

## Random Secretion of Growth Hormone in Humans

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In normal humans, growth hormone (GH) is secreted from a gland located adjacent to the brain (pituitary) into the blood in distinct pulses, but in patients bearing a tumor within the pituitary (acromegaly) GH is excessively secreted in an irregular manner. It has been hypothesized that GH secretion in the diseased state becomes random. This hypothesis is supported by demonstrating that GH secretion in patients with acromegaly can not be distinguished from a variety of linear stochastic processes based on the predictability of the fluctuations of GH concentration in the bloodstream. [S0031-9007(96)00996-9]

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Pulsatile hormone secretion has been demonstrated as an ubiquitous phenomenon in a large number of different hormonal systems with a pulse frequency ranging from approximately 6 to 140 pulses in 24 h [1]. The temporal pattern of pulsatile secretion has been demonstrated to effect the regulation of target cell functions and structure [2–4].

In healthy adult subjects the 24-h time series of growth hormone (GH) concentrations in the blood can be described by stable low GH concentration values which are interrupted by 6 to 12 pulsed releases of GH into the blood [5,6] [Fig. 1(a)]. This temporal pattern of secretion seems to be related to the optimal induction of physiological effects at target organs [7]. In contrast to normal subjects, patients bearing a tumor within the pituitary (a gland located adjacent to the brain) with excessive GH secretion (acromegaly) demonstrate an irregular pattern of GH concentration fluctuations in the bloodstream [Fig. 1(b)] throughout 24 h [8,9]. This pattern of irregular fluctuations of high levels of GH concentration is responsible for many effects such as stimulation of connective tissue growth, cardiovascular and cerebrovascular disease, diabetes mellitus, and arthritis [10].

It has been hypothesized [8] that this irregular pattern of GH concentration fluctuations is caused by random, pulsatile GH secretion from the pituitary gland due to decreased control by the normal orderly secretion of growth hormone-releasing hormone (GHRH) and growth hormone-inhibiting hormone (somatostatin) from the hypothalamus (a structure of the central nervous system, Fig. 2). 24-h time series of GH concentrations in the blood were measured in ten healthy male subjects, and in six patients (three males and three females) with excessive growth hormone secretion (acromegaly) to classify the temporal pattern of fluctuations of GH concentrations. We found significantly higher mean GH concentration levels in the blood in acromegalic patients

than in healthy controls, indicating an excessive GH secretion from the tumor within the pituitary [8,9].

To separate random from deterministic behavior, the technique of time series prediction has been effectively used in a variety of biological time series containing only a very limited number of data points [11–14]. In these studies, the degree to which a time series can be predicted is itself a measure of its deterministic structure. In the recent Santa Fe Institute competition on time series prediction [15], the best predictions for a number of time series from different physical phenomena were achieved by neural network approaches. These approaches have the advantage of broad linear and nonlinear approximation without making specific assumptions of the system under study. In the present study we used feedforward neural networks to predict future values of the time series of GH concentrations as a test for determinism (Fig. 1). The feedforward form of a predictive model was chosen since it is relatively easy to control overfitting by the use of regularization functions and cross validation [16,17].

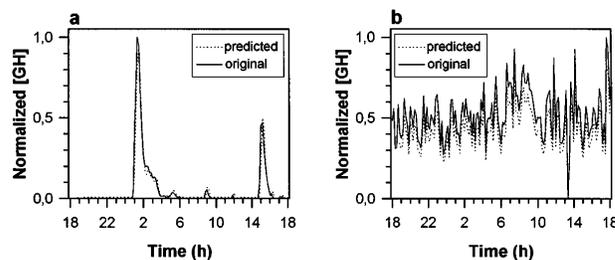


FIG. 1. Original and 10-min ahead-predicted 24-h time series of GH concentrations. (a) Healthy subject. (b) Patient with excessive GH secretion (acromegaly). Blood samples were taken at 10-min intervals. Acromegalic patients were without any medication for four weeks and not cured by previous surgical and/or radiation therapies. The protocol of this study and the hormone measurements have been described in more detail elsewhere [9,20] and some of the data of these studies have been included in the present data set.

