

# Restricting environmental stimulation influences levels and variability of plasma cortisol

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## Summary

Restricting stimulation from the environment has been shown to alter psychological and physiological states. The present study of 27 healthy subjects examines the effects of restricted environmental stimulation technique (REST) on plasma levels of cortisol and variability in plasma cortisol levels across repeated REST sessions.

The REST environment consisted of a 1.2 X 1.2 X 2.4-m ovoid chamber containing 25 cm of saturated MgSO<sub>4</sub> solution (sp gr 1.28) maintained at 34.5°C. The buoyant supinely floating subject experienced a minimum of light, sound, and temperature awareness and spatial orientation.

The non-REST environment was a cushioned reclining chair in a quiet dimly lit room.

The 5-wk protocol consisted of four visits for blood sampling during a 2-wk baseline followed by eight REST or non-REST sessions, 40 min each, with blood samples taken on four non-session days between sessions 5 and 8.

Variability in plasma cortisol was expressed in terms of standard deviation.

REST was associated with across-session decreases of 21.6% in plasma cortisol and 50.5% in plasma cortisol variability, whereas no changes in these measures occurred in non-REST.

**It is concluded that REST influences both static and dynamic aspects of adrenocortical function, possibly altering the feedback monitoring of plasma cortisol.**

## **Introduction**

Increased levels of plasma cortisol in humans have been reported in association with a variety of psychological and physiological stress conditions (13, 16). Conversely, decreases in blood pressure (BP) (3, 7) and plasma levels of several hormones, including cortisol(22) and aldosterone and renin activity (12), have been reported in association with repeated brief exposure to a sensorily restricted environment. One version of this condition is flotation REST (restricted environmental stimulation technique), in which the individual lies supinely in thermoneutral buoyant fluid with minimized photic, auditory, and tactile stimulation (9, 19).

To date, studies of the relationship of restricted sensory input to physiological activity have been limited to point-in-time measurements of given parameters, which give little information on the dynamics of the system. One possible dynamic measure of a system is the variability of its measured parameters (6, 15), and standard deviation (SD) around mean values for a given parameter is a statistic that describes such variability.

The involvement of central nervous mechanisms in the dynamic regulation of physiological systems has been evidenced in several studies of BP regulation. Increased variability in mean arterial BP has been demonstrated in several species after disconnection of baroreceptor input to BP regulation (2, 11, 21). Because flotation REST (henceforth, REST) greatly attenuates the input of sensory information about light, sound, kinesthetics, and temperature, it was of interest to assess the possible impact of REST on dynamic aspects of physiological regulation.

Plasma cortisol was chosen as the monitored parameter for the present study on the bases that REST effects on the activity of this hormone have been previously demonstrated (8,15) and feedback regulation of plasma cortisol has been well researched.

In this study the effect of brief repeated REST on plasma levels of cortisol and their variability is examined. The study is designed to minimize the amount of protocol-related disruption that subjects experienced in their normal daily life.

## **MATERIALS AND METHODS**

Twenty-seven healthy subjects (18 males, 9 females) ranging in age from 21 to 32 yr were recruited. A brief medical history was taken, and subjects were screened for normalcy of sleep-wake cycles and diet and for absence of adrenal-stimulating medications. Three subjects dropped out during the study for personal reasons unrelated to the study. The subjects were pair-matched on the basis of initial midday values of the measured end point, plasma cortisol, and were split into a REST group (n = 15, 5 females) and a non-REST group (n = 12, 4 females). The REST condition consisted of a 1.2 X 1.2 X 2.4-m ovoid chamber (Enrichment Enterprises, Huntington, NY) completely enclosed and filled to a 25-cm depth with saturated MgSO<sub>4</sub> solution (sp gr 1.28)

maintained at 34.5°C. The buoyant supinely floating subject experienced the absence of light and a minimum of sound (~10 dB), temperature awareness, and spatial orientation. The non-REST condition consisted of a cushioned reclining chair, fully reclined in a warm (29°C) quiet (~30 dB) dimly lit (~1 ft cd) room. The 5-wk protocol was identical for each subject and consisted of four visits for blood sampling during a 2-wk baseline followed by eight REST (or non-REST) sessions, one every 3rd day for 3 wk. Blood samples were taken on the day before sessions 5-8. Samples were taken by an experienced phlebotomist via forearm venipuncture into heparinized tubes and were immediately centrifuged. The plasma was frozen and stored at -60°C until assayed for cortisol by radioimmunoassay (kit code KCOD2, Diagnostic Products, Los Angeles, CA).

