

Down-Regulation Models and Modeling of Testosterone Production Induced by Recombinant Human Choriogonadotropin¹

JEAN-MICHEL GRIES, ALAIN MUNAFO, HERVE C. PORCHET, and DAVIDE VEROTTA

Department of Biopharmaceutical Sciences (J.-M.G., D.V.), School of Pharmacy, and Department of Epidemiology and Biostatistics (D.V.), University of California, San Francisco; Ares-Sevono (A.M.), Geneva, Switzerland; and Debio Pharm (H.C.P.), Lausanne, Switzerland

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ABSTRACT

Chorionic gonadotropin (CG) is a glycoprotein hormone, whose action is mediated by the luteinizing hormone/CG receptor. Testosterone concentrations from six pituitary-desensitized, healthy male volunteers were obtained after four different administrations of recombinant-human CG (rhCG). We present a modeling study to provide a possible explanation for the observations that increased exposure to rhCG induces higher and then lower testosterone concentrations and that marked rebound effects are observed at the end of repeated administration of rhCG. We used semimechanistic models (in which flexible functions represent unknown parts of the models) to identify the relationship of rhCG concentrations to the testosterone levels. Based on the results obtained with the semi-

mechanistic models, different mechanistic down-regulation models were devised and tested. The final model uses a one-compartment model to describe the endogenous production rate of testosterone; rhCG affects the production rate with a mechanism consistent with a two-site binding site, with effect proportional to one-site bound concentration. The modeling results indicate that when rhCG concentration increases, the testosterone production rate increases to 45 times the baseline value. However, at an rhCG concentration of more than about 30 IU/liter, the production rate decreases. Simulations showed that both dose and dosing interval profoundly influence testosterone response to rhCG.

Chorionic gonadotropin (CG) is structurally related to the pituitary hormones luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The actions of LH and CG are mediated by the LH/CG receptor, which is a member of the superfamily of G protein-coupled receptors. The binding of LH and CG occurs with similar high affinity, and both hormones have similar potencies and efficacies in stimulating gonadal cells. Thus, at a cellular level, the two hormones are roughly equivalent. In vivo, the main difference is the much longer half-life of CG.

The primary physiological effect of the gonadotropins is the promotion of gametogenesis and/or gonadal steroid production. In the male, endogenous production of CG does not occur, and LH stimulates the de novo synthesis of androgens, primarily testosterone, by Leydig cells. The secreted testosterone is required for gametogenesis and for the maintenance of sexual libido and secondary sexual characteristics. CG is used primarily in females to trigger ovulation or to induce

final follicular maturation before assisted reproduction techniques, and it is used for male infertility and cryptorchidism. Gonadotropins of urinary origin have been used for a long time. Recombinant forms of human LH and CG have been produced (in mammalian cells) and are being tested in clinical studies for human use; recombinant human FSH is available in several countries.

We present the modeling of recombinant human CG (rhCG) in male subjects under pituitary desensitization: the male volunteers previously received a gonadotropin-releasing hormone analog to suppress their secretion of gonadotropins, secondarily decreasing their testosterone secretion. Each volunteer in the study received rhCG via four different routes. The main feature of the data was that all doses induce an effect but that the intravenous route (associated with the highest drug concentrations) leads to the smallest response. To analyze these data, we applied a general approach to model complex drug dynamics (Verotta, 1995; Verotta and Sheiner, 1995) that allows testing for alternative functional forms within a particular model structure. This helps devise final models that appear to be consistent with the physiological characteristics of the LH/CG receptor.

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ABBREVIATIONS: CG, chorionic gonadotropin; LH, luteinizing hormone; FSH, follicle-stimulating hormone (folliotropin); rhCG, recombinant-human chorionic gonadotropin.

We describe the study design; the model for drug dynamics, including a pharmacokinetic model; and the various semi-mechanistic and mechanistic pharmacodynamics models, and we present selected results.

Materials and Methods

Study Design

Six male subjects who were under pituitary desensitization with the use of a depot formulation of gosereline, a gonadotropin-releasing hormone analog (Zoladex; Zeneca Laboratories, Macclesfield, UK), were enrolled in the study. Each volunteer received in a crossover manner 2500 IU rhCG (Ovidrel; Laboratories Serono, Aubonne, Switzerland) by the i.v., i.m., and s.c. routes, with each administration separated by a 2-week washout interval. Two weeks later, each subject received five s.c. injections of 2500 IU of rhCG at 48-h intervals. Extensive blood sampling was performed on preset time points over 8 days after the single administrations of rhCG and over the dosing plus 8 days in the repeated-dose regimen part of the study. hCG and total testosterone levels were assessed in serum by using an immunoradiometric assay (MAIAclone; Serono Diagnostics, Woking, UK) and a radioimmunoassay (Coat-A-Count; Diagnostic Products Corporation, Los Angeles, CA), respectively. The study was approved by an independent ethics committee and was performed in accordance with the guidelines of the Declaration of Helsinki on biomedical research involving human subjects (Hong-Kong revision, 1989). Each volunteer gave written informed consent for participation in the study.

Drug Dynamics Model

The conceptual model for rhCG dynamics is shown in Figure 1; below, we describe the components of the model.

