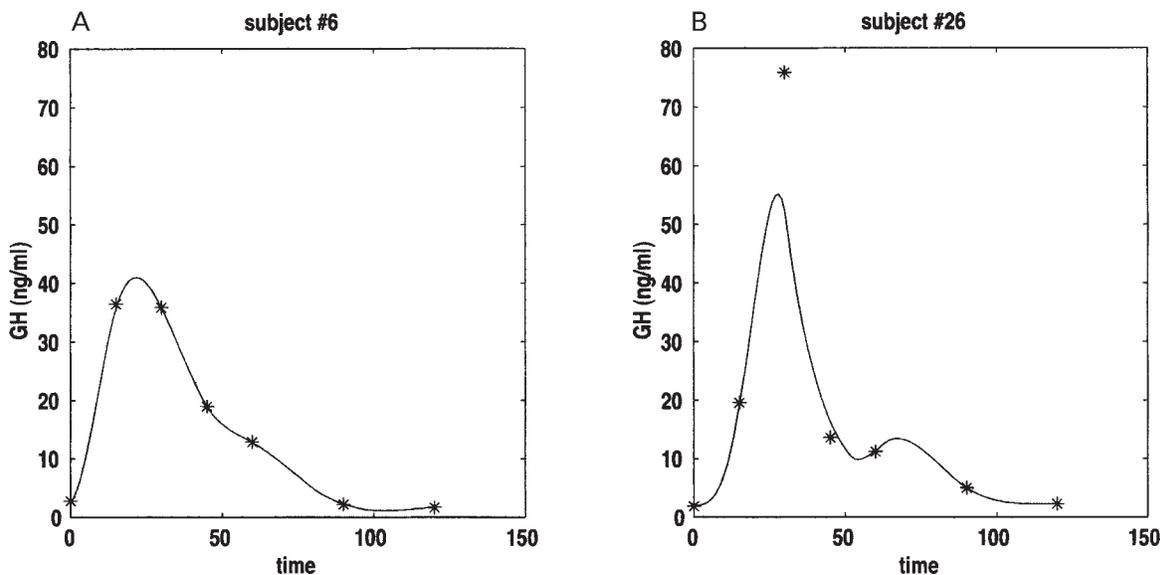
**Standard AUC** An attempt to measure the global effect of the stimulus is to compute the AUC of the plasma concentration samples. The time-range should go from the stimulation to the instant when the effect of stimulation has ceased. Computation can be performed using the trapezoidal rule. In order to discount for the presence of spontaneous secretion that occurred before stimulation, the baseline may be subtracted (5). Usually, constant baselines are assumed, but

exponentially decreasing ones would be more appropriate. In practice, it may be difficult to avoid at least one of the two following shortcomings (see Fig. 2C): (i) the time when the stimulation effect has ceased may be beyond the last sample and (ii) if an extended sampling schedule is adopted the data may also encompass spontaneous post-stimulus secretion episodes whose effect may be superimposed onto the tail of the response to the stimulus.

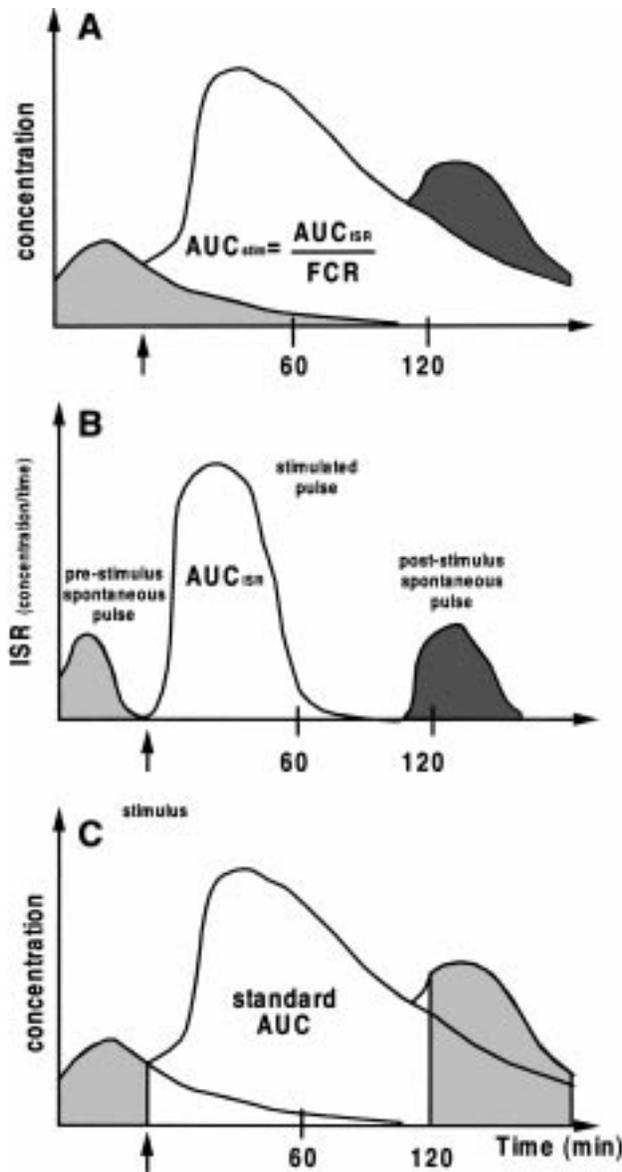
**AUC of the ISR** Since the hormone concentration in plasma is the effect of the glandular ISR combined with hormone clearance, the idea is to assess gland responsiveness via the AUC of the ISR signal. However, the glandular ISR is not directly measurable. The mathematical model linking concentration profile, ISR and clearance is a convolution integral (7), so that deconvolution analysis is needed to reconstruct the ISR profile. Recently, it has been shown that the AUC of the ISR can be accurately obtained with less effort as a linear combination of the plasma hormone concentration samples through proper weights that are specific to the given hormone and sampling protocol. The values of the weights are available for the six pituitary hormones and a number of sampling protocols (4). In practice, the AUC of the ISR is estimated as:

