COMPLEXITY AND NON-LINEAR DESCRIPTION OF DIURNAL CORTISOL AND GROWTH HORMONE SECRETORY PATTERNS BEFORE AND AFTER SLEEP DEPRIVATION

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Objective. The circadian secretory profiles of cortisol and growth hormone (hGH) in normal subjects are interrelated. Slight alterations in cortisol secretion are paralleled by similar ones of hGH secretion. Under physiological conditions an inhibitory effect of glucocorticoids on hGH secretion is more potent than a stimulatory one, while in normal young subjects the nychtohemeral cortisol and hGH levels are lower and higher, respectively, post 24 hours total sleep-deprivation, compared to baseline values. The aim of the present work was to further assess the qualitative characteristics of the 24-hour secretory patterns of these two hormones before and after 24 hours total sleep deprivation, by studying their non-linear profiles using fractal analysis.

Methods. Cortisol and hGH were measured in 24-hour samples drawn from 10 healthy men (mean age±SD: 24±1 yr, mean BMI±SD: 25±1 kg/m^2) before and after 24 hours total sleep deprivation. Twenty-four hour blood sampling was performed serially every 30 min the day before and the day after total sleep deprivation. The 24-hour hormone profiles were analyzed by Fourier spectrum, in order to verify periodicities; the corresponding attractors were drawn and their respective fractal dimensions were calculated using the box counting method.

Results. Diurnal cortisol levels before sleep deprivation gave rise to a fractal attractor with a D_0 fractal dimension of 2.65±0.03, which decreased, post-sleep deprivation, to D_0: 2.18±0.04. Growth hormone before sleep deprivation gave rise to a fractal attractor with a D_0 dimension of 1.96±0.60, which increased to 2.24±0.60 post-sleep deprivation. These post-sleep deprivation changes of the fractal dimensions of cortisol and hGH, suggest that sleep deprivation leads to a more regular secretory profile of cortisol, while it tends to render hGH secretory profile less regular. Additionally, these changes of the fractal dimensions parallell the previously described quantitative overall changes of these hormones.

Conclusions. The post-sleep deprivation decrease of cortisol fluctuation might reflect the mechanism by which sleep deprivation temporarily improves mood in melancholic depression, a condition associated with hyperactivity of the hypothalamic-pituitary-adrenal axis.

Key words: Cortisol – growth hormone – human – sleep deprivation – non-linear analysis

The cortisol and growth hormone (hGH) circadian secretory profiles are interrelated under both physiologic and pathologic conditions and research in humans has indicated that the action of feedback mechanisms may lead to this interrelation. In the acute setting of stress, glucocorticoids stimulate the expression of the hGH gene, leading to enhanced growth hormone secretion, though – with more pro-

longed stress – hGH release is suppressed by corticotropin-releasing hormone (CRH)-induced elevations in somatostatin levels (Casanueva et al. 1990; Raza et al 1998). Ghizzoni et al (1996) have shown, using cross-correlation analysis, that in normal children cortisol and hGH values were negatively correlated over 24 hours, at brief lag times of 0-30 minutes, with cortisol leading hGH. This might be explained by a tentative positive effect of CRH and/or glucocorticoids on the central noradrenergic system and/or somatostatin secretion. These hormones are also positively correlated at long lag times of 12.0-12.5 hours, with cortisol leading hGH. On the other hand, we have recently shown that in normal subjects, nychtohemeral cortisol levels were lower, while hGH levels were higher the day following total sleep deprivation. These changes were attributed to a suppressive and a stimulatory effect of primarily deep sleep following one night of sleep deprivation, on the hypothalamic-pituitary-adrenal axis (HPA) and the hGH axis, respectively (Vgontzas et al. 1999).

The application of complexity and non-linear methods has opened new research fields in the biomedical sciences, with studies on human cardiac electrical function (Goldberger et al. 1988; West 1990), DNA sequences (Peng et al. 1992; Almirantis and Provata, 1997), sleep cycles (Babloyantz 1985) and aging (Lipsitz and Goldberger, 1992), among others. Complex feedback mechanisms control the secretion evolution of hormones. The effect is in general non-linear; usually the secretion of a certain hormone activates or inhibits further the secretion of the same or of other hormones (Murray 1993). Such non-linearities lead to dissipative structures that are characterized by fractality (West 1990). The latter implies that the hormonal levels do not take arbitrary (random) values but they follow certain temporal, self-similar, non-deterministic patterns. To quantify the temporal behavior of the secretion and to determine the degree of self-similarity of the corresponding time series it is thus useful to borrow tools from non-linear dynamics. In this respect, determining the fractal dimension of the phase portrait (attractor) of hormonal level gives a measure of organization in the secretory pattern. Hormonal secretion has recently been the subject of few published reports in humans using such methods (Papavasiliou et al. 1994; Prank et al. 1994; Lenbury and Pacheenburawana, 1991; Noguchi et al. 1998; Brabant and Prank, 2000). Based on the self-similarity of diurnal hormone levels and the interrelation between cortisol and hGH profiles, this study aims to provide a quantitative, non-linear mathematical description of the cortisol and hGH secretory dynamics in healthy subjects. Additionally, we aim to introduce a measure (fractal dimension of the attractor) distinguishing the dynamics before and after total sleep deprivation.