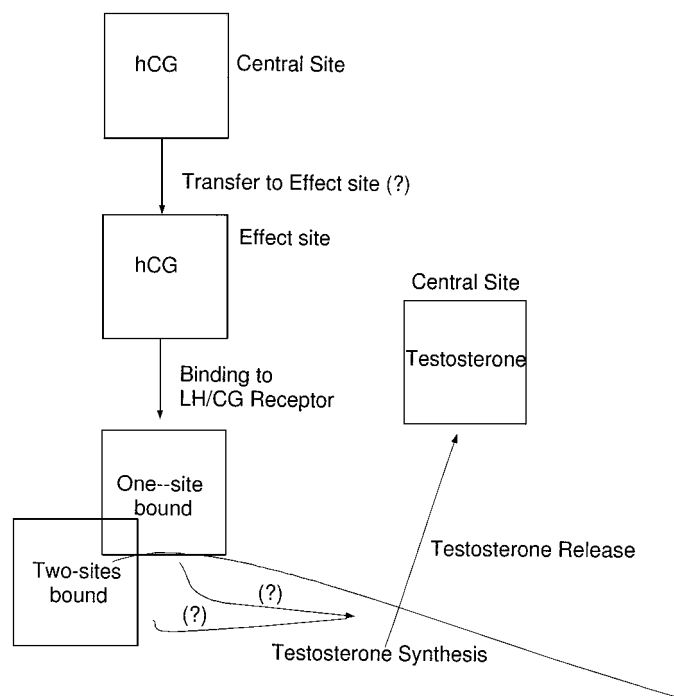


Fig. 1. Drug dynamics model. Hypothetical sequence of events incorporated in the models described in the text. The symbol (?) indicates some of the modeling hypothesis tested by the different models. From the central site, hCG reaches an effect compartment and binds to the CG/LH receptor. The concentration of single- or double-site bound hCG stimulates the synthesis and release of testosterone, causing an increase of concentration of testosterone (extracellular, and, in particular, in the central site).

Pharmacokinetic Model of rhCG. Linear splines were used to represent rhCG-versus-time profiles for the i.v., i.m., s.c., and repeated-dose s.c. (SC×5) routes. Using splines, no assumptions are made on the pharmacokinetics of rhCG, whereas no information is lost from the pharmacokinetics data. Exponentials were also used to represent pharmacokinetics, but doing so resulted in significantly worse fits of the testosterone data. The interested reader can find a short description of splines and of the fit of the rhCG data in *Appendix*. To investigate whether the action of rhCG is kinetically delayed in respect to the observation site, we used an effect compartment (as described by Segre, 1968). The concentration of rhCG in the effect site (C_e) after the different administrations was computed as the convolution of the corresponding linear splines representing rhCG concentration with a monoexponential function:

$$C_e(t) = k_{eo} \int_0^t C(t - \tau) e^{-k_{eo}\tau} d\tau \quad (1)$$

where k_{eo} is the exit rate constant from the effect compartment, and $C(t)$ is a linear spline representing rhCG concentrations (and τ an integration variable).

Pharmacodynamic Model of rhCG. A realistic model for the stimulation of testosterone production should take into account the complex sequence of events that originate at the LC/CG receptor. A simplified list of these events include binding to the receptor, adenylyl cyclase stimulation, cholesterol mobilization and transport to the mitochondrial, testosterone synthesis, and exit from the Leydig's cell (see *Discussion*). The micromodeling of these events is, of course, impossible when only testosterone levels are measured. A feasible model lumps these events into the action of rhCG on the endogenous rate of production (k_o) of testosterone (T). The formula is expressed as follows:

$$k_o\{1 + f[C_e(t)]\} \quad (2)$$

where f represents the change in the production rate of testosterone induced by $C_e(t)$, expressed as a multiple of k_o (the function is zero for $C_e = 0$). Assuming a one-compartment model, the differential equation describing the rate of change of testosterone is expressed by the following:

$$\frac{dT(t)}{dt} = k_o\{1 + f[C_e(t)]\} - k_{el}T(t) \quad (3)$$

where K_{el} is the apparent first order elimination rate constant of testosterone. This structure for hCG action is the best one we could devise to fit the data. An alternative modality of action of $C_e(t)$ will be briefly described after we discuss the choices for f in eq. 3 we investigated.

Semimechanistic Models. As a first step, a natural cubic spline was used to obtain an estimate of the shape of the function f in eq. 3. The advantage of using a spline is that they make no assumptions on the relationship between C_e and the testosterone production rate. The spline was subjected to the following alternative constraints: to be greater than (or equal to) -1 , to be greater than (or equal to) 0 , and to be greater than (or equal to) 0 and unimodal. We will use the symbols ≥ -1 , ≥ 0 , and ≥ 0 /unimodal, respectively, to refer to these models. Model ≥ -1 is the most flexible one: it says that the action of C_e can reduce or increase the production rate, and we only impose that the induced rate of production cannot become negative (for obvious physiological reasons). Model ≥ 0 is less flexible: it says that the action of C_e is to increase the production rate. Model ≥ 0 /unimodal is the least flexible: it says that the action of C_e is to increase the production rate and that the increase cannot have more than one maximum. From a physiological point of view, the ≥ 0 /unimodal model makes more sense. The ≥ -1 and ≥ 0 models are more flexible and are used to test for residual model misspecification.

Down-Regulation Model. We describe in detail the model that obtained the most satisfactory results. The model is based on a binding model for a receptor (R) with multiple (m) sites. We developed this model based on the results of the semimechanistic model and based on the observation that the LH/CG receptor is composed of two subunits (Dufau et al., 1994; McFarland et al., 1989) that can each bind rhCG (see also *Discussion*).