$$AUC_{ISR} = \theta_1 y_1 + \theta_2 y_2 + \dots + \theta_n y_n \quad [1]$$

where  $y_i$ ,  $i = 1, \dots, n$ , are the hormone concentration samples at times  $t_i$ ,  $i = 1, \dots, n$ , and  $\theta_i$ ,  $i = 1, \dots, n$ , are the weights. The weights reported in De Nicolao



**Figure 1** GH plasma concentration samples (symbols) and concentration profiles reconstructed by deconvolution (continuous) in subjects (A) #6 and (B) #26. Panel (A) shows that, as happens in almost all cases, the peak value of the concentration does not coincide with any of the samples. Panel (B) shows that the peak values may be particularly sensitive to experimental errors.



**Figure 2** AUC decomposition. The AUC of the plasma hormone concentration has three components: (i) pre-stimulus AUC (light grey region in panel A) due to spontaneous ISR that occurred before the stimulus (the amount of pre-stimulus secreted hormone is the light grey area in panel B), (ii) stimulated AUC (white region in panel A) due to the stimulus (the amount of stimulated secreted hormone is the white area in panel B) and (iii) post-stimulus AUC (dark grey region in panel A) due to spontaneous secretion that occurred after the stimulus (the amount of post-stimulus secreted hormone is the dark grey area in panel B). The stimulated AUC is a correct index of responsiveness because it is proportional (via the clearance) to the amount of stimulated hormone release. The standard AUC (white region in panel C) is biased because of the superposition of the tails.

*et al.* (14) allow one to compute  $AUC_{ISR}$  relative to the first 60 min after the stimulus. A first definite advantage of such a method is the removal of possible interference (induced by the clearance) between the

effects of spontaneous and stimulated secretion; in Fig. 2, the interference, apparent in Fig. 2C, is removed when considering the ISR plotted in Fig. 2B. A further advantage is that one need not wait for the end of the clearance effects in order to catch the entire secretion response. For instance, in Fig. 2B the ISR response is over after 60 min, whereas in Fig. 2C much more than 120 min are needed to capture the complete tail of the response in plasma (and after a certain time interference may arise with post-stimulus spontaneous secretion). A possible shortcoming is the dependence of the weights on a population model of the hormone clearance, since individual models are hardly ever available. This introduces a source of interindividual error whose assessment is difficult.

**AUC due to the stimulus** In view of the linearity of hormone kinetics, the superposition of effects holds. With reference to Fig. 2A, the hormone concentration curve is the superposition of the response to the stimulus (whose area of  $AUC_{stim}$  is white) and the effects of pre- and post-stimulus spontaneous secretion (whose areas are the light grey and dark grey ones respectively). The distinction between the three components is even clearer in the ISR plot reported in Fig. 2B, because superposition due to the clearance is removed. In Fig. 2B,  $AUC_{ISR}$  is the AUC of the part of the ISR profile due to stimulation. Since  $AUC_{ISR}$  can be regarded as a dose of hormone injected in plasma, the AUC of its effect can be simply computed as:

$$AUC_{stim} = \frac{AUC_{ISR}}{FCR} \quad [2]$$

where FCR is the fractional hormone clearance, i.e. the inverse of the AUC of the plasma concentration  $g(t)$  elicited by a unitary-per-volume hormone bolus.