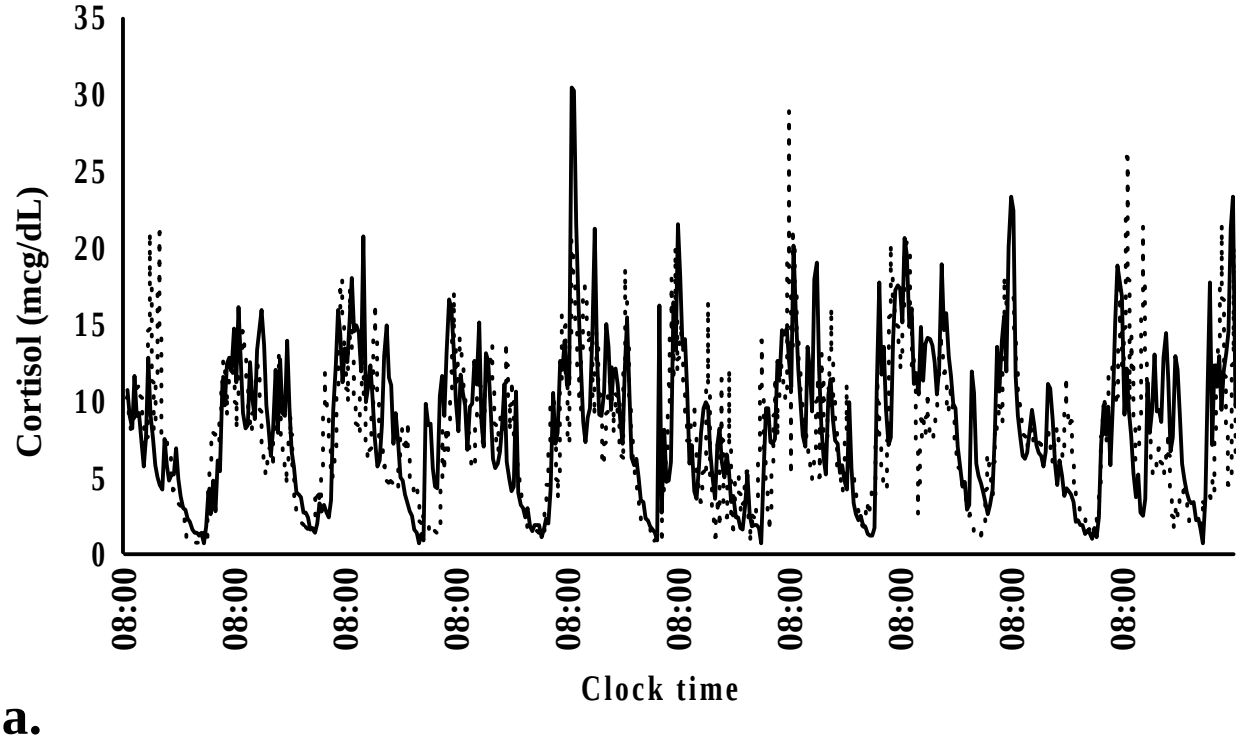
Materials and Methods

Subjects and protocol. Ten men (subjects 1 to 10), healthy volunteers, were studied (mean age±SD: 24±1 years old, mean BMI±SD: 25±1 kg/m^2). The subjects participated in a seven day-long sleep deprivation experiment in the Sleep Laboratory of the Pennsylvania State University. The first night was considered an adaptation period; the following three nights were baseline nights; the fifth night the subjects were deprived of sleep and allowed to sleep again on nights six and seven. The subjects were served two meals a day (at 12:00 and 18:00 h), with no differences in amount or composition between sampling days. A heparinized intravenous catheter was inserted in the antecubital vein 30 minutes before the first blood sampling on the fourth day. Blood samples from each subject were obtained every 30 minutes during 24 hours from 08:00 to 07:30 on the fourth day (pre-sleep deprivation day) and on the sixth day (immediately post-sleep deprivation day). During the sleep period the blood samples were collected outside the subjects' room through a perforation of the wall in their chamber. The samples were immediately centrifuged, the serum was separated and frozen in –70 ^{o}C.

