

**Amendment history:**

- [Correction](#) (August 1993)
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## **Influence of the high-affinity growth hormone (GH)-binding protein on plasma profiles of free and bound GH and on the apparent half-life of GH. Modeling analysis and clinical applications.**

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**Research Article**

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# Influence of the High-Affinity Growth Hormone (GH)–Binding Protein on Plasma Profiles of Free and Bound GH and on the Apparent Half-Life of GH

## Modeling Analysis and Clinical Applications

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### Abstract

The discovery of a specific high-affinity growth hormone (GH) binding protein (GH-BP) in plasma adds complexity to the dynamics of GH secretion and clearance. Intuitive predictions are that such a protein would damp sharp oscillations in GH concentrations otherwise caused by bursts of GH secretion into the blood volume, prolong the apparent half-life of circulating GH, and contribute a reservoir function. To test these implicit considerations, we formulated an explicit mathematical model of pulsatile GH secretion and clearance in the presence or absence of a specific high-affinity GH-BP. Simulation experiments revealed that the pulsatile mode of physiological GH secretion creates a highly dynamic (nonequilibrium) system, in which the half-life of free GH, its instantaneous secretion rate, and the GH-BP affinity and capacity all contribute to defining momentary levels of free, bound, and total GH; the percentage of GH bound to protein; and the percentage occupancy of GH-BP. In contrast, the amount of free GH at equilibrium is specified only by the GH distribution volume and secretion rate and the half-life of free hormone. We conclude that the *in vivo* dynamics of GH secretion, trapping, and clearance from the circulation offer a variety of regulatory loci at which the time structure of free, bound, and total GH delivery to target tissues can be controlled physiologically. (*J. Clin. Invest.* 1993. 91:629–641.) Key words: diabetes mellitus • growth hormone • Laron dwarfism • obesity

### Introduction

Recently a high-affinity, specific, low-capacity growth hormone-binding protein (GH-BP)<sup>1</sup> that circulates in blood has been characterized, purified, and cloned in humans and several animal species (1–7). This high-affinity GH-BP corresponds to the extracellular domain of the tissue growth hormone (GH) receptor (5–9), and contrasts with an incompletely character-

ized low-affinity binding protein that is unrelated to the GH receptor (10, 11). High-affinity GH-BP concentrations are very low or dysfunctional in Laron-type dwarfism (12, 13) and decreased in fetal plasma and in certain GH-resistant populations such as pygmies in Africa or the New Guinea highlands (10, 14–16). Studies in heterologous species (e.g., injection of human GH-BP complexed with human GH into the rat) indicate that the presence of a high-affinity binding protein (BP) can prolong the apparent half-life of GH in plasma (17–19). Moreover, an intuitive prediction is that plasma GH-BP would damp oscillations engendered by the burstlike secretion of GH into the plasma compartment, and contribute to the retention of GH in the circulation (17, 20, 21). Here, we have tested these intuitive inferences by formulating an explicit mathematical model to evaluate the predicted effects of the high-affinity GH-BP in plasma on the half-life of total, bound, and free GH, and the steady-state and nonequilibrium plasma concentrations of free, bound, and total GH as a function of the mode (pulsatile vs. continuous) and amount of GH secreted.

### Methods

*Computer simulations of GH dynamics in vivo.* As an initial approximation of the dynamics of GH secretion and removal in the absence and presence of a GH-BP, we assumed that a single high-affinity, low-capacity, and specific GH-BP contributes predominantly to GH binding in plasma, i.e., that any contribution of other low-affinity BPs is relatively small by comparison, as has been shown (10, 11, 22). In addition, we provisionally assumed that (a) the turnover of the GH-BP is slow compared to that of free GH itself; (b) the intact GH-BP complex is removed at a rate that is slow compared to the clearance of free GH (18); (c) GH and BP mass are conserved; (d) the binding stoichiometry of GH to its BP is equimolar<sup>2</sup>; (e) the native elimination function for removal of GH irreversibly from plasma is a single exponential, which acts only on free GH; (f) GH of 22,000-D molecular mass is measured and constitutes the principal circulating form of GH (23, 24); (g) both bound and free GH are measured as immunoreactive GH (25); (h) GH is secreted in distinct pulses of finite half-duration (duration at half-maximal amplitude), amplitude, and mass; (i) BP concentration/activity is constant over time (26, 27); and (j) the fate of a molecule of GH injected into the bloodstream by the anterior pituitary gland includes entry into the free (protein unbound) compartment, possible association with the BP, dissociation from the BP, and/or irreversible elimination of free hormone by metabolic and excretory mechanisms.

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1. Abbreviations used in this paper: GH-BP, growth hormone-binding protein; IGF-I, insulin-like growth factor I.

2. Recent *in vitro* studies indicate that a single molecule of GH can capture two molecules of recombinant GH-BP through two binding sites; see reference 53. It is not clear at present whether such a 2:1 complex occurs in plasma. Evidence to date has indicated a predominantly 1:1 stoichiometry for the native complex in plasma.

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Based on earlier *in vitro* measurements of the equilibrium association of human GH with its natural high-affinity plasma BP and values averaged from several laboratories (1, 2, 9, 12, 20), we used an apparent equilibrium  $K_d$  of 1.5 nM as a nominal value in most simulations.<sup>3</sup> In addition, we used a nominal  $V_0$  for GH of 7% body weight (28), a putative  $t_{1/2}$  of total GH of 18 min (29), and a mean GH-BP concentration of 1.0 nM (1, 2, 9). From earlier estimates, we assumed that the daily total secretion of GH in humans can span an approximate range of 0.11–1.1 mg/d (5–50 nmol/d), and that a single GH secretory burst has a mass either 2.2 or 22  $\mu$ g (0.1 or 1.0 pmol), unless indicated otherwise (30–33).

The association of GH with its BP has been previously reported (1); its rapidity precludes precise estimation of the association rate constant ( $k_{on}$ ) with available technology. Therefore, the much slower dissociation of the complex was measured. To estimate the unidirectional dissociation rate constant (off rate,  $k_{off}$ ) of GH from the high-affinity GH-BP, pooled adult human serum (9 ml) was preincubated with  $\sim$  9 ng of monomeric [<sup>125</sup>I]-human GH for 2 h at 37°C as described (1). An excess of unlabeled human GH (1 mg/liter) was then added in 0.1 ml of phosphate-buffered saline, pH 7.4, and incubation continued at 37°C. Periodically, aliquots were removed, immediately chilled in an ice-water bath, and free GH was separated from bound GH on Sephadex G-100 columns at 4°C. Peaks corresponding to the high affinity GH-BP complex and free GH were integrated (1), and bound GH expressed as a function of time after initiation of dissociation. A single rate constant of dissociation was estimated from these measurements as 0.037/min (67% confidence interval 0.030–0.043); see Fig. 1. (Attempts to determine the dissociation of the low affinity GH-BP complex [10] in whole plasma were unsuccessful owing to the small percentage of GH bound to that BP, which rendered precise kinetic estimations difficult.)

The following differential equations were formulated to describe the binding, dissociation, and removal of secreted GH from human plasma. Specifically, given a constant secretion rate, the rates of change of concentrations of free and bound GH over time ( $t$ ) are given by:

$$\frac{d[free]}{dt} = -(k_t + k_{on} \cdot ([BP] - [bound])) \cdot [free] + k_{off} \cdot [bound] + \frac{SR}{V_0} \quad (1)$$

$$\frac{d[bound]}{dt} = k_{on} \cdot ([BP] - [bound]) \cdot [free] - k_{off} \cdot [bound] \quad (2)$$

And, if we assume that a pulsed injection or secretory burst of GH enters the bloodstream by way of the free compartment, then

$$\frac{d[free]}{dt} = -(k_t + k_{on} \cdot ([BP] - [bound])) \cdot [free] + k_{off} \cdot [bound] + \frac{S(t)}{V_0} \quad (3)$$

$$\frac{d[free]}{dt} = -(k_t + k_{on} \cdot ([BP] - [bound])) \cdot [free] + k_{off} \cdot [bound] + \frac{1}{V_0} \sum_{i=1}^N \text{ampl}(i) \cdot \exp\left(-\frac{1}{2} \left[\frac{t-t(i)}{\sigma}\right]^2\right) \quad (4)$$

These constructs assume that in plasma GH is either bound or free, where  $[free]$ ,  $[bound]$ , and  $[BP]$  represent the concentrations (M) of

3. A relatively wide range of  $K_d$  values is reported for different *in vitro* incubation and separation conditions of human GH binding in plasma or various buffer systems: viz. from 0.25 nM (Michael J. Waters, Queensland, Australia) to 5.0 nM (reviewed in reference 24). We have the nominal value of 1.5 nM for most of the present work.

