Circadian Rythms of the HPA Axis and Stress

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INTRODUCTION

The term circadian, first described by Franz Halberg (1) , refers to an approximate period of 24 hours (from the latin circa, around; dies or diem, day). A large group of hormones has highly organized and pulsatile patterns of secretion with well-defined amplitude, frequency, orderliness and rhythmicity within the circadian cycle (2, 3) . Such a complex organization is determined by endogenous factors and modulated by exogenous agents. In addition to the key role of light, stress is also among the most important exogenous regulators of circadian rhythms.

The hypothalamic-pituitary-adrenal axis (HPA) is a key hormonal system that has a well-characterized circadian pattern. Under the influence of stress, this pattern is altered and homeostasis of stress-related neuroendocrine function is disrupted, with adverse impact on health.

PULSATILITY OF HPA HORMONE

The adrenal cortex secretes aldosterone, glucocorticoids (GC) and adrenal androgens (predominantly dehydroepiandrostenedione [DHEA], DHEA sulfate and androstenedione). Only the first two hormones are secreted under the influence of corticotropin (ACTH), which is released by the pituitary in response to corticotropin-releasing hormone (CRH). Arginine vasopressin (AVP) and oxytocin also contribute to the release of ACTH, in synergy with CRH. The HPA axis exhibits a prominent daily (approximately 24 h) rhythm that is under the control of the suprachiasmatic nuclei of the hypothalamus (SCN), whose activation, in turn, is regulated by light. Lesions of the SCN block daily rhythmicity (4) (5) . Therefore, circadian rhythms are regulated by light schedules and by food availability.

Circadian variability is observed in all adrenal hormones. Aldosterone levels are elevated during the sleep period (correlated with plasma renin activity), whereas pulse amplitude and frequency are reduced during waking periods (correlated with cortisol) (6) . This pattern suggests that

aldosterone secretion is under the influence of the renin-angiotensin-aldosterone system during sleep, and under the influence of the adrenocorticotropic system during wakefulness (Figure 1).

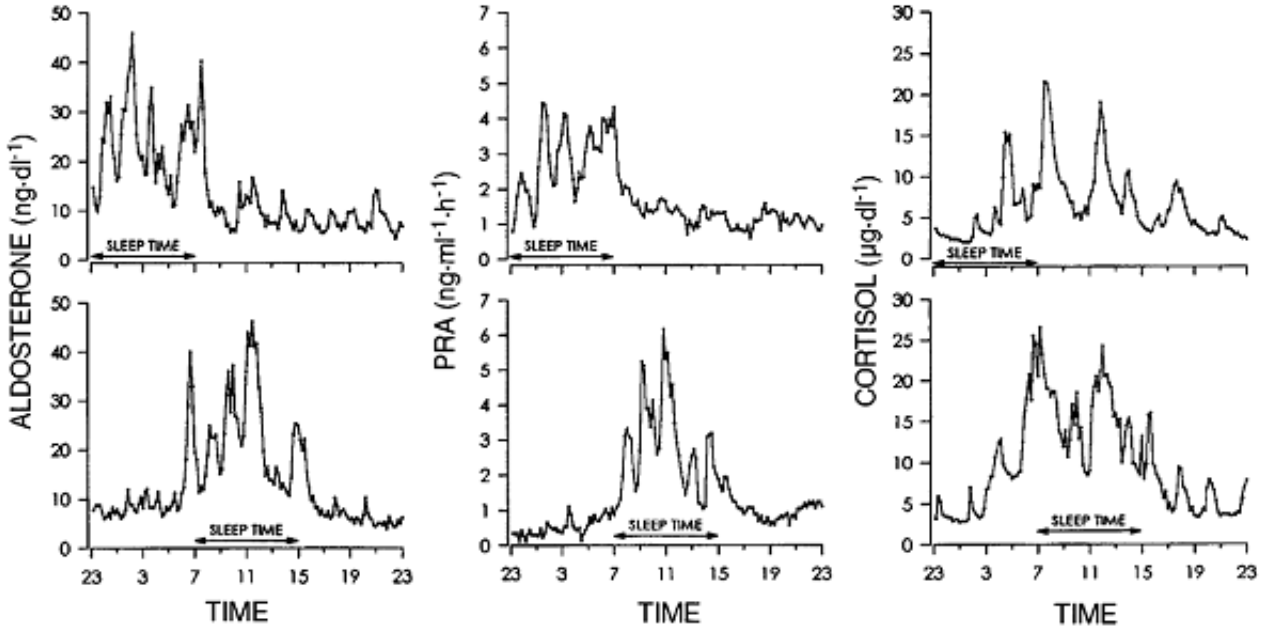

Figure 1:Twenty-four-hour profiles of plasma aldosterone levels, plasma renin activity (PRA), and plasma cortisol levels in a representative subject with normal nighttime sleep (top) and shifted daytime sleep (bottom). From ref. (6) with permission.

Adrenal androgens are also secreted in a circadian pattern (7, 8) , concordant with cortisol secretion and driven not only by ACTH and CRH, but also by other informational molecules such as estrogens, GH, IGF-I and TGF-β (9) .

Among the hormones produced by the adrenal cortex, glucocorticoids are those most well-characterized, due to their key effects on many systems. Cortisol, the main GC in humans, is released by the adrenal cortex in a pulsatile manner, driven by the pulsatile release of CRH and AVP by the hypothalamus, which then leads to the release of ACTH by the pituitary. The trophic and the steroidogenic actions of ACTH on the adrenal cortex are determined by the G protein-coupled melanocortin-2 receptor [6]. Increased GC secretion at the circadian peak depends on increased hypothalamic–pituitary activity and on increased sensitivity of the adrenal cortex to ACTH (10) .

HPA feedback loops occur at different time domains, referred as slow (in response to chronic exposure to glucocorticoids), intermediate and fast feedback (both in response to stress and to circadian events) (11) . In response to the fast and intermediate feedbacks, cortisol levels follow a circadian variability, in which maximum levels are obtained at a 2- to 4-hour window around waking, and subsequently decrease to a nadir at a 2- to 4-hour window around sleeping. The control of HPA circadian rhythmicity depends on hypothalamic–pituitary activity (driven by CRH

and AVP), ACTH secretion, and adrenal responsiveness to ACTH (10) . This activity is ultimately driven by light (at the hypothalamic SCN) and by food (at the ventromedial hypothalamus,) which regulate the expression of the CRH gene.

The GC inhibitory feedback on hypothalamus-pituitary axis is mediated not only by the GC receptors, but also by mineralocorticoid (MC) receptors, expressed in the hypothalamus and pituitary. Studies have demonstrated elevations in basal ACTH and GC after acute administration of MC, but not GC receptor antagonists (12, 13) . Mineralocorticoid receptor knockout mice MR have also elevated circadian nadir GC, which reinforces the important of both receptors in the regulation of the HPA axis (14) .

It has been shown that the MC and GC receptors are also expressed in the hippocampus (15) . Some studies have suggested the stimulation of the hippocampus by MC and GC might inhibit circadian HPA activity (16) .

Within a cycle of 24 hours, there are about 15 to 18 pulses of corticotropin of various amplitudes (17, 18) (Figure 2).

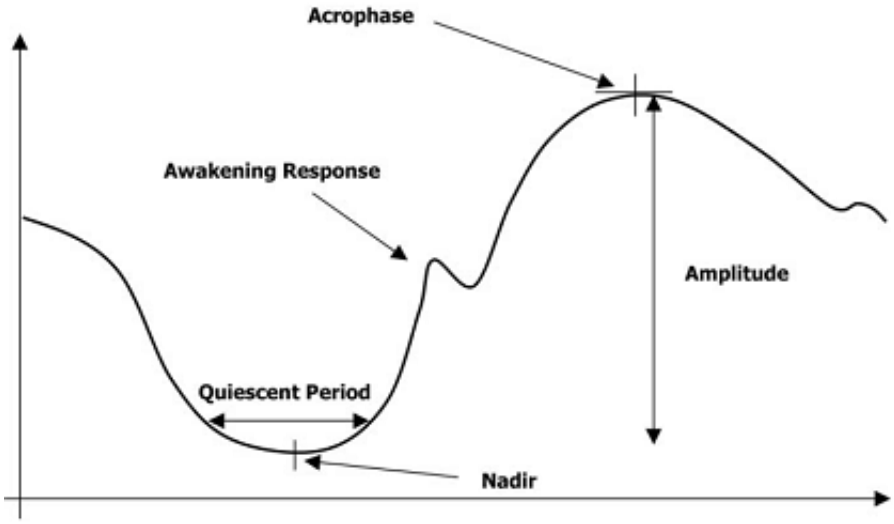

Figure 2:Schematics of the patterns of cortisol secretion. From ref. with permission. Copyright 2005, The Endocrine Society

By collecting blood from healthy individuals every 10 minutes, it has been shown that the adrenal gland secretes cortisol in distinct random bursts which, by amplitude modulation, give rise to GC rhythm (18) (Figure 3).