Ethical approval for the initial human study was obtained from the Institutional Review Board of the Medical College of the Pennsylvania State University.

Assays. Plasma cortisol and serum hGH were measured in all the samples, in one batch, using in-house radioimmunoassays, with 4.6 % and 6.0 %, and 3.3 % and 5.1 % intra- and interassay coefficients of variations, respectively, as previously described (Chrousos et al. 1984; Magiakou et al. 1994).

Time series analyses. The cortisol and hGH circadian profiles and cross correlation analyses have been recently presented (Vgontzas et al. 1999).



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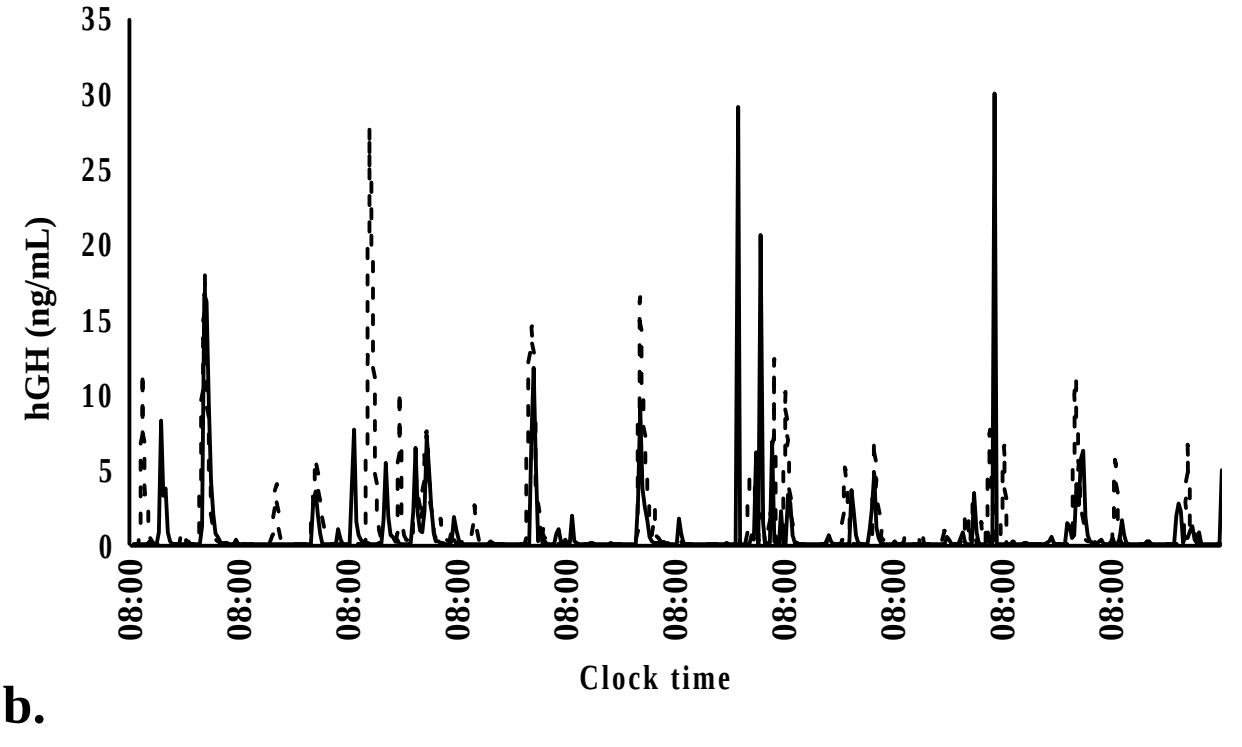