The blood sampling protocol was designed in consideration of the diurnal variation and episodic secretion patterns of cortisol (25) such that, for each blood sampling visit, 1) the subject arrived and sat quietly for 5 min before sampling, 2) blood was drawn between 1200 and 1400 h, and 3) each blood sampling event consisted of two samples, 10 ml each, taken 20 min apart. Thus for each subject there were four sampling sessions in baseline and four in treatment, with a and b samples in each session. Determination of the reproducibility of values in the paired a and b samples provided a means to assess reliability of the cortisol measure in each session, because significant changes in plasma cortisol over 20 min were unlikely under these conditions (1,27). The overall difference between a and b samples was 7.8%, and a and b samples for each session were pooled. Because of potential effects of meals on cortisol release, all subjects were instructed to eat their regular breakfast and not to eat lunch until after the blood sampling session on sampling days. For each treatment session the subject undressed, showered briefly, experienced REST or non-REST for 40 min, showered again (REST only), dressed, and departed. Average total session time was 70 min. Subjects were encouraged to discuss a given session when it was over and were requested to report any unusually stressful experience during the study. A brief subjective report questionnaire was completed by each subject after each treatment phase session.

## RESULTS

Plasma cortisol data are presented in Fig. 1. These data were subjected to two-way repeated-measures analysis of variance. Hypothesis testing for cortisol data was accomplished with the parametric Tukey's test and F test (18). There were no differences among sessions within baseline or among monitored sessions within treatment in either the REST or non-REST groups. In other words, plasma cortisol levels did not change significantly during baseline or during the portion of treatment that was monitored in either REST or non-REST. Plasma cortisol values in REST treatment were lower ( $P < 0.05$ , Tukey's test) than in REST baseline, non-REST baseline, and non-REST treatment. Cortisol values in the latter three were not different. The mean cortisol level (across sessions and subjects) was  $11.29 \pm 0.37$  (SE) pg/dl in REST treatment and  $14.21 \pm 0.82$  in REST baseline. As a dependent variable in this study, variability refers to the classical statistical definition of measures of dispersion and is reported as SD, which is the square root of the variance  $iN SD = \sqrt{C \sum (X_i - \bar{X})^2 / N - i}$   $V \ i=1$ . For each subject, SD was calculated across samples for baseline (4 cortisol values) and treatment (4 cortisol values). These individual SD values were then averaged across subjects in baseline or in treatment to yield SD values as follows:

REST baseline, REST treatment, non- REST baseline, and non-REST treatment. To account for the possibility that lower SD values in a given condition were simply the consequence of plasma cortisol values being lower, the coefficient of variation (SD/Z 100) was determined for each SD. Plasma cortisol variability, reported as SD and as coefficient of variation (Table 1), was subjected to statistical analysis. Although parametric statistics are normally more robust than nonparametric statistics, the latter do not require normalcy of distribution and equal variances in the sample populations to be compared. Because it was hypothesized that variability could be influenced by TABLE 1. Effect of repeated brief REST on variability of plasma cortisol values

|  | REST (n = 15) | Non-REST (n = 12) | Baseline | Treatment | Baseline | Treatment | Plasma cortisol, cLg/dl | SD   | CV    |
|--|---------------|-------------------|----------|-----------|----------|-----------|-------------------------|------|-------|
|  | 14.21         | 11.29             | 13.77    | 14.14     | 3.86     | 1.91*     | 3.51                    | 3.77 | 29.86 |
|  | 18.56*        | 26.85             | 30.06    |           |          |           |                         |      |       |

n, No. of subjs. Values were calculated for 4 samples from each subject and then averaged across subjects in each group. REST, restricted environmental stimulation technique; SD, standard deviation; CV, coeff of variation [(SD of cortisol value/cortisol value) X 100]. \* Different from Baseline (P < 0.005, Wilcoxon matched pairs test). 2012 RESTRICTED ENVIRONMENTAL STIMULI AND PLASMA CORTISOL treatment in this study, data were tested for significant differences by a nonparametric test. The SD and coefficient of variation were 50.5 and 37.9%, respectively, lower in REST treatment than in REST baseline, and the changes in both parameters were significant (P < 0.005, Wilcoxon matched pairs test). In the non-REST group there was no difference in either SD or coefficient of variation between baseline and treatment. Eighty-seven percent of the REST subjects showed decreased plasma cortisol across sessions, and 93% showed decreased SD for cortisol. A Pearson correlation was performed on REST group data, comparing acrosssession percent change in plasma cortisol with acrosssession percent change in the SD of plasma cortisol. These changes were not correlated (r = 0.12; NS, P > 0.05, df = 13).