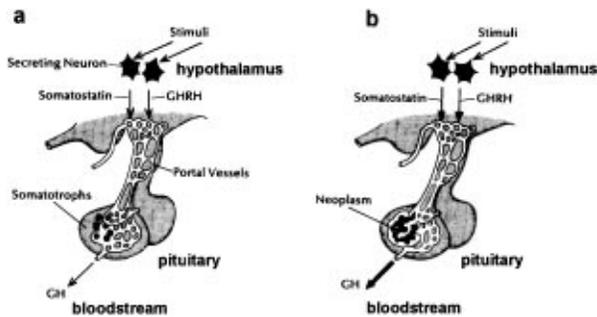


FIG. 2. (a) Normal growth hormone secretion from the somatotrophs (GH producing cells) within the pituitary (a gland adjacent to the brain) into the bloodstream is regulated by growth-hormone-releasing hormone (GHRH) and growth-hormone-inhibiting hormone (somatostatin), which are secreted in response to stimuli acting via the hypothalamus (a part of the central nervous system which is connected to the pituitary and controls GH secretion). The dark regions in (a) are the somatotrophs. In (a) there is no tumor. However, tumorous cells develop from the normal somatotrophs. (b) The more intensive and larger dark region represents such tumorous cells (neoplasm) within the pituitary; they excessively produce and secrete growth hormone. Acromegaly is thought to be a primary pituitary disease with excessive GH secretion due to loss of normal coupling between stimulus and secretion in GH secreting tumor cells within the pituitary neoplasm.

The next value  $x(t_i)$  of each GH concentration time series was predicted from  $m$  past values via an activation function  $f$ :  $x(t_i) = f(x(t_{i-1}), x(t_{i-2}), \dots, x(t_{i-m})) + \epsilon_i$ , where  $x(t_{i-1})$  is the preceding value, and  $\epsilon_i$  corresponds to noise or fitting error. The neural networks were trained (fitting the model  $f$  to data by using a conjugate gradient descent technique) to predict 10 min into the future for either subject group. The training within each group was performed using a “leave one out technique” where the time series being tested for prediction is left out of the training procedure. To predict multiple time steps ahead, the value  $x(t_i)$  predicted one time step ahead was iterated back to predict two steps into the future. This procedure can be repeated in an iterative way to predict any number of time steps ahead. We used the average relative variance (ARV) as prediction error estimate to decorrelate it from the variance of the respective time series:  $ARV = \langle (x_i - x'_i)^2 \rangle / \sigma^2(x_i)$ , where the angle brackets denote the mean over all predicted values and  $\sigma^2(x_i)$  denotes the variance of the measured time series. The simulations were performed on a Sun SPARCstation 20 using customized C code. A large variety of neural network architectures was explored to determine the best predictor within the group of healthy subjects and the group of patients with excessive GH secretion.

Linear correlation can give a time series, even a stochastic one, a degree of predictability that mimics determinism when making use of linear or nonlinear prediction algorithms. To rule out a mimicking of determinism for stochastic systems, we made extensive use of surrogate random data sets [12,18] (Fig. 3) as statistical

controls for the test for determinism. Surrogate data refer to data sets that preserve certain linear statistical properties of the experimental data. Such data serve to test statistically whether our experimental results could be accounted for by a linear stochastic process.

In our study we made use of three different types of surrogate data which all have their specific merits: phase randomized, Gaussian scaled, and Fourier shuffled surrogates. It has been demonstrated that using only phase randomized surrogates one can produce spurious identifications of deterministic structure [19]. Phase randomized surrogate data have a power spectrum, mean variance, and autocorrelation identical to the original experimental time series, but a different amplitude distribution [Figs. 3(a) and 3(d)]. This sort of surrogate data is used to test the null hypothesis that the results can be explained by using data from a Gaussian linear stochastic process. Other types of surrogate data are necessary since this procedure does not preserve the histogram of the signal amplitudes. The Gaussian scaled