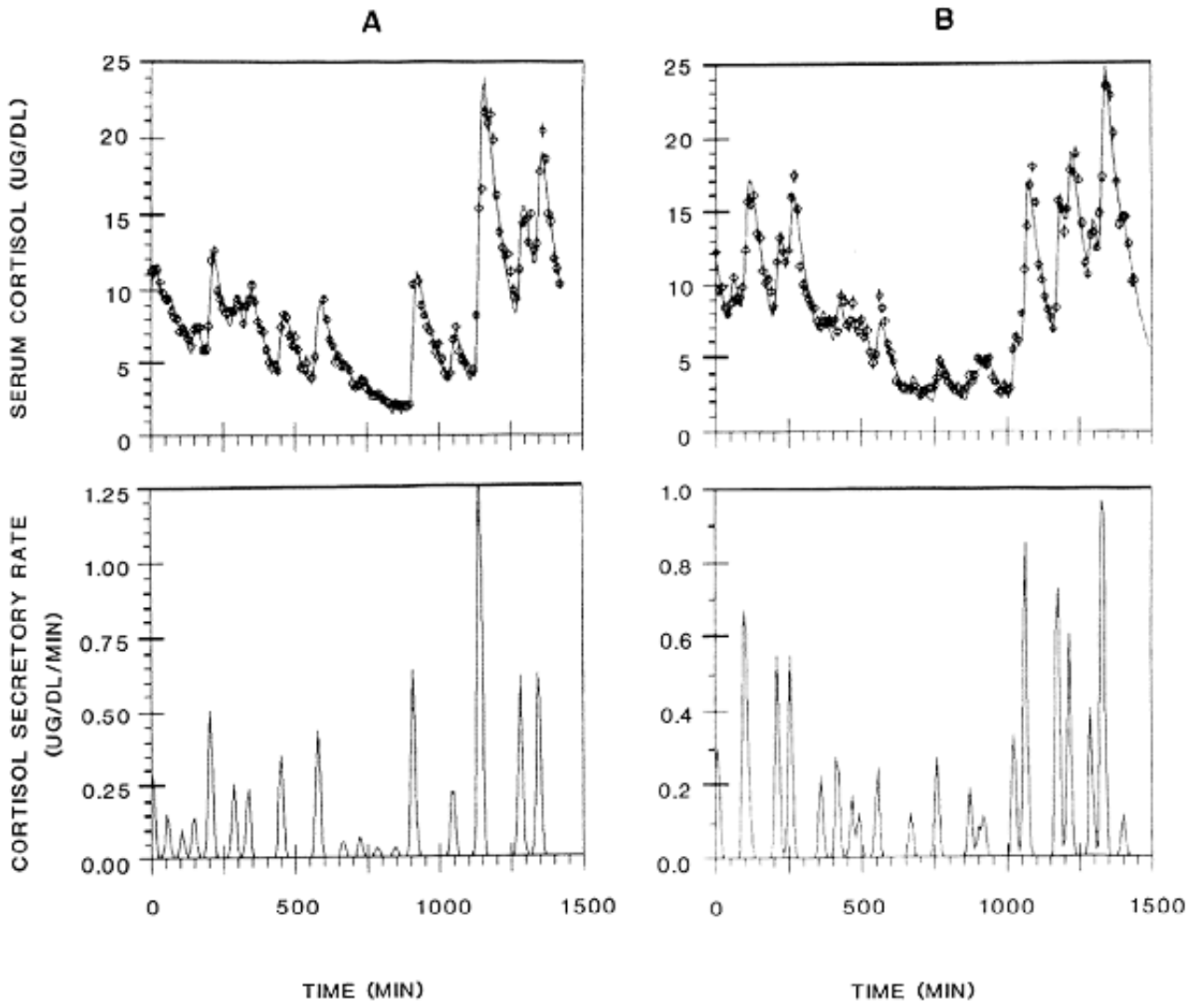

Figure 3:Twenty-four-hour cortisol profiles of two healthy men. Blood was collected every 10 minutes. From ref. (18) with permission.

This pattern of pulsatile secretion is characterized by alternating episodes of HPA activation and inhibition. The adrenocortical activity is regulated not only by light and food intake, but also by stress and cytokines (such as IL-6 and leptin) (10) . Through negative feedback, the corticotropic axis is inhibited by cortisol, which occupies both MC and GC receptors in the brain.

In aging humans, CRH and AVP pulsatility drives ACTH secretion (which is increased at night), and its secretory mass is higher and its shape is reduced by more than 50%. These changes during the 24-hour period are determined not only by changes in CRH and AVP secretion, but also by GC negative feedback on the corticotroph secretory processes (19) .

More recently, studies have discussed the role of the cortisol awakening response (CAR), which is characterized by a sharp increase of cortisol release by about 38 to 75% of awakening levels,

reaching a maximum approximately 30 minutes after awakening (20) . The CAR appears to be a distinct phenomenon superimposing the circadian rhythm of cortisol, and adding a significant incremental effect to the linear trend of increasing cortisol concentrations in the early morning hours (21) . Stress is one of the factors that influence CAR, as are gender, health status, and health behavior (22) . It is suggested that the CAR is associated with the stress-related physical and mental symptoms, particularly in anticipation of upcoming demands (22) .

EVALUATION OF HORMONAL RHYTHMICITY

Pulse analysis

Our group has utilized and optimized several techniques for assessing hormonal circadian rhythms. To analyze hormonal pulses, we have successfully applied cluster analysis (23, 24) , which is a computerized pulse analysis algorithm used to identify statistically significant pulses in relation to measurement error in each hormone time series. The program only detects statistically significant pulses after taking into account both the limit of detection of the assay and a C.V. of 10%. Measurement error (within sample variance) for the number of samples in each series is modeled as a power function of sample concentration. Significantly increased or decreased hormone concentrations (peak margins) are judged by pooled t statistics, which are applied to moving test nadirs and peak clusters that begin with the onset of the experimental series and traversed all points. In all our studies (25-28) , we have identified the following properties of pulsatile hormone concentrations: 1) Pulse frequency (number of significant peaks/24-h); 2) Mean interpeak interval (time separating consecutive peak maxima); 3) Mean pulse duration in minutes; 4) Mean pulse height (maximal leptin concentration in a peak); 5) Mean incremental peak amplitude (differences between peak maximum and preceding nadir) and pulse height, as the percent increase over preceding baseline (100% corresponds to preceding baseline); and 6) Interpulse valley mean (a valley has been defined as a region embracing nadirs without intervening peaks). By using cluster analysis, we have identified the pulsatile behavior of several informational molecules, such as leptin and interleukin-1 (25, 28) . To confirm the results obtained by cluster analysis, we have used Detect (25, 28) , which is an independently formulated, computerized pulse-detection algorithm (29) . Up- and down-strokes of a hormone pulse are identified by line segments that have positive and negative first derivatives, significantly different from zero. In that approach, a pulse is defined as significant down-stroke. (Figure 4).