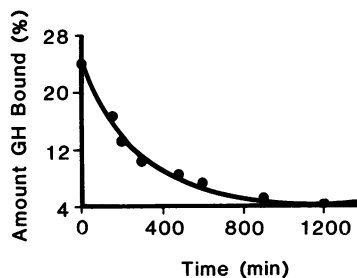


Figure 1. Time course of *in vitro* dissociation of GH from the human high-affinity GH-BP in plasma at 37°C. Observations were fit to a first-order (monoexponential) decay.

free GH, bound GH, and BP, respectively;  $S$  designates the secretory pulse function with a half-duration of sigma times 2.354, and amplitude of  $\text{ampl}(i)$ ;  $SR$  denotes the basal secretion rate of GH (mol/min), if any;  $V_0$  denotes the distribution volume (liters);  $k_t$  ( $\text{min}^{-1}$ ) is the rate constant for GH to BP ( $\text{M}^{-1} \text{min}$ );  $k_{off}$  is the dissociation rate constant (which is related to its  $t_{1/2}$  by  $\ln 2/k_t$ , min);  $k_{on}$  is the association rate constant for GH to BP ( $\text{M}^{-1} \text{min}$ );  $k_{off}$  is the dissociation rate constant ( $\text{min}^{-1}$ ); and  $k_d$  is the equilibrium dissociation constant, which equals  $k_{off}/k_{on}$  (M).

The above differential equations were solved using the Runge-Kutta formulas as implemented for first-order differential equations (34).

## Results

**Equilibrium conditions.** As a first approximation, we investigated GH kinetics under equilibrium conditions, although under physiological circumstances such conditions rarely apply. As shown in Table I, different mean daily GH secretion rates within and exceeding the approximately physiological range observed in the human across various age spans and body weights yield a correspondingly broad range of total, free, and bound GH concentrations in plasma at theoretical equilibrium. Varying the mean secretion rate of GH influences the apparent equilibrium plasma concentrations of free, bound, and total GH, and the percentage occupancy of the BP, but less markedly the percentage of GH bound to the BP. The latter decreases slightly (from 39% to 33%) when the mean GH secretion rate is increased 10-fold above the nominal normal value in humans.

Table I also shows the influence of altering the equilibrium dissociation constant. Using the approximate literature value for the equilibrium dissociation constant, namely a  $k_d$  of 1.5 nM,<sup>3</sup> and our measured  $k_{off}$  of 0.037/min, we observed that BP affinity for GH influences the total and bound concentrations of GH in plasma, the percentage of GH bound, and the percentage occupancy of the GH-BP, but not the concentration of free GH. Alternatively, altering the apparent capacity of the BP modifies the concentrations of total and bound GH and percentage of GH bound at any given moment, but does not influence the concentration of free GH or the percentage occupancy of the BP.

The  $t_{1/2}$  of free GH within the physiological range influences the concentrations of free and bound GH in plasma, the  $t_{1/2}$  of total GH, and the percentage of BP that is occupied, but minimally affects the percentage of GH bound; see Table I.

The above results can also be stated in relation to factors that control free, bound, and total GH concentrations, and the percentages of GH bound and of BP occupied in plasma; see Table I. Specifically, the apparent concentration of free GH at equilibrium is controlled positively by the secretion rate and

Table I. Estimated Molar Concentrations and Percentages of Free and Bound GH in Plasma at Equilibrium in the Presence of Varying Amounts and/or Affinities of GH-BP and Different Simulated Conditions

Simulated BP properties	GH secretion rate <i>pmol/min</i>	Predicted steady-state serum GH concentrations			Percentages	
		Total	Free	Bound	GH bound to protein	BP occupied
		<i>pM</i>				
Nominal*	10	34.2	20.6	13.6	39.8	1.3
Double the BP affinity	10	47.4	20.6	26.8	56.5	2.6
Double the BP concentration	10	47.4	20.6	26.8	39.8	1.3
Halve the BP concentration	10	27.4	20.6	6.8	39.8	1.3
Complete absence of BP	10	20.6	20.6	0	0	0
Nominal but secretion rate doubled	20	68	41.2	26.8	39.4	2.6
Nominal but secretion rate increased 10-fold	100	327	206	121	37.0	12.1
Nominal but GH half-life doubled	10	68.0	41.2	26.8	39.4	2.6
Nominal but GH half-life halved	10	17.1	10.3	6.8	39.4	0.65
Volume of distribution doubled	10	17.1	10.3	6.8	39.8	0.65

\* Assuming: a nominal free GH half-life of 7 min in the absence of BP, which corresponds to a total GH half-life of 18 min in the presence of BP; a GH distribution volume of 4.9 liters or 7.0% in a 70-kg man; a plasma GH-BP concentration of 1 nM with an equilibrium binding constant ( $K_d$ ) of 1.5 nM and an off-rate constant ( $k_{off}$ ) of 0.037  $\text{min}^{-1}$ ; a nominal daily GH secretion rate of 314  $\mu\text{g}$ , which corresponds to  $\sim 10$  pmol/min of GH secretion. Given a GH molecular mass of 22,000 D, then 45 pM is 1  $\mu\text{g/liter}$ .

$t_{1/2}$  of GH and negatively by the value of the  $V_0$ , but is not influenced by the binding properties of the GH-BP. The concentrations of bound and total GH are controlled by the GH secretion rate and  $t_{1/2}$ , and the binding affinity and capacity of the BP. The percentage of GH bound at equilibrium is specified by the BP affinity and capacity, but not the  $t_{1/2}$  or SR within the physiological range. Finally, the percentage of GH-BP that is occupied at steady state is stipulated by the GH secretion rate,  $t_{1/2}$ , and  $V_0$  and by the affinity of the BP but not its capacity.

The above computer-simulation data apply to steady-state conditions, which can be achieved by infusions of exogenous GH, but are rarely if ever attained spontaneously under physiological conditions in vivo (30–33, 35). A plausible pathological exception at least over short time spans (minutes) may be patients with GH-secreting pituitary tumors, since the latter appear to exhibit intervals of tonic or basal secretion upon which may be superimposed high frequency bursts of GH release (36). As shown in Fig. 2, decay from some given steady-state plasma concentration of free, bound, or total GH is influenced by the presence of a GH-binding element in plasma. The absolute value of the rate constant for an approximated monoexponential decay structure depends upon the amount and binding properties of the GH-BP, the  $t_{1/2}$  of free GH removal, and the starting concentrations of free and bound GH in plasma. For example, for three different starting concentrations of total GH in plasma, namely 11, 34, and 101 pM, generated respectively by mean daily secretion rates of GH of 0.11, 0.33, and 1.1 mg/24 h (4, 15, and 40 nmol/24 h), we find by monoexponential approximation the apparent  $t_{1/2}$  values shown in Table II. These values fall within the wide range of measured GH kinetics reported in the available literature (reviewed in references 29 and 33). Fig. 3 gives the monoexponential approximation for bound, total, and free plasma GH half-lives in the presence vs. absence of BP, assuming a range of free GH half-

lives in the absence of a BP. Note that a free GH  $t_{1/2}$  of  $\sim 7$  min in the absence of BP corresponds to a  $t_{1/2}$  of 18 min for total GH when the BP is present in physiological concentrations.

*Nonequilibrium conditions.* We next simulated the more complex nonequilibrium conditions that are more akin those occurring physiologically. The predicted dynamic effects of the BP on the plasma concentrations of free, bound, and total GH over time generated by a secreted pulse of GH are shown in Fig. 4. In response to a single pulse of GH, the plasma concentration of free GH increases rapidly, and decays rapidly initially because of removal by irreversible metabolic clearance as well as by association with BP in plasma. The concentration of bound GH increases almost as rapidly, but decays considerably more slowly because molecules of GH dissociate from the BP at a finite rate while some additional molecules of GH continue to associate with the BP. The plot of total GH concentrations over time is also considerably more extended than the profile of free hormone for the same reason. Of interest, the percentage of GH that is bound to plasma BP begins at  $\sim 39\%$  (assumed equilibrium approximation of previous secretion events), and declines rapidly as the free GH concentration increases during a pulse of GH delivery into the bloodstream. The percentage of GH bound to BP then increases over the succeeding minutes, and thereafter begins to fall as GH dissociates from the specific BP reservoir.