The rate of change of the concentration of single-site-occupied receptor ($[CeR]$) is given by

$$\frac{d[CeR]}{dt} = k_1[R]Ce - (k_{-1} + k_2)[CeR] + k_{-2}[Ce_2R] \quad (4a)$$

where $[R]$ is the concentration of receptor, $[Ce_iR]$ is the concentration of receptor with i sites occupied (we use both $[CeR]$ and $[Ce_1R]$ to indicate the concentration of one-site-occupied receptor), and k_i and k_{-i} are the rates of association and dissociation to the i th site given that $i-1$ sites are occupied, respectively. For the other $m-1$ sites of the receptor, the rate of change of concentration is similar:

$$\frac{d[Ce_iR]}{dt} = k_i[Ce_{i-1}R]Ce - (k_{-i} + k_{i+1})[Ce_iR] + k_{-i-1}[Ce_{i+1}R], \quad i = 2, \dots, m-1 \quad (4b)$$

while for the last site holds:

$$\frac{d[Ce_mR]}{dt} = k_m[Ce_{m-1}R]Ce - k_{-m}[Ce_mR] \quad (4c)$$

The initial conditions of this system are $[R(0)] = R_0$, $[Ce_iR(0)] = 0$, $i = 1, \dots, m$. At steady state, the use of algebra obtains the solution for $[CeR]$ as:

$$[CeR] = \frac{R_0Ce}{k_{-1}/k_1 + Ce + \sum_{i=2}^m \prod_{j=1}^i k_j/k_{-j}Ce^i} \quad (5)$$

Assuming that the action or Ce on the rate of production of testosterone is proportional to $[CeR]$, we obtain the following expression for f :

$$f(Ce) = \frac{\theta_1Ce}{\theta_2 + Ce + \sum_{i=2}^m \theta_{i+1}Ce^i} \quad (6)$$

A more empirical model introduces a sigmoidicity coefficient (0_{m+2}):

$$f(Ce) = \frac{\theta_1Ce^{0_{m+2}}}{\theta_2^{0_{m+2}} + Ce^{0_{m+2}} + \sum_{i=2}^m \theta_{i+1}Ce^{0_{m+2}+i-1}} \quad (7)$$

When m equals 1, eq. 7 reduces to the familiar Hill model:

$$f(Ce) = \frac{\theta_1Ce^{\theta_3}}{\theta_2^{\theta_3} + Ce^{\theta_3}} \quad (8)$$

The fundamental characteristic of the multisite models is that f increases, reaches a peak, and then declines to zero as a function of Ce . The Hill model predicts that $f(Ce)$ asymptotes to θ_1 when Ce increases.

Depending on the model, the parameters k_{eo} , k_θ , k_{el} , and $\theta_1, \dots, \theta_{m+1}$ are estimated from all the data of each individual, subject to non-negativity constraints. To stabilize the estimation of θ_1 and θ_2 ,

we used the parametrization θ_1 and θ_1/θ_2 (Ratkowsky, 1983). We assumed a proportional error model and used the computer program NONMEM (Beal et al., 1992). To select between competing models, we compared objective function values (minus twice log-likelihood) and used the Akaike (AKA) selection criterion (Akaike, 1974). Pooled data fits were also obtained for illustrative purposes.

Models that Do Not Work. Alternative models were also used to investigate alternative modes of action of rhCG. In all the models listed below, the function g represents a spline (estimated by the data fitting). They did not work as well as the models described above and are reported because of their possible use for a different drug/substance: they are not be discussed further in *Results*. We tried models in which rhCG acts on the elimination rate:

$$\frac{dT(t)}{dt} = k_o - k_{ei}\{1 + g[Ce(t)]\}T(t) \quad (9)$$

or where rhCG acts simultaneously on production and elimination rate:

$$\frac{dT(t)}{dt} = [k_o - k_{ei}T(t)]\{1 + g[Ce(t)]\} \quad (10)$$

A different down-regulation model was also used; it takes the following form:

$$\frac{d[CeR]}{dt} = k_1Ce[R] - (k_{-1} + \alpha)[CeR] + g([CeR_{int}]) \quad (11)$$

$$\frac{d[CeR_{int}]}{dt} = \alpha[CeR] - g([CeR_{int}])$$

where $[CeR_{int}]$ is the concentration of receptor internalized in the cell, and $g([CeR_{int}])$ is the rate of reconstitution of the surface concentration of receptor. The model has initial conditions: $[R(0)] = R_0$, $[CeR](0) = 0$, and $[CeR_{int}](0) = 0$, and the effect is proportional to $[CeR]$.

Results

Figure 2 shows the average testosterone (top) and rhCG (bottom) concentration versus time for the four [i.v., i.m., and s.c. (1 dose) and s.c., (multiple doses)] routes of administration. The main feature of the data is immediately apparent: testosterone concentrations after i.v. rhCG increase the least, although rhCG concentrations are the highest. Figure 3 reports the fits of three models to the testosterone data pooled from all the individuals. The models include the same mechanism of action of rhCG (eq. 3) with the function f given by a spline constrained to be unimodal and positive (dashed line), the down-regulation model (eq. 7 with $m = 2$, solid line), and the familiar Hill model (eq. 8, dotted line). The Hill model overestimates testosterone concentration for the i.v. data (Fig. 3, top left).