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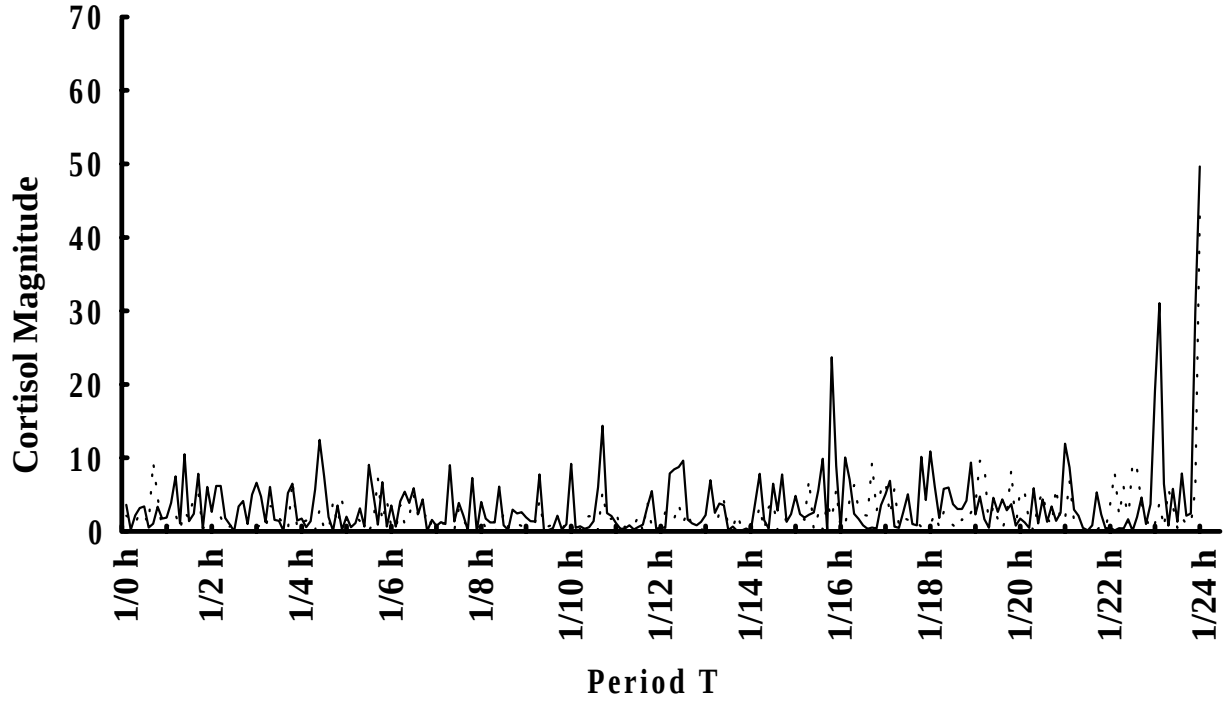
Fig. 1 Composite cortisol (panel 1.a) and hGH (panel 1.b) time series before (solid line) and after (dashed line) sleep deprivation.



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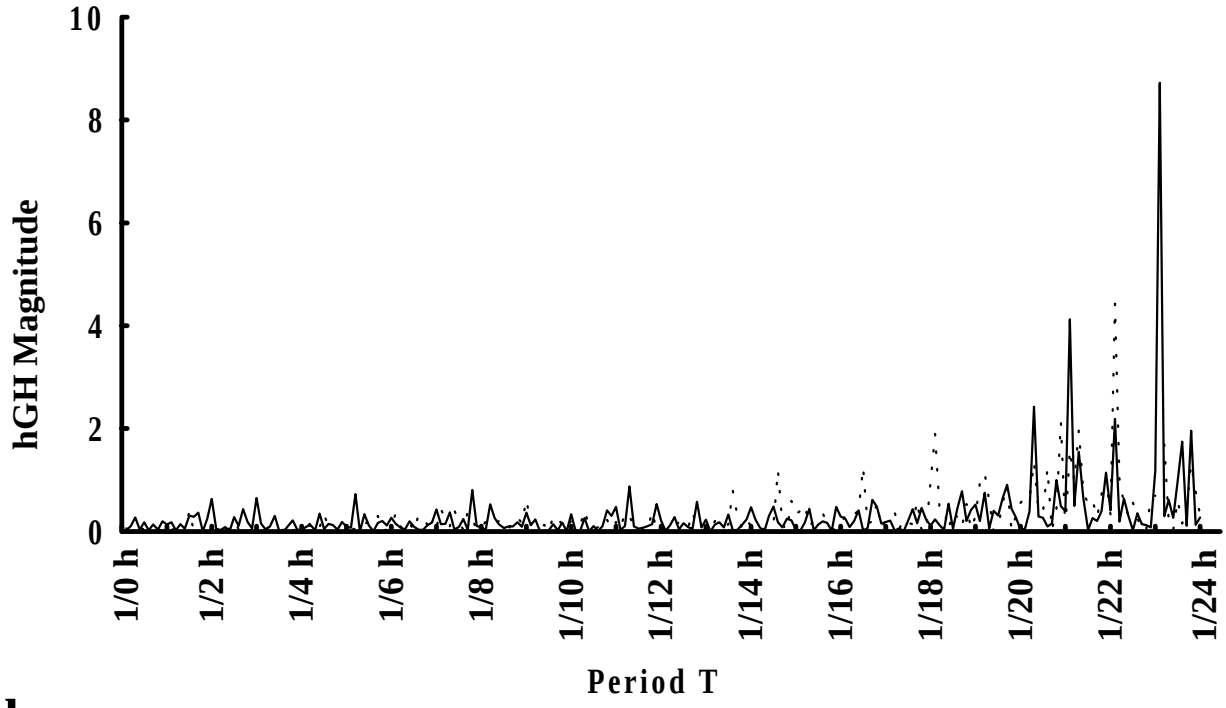