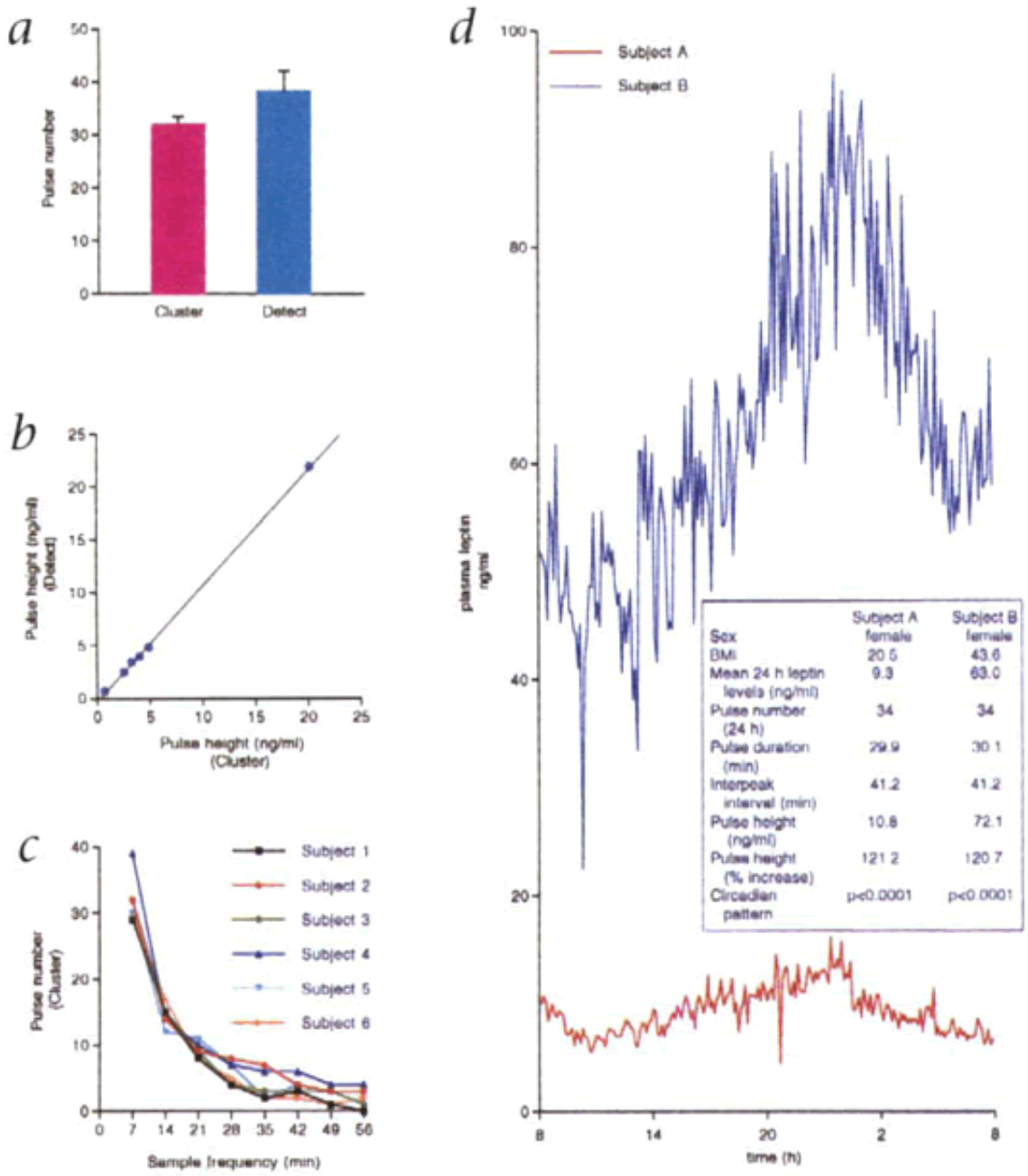

Figure 4:Leptin rhythms evaluated in six healthy men, by collecting blood every 7 minutes during 24 hours. a, number of statistically significant leptin pulses assessed by Cluster (magenta) and Detect (blue). b, pulse height assessed with Cluster (x-axis) and Detect (y-axis) (r=0.9998; P < 8 x 10-8, Pearson correlation). c, assessment of leptin pulse numbers in data sets corresponding to sampling every 7, 14, 21, 28, 35, 42, 49 and 56 minutes, assessed by Cluster. d, comparison of leptin levels and pulse parameters in two Caucasian women (A, normal weight; B, obese). Reprinted by permission from Macmillan Publishers Ltd: Nature Medicine, ref. (25) , copyright 1997.

Spectral (Fourier) analysis

Spectral (Fourier) analysis is used to determine the temporal nature of a hormone, as previously described (25, 27, 30) . Through that approach, our group was the first to describe leptin's twenty-four-hour pulsatility in healthy men and women (25) . (Figure 5)

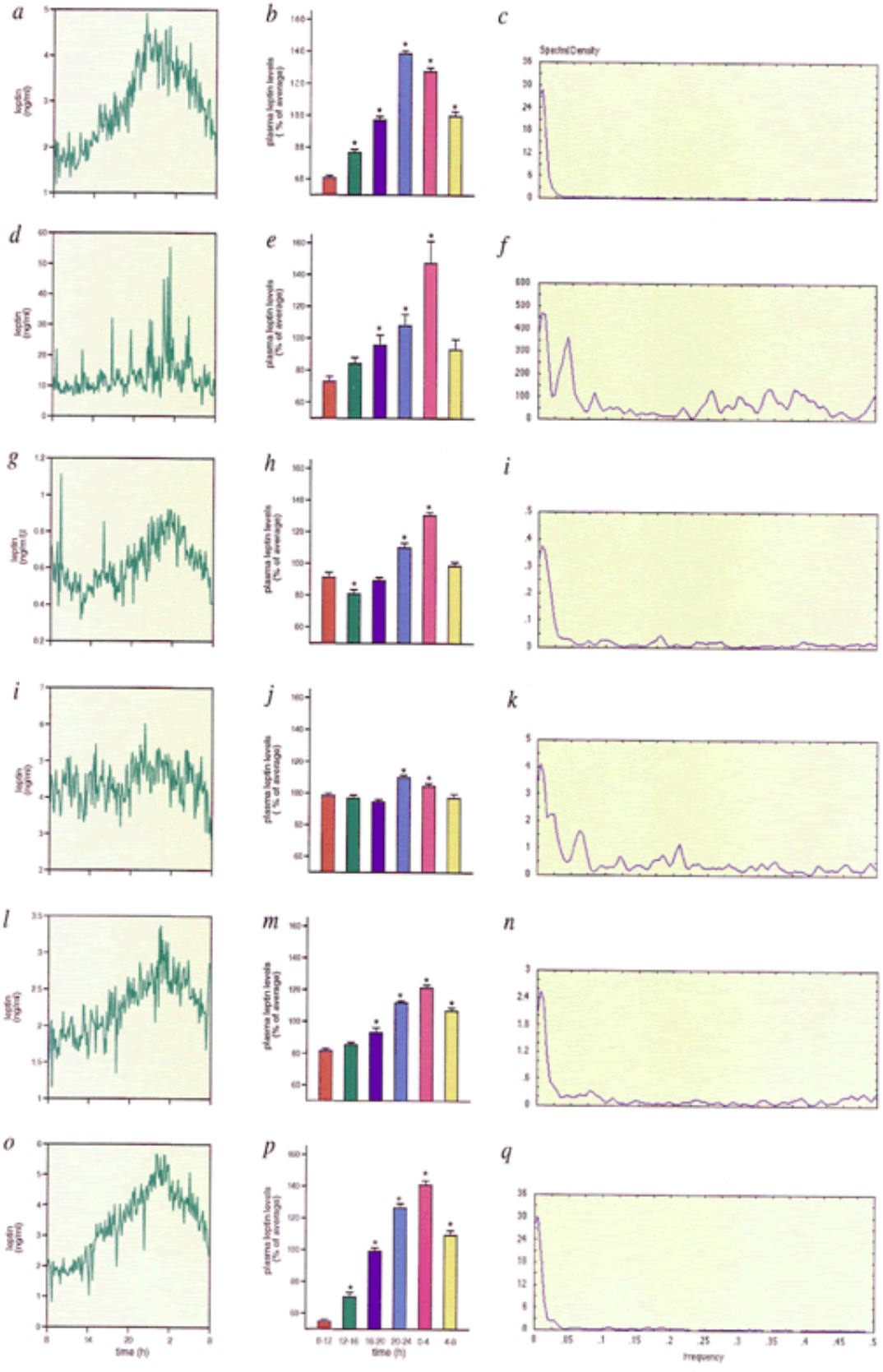

Figure 5:First column; raw plasma leptin levels in six healthy men. Second column; leptin levels were averaged for six 4-h time periods and expressed as percentage of the 24-h average.

Asterisk indicates that leptin levels in a specific time period are statistically different from those at the 8:00-12:00 period. Third column; spectral analysis of the detrended data series showing circadian (24h) periodicity in leptin levels. Reprinted by permission from Macmillan Publishers Ltd: Nature Medicine, ref. (25) , copyright 1997.

Cross-correlation analysis

The fluctuations of a given hormone, in relation to another substance, can be evaluated by cross-correlation analysis (26) . Cross-correlation is calculated after lagging the concentration time series of one hormone relative to the concentration time series of another hormone. Cross-correlation is carried out at variable lags, which are the times in minutes separating consecutive samples in the paired hormone series of interest. Significant cross-correlation values for each subject at any particular lag are tested against the null hypothesis of purely random associations via the one-sample Kolmogorov-Smirnov statistic applied to the z-score-transformed r values, assuming that uncorrelated data show a unit normal z-score distribution with 0 mean. By using that approach, we demonstrated that ultradian fluctuations in leptin levels showed pattern synchrony with those of luteinizing hormone (LH) (26) and of TSH (31) (Figure 6).