For GH, TSH, PRL and ACTH,  $g(t)$  is reasonably approximated by an exponential function:

$$g(t) = \exp(-\alpha t), \quad t \geq 0 \quad [3]$$

Population values of the decay rate  $\alpha$  ( $\text{min}^{-1}$ ) have been estimated in previous studies (7, 14–16): GH:  $\alpha = 0.0779$ ; TSH:  $\alpha = 0.0402$ ; PRL:  $\alpha = 0.0277$ ; ACTH:  $\alpha = 0.0365$ .

For a single exponential, it holds that  $FCR = \alpha$ . For FSH and LH,  $g(t)$  is described as the weighted sum of two exponentials:

$$g(t) = a \exp(-\alpha t) + (1 - a) \exp(-\beta t), \quad t \geq 0 \quad [4]$$

Population values for  $a$ ,  $\alpha$  ( $\text{min}^{-1}$ ) and  $\beta$  ( $\text{min}^{-1}$ ) are as follows (17, 18): LH:  $a = 0.62$ ,  $\alpha = 0.0387$ ,  $\beta = 0.00769$ ; FSH:  $a = 0.52$ ,  $\alpha = 0.0068$ ,  $\beta = 0.0014$ .

In the case of two exponentials:

$$FCR = \left( \frac{a}{\alpha} + \frac{1-a}{\beta} \right)^{-1} \quad [5]$$

Recalling [1] and [2], then:

$$AUC_{stim} = \frac{AUC_{ISR}}{FCR} = \xi_1 y_1 + \xi_2 y_2 \dots + \xi_n y_n \quad [6]$$

where the new weights  $\xi_i$  are obtained as  $\xi_i = \theta_i/FCR$  and  $\theta_i$  are the weights already introduced in [1]. It is worth noting that the population model for hormone kinetics enters both the FCR and the weights  $\theta_i$ , in such a way that its effect tends to be compensated (roughly speaking, the use of the weights  $\theta_i$  amounts to a deconvolution while dividing by FCR is equivalent to a convolution). This means that the dependence on the population model is reduced.

**Weights of the direct formula**

The weights  $\xi_i$  of the explicit formula were computed for all six pituitary hormones and the most common sampling protocols. The resulting weights are reported in Table 1. The weights are adimensional: they do not depend on the adopted measurement

units. They do depend on the hormone kinetics and the sampling protocol. The first weight, corresponding to the stimulation instant 0 min, is always negative. This is consistent with the fact that the concentration at 0 min is representative of the baseline and, as such, has to be discounted for. Moreover, the weights associated with times after 60 min are close to zero. This indicates that, as far as one is concerned with estimating secretion over the first hour, the subsequent samples are practically insignificant.

**Numerical example**

In order to illustrate the use of the proposed method, let us consider the GH response of subject #6 (shown in Fig. 1A). At times 0, 15, 30, 45, 60, 90 and 120 min, the measured samples were 2.8, 36.5, 35.9, 19.0, 12.9, 2.2 and 1.7 U/l. The sampling protocol corresponds to row C for GH in Table 1. Therefore,

**Table 1** Weights of the direct formula to compute the stimulated AUC for the six pituitary hormones and some common sampling protocols, which differ in the sampling times. The concentration at each given time must be multiplied by the corresponding weight and the products summed together to yield  $AUC_{stim}$ . Note that the weights are adimensional.