Fig. 5 (left panels) illustrates the dynamics of two different GH pulse masses, namely 2.2  $\mu\text{g}$  (0.1 nmol) and 22  $\mu\text{g}$  (1.0 nmol). Table III gives the times to maximal values for free, bound, and total plasma GH concentrations in relation to the three different amounts (nanomoles per burst) of GH injected into the bloodstream. As predicted intuitively, free plasma GH concentration peaks first, followed by the bound and total GH concentrations. We also simulated the effect of a nearly instantaneous burst of GH into the bloodstream. Here we assumed a half-duration (duration of the secretion event at half-maximal

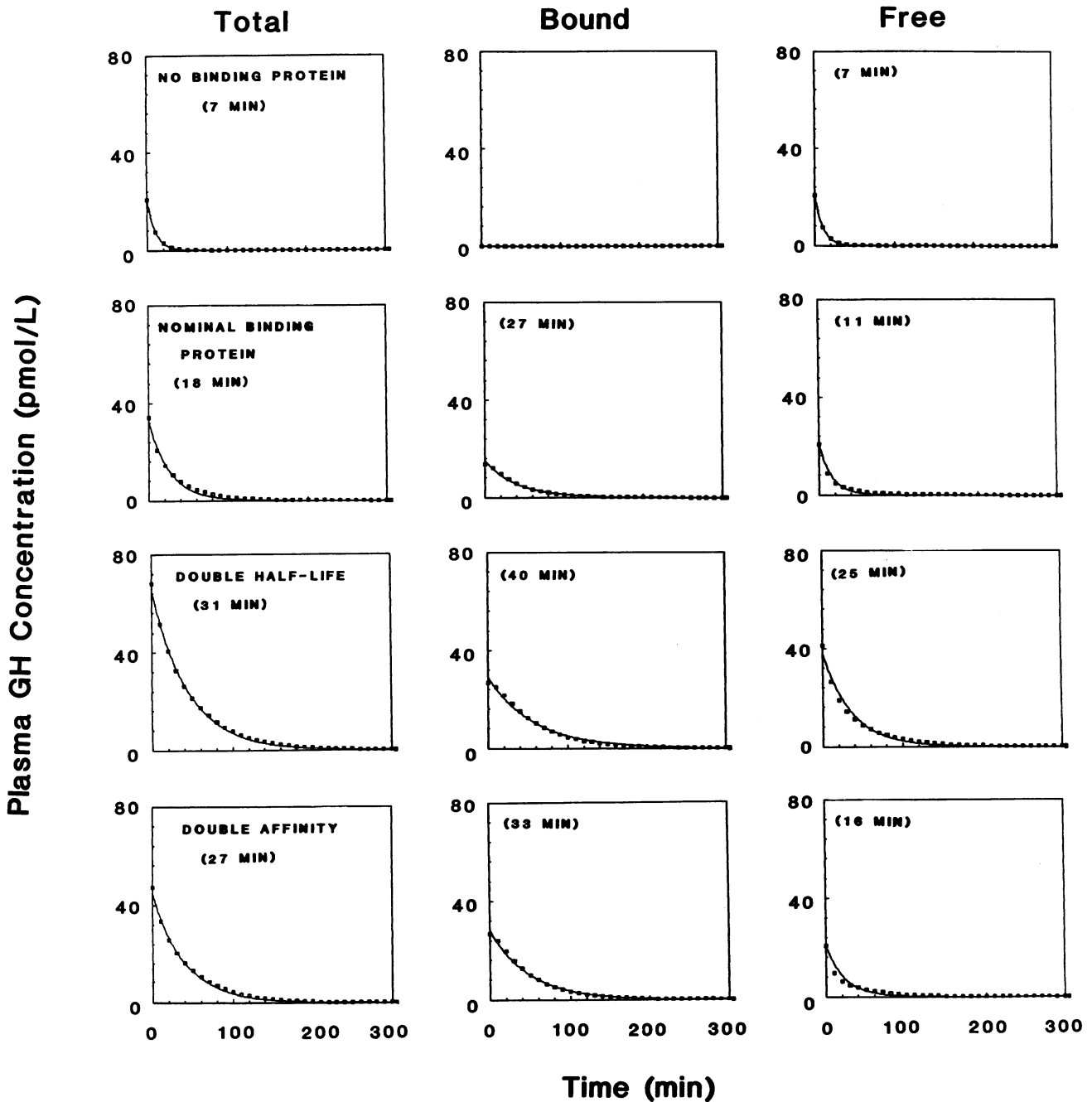


Figure 2. Predicted time courses of total, bound, and free plasma GH concentrations after decay from a steady-state GH infusion rate of 10 pmol/min (314  $\mu\text{g}/\text{d}$ ) assuming a  $V_o$  of 4.9 L and a GH molecular mass of 22,000 D. Four simulations are shown: (top) with no BP present and a free GH  $t_{1/2}$  of 7 min (which would correspond to a total GH  $t_{1/2}$  of 18 min in the presence of BP); (second row from top) in the presence of a BP with  $K_d = 1.5$  nM, capacity 1.0 nM, and  $k_{\text{off}} = 0.037/\text{min}$ ; (second row from bottom) a similar BP but with a  $t_{1/2}$  of free GH of 14 min (total GH  $t_{1/2}$  31 min); and (bottom) a similar BP with a  $K_d$  of 0.75 nM (double the affinity). The  $t_{1/2}$  values shown on the figure are from approximated monoexponential fits of the simulated decay data. These theoretical curves are subject to the assumptions of conservation of GH mass, irreversible metabolic removal of free hormone only, and a 1:1 stoichiometry of GH binding to its BP at a concentration of 1.0 nM. As a first approximation, the GH-BP complex was assumed to be formed ( $k_{\text{on}} = 2.47 \times 10^7 \text{ M}^{-1}\text{min}$ ), remain intact, or dissociate ( $k_{\text{off}} = 0.037/\text{min}$ ), but not undergo irreversible metabolic removal.

amplitude) of one minute. One-tenth of the daily production rate of GH was "injected" in this single pulse (22  $\mu\text{g}$  or 1 nmol, as above). Note that the plasma concentrations of free and bound GH under these conditions increase to considerably higher absolute values, and that the duration of the total

plasma GH concentration pulse is abbreviated. Comparison of a 1- and 30-min secretory burst is shown in Fig. 5 (right panels).

We next evaluated the effects of four different half-lives of free GH on the profiles of free, bound, and total GH concentra-

Table II. Approximated Monoexponential GH Half-Lives and Steady-State Plasma GH Concentrations after Continuous Secretion of GH

	Estimated half-lives*			Concentrations at steady-state		
	Total	Bound	Free	Total	Bound	Free
		<i>min</i>			<i>pM</i>	
Secretion rates <sup>‡</sup>						
314 $\mu\text{g}/\text{d}$ (10 pmol/min)	18	29	9	[34]	[13]	[21]
104 $\mu\text{g}/\text{d}$ (3.33 pmol/min)	18	29	9	[11]	[4.5]	[6.5]
942 $\mu\text{g}/\text{day}$ (30 pmol/min)	18	29	9	[101]	[39]	[62]
Half-lives <sup>†</sup>						
3.5 min	13	25	4.3	[17]	[7]	[10]
7.0 min	18	29	9	[34]	[14]	[21]
14 min	29	43	22	[68]	[27]	[41]
GH-BP						
Absent	7	None	7	[21]	[0]	[21]
Twice the capacity	25	35	16	[48]	[27]	[27]
Twice the nominal affinity <sup>‡</sup>	25	35	16	[48]	[27]	[21]

\* Values are fitted GH half-lives in the indicated compartments after abrupt cessation of equilibrated continuous secretion of GH at the indicated rate (or otherwise, 314  $\mu\text{g}/\text{d}$  = 10 pmol/min). <sup>‡</sup> Theoretical decay half-time of free hormone in the absence of BP was assumed to be 7 min or as indicated. Also assumed are a distribution volume of 4.9 liters, a GH-BP concentration of 1.0 nM and  $K_d$  of 1.5 nM. Note that once a BP is introduced, the half-life of free GH is controlled by both the removal rate of unbound GH and the binding properties of the BP.

tions in plasma. We used nominal half-lives of free GH of 4, 9, 18, and 36 (Fig. 6). The corresponding approximate half-lives of total GH are 16, 21, 37, and 63 min, values which cover the range reported in the literature for physiological and pathological conditions. One-tenth of the daily GH secretion rate was injected as a single pulse in these simulations (22  $\mu\text{g}$  or 1 nmol). Note that the times to maximum and the maximal values of the plasma concentrations of free, bound, and total GH are strongly controlled by the apparent half-life of free GH in plasma; see Table III.