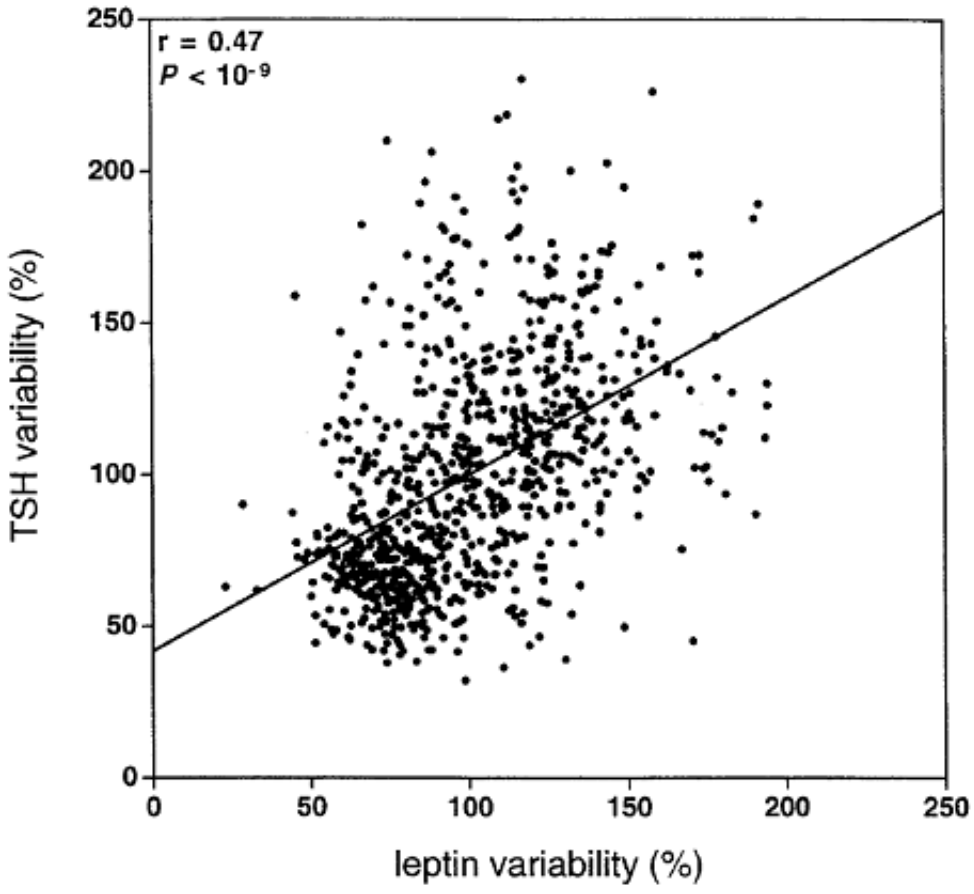

Figure 6:Cross-correlation analysis of TSH and leptin levels in five young healthy Caucasian men. From ref. (31) with permission. Copyright 2001, The Endocrine Society.

Bayesian and cosinor analysis

Bayesian and cosinor analysis can also be used to quantitate hormonal nyctohemeral rhythmicity. A Bayesian nonlinear, hierarchical model is useful for examining differences in the baseline levels and diurnal pulsatility of a given hormone between different populations (32) . The cosinor analysis represents a linear reduction of sinusoidal regression, and is performed by using Chronolab (, Bioengineering & Chronobiology Lab., http://www.tsc.uvigo.es/BIO) (33, 34) . Parameters of the sinusoidal regression such as MESOR (midline estimating statistic of rhythm or rhythm-adjusted mean), acrophase (the time lag expressed in radians from a reference time point of the top point in the fitted curve), telophase (the time lag from the reference point of the lowest point in a fitted curve), and amplitude (the difference between the peak and the MESOR of the fitted curve) are obtained for a 24-h period. Rhythm detection is sought by testing the null hypothesis of zero amplitude with an F-test using the Chronolab program (34) . The percentage of rhythm (PR) is the percentage of the total variability explained by the model. The combination of both approaches has allowed us to better describe the rhythmicity of various hormones, such as leptin and TSH (31, 35) (Figure 7).

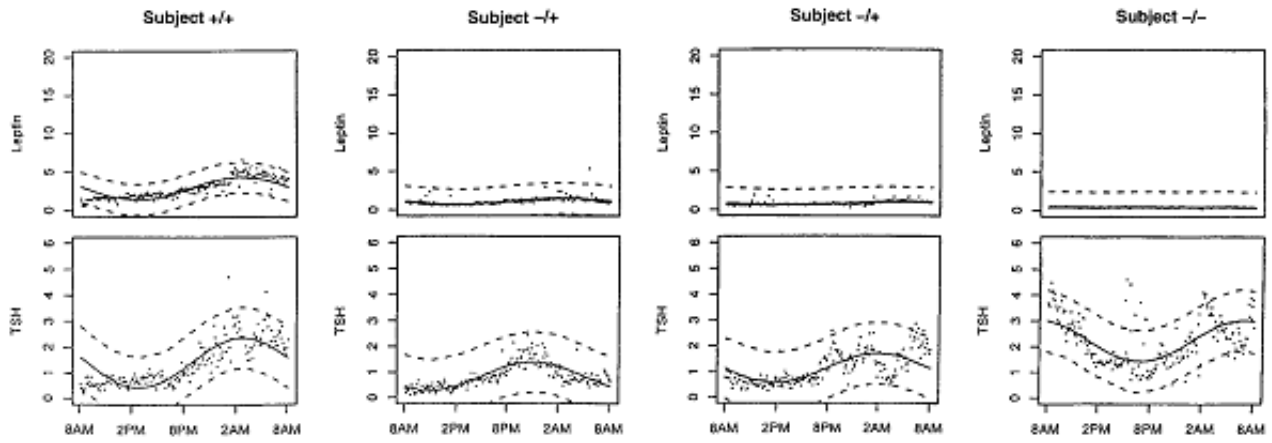

Figure 7:Bayesian model: diurnal behavior of leptin and TSH in normal subjects (+/+), in heterozygous patients for a mutation in the leptin gene (+/-) and leptin-deficient patients (-/-). From ref. (31) with permission. Copyright 2001, The Endocrine Society.

Deconvolution analysis

Multiparameter deconvolution analysis can be used to transform plasma concentrations (i.e.,

hormone quantity per volume) into quantitate pulsatile hormonal secretion rates (i.e., hormone output over time) with concomitant estimation of hormonal half-life (36-38) . For example, daily pulsatile GH secretion is the product of secretory burst frequency and the mean mass of GH released per pulse (39) . Basal hormone secretion represents the time-invariant interpulse component of the release profile. Secretory pulse identification requires that hormone secretory-burst amplitudes exceed 95% statistical confidence intervals (39, 40) . By deconvolution of frequently sampled plasma concentrations of glucose, insulin and C-peptide, we have simultaneously modeled over 24 h the time courses of pancreatic insulin secretion rates, hepatic extraction of insulin, and post-hepatic delivery rates of insulin during standardized meals over 24 hours, as well as insulin sensitivity, and applied that comprehensive modeling to a leptin-deficient patient (41) (Figure 8).