| Protocol    | 0 min     | 15 min  | 20 min  | 30 min  | 45 min  | 60 min   | 90 min  | 120 min |
|-------------|-----------|---------|---------|---------|---------|----------|---------|---------|
| <b>GH</b>   |           |         |         |         |         |          |         |         |
| A           | -5.1510   | 14.8151 | —       | 14.7671 | 16.1873 | 19.6346  | -0.2532 | —       |
| B           | -5.1531   | 14.8238 | —       | 14.7345 | 16.3190 | 19.3748  | —       | -0.0990 |
| C           | -5.1508   | 14.8145 | —       | 14.7697 | 16.1769 | 19.6594  | -0.3217 | 0.0520  |
| D           | -5.1546   | 14.8302 | —       | 14.7100 | 16.4180 | 19.1964  | —       | —       |
| <b>PRL</b>  |           |         |         |         |         |          |         |         |
| A           | -33.8276  | —       | 40.3011 | —       | —       | 53.5265  | —       | —       |
| B           | -21.7422  | —       | —       | 33.1554 | —       | 48.5868  | —       | —       |
| C           | -24.0366  | —       | 5.8993  | 29.1373 | —       | 49.0000  | —       | —       |
| D           | -28.4231  | 14.8391 | —       | 14.7213 | 16.2929 | 42.8319  | -0.3194 | 0.0572  |
| E           | -28.4319  | 14.8737 | —       | 14.5997 | 16.7523 | 42.2062  | —       | —       |
| <b>LH</b>   |           |         |         |         |         |          |         |         |
| A           | -82.7796  | 19.5738 | —       | 20.8327 | 25.8827 | 76.8598  | -0.3693 | —       |
| B           | -80.3055  | 9.8213  | —       | 42.7292 | —       | 88.5778  | —       | -0.8228 |
| C           | -82.7767  | 19.5700 | —       | 20.8488 | 25.8256 | 76.9820  | -0.5446 | 0.0948  |
| D           | -82.7825  | 19.6330 | —       | 20.6414 | 26.6355 | 75.8726  | —       | —       |
| <b>FSH</b>  |           |         |         |         |         |          |         |         |
| A           | -447.7681 | 24.3369 | —       | 24.6335 | 28.2223 | 430.9155 | -0.3400 | —       |
| B           | -444.8219 | 12.8837 | —       | 49.4285 | —       | 443.2639 | —       | -0.7541 |
| C           | -447.7660 | 24.3297 | —       | 24.6586 | 28.1286 | 431.1030 | -0.5465 | 0.0926  |
| D           | -444.4107 | 11.3203 | —       | 52.3353 | —       | 440.7551 | —       | —       |
| E           | -447.7870 | 24.4144 | —       | 24.3644 | 29.2291 | 429.7790 | —       | —       |
| <b>ACTH</b> |           |         |         |         |         |          |         |         |
| A           | -18.2914  | 9.2116  | —       | 27.5611 | —       | 43.1979  | -2.0457 | 0.3665  |
| B           | -19.7185  | 14.8360 | —       | 14.7274 | 16.2782 | 34.1405  | -0.3212 | 0.0575  |
| C           | -15.4425  | —       | 6.7887  | 27.6810 | —       | 41.5233  | —       | -0.5505 |
| D           | -19.7262  | 14.8663 | —       | 14.6200 | 16.6869 | 33.5529  | —       | —       |
| E           | -15.3857  | —       | 6.2857  | 28.5994 | —       | 40.5006  | —       | —       |
| <b>TSH</b>  |           |         |         |         |         |          |         |         |
| A           | -21.8784  | —       | 39.1512 | —       | —       | 42.7272  | —       | —       |
| B           | -12.8871  | —       | 6.4498  | 28.3783 | —       | 38.0590  | —       | —       |
| C           | -12.9386  | —       | 6.9075  | 27.5374 | —       | 39.0514  | —       | -0.5577 |
| D           | -17.1964  | 14.8345 | —       | 14.7304 | 16.2711 | 31.6247  | -0.3218 | 0.0575  |
| E           | -17.2037  | 14.8631 | —       | 14.6285 | 16.6603 | 31.0517  | —       | —       |

the estimated secretion over 60 min is obtained as:

$$\begin{aligned} \text{AUC}_{\text{stim}} &= -5.1508 \times 2.8 + 14.8145 \times 36.5 \\ &+ 14.7697 \times 35.9 + 16.1769 \times 19.0 \\ &+ 19.6594 \times 12.9 - 0.3217 \times 2.2 \\ &+ 0.0520 \times 1.7 \\ &= 1617(\text{U}_{\text{min}})/\text{l} \end{aligned} \quad [7]$$

Since the estimated secretion depends linearly on the measurements, one can immediately derive confidence intervals accounting for the variability induced by measurement error. Again with reference to GH secretion for subject #6, assuming a constant coefficient of variation (CV) equal to 6%, the variance of the k-th measurement is approximately equal to  $(0.06y_k)^2$  so that

$$\text{Var}[\text{AUC}_{\text{stim}}] \cong 0.0036 \sum_{k=1}^n (\xi_k y_k)^2 = 2637 \quad [8]$$

Therefore, as far as measurement error is concerned, the 95% confidence interval is  $1617 \pm 101$  (U/min)/l. In this example, the CV (calculated as  $\text{Var}[\text{AUC}_{\text{stim}}]^{1/2} / \text{AUC}_{\text{stim}}$ ) for secretion assessment is equal to 3%, that is twice as small as the CV of the measurement error.

## Results

The gland responsiveness in the 174 tests was assessed using three alternative methods illustrated in the Materials and methods section: peak value, standard AUC between 0 and 60 min, and stimulated AUC. The population statistics are summarized in Table 2.