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Fig. 2 Fourier transform for cortisol (panel 2.a) and hGH (panel 2.b) composite series before (solid lines) and after (dashed lines) sleep deprivation. The magnitude is plotted as a function of period T.

Two composite time series were made for each hormone, concatenating each individual's profile (with 48 measurements), one for day 4 - before sleep deprivation - and one for day 6 – after sleep deprivation. The creation of each time series was based on the assumption that they represent a single individual's cortisol and hGH values over 10 consecutive days. This assumption allows the application of the mathematical procedures implemented; the techniques have been previously extensively described by PAPAVASILIOU et al. (1995) and applied in the description of diurnal variations of prolactin secretion in humans. The resulting composite cortisol time series, both before and after sleep deprivation, were smooth in appearance (Fig 1a). On the contrary, the resulting composite hGH time series were not smooth in appearance, due to the individual hGH profiles which are characterized by isolated spikes (Fig 1b). However, in order to apply the same mathematical procedures for hGH as for cortisol, we created the hGH continuous time series in a similar way.

Similarities and differences between the composite time-series before and after sleep deprivation were assayed, implementing the following mathematical procedures:

a. Fourier spectrum. The four composite series (cortisol and hGH before and after total sleep deprivation) were examined using Fourier transform to determine regularities and repetitive (oscillatory) behavior of hormone secretion over time. This method unravels hidden periodicities in the hormonal secretory profile.

b. Fractal analysis. The same composite time series of hormone values is reported on the axes *x, y, z* of a triaxial system. The resulting triaxial representation is called "attractor". Each point of the attractor is defined by: *x* value=n(t), *y* value=n(t+1) and *z* value=n(t+2), where t indicates a certain sampling time while n(t), n(t+1) and n(t+2) stand for temporally consecutive hormone values of the composite series.

To unravel the differences between the day before and the day after sleep deprivation, we calculated the fractal dimension D_o of the attractor of each hormone before and after sleep deprivation. The calculation of D_o is based on the box-counting method. According to this method the space taken by the attractors is covered with cubic cells («boxes») of linear size s. The number N of non-empty boxes (cells that contain at least one point of the attractor) is plotted as a function of the size s of the boxes on a double logarithmic scale (the log s is represented in the x-axis while the log N is represented on the y-axis). The size of the cell is changed to s', s'', s''', etc (where s'< s''< s''' etc) and the procedure is repeated. The slope ß±SD of the resulting fitted line is the fractal dimension D_o of each attractor.

The analyses implemented were done with the *Statistica* statistical software package (1999, StatSoft, Tulsa, OK) and standard fractal analysis using box-counting methods (VICSEK T, 1992).

Results

Analyses of cortisol time series

a. Cortisol Fourier spectrum. Fast Fourier transform and analysis of the cortisol period yielded a distinct peak at the 24th h period time of the analysis (48th sample), both before and after sleep deprivation (Fig 2.a).

b. Cortisol fractal analysis. The points of the corresponding attractors cover almost uniformly the tridimensional space (Fig. 3). The fractal dimensions of the attractors before and after sleep deprivation were D_0=2.65±0.03 (Fig. 3.a) and D_0=2.18±0.04 (Fig. 3.b), respectively.