The pathophysiological impact of decreased plasma GH-BP concentrations on pulsatile free, total, and bound GH concentrations over time is illustrated in Fig. 7 (*left panels*), which compares GH profiles over 24 h in a normal male (nominal GH-BP concentration 1.0 nM) and a patient with insulin-dependent diabetes mellitus (mean GH-BP concentration 0.725 nM). Nonlinear curve-fitting of the total plasma GH concentrations measured in samples collected at 10-min intervals for 24 h showed significant increases in GH secretion rates and free GH concentrations in diabetes mellitus, and a decrease in total

but not free GH half-life as predicted by the kinetic model. Conversely, the effects of increased serum GH-BP concentrations are shown for comparison in Fig. 7 (*right panels*), which depicts 24-h serum GH concentration profiles obtained by sampling blood at 10-min intervals for 24 h in an obese middle-aged man and a nonobese age-matched control. Assuming a blood GH-BP concentration of 1.7 nM in the obese (vs. 1.0 nM in the normal weight) subject, we used deconvolution analysis to determine the half-life of total and free GH, the time-course of plasma GH secretion, and the resultant plasma concentrations of free GH, bound GH, and total GH. The last-mentioned values were compared to measured concentrations of total GH; see Fig. 7 (*right panels*).

The dynamic changes in GH binding over 24 h in the above subjects are illustrated in Fig. 8. Note that in the healthy, obese, and diabetic men, the percentage of GH bound to high-affinity BP varies from 10% to 80% over the course of 24 h. Of particular interest, during intervals of secretory quiescence (i.e., between successive GH secretory bursts), the percentage of GH bound increases progressively, which reflects the reservoir-like and buffering capacity of GH-BP. Conversely, during individual GH secretory bursts, the free concentration of GH increases rapidly and the percentage of bound GH falls with a consequently increased availability of GH to metabolic removal. These dynamics apply to the normal, diabetic, and obese profiles.

## Discussion

To evaluate the dynamics of GH secretion into, removal from, and partitioning within plasma, we formulated a model that assumed either the attainment of equilibrium plasma GH concentrations (e.g., achieved by constant infusion of exogenous GH, or tonic GH secretion in patients with GH-secreting pitu-

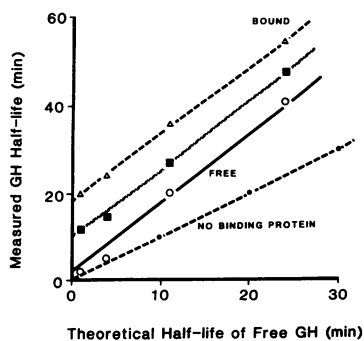


Figure 3. Monoexponential approximation of GH half-lives for bound, total, and free GH given the nominal GH-BP model summarized in Fig. 2, and assuming various theoretical half-lives of free GH in the absence of a BP. The line with the filled squares corresponds to total GH.

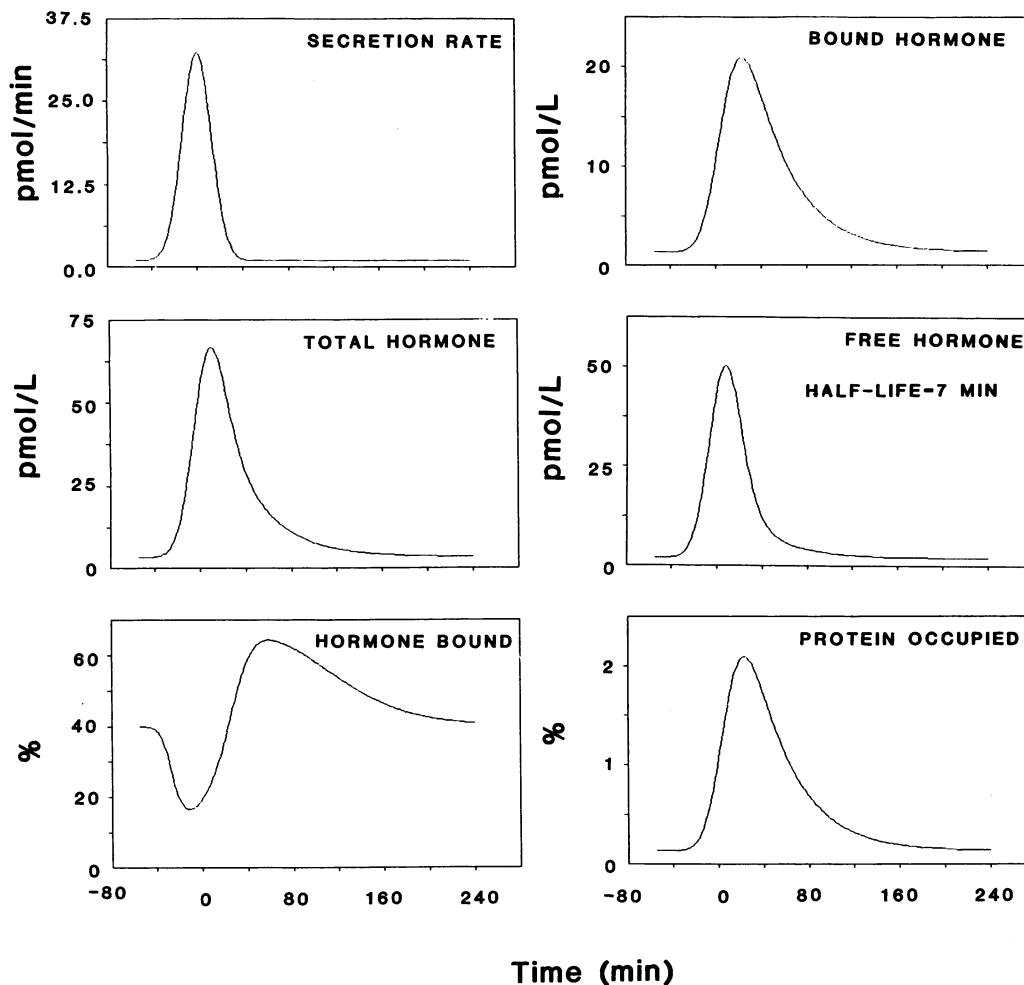


Figure 4. Time courses of calculated GH secretory rate, total, bound and free plasma GH concentrations, and percentages of GH bound and protein occupied assuming: (a) a high-affinity plasma GH-BP ( $k_d = 1.5$  nM, capacity = 1.0 nM); (b) a distribution volume of 4.9 liters; (c) a total GH  $t_{1/2}$  of 18 min; (d) a molecular mass of GH of 22,000 D; (e) a  $k_{off}$  of 0.037/min; and (f) a symmetric (Gaussian) GH secretory burst with a half-duration (duration at half-maximal amplitude) of 30 min centered about zero and a mass of 1 nmol (22  $\mu$ g); see Methods. For ease of visualization, the curves have been elevated to reflect a low rate of constant basal secretion (1 pmol/min).

itary tumors), or a physiologically pulsatile mode of GH release, characterized by abrupt bursts of GH release into the bloodstream. To this end, we measured the unidirectional rate constant for GH dissociation from its BP in vitro ( $\sim 0.037$ /min), and used available earlier estimates of the equilibrium dissociation constant of GH from its BP,<sup>3</sup> the total binding capacity of the GH-BP in human plasma, and an assumed 1:1 stoichiometry for GH binding.<sup>2</sup> For quantitative purposes, we carried out computer simulations to define the equilibrium as well as the nonequilibrium characteristics of GH binding and disposal over time in the presence or absence of a specific high-affinity GH-BP, given various assumed half-lives for the removal of free GH from plasma, plausible GH secretion rates and secretory pulse waveforms, different BP affinities and capacities, and different apparent  $V_0$ 's for GH.