**Table 2** Population statistics of three responsiveness indices in normal subjects. The average of stimulated AUC is always greater than standard AUC because the standard AUC does not take into account the tail of the response lying beyond the last sample. In fact, the difference between the two AUCs increases for hormones with longer half-lives, such as LH and FSH.

|      | Mean peak value    | Standard AUC               | Stimulated AUC             |
|------|--------------------|----------------------------|----------------------------|
| GH   | 28.1 ± 16.5 (mg/l) | 1069.4 ± 648.9 (mg/min/l)  | 1195.6 ± 749.7 (mg/min/l)  |
| PRL  | 73.9 ± 34.0 (mg/l) | 3306.4 ± 1437.1 (mg/min/l) | 3968.7 ± 1735.6 (mg/min/l) |
| LH   | 29.0 ± 15.7 (U/l)  | 1364.0 ± 730.7 (U/min/l)   | 3357.9 ± 1832.5 (U/min/l)  |
| FSH  | 11.7 ± 6.6 (U/l)   | 575.2 ± 318.1 (U/min/l)    | 3217.1 ± 1904.8 (U/min/l)  |
| ACTH | 40.4 ± 16.4 (ng/l) | 1882.0 ± 815.3 (ng/min/l)  | 2498.4 ± 1065.7 (ng/min/l) |
| TSH  | 1.9 ± 3.9 (mU/l)   | 512.2 ± 163.6 (mU/min/l)   | 638.3 ± 203.2 (mU/min/l)   |

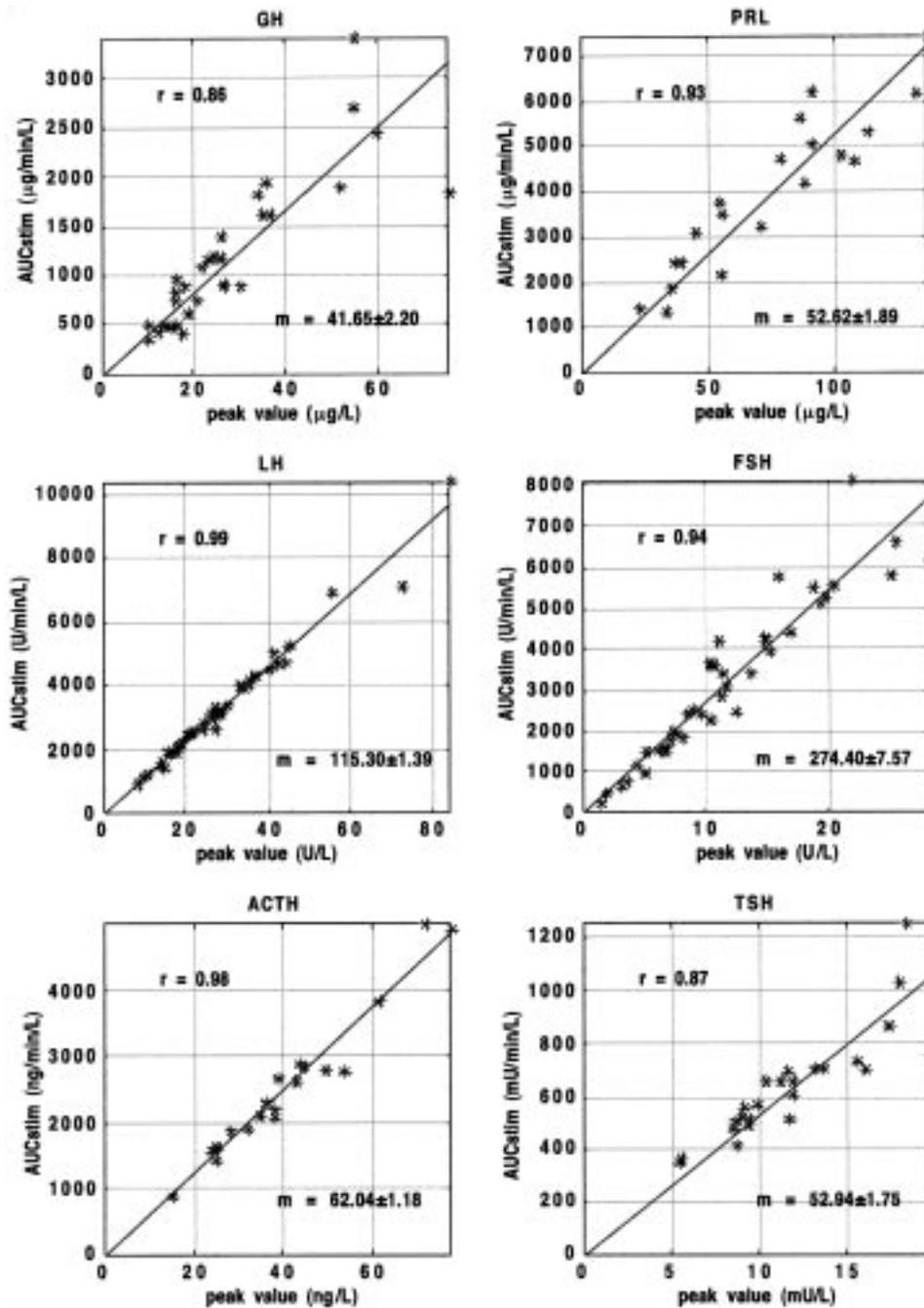
The relationship between the peak value and the stimulated AUC is illustrated through the scatter plots reported in Fig. 3. The correlation coefficients (r) were computed. They range from 0.86 for GH to 0.99 for LH. Inspection of the scatter plot for LH (Fig. 3) shows that responsiveness ranking is almost equivalent irrespective of the two criteria. In other words, if a subject has a higher peak value than another subject, then the same inequality holds for the stimulated AUC. However, in the other hormones, scoring lower correlations (in particular GH and TSH), the ranking is more dependent on the adopted criterion. Given that the stimulated AUC has a sounder physiological basis, this implies that the examination of the peak value alone might be misleading when classifying subjects. The regression of stimulated AUC over peak value was computed as  $\text{AUC}_{\text{stim}} = m \times \text{peak}$ , and the values of m are reported in Fig. 3 with their s.d.