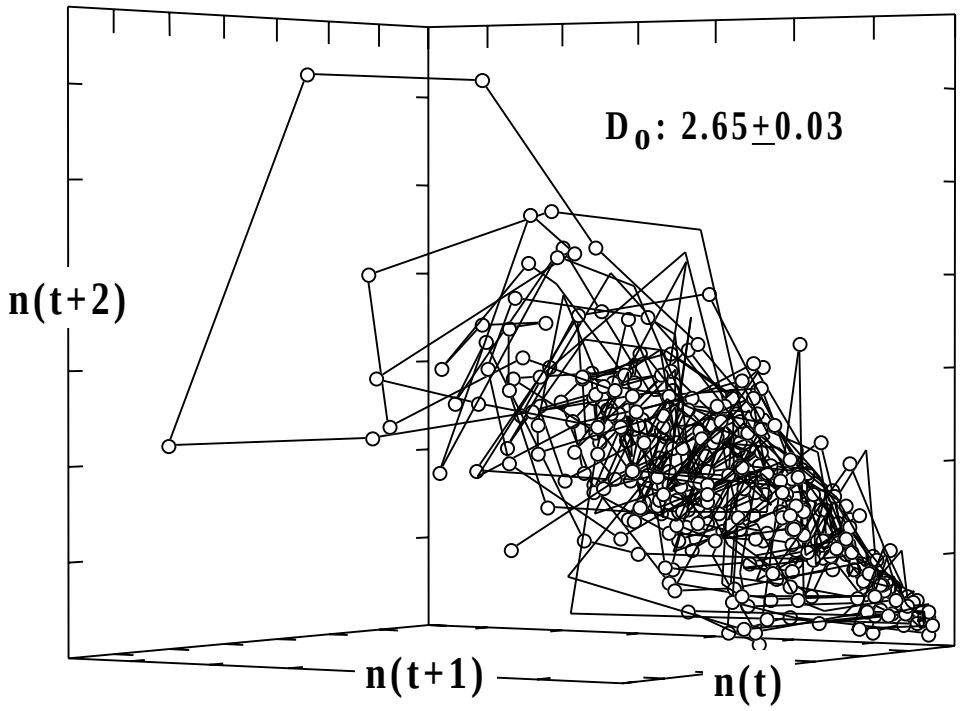
Analyses of hGH time series

a. hGH Fourier spectrum. Peaks were noted in hGH Fourier transform both before and after sleep deprivation, though not clearly distinct from noise except a number of peaks between the 21st and 23rd h period time of the analysis (42nd-46th sample), both before and after sleep deprivation (fig 2.b).

b. hGH fractal analysis. Most of the points of the attractor resulting from the composite time series before sleep deprivation are concentrated around the origin of the axes, while a few greater values are scattered (Fig. 4). The fractal dimensions of the attractor before and after sleep deprivation were D_0=1.96±0.60 (fig 4.a) and D_0= D_o=2.24±0.60 (Fig 4.b), respectively.

Discussion

In the present study we found that cortisol secretion presents regular periodicity before and after sleep dep-



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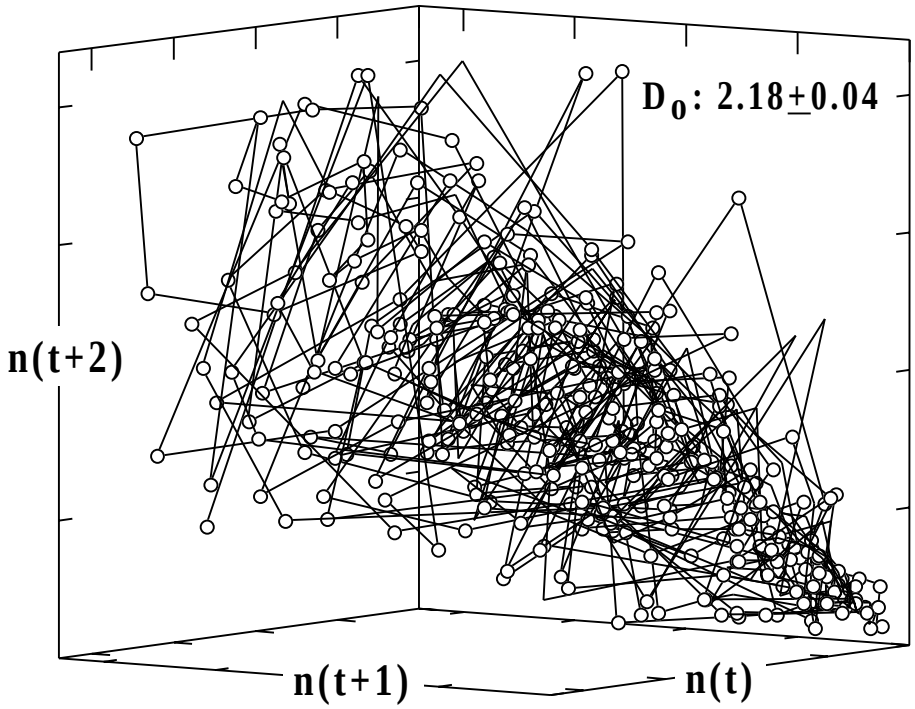