Our analyses demonstrate that certain intuitive predictions of the effects of GH-BP on the dynamics of GH secretion, distribution, and clearance can be corroborated quantitatively. Indeed, we identified specific predictors of plasma concentrations of free, bound, and total GH, the percentage of GH bound to plasma BP at any given instant, and the percentage of BP occupied by GH. The steady-state predictions given here correspond simply to those of a two-compartment kinetic model, and are to be anticipated only during continuous (exogenous) GH infusions, or in selected pathophysiology (e.g.,

some patients with acromegaly who have high rates of basal GH secretion with no or few superimposed pulses). In addition, we posited a model combining pulsatile (i.e., nonequilibrium) GH secretion and stable amounts of the GH-BP in plasma in order to mimic physiological conditions of burstlike pituitary GH secretion. This construct of pulsatile ligand secretion and association with and dissociation from a high-affinity BP in plasma is schematized in Fig. 9, and offers novel predictions that are relevant to any protein, metabolite, or substrate injected into the bloodstream with a defined time course, when that ligand can bind to one or more specific (high or low affinity) BPs in the plasma compartment. Moreover, our dynamic model can be expanded to conditions in which either the unoccupied BP or ligand-BP complexes are themselves removed from the circulation, and/or to situations where higher-order exponential kinetics exist for the removal of free, bound, or total ligand from plasma.

Under the simplifying assumptions of full equilibration, conservation of mass, and clearance of free hormone only, we found that the  $V_0$ , mean GH secretory rate, and the  $t_{1/2}$  of free hormone all substantially affect the steady-state concentrations of free and bound (and total) GH, as well as the percentage of BP occupied. These parameters influence the percentage of GH bound considerably less. In addition, whereas the BP affinity influences the amount (concentration and percentage) of

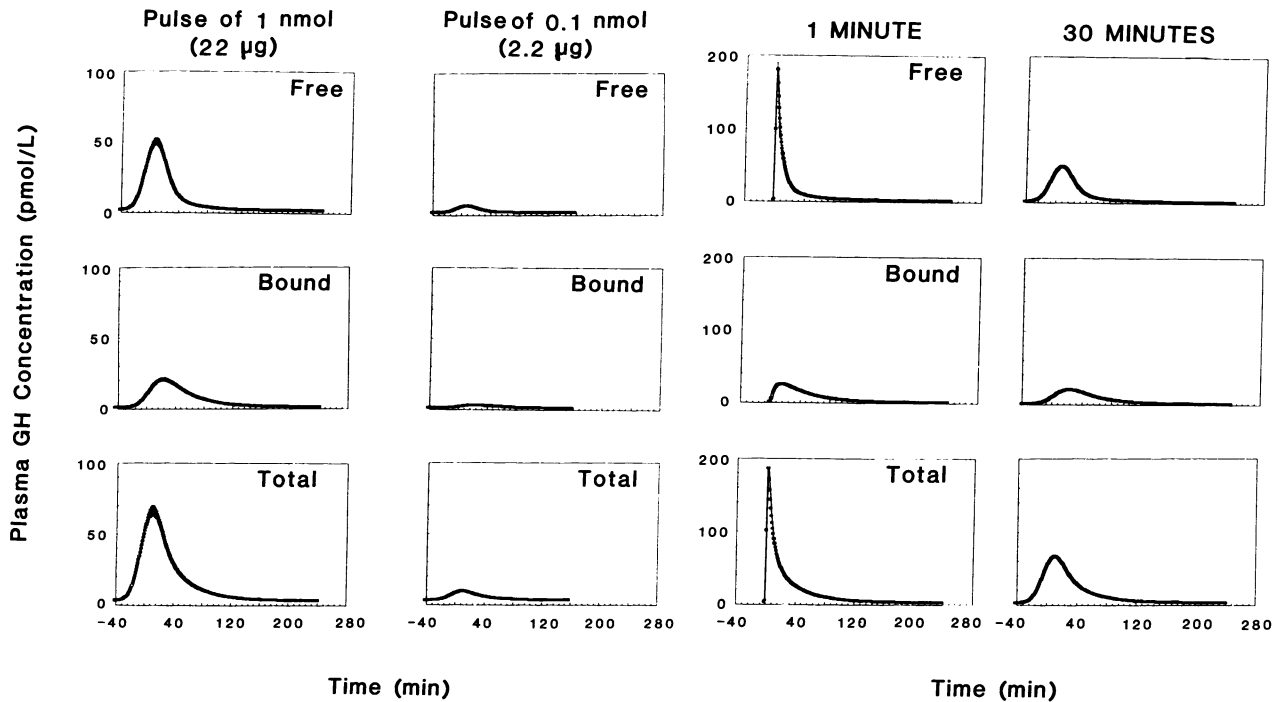


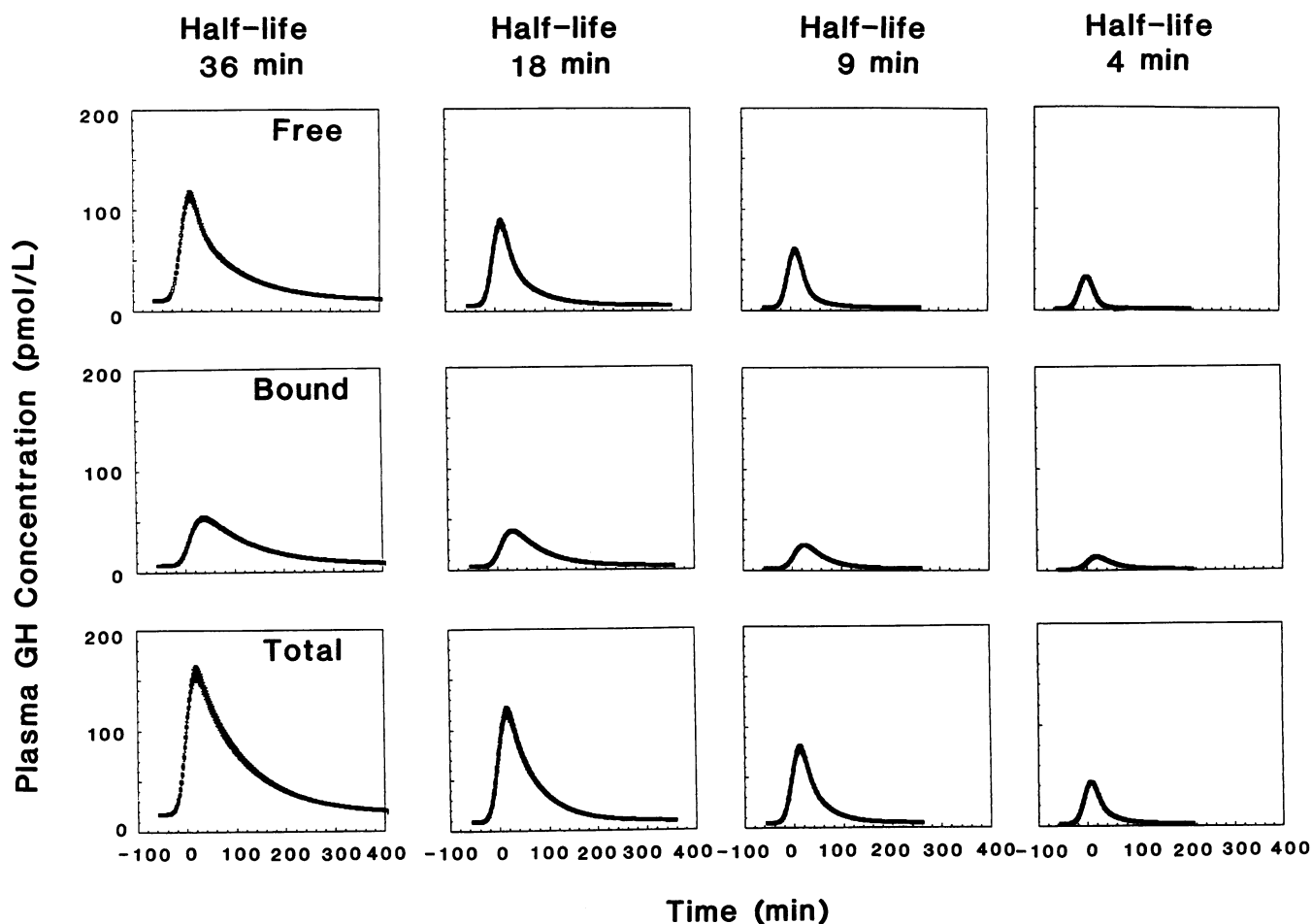
Figure 5. (Left panels) Computer simulation of a hypothetical GH secretory burst containing specified amounts of GH within the presumptive physiological range. A single burst of GH secretion was simulated and centered about time zero (abscissa). The total mass of GH delivered in the burst was either 1 nmol (22 µg, left column), or 0.1 nmol (2.2 µg, right column). The GH secretory burst was simulated algebraically by a Gaussian distribution of GH secretion rates, in which the half-duration (time in minutes elapsing in half-maximal amplitude) was 30 min. The vertical axis gives the calculated plasma GH concentration in pmol/L (pM). In each column three curves are shown to denote the expected time courses of free, bound, and total GH assuming the nominal parameters given in Fig. 4. (Right panels) Time course of predicted plasma GH concentration profiles after a presumptively physiologic secretory burst (right column) compared to a nearly instantaneously secretory event (left column), which were assigned respective half-durations (duration in minutes of the secretion episode at half-maximal amplitude) of 30 min or 1 min. Results are presented otherwise as described for left panels.