In the example reported in the previous section, it has been shown that the CV of  $\text{AUC}_{\text{stim}}$  can be significantly lower than that of the peak value, meaning that the former is less sensitive to measurement errors because it is based on more than one sample. The ratio between the CV of  $\text{AUC}_{\text{stim}}$  and the CV of the peak value has been computed in all the 174 tests and the average values for each hormone are reported in Table 3. In all cases except FSH, the precision of  $\text{AUC}_{\text{stim}}$  improves on that of the peak value. On the other hand, using the peak value for FSH is completely meaningless, because, due to the very long half-life of the hormone, the tail of the baseline declines very slowly. If a measure related to the peak had to be used, it would be imperative to compute the difference between the peak value and the baseline concentration. The ratio between the CV of  $\text{AUC}_{\text{stim}}$  and the CV of such a difference turns out to be 0.896, showing an improvement in this case as well.

The relationship between the standard AUC and the stimulated AUC is illustrated through the scatter plots reported in Fig. 4. The correlation coefficients (r) were computed. They range from 0.90 for FSH to 0.99 for GH and LH. Inspection of the scatter plots for GH and LH (Fig. 4) shows that, for these hormones,

**Table 3** Average ratio between the CV of  $\text{AUC}_{\text{stim}}$  and the CV of peak value. Being based on a weighted average of samples,  $\text{AUC}_{\text{stim}}$  is less sensitive to measurement errors. The only apparent exception is FSH which is discussed in the text.