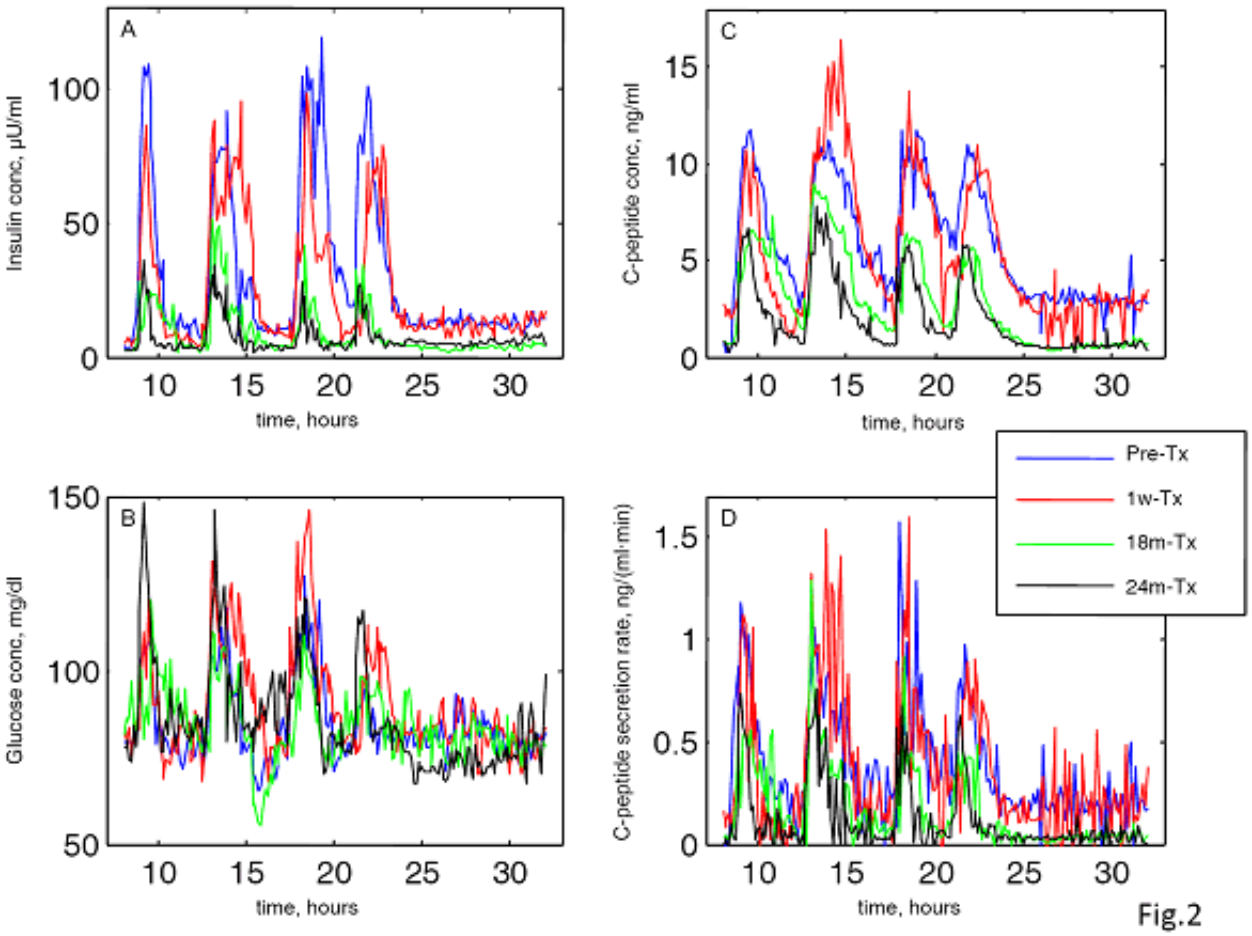

Figure 8:Time courses of insulin (A), glucose (B), C-peptide plasma levels (C) and C-peptide secretion rate in a leptin-deficient man, before, 1 week, 18 months and 24 months after the initiation of leptin replacement. From ref. (41) with permission.

APPROXIMATE ENTROPY AND CROSS-APPROXIMATE

ENTROPY

Approximate entropy (ApEn) corresponds to the serial orderliness of hormone measurements. Larger ApEn values correspond to greater randomness (irregularity). Technically, ApEn measures the logarithmic likelihood that runs of patterns that are similar remain so on next incremental comparison. The basic derivation and calculation of ApEn have been previously reported (42, 43) . ApEn can be used as a scale- and model-independent statistic, which is complementary to cosine fitting and deconvolution analysis (44) . ApEn is stable to small changes in noise characteristics and infrequent albeit significant outliers (43, 45) . This statistic evaluates a variety of dominant and subordinate patterns in the data; for example, ApEn can detect and quantify changes in underlying regularity of hormone release that are not necessarily reflected in changes in peak frequency or amplitude (43) . ApEn identifies consistency of point-by-point variations in the data, rather than macroscopic patterns or diurnal trends. Indeed, the latter are removed by first-differencing of the data. Additionally, ApEn provides a barometer of feedback changes in many coupled systems (43, 46) .

Cross-ApEn is algorithmically similar to ApEn, although it directly addresses network, and not only nodal, but also signal quantification (46, 47) . This measure quantifies the conditional regularity or synchrony of point-by-point variations across two time series. It is distinct from cross-correlation analysis because cross-ApEn is independent of lag. In leptin-deficient patients under replacement therapy with recombinant methionyl human leptin (r-metHuLeptin), ApEn analysis of leptin levels showed that the level of regularity of plasma leptin concentrations increased markedly with leptin replacement. Concomitantly, irregularity of testosterone and LH increased, and their pulse heights were more pronounced. These results indicated that leptin administration had a profound effect on the dynamics of sex hormone concentrations while concurrently causing the onset of puberty in a previously hypogonadic male (48) .

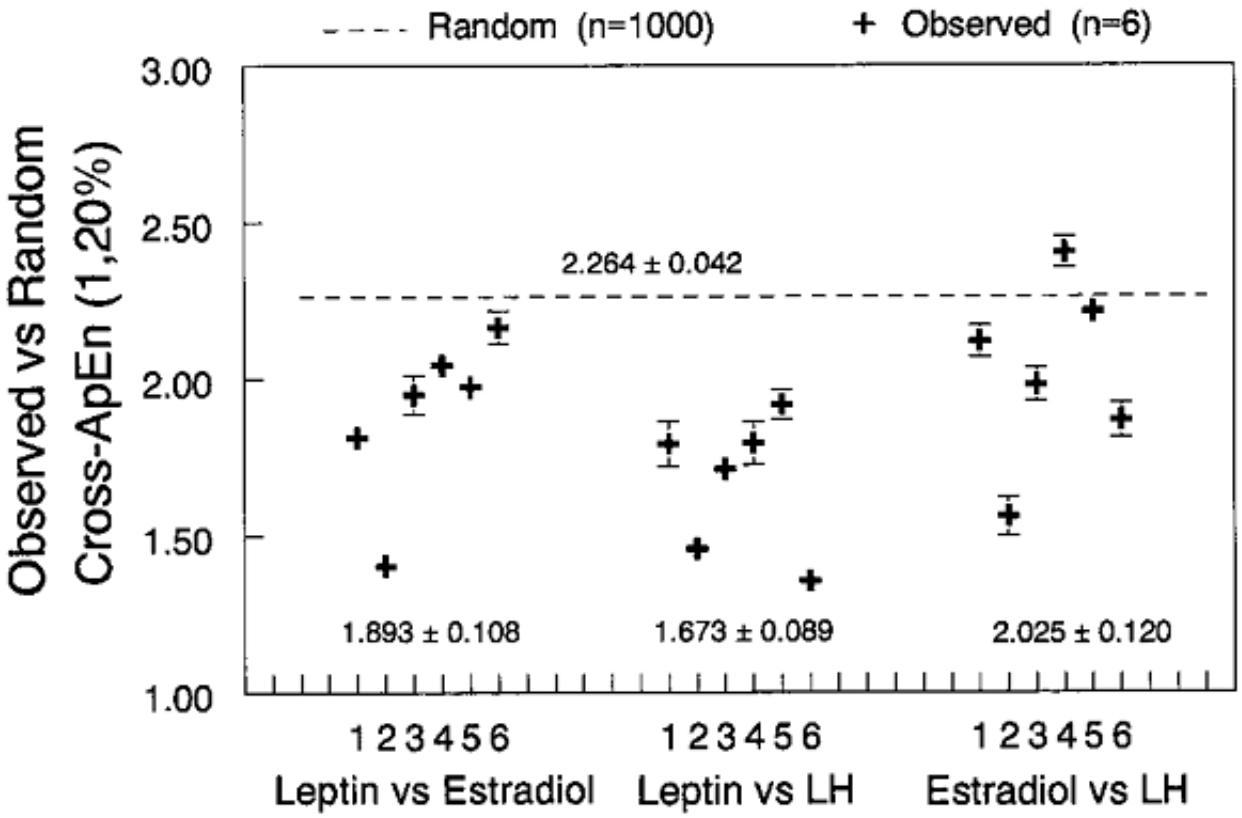

Figure 9:Individual values of cross-ApEn in six women who had their blood sampled at 7-min intervals for 24 hours. Each woman's cross-ApEn is plotted, with a SD denoting the Monte Carlo-predicted experimental uncertainty in the cross-ApEn calculation, given the dose-dependent within-assay errors. Below each set of cross-ApEn values is a mean ± SEM for the group of six individuals. The interrupted line denotes the mean global random cross-ApEn. Lower cross-ApEn values indicate greeter pattern repetition or conditional synchrony in the point-by-point release profiles of the two hormones. From ref. (26) with permission.

ROLE OF LEPTIN IN HPA RHYTHMICITY

The effects of leptin on the HPA rhythmicity have also been evaluated using the same approaches. In the absence of leptin, cortisol dynamics was characterized by a higher number of smaller peaks, with smaller morning rise, increased relative variability, and increased pattern irregularity. After the initiation of leptin replacement in leptin-deficient adults, cortisol dynamics was characterized by higher 24-h mean concentrations, with fewer pulses of greater height, including a greater morning rise. ApEn and relative increment decreased after treatment. These data suggest that leptin has a role in organizing the dynamics of human HPA function (48) (Figure 10).