To more formally select between competing models for the function f and to obtain individual subject parameter estimates, we fitted the semimechanistic models, the down-regulation model eq. 7 (with $m = 2, 3$ or 4), and the Hill model to the individual subject data. In all cases we fitted the models, with or without an effect compartment, to the testosterone data for all routes of administration.

The semimechanistic models provided similar results. In particular, the ≥ -1 and ≥ 0 versions gave always almost identical estimates of f , whereas the ≥ 0 unimodal obtained a slightly different estimates and higher objective function (mi-

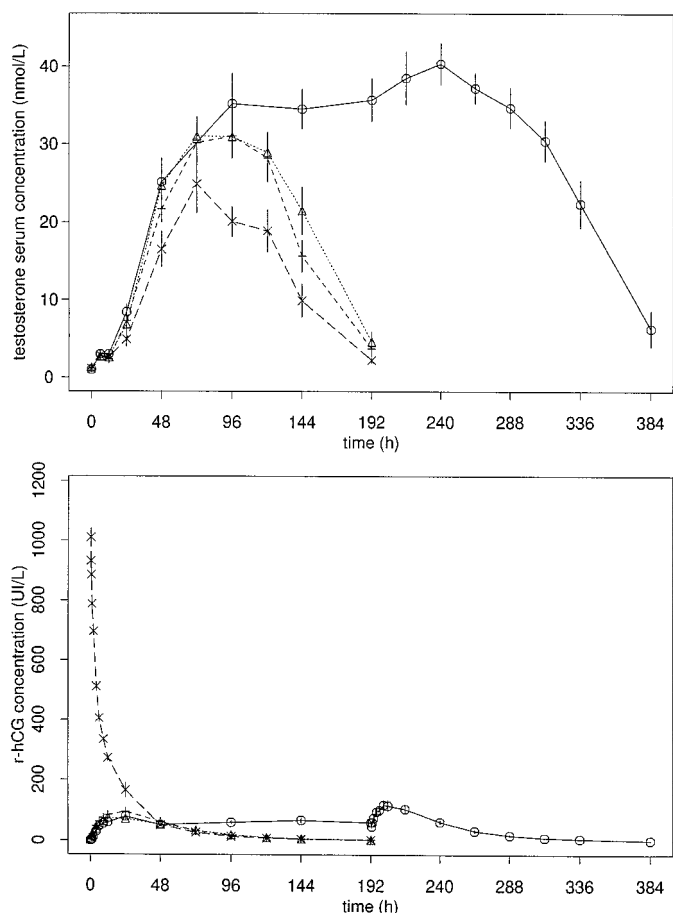


Fig. 2. Mean testosterone and rhCG serum concentrations after 2500 IU of rhCG i.v., i.m., s.c., and s.c. repeated five times (every other day). Top, testosterone. Bottom, rhCG. The solid line (and circles) is for the s.c. dose repeated five times, the dotted line (and triangles) is for the single s.c. administration, the broken line (and plus) for the i.m. administration, and the long dashed line (and crosses) for the i.v. administration. The error bars represent ± 1 S.E.M.

nus twice log-likelihood). The down-regulation models provided objective function values similar to those of the semi-mechanistic models. The model with $m = 2$ provided the best (smallest) value of the selection criterion in all subjects. The Hill equation provided the worst objective function and selection criterion values. (To give an idea of the size of the difference, the values of the objective function for each subject in the case of the Hill model are: 963, 850, 1053, 957, 1066, and 954; for the down-regulation model with $m = 2$, which has only one additional parameter, the values are 174, 131, 160, 174, 179, and 165. For a difference of one parameter, the AKA selection criterion requires a difference of 2 for a model to be significantly better than the competitor.) For the down-regulation model (with $m = 2$), inclusion of an effect compartment decreased the objective function value only in two subjects and did not decrease the selection criterion value in any subject.

Based on these results, we selected the down-regulation model with $m = 2$, without an effect compartment as a final model. Table 1 reports the mean estimates and standard deviations of the parameters of the selected model obtained from each of the individual subjects (for comparison, the estimates corresponding to the Hill model are given in parentheses). Notice the relatively small interindividual vari-