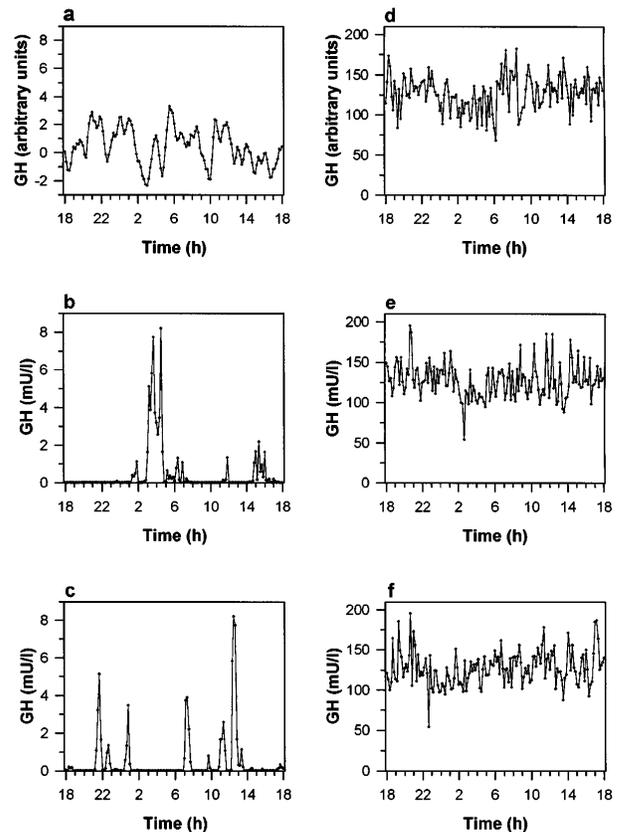


FIG. 3. Three types of random surrogate data sets corresponding to original time series in Fig. 1. Healthy subject: (a) Phase randomized, (b) Gaussian scaled, and (c) Fourier shuffled. Patient with excessive GH secretion (acromegaly): (d) Phase randomized, (e) Gaussian scaled, and (f) Fourier shuffled. Ten random surrogate data sets of each type were generated for each experimental 24-h GH time series. The construction of these types of surrogates is given in more detail in Ref. [12]. A full listing of the MATLAB (The Mathworks, Inc., Natick, MA) source code can be found in Ref. [21].

TABLE I. Prediction length of 24-h GH time series vs random surrogate data (in min). Ten realizations of three types of random surrogate data (as shown in Fig. 3) were constructed for each original time series of GH blood concentration. One- and multiple-step-ahead prediction were performed for each individual 24-h GH time series as well as for ten surrogate data sets of each type using the same network architecture trained with the "leave-one-out method" (see caption of Fig. 1). The prediction length is given in min (mean  $\pm$  standard deviation) when the prediction error ARV exceeds a value of 0.5. This prediction criterion is a measure for the predictability of the system dynamics by the neural network. A prediction error ARV of 1.0 may be achieved simply by always guessing the mean of the time series under study as the predicted value. A predictor that meets our criterion for the prediction length is on average twice as good as a predictor that completely ignores the dynamics of time series. Probability values are based on a two-sided paired  $t$  test. A significant difference between the predictability of the original GH concentration time series and the corresponding surrogate data sets is assumed for values of  $P < 0.05$ .

Group	Original	Phase randomized	Gaussian scaled	Fourier shuffled
Healthy subjects ( $n = 10$ )	$30 \pm 7$	$20 \pm 0$ $P < 0.05$	$10 \pm 0$ $P < 0.005$	$16 \pm 5$ $P < 0.01$
Patients ( $n = 6$ )	$20 \pm 9$	$17 \pm 5$ $P = 0.175$	$15 \pm 5$ $P = 0.076$	$13 \pm 5$ $P = 0.102$

surrogates are used to test the null hypothesis that the results can be explained by data from a nonlinear static transformation of a linear stochastic process [Figs. 3(b) and 3(e)]. The Fourier shuffled surrogates in some cases more closely approximate the power spectrum than the Gaussian-scaled surrogates while preserving the amplitude distribution [Figs. 3(c) and 3(f)].

Using time series prediction in conjunction with surrogate data as statistical controls, we found no evidence of deterministic temporal structure in the 24-h time series of GH concentration in the group of patients (Table I). In contrast, the 24-h GH time series in healthy subjects were predictable significantly further into the future compared to their corresponding surrogate data sets; a sign for highly regulated deterministic secretion (Table I). Our findings support the suggestion of random secretion of GH in patients bearing a tumor with excessive GH secretion. Whether the regulatory mechanisms controlling GH secretion operate stochastically or deterministically in the normal and diseased situation must be a fundamental feature of any theoretical model that can explain how this hormonal subsystem processes information. Furthermore, a realistic model for the regulation of GH secretion has to describe the conditions under which such hormonal control mechanisms might switch between stochastic and deterministic behavior.

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