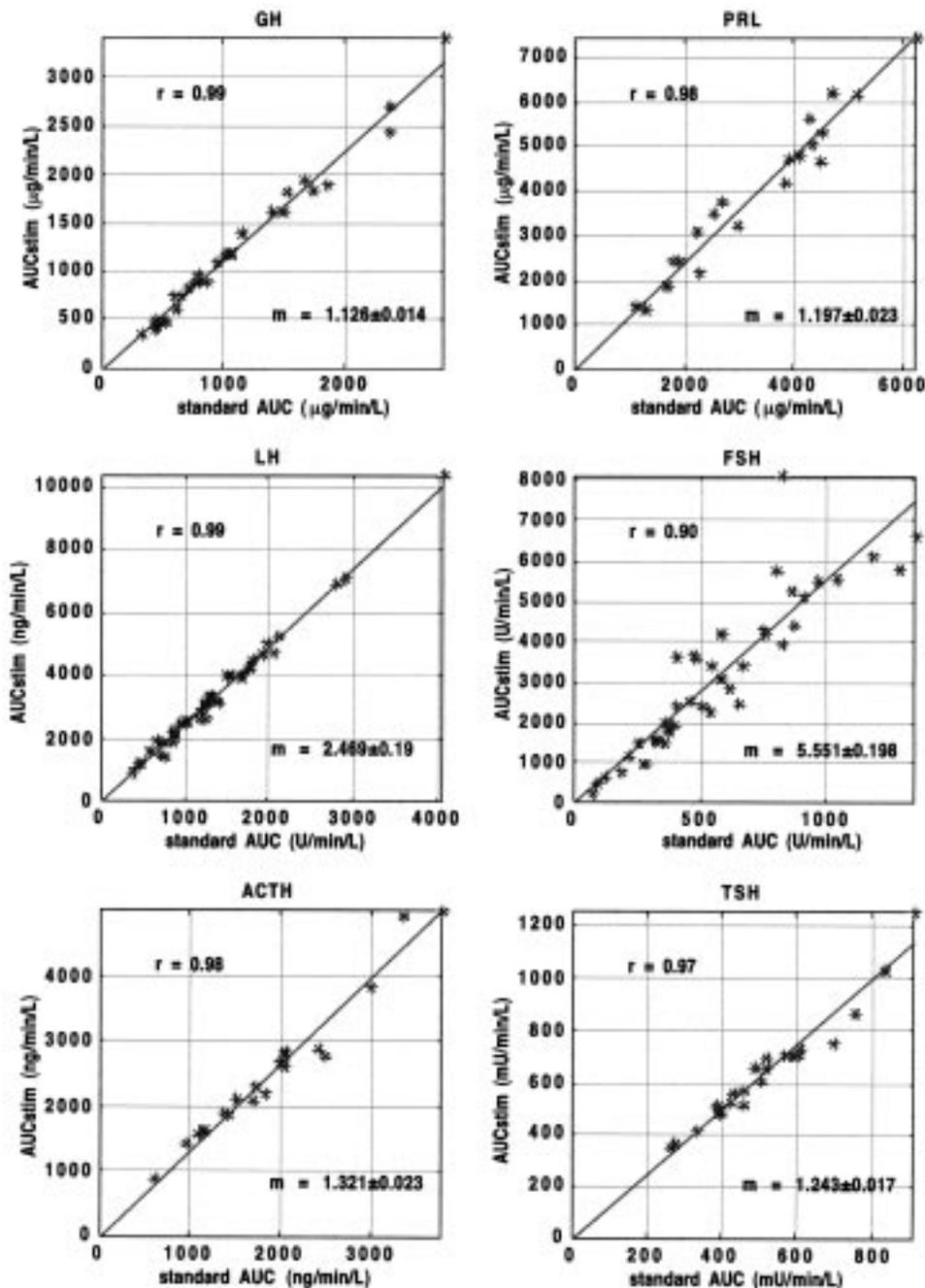
|      | CV( $\text{AUC}_{\text{stim}}$ )/CV(peak) |
|------|---|
| GH   | 0.5462 ± 0.0463                           |
| PRL  | 0.5984 ± 0.0338                           |
| LH   | 0.6516 ± 0.0468                           |
| FSH  | 1.8095 ± 0.4134                           |
| ACTH | 0.7282 ± 0.0316                           |
| TSH  | 0.5411 ± 0.0142                           |



**Figure 3** Scatter plots of stimulated AUC against peak value. Regression lines passing through the origin are plotted and the regression coefficients are reported together with the correlation coefficients. The two indices are correlated but not equivalent.

the two criteria yield almost equivalent responsiveness rankings, in accordance with the high correlation coefficient. In all other cases, equivalence does not hold, showing that standard AUC is not a valid surrogate of  $AUC_{stim}$ . In any case, it shows that the standard AUC is more concordant with  $AUC_{stim}$  than the peak value for GH, PRL, ACTH and TSH, whereas it is less concordant for FSH. The behaviour of FSH is not

surprising because standard AUC does not discount the initial baseline, whose effect does not vanish when the hormone half-time is particularly long as it is for FSH. The regression of stimulated AUC over standard AUC was computed as  $AUC_{stim} = m \times AUC$ , and the values of  $m$  are reported in Fig. 3. Note that the values of  $m$  are always greater than one. With reference to Fig. 2, this means that the area under the tail



**Figure 4** Scatter plots of stimulated AUC against standard AUC. Regression lines passing through the origin are plotted and the regression coefficient are reported together with the correlation coefficients. Regression coefficients are all smaller than one, confirming that the standard AUC underestimates the part of the AUC induced by the stimulus. The two indices are correlated but not equivalent.

of the baseline (accounting for secretion occurring before the stimulus) is smaller than the area of the tail after 60 min, so that the standard AUC underestimates the AUC elicited by the stimulus. The phenomenon is more apparent for FSH which has the longest half-life.

## Discussion

In the paper, the problem of assessing the pituitary responsiveness to exogenous stimuli has been investigated. The most widespread approach is to consider the peak value of the hormone concentration curve

following stimulation. The shortcomings of this approach are apparent (see Fig. 1): being based on a single measurement, this index is sensitive to measurement error and, moreover, in view of the sparse sampling, it is possible that the true peak value will lie in between two consecutive samples.