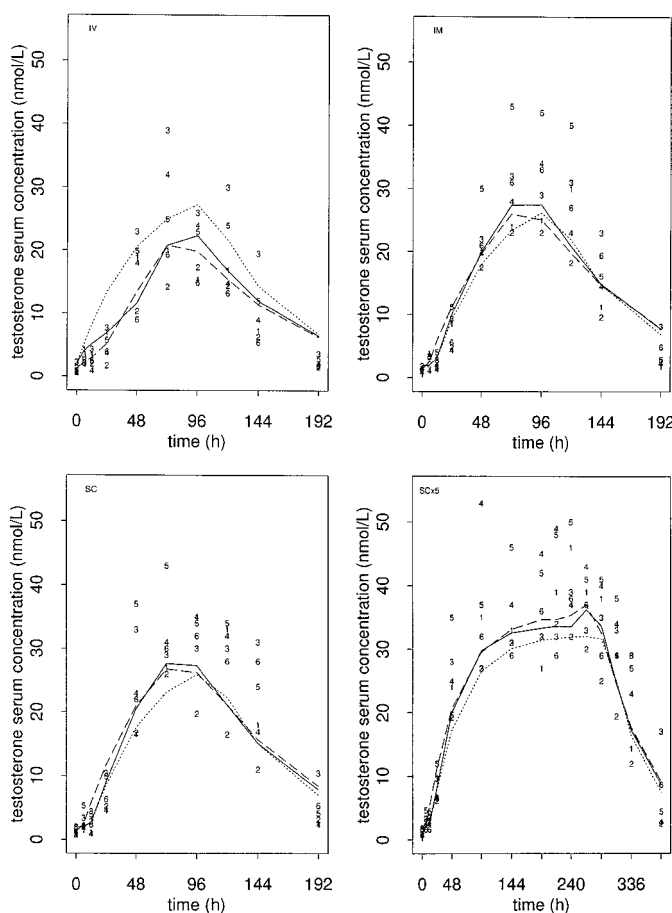


Fig. 3. Pooled data fit. Solid line, fit of the down-regulation model; dotted line: Hill model; dashed line: semimechanistic model. The numbers represent observations from different individuals. Top left, data and fitted values versus time for the i.v. experiment. Top right, i.m. experiment. Bottom left, s.c. experiment. Bottom right, repeated s.c. experiment.

TABLE 1
Mean parameter estimates for the down-regulation model

Parameter	Mean	S.D.	CV
k_{el}	0.0143 (0.0283)	0.0023 (0.011)	16
k_o	0.0151 (0.010)	0.0079 (0.0070)	53
θ_1	56.6 (17.9)	18 (10.5)	32
θ_2	18.9 (10.5)	2.1 (3.5)	11
θ_3	6.09 (33.9)	1.19 (14.1)	31
θ_4	0.00675	0.0038	56
Max (f)	45 (17.9)	8.55 (10.5)	19

Shown in parentheses are mean and S.D. values for the Hill model. Max (f), maximum fold increase in production rate (i.e., maximum of the function f). For the Hill model, max (f) = θ_1 .

ability with all parameters showing less than 60% coefficient of variation.

Figure 4 shows the mean estimates of the f functions: the spline (dashed line), the down-regulation model (solid line), and the Hill model (dotted line). Note the similar estimates obtained by the spline and the down-regulation model, which are both characterized by an increase followed by a decrease

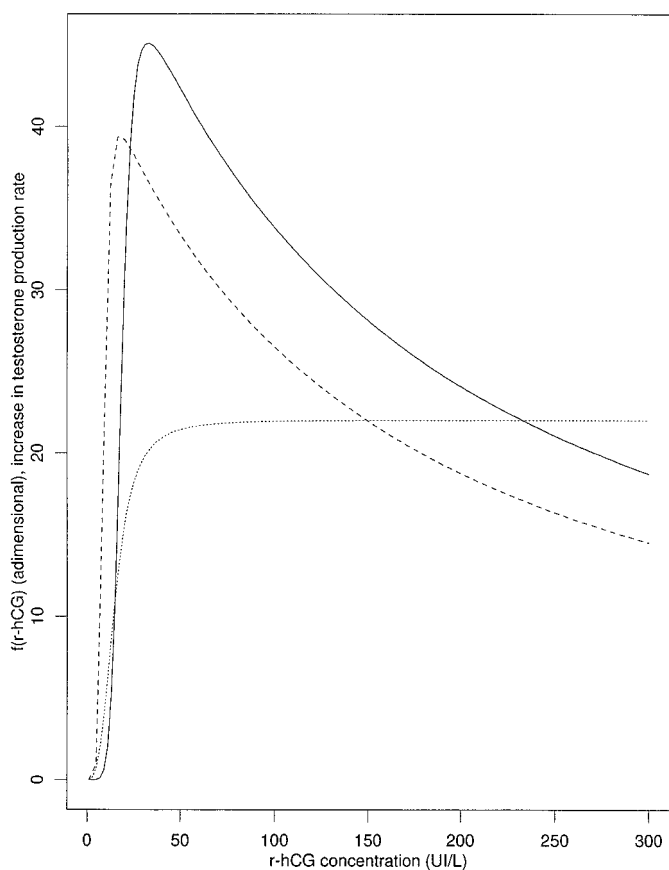


Fig. 4. Mean estimates of the stimulation of testosterone production induced by hCG (f function). Solid line, mean f function estimated by the down-regulation model; dotted line; Hill model; dashed line: semimechanistic model.

in the stimulation of production rate of testosterone. The maximum increase in testosterone production estimated by the down-regulation model is on average 45-fold obtained at around 30 UI/liter rhCG (these values are obtained numerically from the estimated mean f function).

Simulations. We simulated the testosterone production after one or multiple s.c. administrations using the mean parameter estimates reported in Table 1. (It is, of course, possible to simulate a sample from a population using the parameters estimates in Table 1.) Figure 5 shows the response corresponding to five different doses (500, 1000, 2500, 5000, and 10,000 IU) of rhCG given daily, every other day, every 4 days, or weekly (top left, top right, bottom left, and bottom right, respectively). Note how the higher doses (5000 and 10,000 IU) produce a lesser testosterone concentration than the 2500 IU dose for the daily, every other day, and every 4 days schedule and the rebound in testosterone concentration that could be observed in the multiple dose experiment at the same doses. This rebound can be seen in the mean observed data in Figure 1 for the SC \times 5 (2500 IU) dose: the peak concentration was obtained 2 days after the last dose.