Table III. Peak GH Secretory Rates, Times to Maximal Values, and Maximal Plasma GH Concentrations after a Single Intravenous Pulse of GH

	Peak secretory rate <i>pmol/min</i>	Times to maximal values			Maximal plasma GH concentrations		
		Free	Bound	Total	Free	Bound	Total
		<i>min</i>			<i>pM</i>		
Mass of GH pulse, µg (nmol)							
22 (1.0)	32	7	23	10	[50]	[21]	[67]
2.2 (0.1)	3.2	7	23	10	[5]	[2]	[7]
0.22 (0.01)	0.32	7	23	10	[0.5]	[0.2]	[0.7]
Free GH half-life (total GH half-life)							
4 (16)	32	4	19	7	[33]	[13]	[43]
7 (18)	32	7	23	10	[50]	[21]	[67]
9 (21)	32	9	25	11	[59]	[25]	[79]
18 (37)	32	12	31	15	[86]	[39]	[117]
36 (63)	32	16	38	20	[114]	[54]	[158]
GH secretory burst half-duration (min)							
1	880	1	16	1	[180]	[26]	[190]
30	32	7	23	10	[50]	[21]	[67]
50	18	9	27	13	[34]	[17]	[49]

Values are peak secretory rates and times (min) to maximal values of free, bound, or total plasma GH concentrations assuming a single Gaussian secretory burst centered at time zero with a half-duration (duration at half-maximal amplitude) of 30 min, a mass of 22 µg (1 nmol), a distribution volume of 4.9 liters, a half-life of total GH of 18 min (corresponding to a free GH half-life in the absence of BP of 7 min), and a BP concentration of 1.0 nM and equilibrium  $K_d$  of 1.5 nM, except where indicated otherwise. Note that the time-to-peak values differ for free, bound, and total GH, and hence the maximal values given in the last three columns do not simply sum.



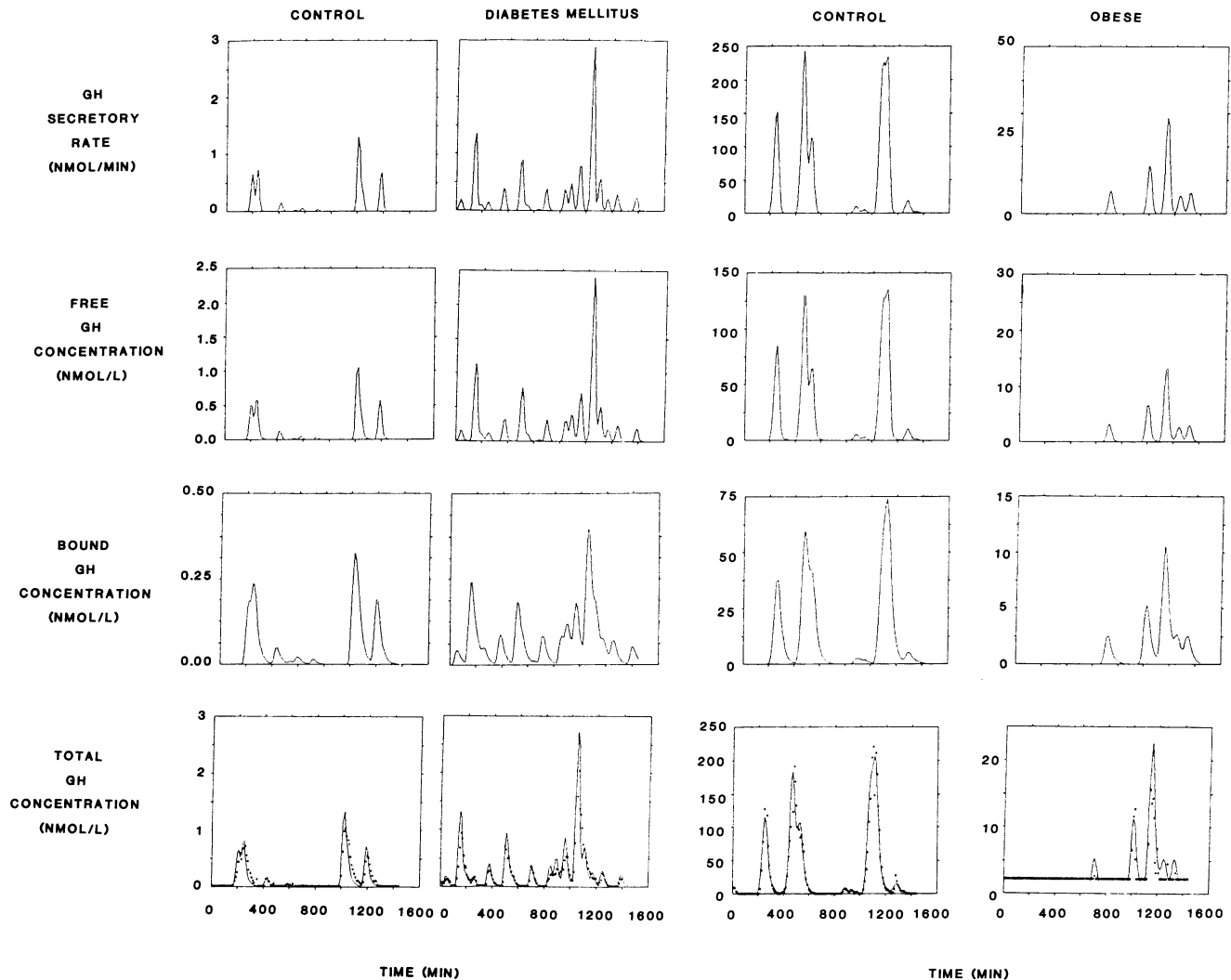


**Figure 6.** Influence of different free GH half-lives on the profiles of plasma free, bound, and total GH concentrations following a hypothetical GH secretory burst. The hypothetical GH secretory burst with a mass of 1 nmol (22  $\mu$ g) is described in Fig. 5 (left panels). The four columns of panels depict the anticipated plasma concentrations of free, bound, and total GH assuming monoexponential decay rates of free GH in the absence of BP corresponding to half-lives of 36, 18, 9, or 4 min which correspond to total GH half-lives of 63, 37, 21, and 16 min, respectively. The last two values bracket the physiological range for published half-lives for total GH in humans.

hormone bound and also the percentage of BP occupied by hormone, the free concentration of ligand at equilibrium is not controlled by the BP affinity. In contrast, the capacity (i.e., concentration) of the BP affects the concentration and percentage of hormone bound, but does not modify the steady-state free hormone concentration or the percentage of BP occupied by its ligand. These general inferences apply qualitatively to other ligand-circulating receptor interactions, under similar simplifying assumptions.