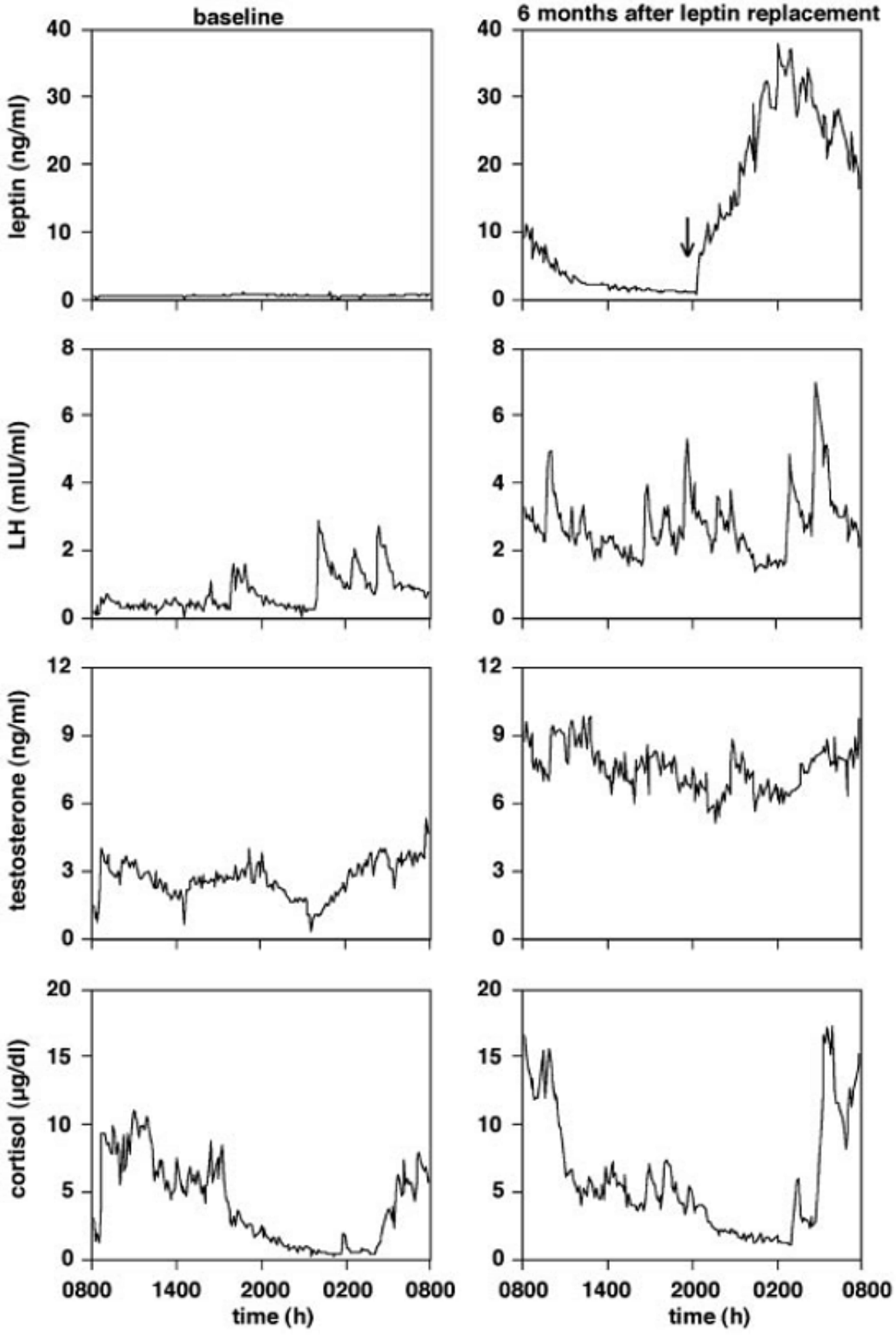
baseline
6 months after leptin replacement
leptin (ng/ml)
LH (mIU/ml)
testosterone (ng/ml)
cortisol (µg/dl)
0
10
20
30
40
2
4
6
8
3
9
12
5
15
0800
1400
2000
0200
0800
time (h)

Figure 10:Circadian rhythms in a leptin-deficient male before and six months after the initiation of leptin replacement. The arrow indicates leptin injection. From ref. (48) with permission.

HPA DYSREGULATION BY DISEASE

These approaches can be used to evaluate the effects of several diseases on the HPA axis. In sleep-deprived individuals, the circadian nadir GC levels are elevated, and the quiescent period is shorter, compared with extended sleep periods (49) . As a result, mean cortisol levels are elevated after sleep deprivation (50) . In insomnia, cortisol is increased, particularly in the evening and the first part of the nocturnal sleep period (17) .

The function of the HPA axis is also abnormal in many depressed patients (51, 52) . About sixty-six percent of patients with melancholic disorders exhibit nonsuppression of cortisol secretion after administration of the dexamethasone suppression test (53) . These alterations in the HPA axis are attributed to the larger production of CRH in the brain, and to the impairment of the negative feedback by glucocorticoids. The changes in the HPA axis of depressed patients seem to be sex-dependent, as a study has shown that depressed men had more ACTH pulses over 24h than matched control men, and men showed a smaller area-under-the-curve ACTH than women (54) (Figure 11). Deconvolution analysis of 24-h pulsatile secretion, approximate entropy (ApEn) estimation of secretory regularity, cross-ApEn quantitation of forward and reverse ACTH-cortisol synchrony, and cosine regression of 24-h rhythmicity of normal and depressed patients have shown several alterations of the HPA axis of depressed patients, such as increased ACTH secretion (regardless of hypercortisolemia), and elevated cortisol secretion in a highly irregular pattern (55) .

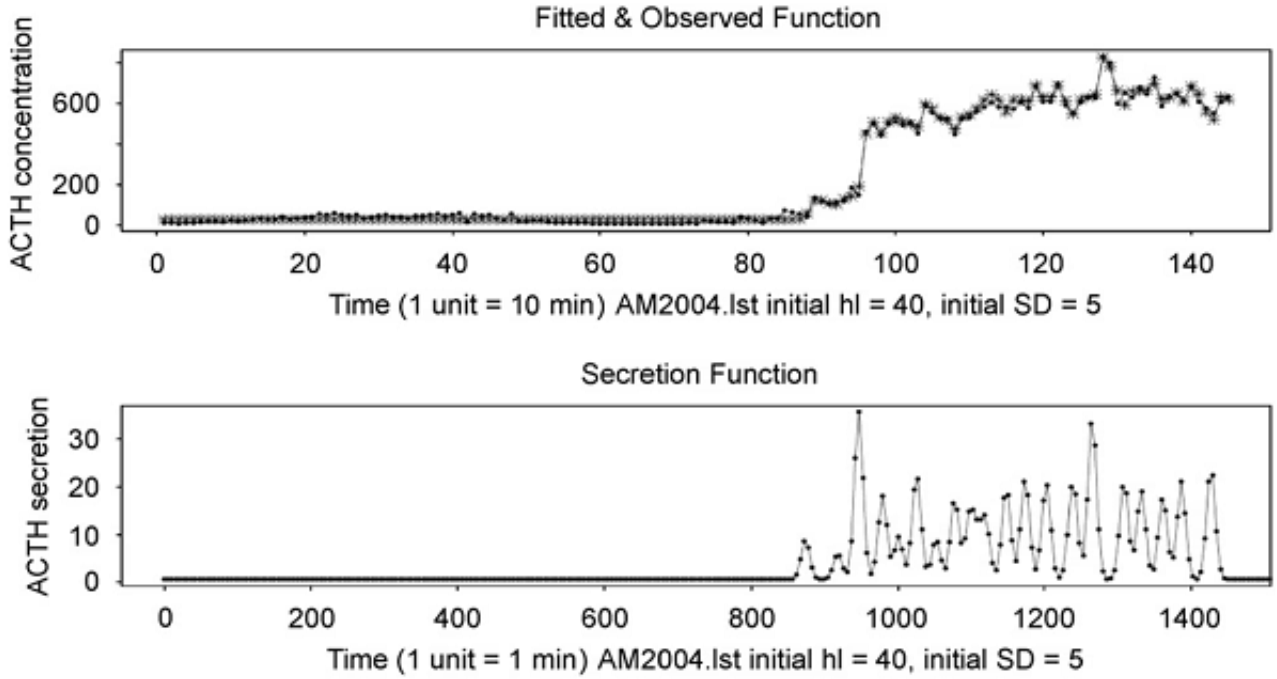

Figure 11:ACTH levels in a depressed patient on metyrapone. Output from deconvolution program demonstrated more ACTH pulses over 24h than matched control men. From ref. (54) . Copyright 2007, with permission from Elsevier.

In Cushing's disease, mean plasma ACTH and cortisol levels are elevated, with a more than 2-fold increase in mean pulsatile ACTH amplitude. The timing of the acrophases and nadirs for cortisol are not significantly altered compared to that in the normal group, and the normal postprandial elevation in cortisol is depressed or absent in the CD group, after the noon meal (56) . In patients with Cushing's disease or Cushing's Syndrome, the diurnal pattern of cortisol secretion is lost, as nocturnal levels are usually increased (57) . Based on these findings, the differential diagnosis of hypercortisolism relies on the circadian patterns of cortisol secretion.

Stress

Stress is defined as any actual or perceived threat to well-being, and the activation of stress systems aims to improve the chances of survival. The stress response is determined by the integration between complex systems located in the central nervous system (CNS) and peripheral signals, such as visceral, nociceptive, somatosensory, visual and auditory stimuli (58) . Psychological stress occurs when an individual perceives that environmental demands exceed his or her adaptive capacity, threatening homeostasis (59) . Not only psychological, but also physical stressors (such as blood loss, hypoglycemia, infection, or tissue damage) are potential disruptors of homeostasis; therefore, both types of threats are responsible for the activation of many organ systems. Among these systems, the circadian pattern of the HPA axis is the most affected when the body is under stress. Interestingly enough, the anticipation of stressful

situations, even in the absence of an actual aggression to the body, may also affect the HPA axis, demonstrating the importance of perceived or psychological stress (60) . Glucocorticoids play an important role in both the onset and the termination of the stress–response.