Discussion

The results offered by the present data analysis can be summarized as follows: 1) the semimechanistic models indicated that the production rate of testosterone increases in

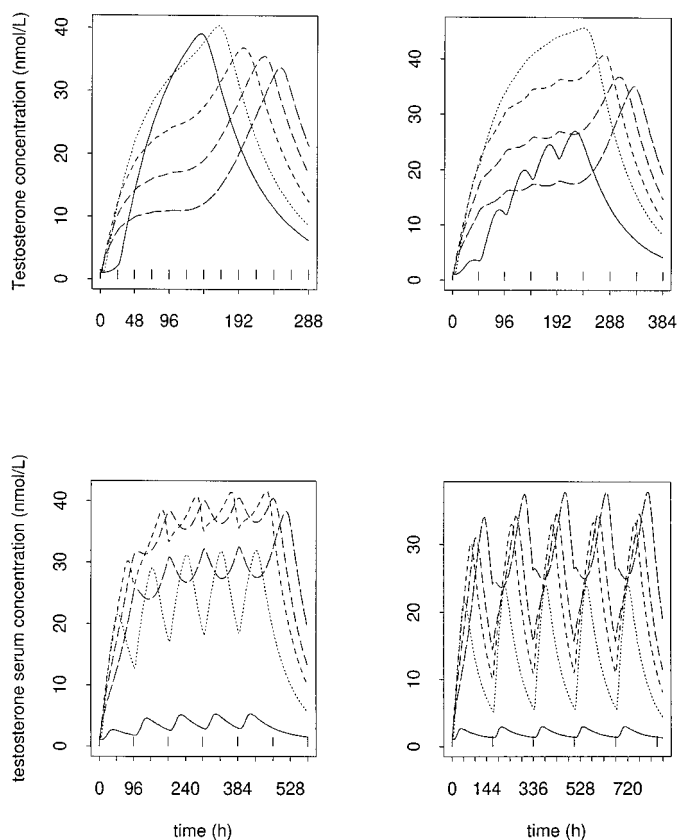


Fig. 5. Simulation of testosterone production in response to different doses and schedules of rhCG (SC \times 5 route) obtained with the down-regulation model. Five s.c. administrations are given daily (top left), every other day (top right), every 4 days (bottom left), and weekly (bottom right). The solid, dotted, broken, long dashed, and longer dashed lines mark the concentration time course for 500, 1000, 2500, 5000, and 10,000 IU, respectively; the short (solid) bars indicate the time of dose.

presence of rhCG, but the increase does not asymptote to a maximum level but instead decreases at higher concentrations of rhCG; 2) the down-regulation model introduced here performed as well as the semimechanistic models, indicating that no residual model misspecification is present; and 3) as expected, the Hill model performed worse than both the semimechanistic and down-regulation models.

In the final model we selected, the pharmacokinetics of rhCG for the repeated s.c. administration are assumed to be linear in respect to the dose; we make no additional assumptions for the other routes of administration (see *Appendix*). An effect compartment is not present in the final model. Testosterone is released at a rate dependent on rhCG concentration and is eliminated at a constant rate independent of rhCG concentration. Last, the relationship of rhCG versus the production rate of testosterone is by means of a rather complex binding model, which is consistent with a two-site binding receptor with an effect proportional to one-site bound concentration.

The binding model allows at least two interpretations: the receptor is either deactivated (i.e., stimulation of production rate decreases) or internalized (and stimulation of production rate ends) when more than one molecule binds to it. The study design did not allow separation of a concentration effect at the receptor level or receptor internalization (or a combination of the two), and it is questionable whether an in

vivo study aimed at answering this point is feasible. One of the reviewers pointed out a third possibility: a refractory period before the α subunit of the G protein recombines with the $\beta\gamma$ portion.

In general, the Hill and the down-regulation models behave similarly for the routes with low exposure (i.m., s.c.); the major differences between the two model predictions can be observed in the high exposure route (i.v.) and, in particular, in the first 72 hours (see Fig. 3). The down-regulation model predicts the decrease in the stimulation of endogenous testosterone production at around 30 UL/liter rhCG (see Figure 4), which, of course, the Hill model cannot reproduce. As a consequence, the Hill model would obtain a decreased estimate of the maximum response (19-fold increase instead of 45-fold increase), a decreased value of O_2 (10.5 instead of 18), and an extremely high value for the Hill coefficient (33.9 instead of 6.09), leading to incorrect conclusions on the size of the testosterone rate of production induced by rhCG.

We used a class of semimechanistic models described by Verotta (1995), and Verotta and Sheiner (1995). Semimechanistic models allowed us to 1) identify the shape of the relationship between active substance (rhCG) and parts of the model characterizing the response, and 2) quantify the presence of residual model misspecification within a particular model structure. Shape identification is a rather straightforward process and consists of using a flexible function (in our case, a spline) to represent a part of the model. Here, we used shape identification to identify the pattern of action of rhCG on the production rate of testosterone. Residual model misspecification detection is a more subtle data analysis feature offered by constrained splines. Given a model structure (e.g., effect on production rate), it consists of considering models with different constraints, which in turn correspond to different physiological interpretations. In our case, the least constrained model (≥ -1) had the least likely physiological interpretation (testosterone production can decrease, reach a minimum, increase, reach a peak, and then decline to zero as a function of rhCG), and it turned out to be rejected by the data.

