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Measuring Stress using Saliva Data Sets

Mrs.M.Anusha and Mrs.G.SriNaganya

** Programmer, Department of MCA, Shrimati Indira Gandhi College, Tiruchirappalli, Tamil Nadu, India.*

INTRODUCTION

Stress:

Stress is a popular topic these days. There is rarely a week that passes without hearing or reading about stress and its deleterious effects on health. Given the negative impact of stress on human health, many types of stress management therapies have been put forward in order to decrease stress and to promote well being. However, there is a great paradox in the field of stress research, and it relates to the fact that the popular definition of stress is very different from the scientific definition of stress. This inconsistency has left a multitude of people and experts talking about, and working on very different aspects of the stress system.

Indeed, if this were true, every individual would feel stressed when pressured by time. However, many of us know that while there are some people who are extremely stressed by time pressure, there are others who thrive under time pressure. This shows that stress is a highly individualistic experience that does not depend on a particular event such as time pressure, but rather depends on specific psychological determinants that trigger a stress response.

The stress response

When a situation is interpreted as being stressful, it triggers the activation of the hypothalamic-pituitary-adrenal (HPA)[1] axis whereby neurons in the hypothalamus, a brain structure often termed the "master gland", releases a hormone called corticotropin-releasing hormone (CRH)[2]. The release of CRH triggers the subsequent secretion and release of another hormone called adrenocorticotropin (ACTH) from the pituitary gland, also located in the brain.

When ACTH is secreted by the pituitary gland, it travels in the blood and reaches the adrenal glands, which are located above the kidneys, and triggers secretion of the so-called stress hormones.

There are two main stress hormones, the glucocorticoids (called corticosterone in animals, and cortisol in humans), and the catecholamines (epinephrine and norepinephrine).

Under normal (non-stressed) conditions, cortisol[3] secretion shows pronounced circadian rhythmycity, where concentrations are at their highest in the morning (the circadian peak), progressively decline from late afternoon to early nocturnal periods (the circadian trough), and show abrupt elevations after the first few hours of sleep.

The acute secretion of glucocorticoids and catecholamines constitutes the primary mediators in the chain of hormonal events triggered in response to stress. When these two hormones are secreted in response to stress, they act on the body to give rise to the fight-or-flight response whereby one would, for instance, experience an increase in heart rate and blood pressure.

Keywords: glucocorticoids, catecholamines, cortisol, hormones.

1. Measuring Stress

1.1 Physiological Measures of Stress

The interpretation of a situation as being stressful leads to the activation of the hypothalamic-pituitary-adrenal (HPA) axis, and to the ultimate secretion of cortisol and catecholamines[4] in humans. The end products of HPA activation (cortisol and catecholamines) are easily measurable in blood, urine and saliva.

This is not the case for other markers of HPA activation such as ACTH and CRF levels. ACTH can only be measured in blood and CRF can only be measured in cerebrospinal fluid. Given the ease with which one can assess the end products of HPA activation, several studies are now using cortisol and proxy measures of sympathetic activation (catecholamine) as validated physiological measures of stress in humans.

Cortisol and catecholamines can be measured in blood. However, new studies now show that cortisol can also be sampled in saliva data set, a technique that has been preferred by researchers for its non-invasive advantage.

1.1.1 Autonomic measures

Salivary cortisol as a biomarker of stress

Cortisol measured in saliva reflects the fraction of cortisol that is “free” or “unbound” (to carrier proteins), the portion that crosses the blood-brain-barrier to affect different brain structures. This mechanism is believed to be at the basis for alterations in higher-order cognitive functions and behavior. This free fraction of cortisol, after crossing the blood-brain-barrier, binds to receptors in brain structures that are known to be involved in learning, memory, and emotional processing.

2. Aspects to measure HPA axis

2.1. Diurnal cortisol secretion

Under natural unstimulated conditions, the secretion of cortisol follows a circadian rhythm characterized by a peak in the early morning hours, followed by declining cortisol concentrations throughout the day, reaching the lowest levels during the late evening. This rhythm is influenced by altered sleep patterns and exposure to daily life

stressors. While this pattern of diurnal cortisol secretion has been widely published, it has been shown that individuals may deviate from this "typical pattern". Indeed, both inter-individual differences and intra-individual differences in the diurnal pattern of cortisol secretion have been reported.

2.2. Calculation of diurnal cortisol subgroups

Below calculations allow one to assess stability and characteristic of the diurnal cortisol slope over a 2-day period. Specifically, based on a 2-day sampling period, individuals may be categorized into one of three possible subgroups: the Typical diurnal subgroup, in which the individual displays the typical cortisol peak and decline throughout the day (as described above); the Flat diurnal subgroup, in which evening cortisol levels fail to decline to the common nadir phase and remain relatively elevated; finally, the Inconsistent subgroup, in which individuals display both the Typical and Flat pattern on alternate days (e.g. flat on day one and typical on day two).