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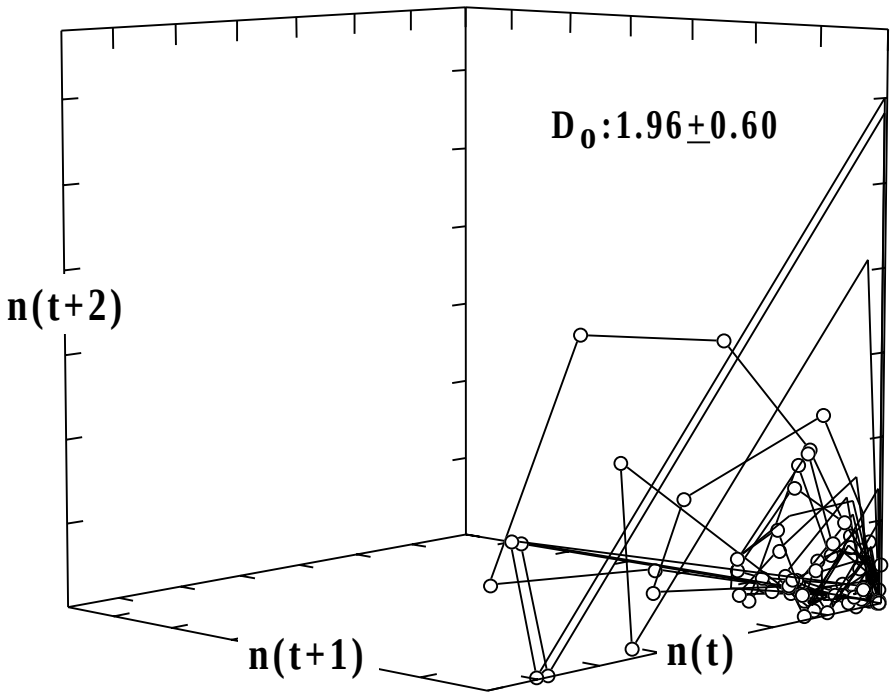
Fig. 3 Cortisol attractor before (panel 3.a) and after (panel 3.b) sleep deprivation in tridimensional time-phase space with axes n(t), n(t+1) and n(t+2); t indicates a certain sampling time while n(t), n(t+1) and n(t+2) stand for temporally consecutive hormone values of the composite series. Note the decreased fluctuation around the mean value after sleep deprivation.



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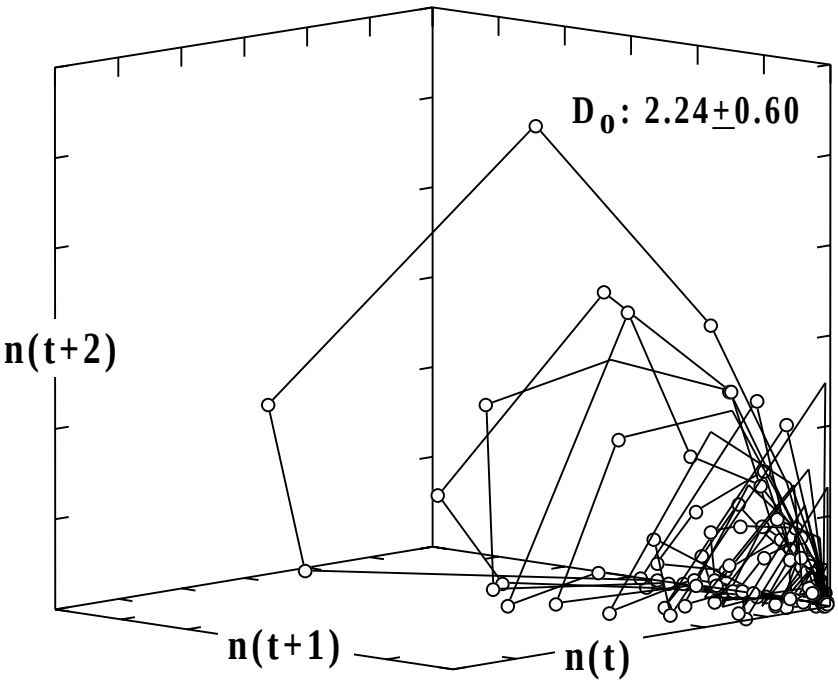

b.