We now discuss in more detail the physiology of the LH/CG receptor in relation to the model findings. The LH/CG receptor is a member of the G protein-coupled receptor family that differs by the presence of a large extracellular domain (Bardin et al., 1995; McFarland et al., 1989). It is composed of two subunits (Dufau et al., 1994; McFarland et al., 1989) that can each bind rhCG. However, although occupancy by an agonist is not necessary for dimer formation, occupancy of only one of the receptor subunits may favor dimerization, leading to the initiation of signal transduction by the hormone (Dufau et al., 1994). The receptor can bind to G_s and G_q proteins, stimulating the adenylate cyclase but also the turnover of phosphoinositides that leads to an increase in intracellular Ca^{2+} (Minegishi et al., 1993). The receptor gene expression is related to agonist exposure (Minegishi et al., 1993); at a low level of hCG, the receptor expression is increased, and at a high level, it is depressed. Only a small fraction of the approximately 20,000 cellular receptors (Knobil and Neill, 1988) need to be occupied to obtain the maximum rate of testosterone synthesis (Yen and Jaffe, 1991).

The time course of the LH/CG receptor activity after stimulation is complex and is a function of the duration of the stimulation (Segaloff and Ascoli, 1993). For a long stimula-

tion at a high concentration of agonist, this time course can be split into three parts. During the first minutes, the receptor exhibits agonist-induced changes in functional properties without diminution of the total number of receptors (also known as receptor uncoupling). In a second step (which last from a few minutes up to 4 h), a slow decrease in the number of receptors can be seen (-50% at 1 h, -80% at 4 h). This decrease is caused by the recycling cycle of the receptor. However, almost 50% of the internalized receptors are degraded at each cycle, leading to the decrease in the total number of receptors. After 4 h, a third process can be seen: the receptor gene transcription is decreased as stated by the diminution of 50% of the receptor mRNA (this adds to the 95% reduction in receptor due to recycling). For shorter stimulations or lower concentration of agonist, only some of these steps are involved.

It is well known that hCG may induce testicular steroidogenic desensitization (Saez and Forest, 1979). Desensitization is apparently due to 1) estradiol-induced inhibition of enzyme activity and 2) receptor down-regulation in the testis (Saal et al., 1991a). However, because estradiol increase is correlated with hCG dose/serum level (Padron et al., 1980), the present study design does not allow differentiation of the effects of the increase in estradiol or in hCG serum levels.

It has been reported that the ID_{50} of hCG for receptor down-regulation is about 10^{-10} M (Chuzel et al., 1994). Considering an approximate molecular mass of 38,000 Da for hCG and a specific activity of about 20,000 IU/mg, this corresponds to an ID_{50} of 78 IU/liter. This compares extremely well with the estimated peak around 45 IU/liter obtained in the estimate of f . There seems to be a disparity in testosterone response to hCG stimulus between eugonadotropic subjects and patients with (isolated) gonadotropin deficiency. The latter tend not to show the biphasic secretion observed in healthy subjects but only the delayed increase in serum testosterone concentration (Smals et al., 1980). This is in keeping with the concept that the late response represents restimulation of Leydig's cell by residual hCG as they emerge from the refractoriness induced by the initial stimulus (Saal et al., 1991a). Noteworthy, doses ranging from 750 to 1500 IU of hCG, divided into one to three weekly doses, were necessary to restore and maintain normal testosterone serum levels in hypogonadotropic hypogonadic male subjects (Saal et al., 1991b). This illustrates the large intersubject variability and highlights the deserved caution in extrapolating the present results and simulations from healthy (albeit down-regulated) subjects to hypogonadic patients.

The model we developed allows us to simulate arbitrary dosing schemes. The example we provide shows an informal way to obtain a maximum response while using the minimum amount of drug. The simulated testosterone levels show that to reach a target testosterone concentration of 25 nmol/liter, a dose of 1000 IU of rhCG every other 4 days would be sufficient. A higher 2500 or 5000 IU dose would produce a slightly higher response, but the highest dose will produce a lesser response according to the model. Clearly, the predicted pattern of decreased response at high doses and the pronounced rebound effect at treatment cessation is intriguing. The extrapolation to a clinical setting certainly deserves confirmation.

Acknowledgments

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Appendix

Splines. Briefly, a polynomial spline (DeBoor, 1978) is characterized by a sequence of distinct and nondecreasing real numbers called breakpoints. The polynomials making up a spline join at the breakpoints and satisfy certain continuity conditions. For example, for a linear spline the polynomials, simply join at the breakpoints. For a cubic spline, the polynomials join, and so do their first and second derivatives. To reduce the variance of the prediction at the boundaries (Hastie and Tibshirani, 1990), natural splines were used. For natural splines, the number of breakpoints equals the number of the parameters to be estimated. The breakpoints of the splines used in this report are put at the quantiles of the empirical distribution (see Bickel and Doksum, 1977, page 108 for definition) of the predictor variable (Stone, 1985), time or concentrations, respectively, for pharmacokinetics and pharmacodynamics modeling.

Pharmacokinetics of Repeated s.c. Administration.