The neuroendocrine response to stressors is originated at the parvocellular cells of the paraventricular nucleus of the hypothalamus. Inputs from limbic circuits and brain stem centers activate those cells, when psychological and physical stressors are present (16) . Stress stimuli lead to a rapid increase in c-fos (61) followed by increased gene activation, such as CRH and AVP, which are crucial for the activation of the HPA axis (62) .

There seems to be a difference in response to chronic and acute stress. In chronic stress, the central activation of HPA activity is taken over by a predominant AVP rather than CRH drive (63) . Nevertheless, the stress signals increase the amplitude and frequency of the CRH and AVP pulses. Other substances, such as serotonin, angiotensin II, cytokines and mediators of inflammation are also secreted and potentiate the activity of the HPA axis. In stressful situations, there seems to exist dissociation between the secretion of ACTH and cortisol, as other substances become important stimulatory factors for cortisol secretion (64-66) .

In severe acute physical stress, such as the one observed during septic shock, the normal feedback system within the HPA axis might be impaired, as shown by lesser suppressibility of hypercortisolism by dexamethasone (67) . The normal circadian rhythm of cortisol secretion is also disrupted (especially during the night), whereas pulsatility persists. Moreover, in acute illness, the biologic effects of cortisol increase due to a decrease in cortisol binding globulin and an increase in receptor concentration and in its sensitivity (68, 69) .

Prolonged stress and chronic GC release may lead to increased CRH mRNA expression in the PVN (paraventricular), altered glucocorticoid receptor (GR) expression in limbic brain regions, adrenal hypertrophy (due to elevated ACTH) (70) , and thymic atrophy (due to GC elevation) (71) . In the prolonged stress caused by critical illnesses, the regulation of the adrenal cortex is mediated not only by ACTH, also by other substances, such as cytokines, endothelin 1, atrial natriuretic peptides, and pro-adrenomedullin.

Psychological stress also affects the HPA axis, leading to an exaggerated response to the event. After a stressful event (physical and/or psychological), enhanced activity of the HPA axis may be observed as long as 24 hours after the last stress exposure, indicating that the HPA axis has undergone long-term upregulation. In such situations, CRH synthesis and secretion are increased, but CRH receptor binding is decreased, as a form of downregulatory adaptation to increased CRH secretion. Patients with post traumatic stress disorder (PTSD) have low cortisol levels (72) , enhanced cortisol suppression following dexamethasone administration (73) and an increased number and reactivity of GC receptors in lymphocytes (74) . Those findings reflect an increased negative feedback mechanism of the HPA system of patients with PTSD, when put under stressful conditions. In addition, childhood parental loss is also associated with increased cortisol responses to the dexamethasone/corticotropin-releasing hormone test (75) . Early exposure to stress seems to be particularly important in males. A recent study has shown that in that subpopulation, cortisol response to a stressful situation is blunted (76) .

The HPA axis response to stress is highly variable, according to age, sex, prenatal programming, and even ethnicity (77) . The genetic background plays an important role on the response to stress, as it has been recently demonstrated that girls who were homozygous for the s allele of the promoter region of the serotonin transporter gene produced higher and more prolonged levels of cortisol in response to a stressor (78) . In addition, polymorphisms of brain mineralocorticoid (MR) and glucocorticoid receptors (GR) also determine different individual responses to stress (79) .

In addition to intrinsic factors, extrinsic factors can also modulate the HPA axis responses to stress. For example, psychological manipulation prior to the pharmacological stimulation of the HPA axis can significantly reduce HPA activation in challenge paradigms (80) .

PATHOPHYSIOLOGY OF ALTERED CIRCADIAN HPA ACTIVITY

In order to avoid the genesis of stress-associated GC excess, it is important that the circadian rhythm not be disrupted. Physiologically, the increase in GC after stressful stimuli is beneficial to improve chances of survival. However, prolonged GC excess leads to myriad prejudicial effects. Changes in the GC nadir that occurs close to the onset of sleep alters the regulation of HPA function and other GC-sensitive endpoints, leading to inadequate GC secretion. To avoid GC excess, the nadir must be maintained for 4 to 6 hours (10) . However, not only the levels of GC are important, but also the patterns of pulsatility plays an important role, as studies have shown that different pulse sizes and frequencies determine different effects on MR and GR binding and also probably on MR and GR homodimer and heterodimer formation (81, 82) .

It has been shown that the disruption of the GC nadir, not the elevated 24-h GC levels, is the main factor associated with the detrimental outcomes on the parameters of metabolic syndrome (83) (Figure 12). The effects of elevated GC secretion at the circadian peak are less well-defined. In humans, increased cortisol responses to stress also have been correlated with diminished amplitude of the circadian rhythm (84) .

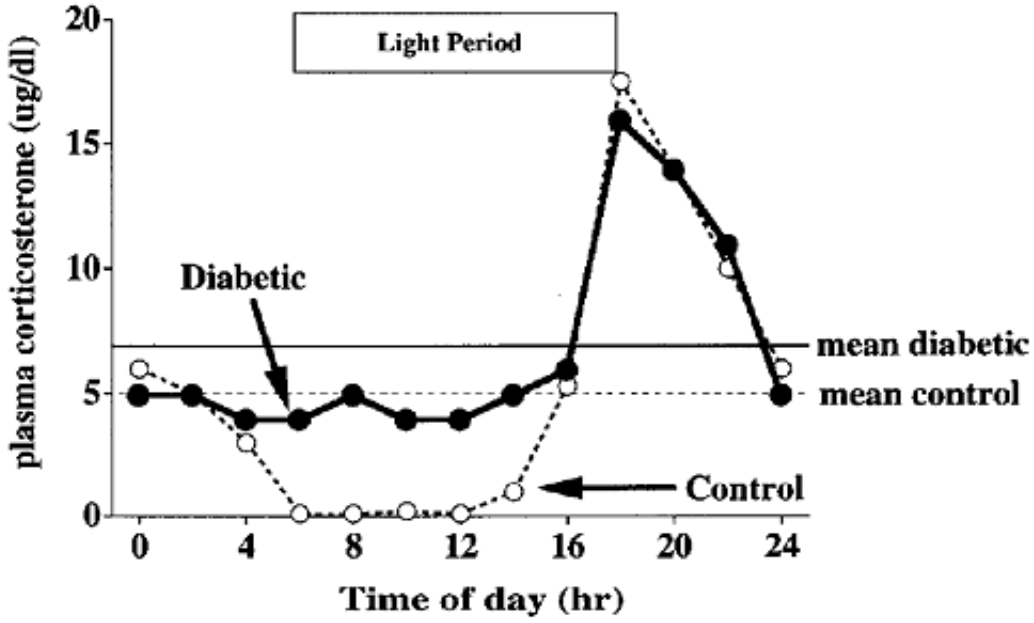

Figure 12:Plasma corticosterone circadian rhythms in normal rats (open symbols, dashed line) and in a diabetic rat under mild stress (solid symbols, solid line). There is not a significant increase in mean daily levels of corticosterone in the diabetic rat. However, during the period of inactivity, the circadian nadir does not occur. Reprinted by permission from Macmillan Publishers Ltd: International Journal of Obesity, ref (83) , copyright 2000.

In addition, different types of stressors lead to different changes in HPA axis rhythmicity. Although increasing stressful experiences (such as PTSD) have been associated with elevated cortisol response, relatively severe trauma has been associated with a blunting of the morning cortisol response. This may be coupled with an increase of the afternoon cortisol levels, which leads to the flattening of the diurnal cortisol release, as described in a recent meta-analysis (85) . According to that analysis, the stress chronicity was fundamental in predicting cortisol changes. Acute stress determined more important changes in the HPA axis, and previous exposition to the stressor minimized those changes. Stressor type, stressor's controllability, and contextual factors were also confounders of the cortisol changes.

During stressful situations, the activation of the HPA axis is sensitive to prior levels of GC receptors occupancy achieved during routine, unstressed HPA activity. This is particularly true when adrenalectomized rats are put on GC replacement in a circadian pattern rather than at a constant level, allowing more efficient termination of ACTH responses to stress.

Stressful stimuli, either physical or psychological, are capable of disrupting the HPA axis homeostasis. Those stimuli can be originated from extra-hypothalamic sites, such as the cathecolaminergic cell groups throughout brainstem, the spinohypothalamic-spinothalamic-spinoreticulothalamic pain pathways, pro-inflammatory cytokines originated from the immune

system, and psychogenic inputs from the medial prefrontal cortex and from the hippocampus. Among the intra-hypothalamic sites responsible for regulating the HPA axis, are the arcuate complex/melanocortin/NPY systems, the periventricular hypothalamic network that functions as a hypothalamic visceromotor pattern generator (constituted by nodes that include five preoptic nuclei and the dorsomedial nucleus of the hypothalamus). This intrahypothalamic pattern generator seems to play an important role on coordinating neuroendocrine, autonomic and behavioral outflows to circadian, immune, and psychogenic stimuli (86) .