If the concentration of free hormone is primarily responsible for hormone action in target tissues, then factors that control the free hormone concentration in plasma are of particular interest. We observed that only the secretion rate,  $t_{1/2}$ , and  $V_0$  of the hormone contribute substantially to the equilibrium concentration of free ligand in plasma. BP affinity or capacity do not markedly influence the free hormone concentration at steady state within physiological ranges of hormone secretion rates or half-lives. Indeed, complete absence of the BP does not modify the free hormone concentration at steady-state for any given secretion rate. This observation has interesting implications in the interpretation of hormone concentrations in condi-

tions of inborn deficiency of a BP. For example, in Laron dwarfism, there is virtual absence of the GH-BP in plasma, while serum GH concentrations are very high (12, 13, 37, 38). This increase in mean serum GH concentrations indicates mathematically that either the secretion rate and/or the  $t_{1/2}$  of GH are increased (or the  $V_0$  for GH is decreased) in Laron dwarfs. Available data suggest that increased secretion rather than a prolonged  $t_{1/2}$  of GH occurs in this clinical model of GH receptor/BP deficiency (39), in that the predicted (see Fig. 3) and measured half-lives of GH are actually decreased in the absence of BP (17–19, and Z. Laron, M. L. Johnson, and J. D. Veldhuis, unpublished data). Such an increase in GH secretion rate presumably results from the failure of GH-dependent negative feedback mechanisms (such as generation of insulin-like growth factor I, or IGF-I) to suppress GH secretion (40). Of note, Laron-type dwarfism is not simply a GH-BP deficiency state. The fact that GH receptors are also absent (38, 41) is likely to influence free and total GH  $t_{1/2}$  independently of the BP, as at least part of the clearance of GH proceeds through receptor-mediated internalization into cells and lysosomal degradation. Thus, the combined absence of plasma GH-BP



**Figure 7.** Dynamic features of GH secretion into free and bound compartments and the resultant effects on total serum GH concentrations (*left panels*) in a healthy young male (control) and a man with type 1 diabetes mellitus with reduced plasma GH-binding protein concentrations, or (*right panels*) a middle-aged male control and obese counterpart. Each subject underwent blood sampling at 10-min intervals for 24 h, and the resultant sera were subjected to immunoradiometric assay to estimate total serum GH concentrations (*lowermost panels*). The GH secretory rate over time was then determined by multiparameter deconvolution analysis (33) (*uppermost panels*). The free and bound serum GH concentrations were defined by conservation of mass, estimated GH-binding protein capacities of respectively 1.0, 0.725, and 1.7 nM in the control, diabetic, and obese individuals, a GH distribution volume of 4.9 liters, and an equilibrium  $K_d$  of 1.5 mM. Nonlinear curve-fitting of the observed total serum GH concentrations was carried out to estimate the half-life of free GH, which was  $\sim 3.25$  min in the young normal and diabetic men compared to 2.0 and 1.75 min in the middle-aged normal and obese men. (These individual values fall within the broad normal range of 2–12 min predicted in Fig. 3.) The total GH half-life was 24 min in the normal young subject and 16 minutes in the diabetic man, which compares with 14 and 10 min in the nonobese and obese middle-aged volunteers. Note that the numbers of detectable GH secretory episodes were 9 and 17, respectively (control vs. diabetes mellitus), with corresponding GH interburst intervals of 123 and 84 min, and daily GH secretion rates of 0.573 vs. 1.42 mg. Similar deconvolution estimates of episodic GH secretion were obtained in six other control and diabetic individuals. In the obese and normal weight middle-aged men, there were, respectively, 5 and 9 detectable GH secretory bursts per 24 h (interburst intervals of 156 and 137 min), and daily GH secretion rates of 0.013 and 0.22 mg. The continuous curves drawn through the total serum GH concentrations are predicted values, which agree well with the observed measurements (also shown).

(which normally prolongs  $t_{1/2}$ ) and tissue GH receptors (which normally shorten  $t_{1/2}$ ) may have unpredictable net effects on GH clearance in that condition (42–45).

The GH-BP complex is known to exert complex biological effects on target tissues. For example, addition of unoccupied BP *in vitro* impairs GH action (44, 45), yet *in vivo* the BP can enhance GH action in a dose-dependent fashion (46, 47). This

seeming paradox is most likely explained by the opposing effects of competition for ligand on the one hand (42–44) and prolongation of  $t_{1/2}$  on the other (17–19). Hence, subject to the assumption that unoccupied BP has no intrinsic effects itself (44), regulation of the amount of unoccupied BP in plasma or in tissues can provide a plausible mechanism for modulating the plasma retention time and local tissue actions of GH. Our

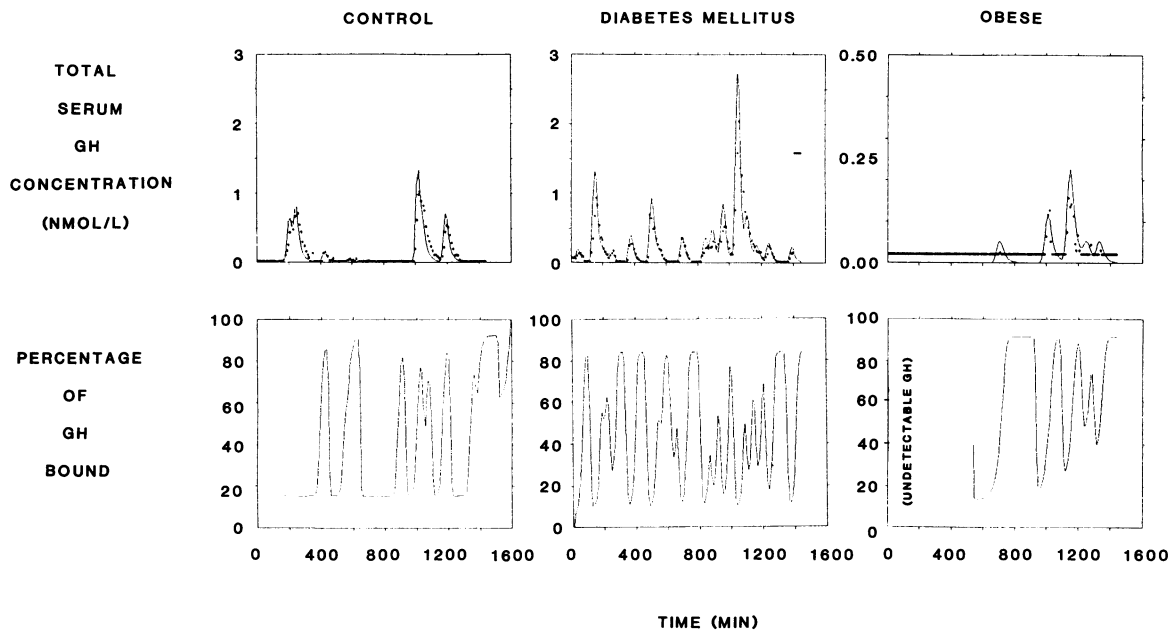


Figure 8. Total serum GH concentrations and percentage GH bound to GH-BP in a healthy (control), an insulin-dependent diabetic patient, and an obese man, all of whom underwent blood sampling as described in the legend to Fig. 7. (*Upper panels*) The predicted curves for measured total serum GH concentrations are reproduced from Fig. 7. (*Lower panels*) The percentage of GH bound to its high-affinity plasma BP is depicted. Note that the percentage of GH bound to the transport protein is not in steady state but varies from ~ 10% to 80% over 24 hs. Occasional high “plateau” regions of the plot of percentage GH bound over time denote indeterminate estimates, in that serum GH concentrations at these times fell below the limit of detection of the particular GH IRMA (0.5 ng/ml, = 0.0225 nM). This also occurred at the outset of sampling in the obese subject.