In order to obtain these three subgroups within a given population, 3 calculation steps are followed:

- STEP 1. Determine the cortisol slope of Day 1 and Day 2: First, one must log-transform all cortisol values for both sampling days. Once values are transformed, the slope for Day 1 and Day 2 must be calculated.
- STEP 2. Determine which individuals display a consistent profile (flat or typical) and which individuals display an inconsistent profile: In order to do this, one must take the difference score between the slopes of Day 1 and Day 2 (i.e. slope Day 2 – slope Day 1). From this difference score, calculate the standard deviation (SD). If the absolute difference score is greater than 1 SD, the diurnal cycle for that individual is characterized as Inconsistent. Why? Because there is a significant difference (greater than 1 SD) between their Day 1 and Day 2 slope.
- STEP 3. For the remaining individuals (i.e. those who have not been categorized as Inconsistent), determine which individuals display a Typical cycle and which individuals display a Flat cycle: In order to do this, one must obtain the average of the two slopes. From the average score, those who display a slope that is more positive than the (-)SD are labeled as Flat (i.e. less of a decline in cortisol over time) and those who display a slope that is more negative than the (-)SD are labeled Typical (i.e. more of a decline in cortisol over time).

Below is an example of calculations: Five saliva data sets were collected for Day 1 and Day 2. On both days, samples were collected at 9:00AM, 12:00PM, 3:00PM, 5:00PM. Table 1, shows the log-transformed concentrations on each day.

Table 1:. Samples on both days.

	Day one				Day Two			
Code	9 AM	12 PM	3 PM	6 PM	9 AM	12 PM	3 PM	6 PM
F01	0.4	0.58	0.8	0.43	0.65	0.54	0.27	0.22
F02	0.53	0.8	0.46	0.2	0.72	0.59	0.47	0.23
F03	0.81	0.64	0.4	0.28	0.79	0.47	0.39	0.13
F04	0.74	0.76	0.57	0.59	0.64	0.75	0.42	0.56
F05	0.72	0.45	0.27	0.21	0.52	0.49	0.3	0.35

The cortisol slopes for Day 1 and Day 2 in Table 2.

Table 2: Calculation of slope. Calculation of Difference between slope 1 and slope Day 1

Day 1 Slope	Day2 Slope	Difference	Inconsistent/ Consistent?	Average Slope	Typical/ Flat?	Subgroup
0.010333	-0.052	0.062333	Inconsistent	-0.02083		Inconsistent
-0.04433	-0.053	0.008667	Consistent	-0.04867	Flat	Flat
-0.0418	-0.06867	0.026867	Consistent	-0.05523	Typical	Typical
-0.02133	-0.019	-0.00233	Consistent	-0.02017	Flat	Flat
-0.057	-0.02333	-0.02017	Consistent	-0.04017	Typical	Typical
		SD=0.035552			Typical	Typical

The slopes for Day 1 and Day 2, were calculated using the difference score (3rd column Table 2). Then, calculate the SD of the difference scores (=0.035552). If the absolute value of the difference score is greater than the SD, then the person is labeled as Inconsistent (4th column Table 2).

Flat or Typical were calculated using Inconsistent. By, calculating the average of the Day 1 and Day 2 slopes (3^{rd} and 5^{th} columns of Table 2), In this the data sets, a more negative (average) slope were labeled Typical and a more positive (average) slope were labeled Flat (2^{nd} and 4^{th} column Table 2).

3. Analyzing

Cortisol reactivity can be computed many different ways, e.g. as increments, percentage, ratio. Below is an example 5 data sets, those were exposed to a 10-minute stressor (between 0 and 10 min). Saliva data sets for cortisol assay were collected at baseline (0 min), right after the stressor (10 min), and at +20, +35 and +50 min after the end of the stressor[5].

Table 3: Increment, percentage and ratio cortisol response

CODE	Minutes					Increment	%	Ratio
	0	10	20	35	50			
F01	0.25	0.56	0.80	0.43	0.38	0.55	220.0	0.3
F02	0.31	0.47	0.64	0.46	0.23	0.33	106.5	0.5
F03	0.28	0.32	0.63	0.54	0.31	0.35	125.0	2.3
F04	0.34	0.44	0.58	0.65	0.37	0.24	70.6	0.6
F05	0.22	0.32	0.70	0.54	0.28	0.48	218.2	0.3

3.1. Increment

The data set F03 shows, the increment or change in cortisol from baseline (0 min) to 10 minutes after the end of the stressor (20 min) would be: Increment = concentration at 20min – concentration at 0min = 0.63 μg/dL – 0.28 μg/dL = 0.35 μg/dL

The data set F03 showed an increase of 0.35 μg/dL in cortisol, 10 minutes after exposure to the stressor.

3.2. Percentage

For the same data set (F03), the percentage of increase from baseline (0 min) to 10 min after the stressor (20 min) would be: Percentage = 100 x (concentration at 20min – concentration at 0min)/concentration at 0min = 100 x (0.63 μg/dL – 0.28 μg/dL)/ 0.28 μg/dL = 125.0%

The data set F03 showed a 125.0% increase from baseline to 10 minutes after the end of the stressor.

3.3. Ratio

The increment and the percentage of increase should be distinguished from the ratio, which is calculated using the following formula: Ratio = concentration 0 min / concentration 20 min.For instance, for the same data set F03, Ratio = 0.63 μg/dL / 0.28 μg/dL = 2.3-fold increase

Thus, In the data set F03 showed an increase of 0.35 μg/dL or an increase of 125.0% in free salivary cortisol from baseline to 10 min after the end of the stressor, it showed a 2.3-fold increase in cortisol during that period.

Conclusion

Based on the above result, it has been identify that a 1-fold increase would mean that there was no change in cortisol concentrations between these two time points. 0% increase would mean that there was no change in cortisol between these two time points.

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