Acute stress can change the HPA circadian rhythms, which are sustained for a short period of time. In response to acute stress (tube restraint), rats present a rapid increase in ACTH, which peaks after 10 to 20 minutes after the onset of stress. Then, ACTH tends to fall off towards 60 min, and even further by 120 min, where they often have returned to baseline, despite continued restraint. The adrenal response is more prolonged, due to its saturable response (86, 87) (Figure 13).

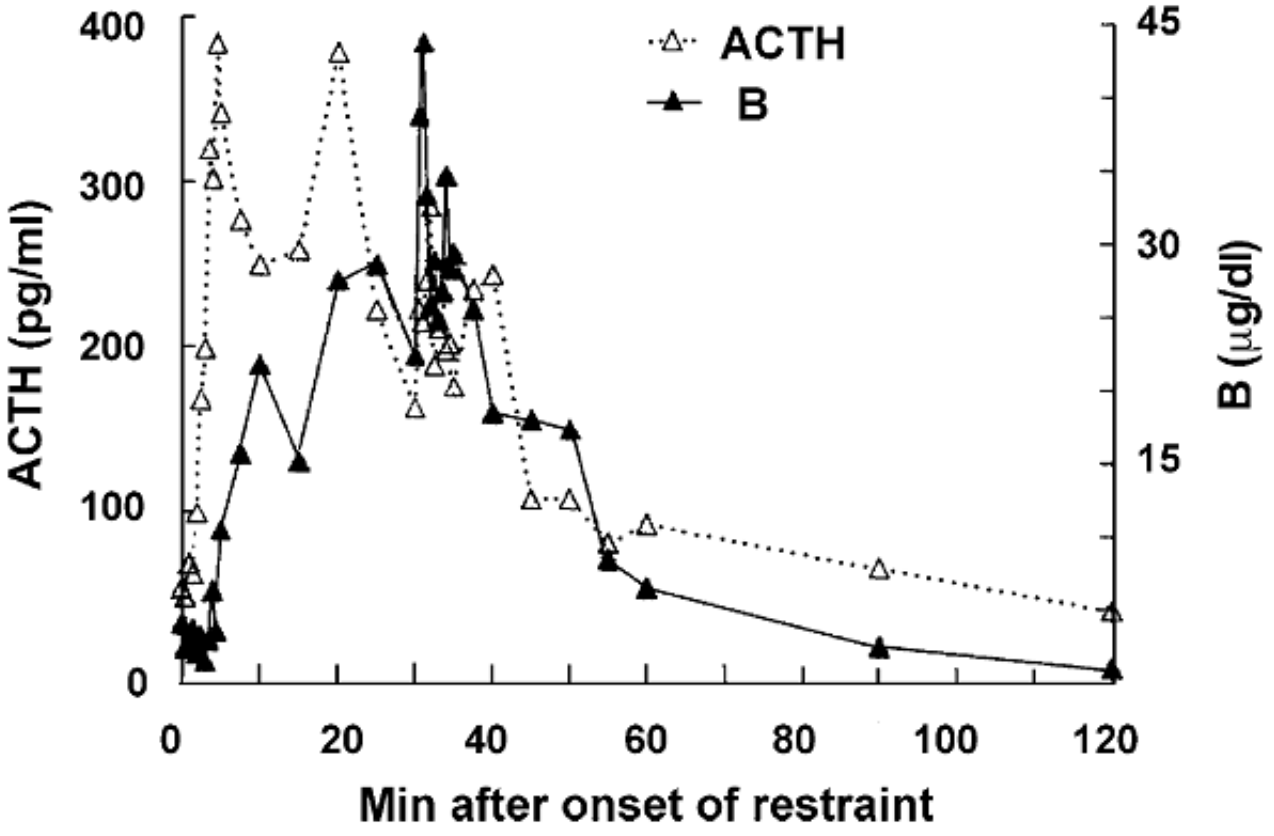

Figure 13:Profile of ACTH and corticosterone (B) responses to stress (restraint). The ACTH is rapidly elevated within minutes of restraint, peaking between 5 and 20 minutes before being trimmed by the slower adrenocortical response that closely follows. Reprinted from ref. (86) , Copyright 2006, with permission from Elsevier.

Acute stress determines increases in the amplitude and the synchronization of the PVN CRH and AVP pulsations. Also, with strong physical stress, secretion of AVP from the magnocellular neurons is increased (88) . Depending on the stressor, other factors, such as angiotensin II, various cytokines, and lipid mediators of inflammation, are secreted and act on hypothalamic, pituitary, or adrenal components of the HPA axis, mostly to potentiate its activity (89) .

Animals under chronic stress have a markedly altered regulation of the circadian and ultradian rhythms. The circadian rhythm is flattened or lost, and the frequency of corticosterone pulses almost doubles (90) .

There seems to be great variability regarding responsiveness to stress, which is attributed to genetic and neonatal influences. Lewis (LEW/N) rats are highly susceptible to the development of rheumatoid arthritis after stimulation of the HPA axis by inflammatory factors. On the other hand, Fischer (F344/N) rats resistant to the development of rheumatoid arthritis (91) . This difference is explained by their unique HPA axis. Lewis rats show clear circadian variation in both pulse frequency and height. Fischer rats exhibit pulses of similar frequency and height to those in Lewis rats during the evening, but show no circadian variation, resulting in higher mean daily corticosterone concentrations. Stress led to different HPA axis responses between the animals, resulting in higher corticosterone levels in the Fischer rats (92) .

In addition to genetic factors, neonatal programming plays an important role on the adult HPA axis ultradian rhythmicity (93) . The administration of neonatal endotoxin results in both an increased frequency of pulses and increased corticosterone pulse amplitude at adulthood (94) . Other factors, such as circulating androgens during gestation can program the activity of the HPA axis (95) .

Cytokines, including the interleukins, interferons, colony-stimulating factors, and tumor necrosis factor, also seem to play a role on the regulation of the HPA axis under stress. Cytokines are secreted as a result of immune and nonimmune stimuli. Interleukin 6 (IL-6) recently has been shown to contribute significantly to adrenocortical activity (96) . IL-6 receptors are present on pituitary corticotrophs and adrenocortical cells, and mice deficient in CRH action have impaired GC in response to stress, glucocorticoid production in response to psychological and metabolic challenge, but near normal responses to stressors that lead to increases in IL-6 after the activation of the immune system. IL-6 stimulates secretion not only of ACTH, but also of AVP by the magnocellular cells, which is associated with acute stress (97) . Besides IL-6, other inflammatory cytokines, such as IL-1 and tumor necrosis factor-alpha can also stimulate the HPA axis (98) .

Leptin, an adipocyte hormone, is a major regulator of neuroendocrine function. Leptin has an overall inhibitory effect on HPA activity (99) , aimed at suppressing the appetite-stimulating effects of GC. Leptin’s effects are explained by its ability to inhibit CRH release, during normal or stressful situations (100) . The decrease in leptin levels caused by starvation, a stressful situation, leads to increases in HPA activity (101) . In leptin-deficient adults, cortisol dynamics is characterized by a higher number of smaller peaks, with smaller morning rise, increased relative variability, and increased pattern irregularity. Replacement treatment with recombinant human methionyl leptin (r-metHuLeptin) organized the dynamics of human HPA in these patients (48) .

In patients with anorexia nervosa, or in highly trained athletes, elevated cortisol levels are probably a consequence of a relative, functional leptin deficiency induced by the paucity of adipose tissue in conjunction with food restriction and intensive energy expenditure.

More recently, it has been suggested that the mere anticipation of a new day can alter the HPA axis. In particular, this anticipation can alter the cortisol awakening response (CAR), which is distinct feature of the HPA axis, superimposing the circadian rhythmicity of cortisol secretion (22) . The consequences of these alterations remain to be understood.

CONCLUSIONS

Stress-induced changes in HPA axis are important to facilitate survival against external or internal stressors. Those changes vary across individuals, as determined by myriad factors, such as genetic make-up, neonatal and early-life environment, and previous experiences. In addition, many factors regarding the type of stress play a major role on eliciting the HPA axis response.

The stress-induced responses of the HPA axis overcome the negative feedback provided by endogenous cortisol levels. It has been extensively shown that not only the GC levels, but also their pulsatility and rhythmicity influence the outcomes determined by hypercortisolism. Stress disrupts the HPA axis rhythmicity by altering the hormonal pulses' amplitude and frequency as well as its circadian cycles.

Rapid social and economic changes with high levels of stress are the hallmarks of contemporary western and global civilizations. It is therefore critical to fully elucidate stress-induced alterations of HPA axis rhythmicity and their effects on health and disease.

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