**DISCUSSION** Both the concentration and the variability in concentration of cortisol in plasma were decreased across sessions in the REST group, whereas no changes occurred in the non-REST group. These data suggest a REST-specific effect on the activity of the adrenal cortex or the clearance of cortisol or both. The present study does not differentiate between these possibilities. Although the metabolic clearance rate (MCR) for cortisol has been shown to increase 19-30% after exogenous pharmacological- dose ACTH administration (27), it appears unlikely that cortisol clearance changes significantly in unstressed individuals (1, 20). In the present study of unstressed healthy subjects, mean plasma cortisol decreased 21.6%. The decrease in plasma cortisol levels was not surprising, because previous studies have demonstrated decreased plasma cortisol across REST sessions in normal subjects (22). The decrease in variability around the mean value of plasma cortisol from baseline to treatment in the REST group, but not in the non-REST controls, suggests that the conditions of REST can influence the dynamic state of cortisol regulation. The coefficient of variation data demonstrate that the decrease in variability is not due simply to smaller SD values accompanying smaller absolute cortisol values. In fact, the results of the Pearson correlation show that changes in plasma cortisol and changes in the SD of mean plasma cortisol were not significantly correlated. This suggests that the effects of REST on absolute cortisol levels can occur independently of the REST effects on the variability of cortisol levels. It is known that cortisol exhibits episodic pulsatile release (8), with considerable variation occurring within and between individuals (10,25). Also, it has been shown that there is a cortisol peak associated with mealtimes (4). These factors may have contributed a significant “noise” component to the variance data. However, it appears that the REST effect was robust enough to have a discernible impact on variability, because SD values were decreased across treatment in REST but not in non- REST. Sampling

was too infrequent in the present study to determine whether changes in pulse frequency or pulse height were associated with REST. A large number of blood samples taken 15-20 min apart would be necessary for analysis of pulsatile release characteristics (23, 24). Such analysis would require the use of an in-line venous catheter. This approach was not chosen for this study, because it precluded assessment of the effect under "normal" circumstances. The present study was designed to minimize the amount of disruption that subjects experienced in their normal daily life. This was done to monitor the dynamic physiological system across time under "natural" everyday conditions. A more radical monitoring such as in-line blood sampling over many hours might confound the interpretation of the treatment effect. Although the control condition could potentially obviate this problem, there is the possibility that the stress associated with extended intravenous monitoring would wash out the REST effect. The present study is, to our knowledge, the first report of external environmental conditions influencing the variability of plasma cortisol levels. In the conceptual framework of cybernetic theory, variability is one measure of the dynamic state of a negative feedback loop (15, 26). Plasma cortisol is one component of an integrated negative feedback loop. Thus plasma cortisol variability is one reflective measure of the dynamics of physiological regulation. In the present controlled study, the intervention (REST) was associated with a change in plasma cortisol levels and variability over time; i.e., REST influenced both the total output and the dynamics of the physiological system. This result is consistent with other studies in which variability in a measured parameter was influenced by a central nervous system-mediated intervention. For example, Cowley et al. (2) observed increased variability in mean arterial pressure after BP baroreceptor disconnection in dogs, various mental and physical stressors have been shown to influence BP variability in humans (5,17), and Porges (14) reported decreased variability in heart rate in association with increased attention to a reaction time task. It should be noted that these studies examined immediate response dynamics, whereas the present study examined longer-term dynamics. It has previously been reported that repeated REST can be associated with cortisol changes that persist for days beyond the REST sessions (22). Likewise, REST effectiveness in BP reduction in essential hypertensives may continue for weeks to months beyond cessation of treatment (3, 12). This raises the possibility that REST may contribute to reorganization of set points for the operation of physiological feedback loops. The cortisol variability data in the present study are consistent with such a hypothesis, although they do not address either the mechanism by which REST affects plasma cortisol levels or whether REST specifically facilitates or improves feedback regulation of plasma cortisol levels.

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