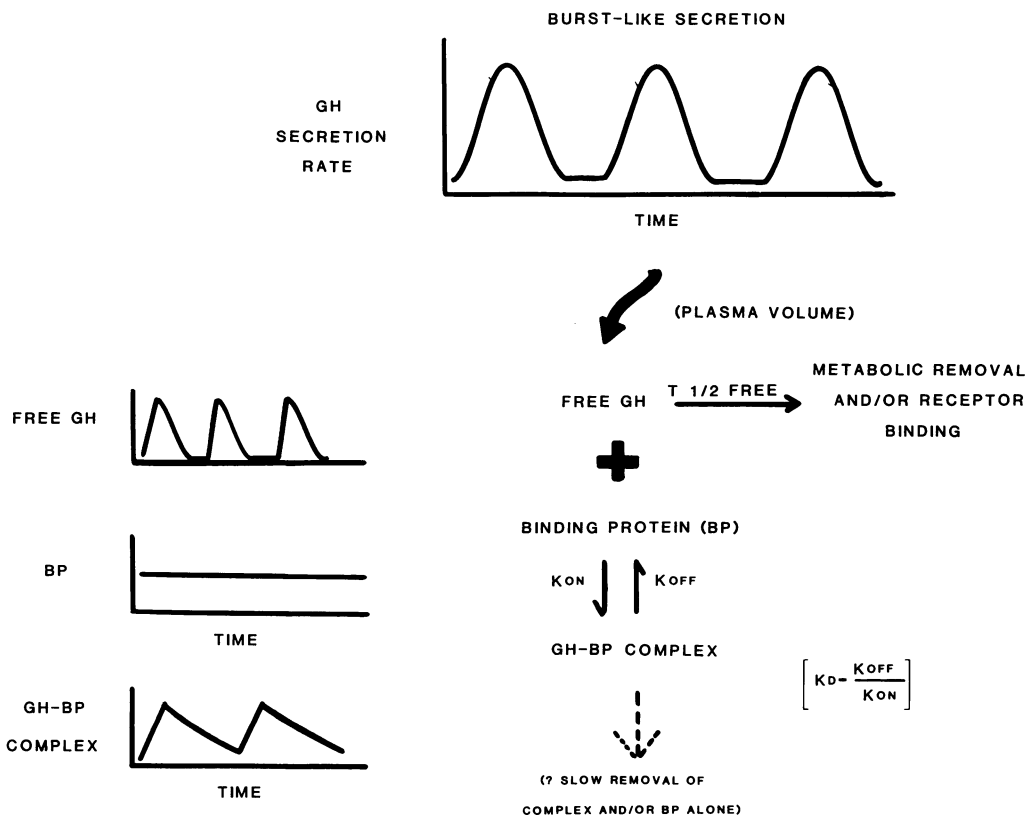


Figure 9. Schema illustrating the nonequilibrium kinetic behavior of the GH axis, assuming the presence of a high-affinity GH-BP in plasma, metabolic clearance of GH from the free compartment, and an episodic burstlike mode of GH secretion.

computer simulations indicate that *ceteris paribus* the amount of unoccupied BP is controlled by the  $V_0$  and the secretion rate of GH (at high secretion rates, there is a reduced amount of unoccupied BP), as well as the free GH half-life (if  $t_{1/2}$  is prolonged, fewer binding sites on BP will remain unoccupied). Similarly, the affinity of the BP regulates the availability of unoccupied binding sites.

Clinical correlations indicate that the GH secretion rate is inversely related to the level of GH-BP in plasma in healthy boys (46). Thus, children with low BP levels tend to have high GH secretion rates, and vice versa. Our computer simulations predict that this physiological relationship in a group of subjects would tend to damp pulsatile free GH availability to target tissues. A buffering effect of constant amounts of high-affinity GH-BP also occurs in individuals over minutes and hours, as shown in Fig. 8 in a normal, diabetic, and obese subjects. In all three conditions, the percentage of GH bound to high-affinity GH-BP varies remarkably over 24 h; viz., from 10% to 80% during the course of minute-to-minute pulsatile GH release (Fig. 8). Of particular interest, the relative retention of GH in plasma by GH's complexing with BP prevents plasma GH concentrations from falling to zero between successive GH secretory bursts. Indeed, the dissociation rate constant of 0.037/min equates to a half-life of GH dissociation of 18.7 min, which is considerably longer than our estimated free GH half-life of  $\sim 7$  (2–12) min. Thus, the percentage of GH bound to BP will tend to rise following a secretory burst, as free GH is removed more rapidly than bound. Dissociation of GH from its BP at these times of low or absent GH secretion maintains some free GH in plasma bathing the target tissues, which in circumstances of decreased GH secretion (obesity) may be physiologically relevant. However, whether such low but non-zero concentrations of free GH or GH-BP complex are important in maintaining tissues responsiveness to GH is not known.

Assuming that free hormone is the major (but not necessarily exclusive) moiety subject to irreversible metabolic removal, then differences in the  $t_{1/2}$  of free hormone among different individuals will influence the absolute amounts of free and bound GH in plasma, and the percentage occupancy of the GH-BP. Up to fourfold variability has been recognized in the  $t_{1/2}$  of exogenously infused GH, and more recently of endogenously secreted GH in healthy individuals (reviewed in references 29 and 33). Based on Fig. 3, we therefore can predict free GH half-lives of 2–12 min in normal adults. Thus, whether free and/or bound GH acts on target tissues, this variability in  $t_{1/2}$  has the potential to modify tissue actions of GH significantly.

In obese men, the calculated  $t_{1/2}$  of endogenous GH appears to be somewhat reduced (31). Such calculations assume that the  $V_0$  for GH is not significantly increased in obesity. Similarly, in obese orchidectomized Rhesus monkeys, the metabolic clearance rate of human GH infused at a constant rate is significantly higher (47). A decrease in GH  $t_{1/2}$  (or increase in metabolic clearance rate) would decrease the amount of free and bound GH bathing target tissues, and thereby contribute to tissue GH deficiency in obesity. Decreased GH  $t_{1/2}$  is not due to evident alterations in BP affinity or capacity, since the estimated serum GH binding capacity is either high-normal or moderately increased in obese men (31, 48). Thus, alternative mechanisms for the decrease in apparent GH  $t_{1/2}$  in obese individuals should be considered, and might include increased re-

moval of GH by receptor and/or nonreceptor-mediated mechanisms and/or an increased  $V_0$  for GH.

Our measurements of GH-BP concentrations in type I (insulin-dependent) diabetes mellitus reveal a significantly reduced GH-BP capacity (49). In conjunction with increased GH secretion in type I diabetic patients, the decrease in GH-BP capacity amplifies the already high free GH concentration in plasma and would tend to shorten the half-life of total GH (see Fig. 7, *left panels*). These observations are consistent with the free GH hypothesis, i.e., metabolic removal of GH proceeds largely or predominantly via free GH rather than by way of complexes of GH-BP.

Our computer simulations also provide a possible basis for interpreting some apparent differences in the reported GH  $t_{1/2}$  in the literature. For example, estimating GH  $t_{1/2}$  before steady state is attained or in response to infusions of pharmacological amounts of GH sufficient to exceed BP capacity would produce a preferential increase in free hormone and yield initial phase kinetic estimates reflecting predominantly the short  $t_{1/2}$  of free hormone. However, measurements of the GH  $t_{1/2}$  within the physiological GH concentration range of 50 ng/liter to 50  $\mu\text{g/L}$  ( $\sim 2.3$  pM to 2.3 nM) would reflect free GH  $t_{1/2}$  only if the laboratory technique did not measure bound hormone—an unlikely possibility in the case of immunoassays (25). Indeed standard immunochemical assays typically measure total GH in plasma (25). Thus, the half-lives derived from such measurements reflect the damping effect of the GH-BPs, the concentration of which varies among individuals (11, 14). The variability in BP levels may in part explain individual differences in GH clearance. Of course, other factors can introduce variability in estimates of GH  $t_{1/2}$ , including measurement technique, assay specificity, buffer and matrix, choice of GH standards, sampling frequency, and the extent to which endogenous GH secretion is suppressed when the  $t_{1/2}$  of exogenous GH is computed.

The dynamic model of GH secretion and GH partitioning between BP and metabolic clearance presented here can be used to predict the time course of plasma GH-BP complex and free GH. Although the time course of total, free, and bound GH in blood can be predicted, the exact temporal profiles of free and bound GH to which tissue GH receptors are exposed are not known, since the relative extent to which free GH and GH-BP complex permeate various target tissues has not been defined. An additional unanswered question is: "What time-configuration of free GH (or GH-BP complex) in blood most effectively initiates or modifies tissue responses to GH?" For example, under some conditions, a pulsatile rather than a continuous mode of GH delivery preferentially enhances tissue responses, such as linear growth and weight gain in the rat and the induction of epiphyseal cartilage and muscle IGF-I mRNA (50). In contrast, in acromegaly, the nadir (or mean or integrated) serum GH concentration is the strongest single correlate of serum IGF-I concentrations (36). Moreover, the induction of a "feminized" pattern of certain hepatic enzymes (e.g., carbonic anhydrase in the rat) is best achieved by continuous GH delivery (51). Near-continuous GH replacement may also be optimal for generating IGF-I in GH-deficient humans (52).

In conclusion, assuming that plasma GH concentration profiles are transferred to tissue receptor sites, the dynamic interplay among pulsatile GH secretion, intravascular GH partitioning, distribution, high-affinity binding, and irreversible

metabolic clearance offers significant regulatory control over tissue GH actions within the somatotrophic axis in health and disease.

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