Fig. 4 hGH attractor before (panel 4.a) and after (panel 4.b) sleep deprivation in tridimensional time-phase space with axes n(t), n(t+1) and n(t+2); t indicates a certain sampling time while n(t), n(t+1) and n(t+2) stand for temporally consecutive hormone values of the composite series. Note the increased fluctuation around the mean after sleep deprivation.

rivation while its D_0 fractal dimension is reduced after sleep deprivation. Growth hormone secretion was found to be under non-regular dynamics before and after sleep deprivation. Its D_0 fractal dimension increased after sleep deprivation. In the past, basal cortisol secretion has been proposed to arise via non-linear mechanisms. LENBURY and PACHEENBURAWANA (1991) have presented elegant mathematical models, in which cortisol secretion is described implementing non-linear differential equations with exponential feedback terms. However, to the best of our knowledge this is the first time that mathematical methods based on non-linear/fractal analysis have been used in the experimental study of the complex mechanisms underlying the concomitant circadian secretion of cortisol and hGH.

Sleep disturbances appear to be associated with HPA and hGH axes changes (VGONTZAS et al. 1998). In a recent study we have shown that mean plasma and time-integrated (AUC) cortisol levels are lower during the post-sleep deprivation nighttime period than on the pre-sleep deprivation night-time period. Moreover, hGH levels after sleep deprivation were found to increase primarily during the post-sleep deprivation night (VGONTZAS et al. 1999). In that study, the dynamics of cortisol and hGH secretion studied with cross-correlation analysis did not change after sleep deprivation. In fact, cortisol and hGH were negatively correlated with each other over time, at 0-30 min lag time, with cortisol leading hGH, both before and after sleep deprivation. This reciprocal relation may be mediated through the negative action of glucocorticoids on the central noradrenergic system (CHROUSOS and GOLD, 1992). Additionally, our findings are in accord with a recent study of six hours-long sleep deprivation (NOGUCHI et al. 1998).

The D_0 fractal dimension describes the dynamic mechanisms underlying hormonal secretion. The SD of these fractal dimensions reflects short-range fluctuations around the mean. The decrease of the fractal dimension of cortisol, found after sleep deprivation, goes along the quantitative decrease of this hormone, reported before (VGONTZAS et al. 1992), apparently resulting from changes of its underlying secretory mechanisms. The increase of the fractal dimension of hGH after sleep deprivation goes along with the increase in its levels, after sleep deprivation (VGONTZAS et al. 1992). These changes of cortisol and hGH secretion, after sleep deprivation, might reflect the activation of an error-correcting physiologic mechanism. Certain limitations should be taken in consideration in the overall interpretation of the fractal analysis of these data: the sketching of attractors was based on composite time series resulting from 10 individuals' serial measurements. This approach has been used by other researchers, without a significant loss of accuracy (PAPAVASILIOU et al. 1995). Such an approach is based on the *a priori* assumption that these normal subjects' measurements follow a similar, 24-hour, diurnal variation. Although this assumption seems valid for cortisol secretion, the composite hGH raw data (Fig. 2b) present significant variance among different individuals.

Therefore, we can conclude that sleep deprivation, by lowering the variation of cortisol around its mean value, may lead to a more regular secretory profile, whereas by tending to increase the variation of hGH around its mean value it may lead to a less regular secretory profile of this hormone. Since cortisol secretion reflects hypothalamic CRH neuron activity, the post-sleep deprivation decrease of cortisol variation might be the mechanism by which sleep deprivation improves mood in major depression (GOLD et al. 1988; MEYER et al, 2001).

It is known that fractal dimensions calculated by different algorithms may give different – but consistent – numerical values (JELINEK and FERNANDEZ, 1998). The accuracy of our results is strongly limited by the size of the time series, i.e., by the number of subjects studied and the sampling period. Moreover, among other limitations we have to note that we did not separate the waking from sleeping phases in the analyses, which would have added another important factor in the interpretation of data (MURCK et al, 1999). However, it has been shown that as far as hGH secretion is concerned, temporal associations between hGH and sleep phases are not reliable after sleep deprivation (DAVIDSON et al, 1991). Although the experimental/mathematical methods used are, with actual standards, still at birth, in the future non-invasive methods, such as salivary or transdermal estimations of hormone levels could render the non-linear description of the diurnal variations of hormones more feasible. In that way, the clinical observation of the continuous rhythm of hormonal secretion, instead of the study of isolated values, might contribute to our understanding of the dynamic changes in different pathologic situations.

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