To mimic the time course of a repeated dose using the semi-parametric approach for the observations from the SC×5 experiment, we opted for the following approach:

Let t_{last} be the latest observation time after the last dose. The disposition function (F) for this experiment is the following function:

$$F(t) = \begin{cases} f(t) & t \leq t_{last} \\ f(t_{last})e^{f'(t_{last})(t-t_{last})} & t > t_{last} \end{cases}$$

where $f(t)$ is a linear spline representing the concentration-versus-time course, $f'(t_{last})$ is the first derivative of f at t_{last} , and $f(t_{last})e^{f'(t_{last})(t-t_{last})}$ is an exponential extrapolation for $t > t_{last}$. The spline defining F had the breakpoints fixed at all the observed time points after the last dose. We used the superposition principle to compute the predicted concentrations for the SC×5 experiment.

References

Akaike H (1974) A new look at the statistical model identification problem. *IEEE Trans Automat Contr* **19**:716–723.
Bardin CW, Hardy MP, and Catterall JF (1995) Androgens, in *Reproductive Endo-*

crinology, Surgery and Technology, (Adashi EY, Rock JA and Rosenwaks Z eds), vol. 1, pp 510–511, Lippincott-Raven, Philadelphia.
Beal SL, Boeckmann AJ and Sheiner LB (1992) *NONMEM Users Guides*, NONMEM Project Group, UCSF, San Francisco.
Bickel PJ and Doksum KA (1977) *Mathematical Statistics*, Oakland, Holden-Day, Inc.
Chuzel F, Schteingart H, Avallet O, Vigier M and Saez JM (1994) Induction by LH/hCG of an increase in the rate of pig Leydig cell receptor mRNA degradation, in: *Function of Somatic Cells in the Testis*, Bartke A, ed., pp 286–292, Springer-Verlag, New York.
DeBoor C (1978) *A Practical Guide to Splines*, Springer-Verlag, New York.
Dufau ML, Morris CHT, Hu ZZ, Geng Y, Xie X, Zhang R and Buczko E (1994) The luteinizing hormone receptor, in: *Cell and Molecular Biology of the Testis*, Dufau ML, Fabbri A and Isidori A, eds, pp 51–60, Ares-Serono Symposia Publications, Rome.
Hastie TJ and Tibshirani RJ (1990) *Generalized Additive Models*, Chapman and Hall, Monographs on Statistics and Applied Probability 43, New York.
Knobil E and Neill JD (1988) Testicular steroid synthesis, *The Physiology of Reproduction*, p 990–994, Raven Press, New York.
McFarland KC, Sprengel R, Phillips HS, Kohler M, Roseblit N, Nikolics K, Segaloff DL and Seeburg PH (1989) Lutropin-choriogonadotropin receptor: An unusual member of the G-coupled receptor family. *Science* **245**:494–499.
Minegishi T, Nakamura K and Ibuki Y (1993) Structure and regulation of LH/CG receptor. *Endocr J* **40**:275–287.
Padron RS, Wischusen J, Hudson B, Burger HG and de Kretser DM (1980) Prolonged biphasic response of plasma testosterone to single intramuscular injections of human chorionic gonadotropin, *J Clin Endocrinol Metab* **50**:1100–1104.
Ratkowsky DA (1983) *Nonlinear Regression Modeling*, Marcel Dekker, New York.
Saal W, Glowania HJ, Hengst W and Happ J (1991) Pharmacodynamics and pharmacokinetics after subcutaneous and intramuscular injection of chorionic gonadotropin, *Fertil Steril* **56**:225–229.
Saal W, Happ J, Cordes U, Baum RP and Schmidt M (1991) Subcutaneous gonadotropin therapy in male patients with hypogonadotropic hypogonadism. *Fertil Steril* **56**:319–324.
Saez JM and Forest MG (1979) Kinetics of human chorionic gonadotropin-induced steroidogenic response of the human testis. I. Plasma testosterone: Implications for human chorionic gonadotropin stimulation test. *J Clin Endocrinol Metab* **49**:278–282.
Segaloff DL and Ascoli M (1993) The lutropin/choriogonadotropin receptor. . . 4 years later. *Endocr Rev* **14**:324–347.
Segre G (1968) Kinetics of interaction between drugs and biological systems. *II Farmaco* **23**:907–918.
Smals AGH, Pieters GFFM, Kloppengorg PWC, Lozekoot DC and Benraad TJ (1980) Lack of a biphasic steroid response to single human chorionic gonadotropin administration in patients with isolated gonadotropin deficiency. *J Clin Endocrinol Metab* **50**:879–881.
Stone CJ (1985) Additive regression and other nonparametric models. *Ann Stat* **13**:689–706.
Verotta D (1995) Semi-parametric direct and indirect action models for pharmacokinetics/pharmacodynamic data. Proceedings Society Computer Simulation Western Multiconference, Las Vegas.
Verotta D and Sheiner LB (1995) A general conceptual model for non-steady state pharmacokinetic/pharmacodynamic data. *J Pharmacokinetic Biopharmacol* **23**:1–4.
Yen SSC and Jaffe RB (1991) Endocrine regulation of the reproductive system: The hypothalamic-pituitary-testicular axis. *Reproductive Endocrinology: Physiology, Pathophysiology and Clinical Management*, pp 420–422. WB Saunders, Philadelphia.

Send reprint requests to: D. Verotta, Ph.D., School of Pharmacy, Department of Biopharmaceutical Sciences, Box 0446, University of California, San Francisco, CA 94143-0446. E-mail:davide@ariel.ucsf.edu.