A more physiological index of responsiveness would be the total release of hormone caused by the stimulus. This is just the AUC of the so-called ISR (see Fig. 2) which, however, is not directly measurable. In fact, it is only possible to measure the hormone concentration in plasma, which can be described as the convolution integral of the ISR with the hormone elimination function (7). Starting from the late eighties, the reconstruction of the ISR through deconvolution has been the subject of several investigations, covering the case of spontaneous pulsatile secretion (7–9, 19) as well as that of stimulated secretion (10–13). The response assessment made through deconvolution has two drawbacks: (i) in general, in provocative tests the individual kinetic parameters are not available and population values have to be used and (ii) specific software programs are needed (see 20 for example), which are not yet commercial, and whose use may not be straightforward for the clinician. The latter problem has been overcome with the introduction of explicit formulas that allow the calculation of the cumulative ISR as the linear combination of the measured samples through a proper set of weights, specific for each hormone and each sampling protocol (14).

A natural surrogate for the AUC of the ISR would be the AUC of the hormone concentration in plasma, which is easy to compute and has already been used in some studies (5). Unfortunately, it does not provide a satisfactory estimate because of the tails due to pre-stimulus spontaneous secretion (this tail is included but should be discounted for) and due to the part of the response extending after the last sample (this tail is truncated but should be included) (see Fig. 2). Note that using longer sampling protocols, besides increasing costs and discomfort, does not solve the problem because there would be an increased probability of including post-stimulus spontaneous secretion episodes not strictly related to the stimulation.

In this paper, we propose an alternative solution that is based on the superposition principle: due to linearity, the AUC is the sum of three contributions: the tail due to pre-stimulus spontaneous secretion, the AUC caused by the stimulus, namely  $AUC_{stim}$ , and a possible tail due to additional post-stimulus spontaneous secretion episodes (see Fig. 2). In view of an elementary kinetics result,  $AUC_{stim}$  is proportional through the hormone clearance to the AUC of the ISR elicited by the stimulus. Although it may seem difficult to separate the three components, exploiting the linearity of the model along the rationale introduced by De Nicolao *et al.* (14), it turns out that  $AUC_{stim}$  can be estimated as a linear combination of the measured samples through

a proper set of weights, specific for each hormone and each sampling protocol (Table 1). Differently from the method presented in De Nicolao *et al.* (14), the dependence on the clearance (whose individual values are usually not available) is less critical, because what is actually estimated is the AUC of the measured concentration, and the clearance affects only the corrections introduced to discount for the pre-stimulus secretion tail and to re-integrate the tail after the last measured sample that would be otherwise truncated (see Fig. 2).

In computing the weights for the six pituitary hormones, it has been assumed that the glandular hormone release elicited by the selected secretagogues takes place within 60 min from stimulation, as verified from the inspection of the 174 ISR curves computed by deconvolution (14). If other secretagogues are employed, it may be necessary to consider a wider secretion window and recompute the weights accordingly. Inspection of the weights in Table 1 shows that the measurements taken after 60 min affect  $AUC_{stim}$  only marginally. Though useful in the exploratory study in order to assess the ISR profile, they are not strictly needed to calculate  $AUC_{stim}$ .

The new method was applied to 174 tests and the results compared with the use of the peak value as well the use of the standard AUC. A first notable result is that, as a rule,  $AUC_{stim}$  is less sensitive to measurement error than the peak value (Table 3). This is not surprising as  $AUC_{stim}$  averages over multiple measurements, whereas the peak value relies on a single measurement. The only exception seems to be FSH, but, as already explained in the Results, in this case the peak value alone is meaningless.

Even if we restrict our attention to the ranking between subjects, using  $AUC_{stim}$  as a responsiveness index is neither equivalent to the use of the peak value nor to that of standard AUC. In particular, a subject who is regarded as less responsive than another subject according to the peak may result in being more responsive according to  $AUC_{stim}$  and vice versa. This demonstrates that the choice of the correct index is a substantial issue in the definition of guidelines for the diagnosis of hormone deficits. On the other hand,  $AUC_{stim}$  and the standard AUC are much more concordant and in most cases it is improbable that the modest difference would impact on the appropriate clinical designation of an individual patient. Nevertheless, such similarity holds only for sampling protocols well suited to the hormone kinetics and is not guaranteed in general, as demonstrated by the FSH data shown in Fig. 4. A possible future development may regard the application of the new responsiveness index to specific classes of subjects, such as short children suspected of GH deficiency, moving towards a critical reconsideration of the criteria currently used for diagnosis.

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