

## CORTISOL LEVELS AND AUTONOMIC CONTROL OF HEART RATE IN HEALTHY SUBJECTS

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### Abstract

**Introduction:** There is an increase in level of stress in the general population because of the social, personal and professional demands. Currently, there are only simple tools that can safely measure this stress such as levels of cortisol and heart rate variability (HRV).

**Objective:** To analyze the relationship between salivary cortisol and the cardiac autonomic modulation. Methods: A total of fifty-one male and female subjects between 18 and 40 years old were evaluated. Saliva collection was achieved for the salivary cortisol dosage. The collection was performed through the Salivette® tube. After this collection, the median cortisol levels (0.24 ug/dl) were analyzed and the volunteers were divided into two groups: i) cortisol below the median ii) cortisol above the median. After this division, each group consisted of 25 volunteers and then was verified the following information: age, gender, weight, height, body mass index (BMI), blood pressure. Shortly thereafter was assessment of cardiac autonomic modulation por meio da HRV. The Polar RS800cx heart rate receiver was placed on the chest of the volunteers, in the vicinity of the distal third of the sternum. The volunteers were instructed to remain in rest with spontaneous breathing in dorsal position for 20 minutes. HRV analysis included geometric, time and frequency domain indices.

**Results:** There were no statistical differences for the two groups regarding systolic and diastolic blood pressure, heart rate, RR intervals or linear and frequency indices for the volunteers. In addition, also there was no correlation the cortisol with the analyzed variables (SAP,  $p=0.460$ ; DAP,  $p=0.270$ ; HR,  $p=0.360$ ; RR,  $p=0.380$ ; SDNN,  $p=0.510$ ; rMSSD,  $p=0.660$ ; pNN50,  $p=0.820$ ; RRtri,  $p=0.170$ ; TINN,  $p=0.470$ ; SD1,  $p=0.650$ ; SD2,  $p=0.500$ ; LF [ $ms^2$ ],  $p=0.880$ ; LF [nu],  $p=0.970$ ; HF [ $ms^2$ ],  $p=0.870$ ; HF [nu],  $p=0.960$ ; LF/HF,  $p=0.380$

**Conclusion:** Heart rate variability autonomic control was unchanged in healthy subjects with physiological distribution of salivary cortisol levels. There was no association between normal salivary cortisol and resting autonomic regulation of heart rate.

**Key words:** cardiovascular system; autonomic nervous system, salivary cortisol.

### INTRODUCTION

Contemporary lifestyles generate a high level of psychological stress that causes marked subjective and biological responses (1). In chronic cases, it can initiate deficiencies in physical and mental health (2,3), and increase the biological aging processes (3).

Contrary to this background, there has been an increase of the psychological disorders such as anxiety and depression, and these disorders are associated with increased cardiovascular risk (4). Deviations in homeostasis of autonomic function with an imbalance between the sympathetic and parasympathetic autonomic nervous systems could be reasons involved in this increased cardiovascular risk (5,6).

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Physical or emotional stress stimulates the hypothalamus to synthesize corticotrophin releasing hormone (CRH), which thereby stimulates the pituitary gland to synthesize the adrenocorticotrophic hormone (ACTH), which permeates through the bloodstream to the adrenal glands and stimulates the synthesis of cortisol (7). Concurrently, activation of the sympathetic nervous system occurs and catecholamine's (noradrenaline and adrenaline) are released, which activate an increase in the frequency and strength of cardiac contraction, cardiac output and blood pressure (8). Cortisol is the primary effector hormone of the hypothalamic-pituitary-adrenal axis stress response system (9).

Thus, a technique that has been revealed to be effective in measuring stress and anxiety is the quantification of cortisol in saliva. As there is a direct relationship between the levels of some hormones and medications in saliva and blood, the salivary cortisol dosage has been applied owing to the simplicity in sample collection and the sensitivity of the technique (7).

The regulation of the cardiac function, stimulated by the autonomic nervous system (ANS), allows us to evaluate the imbalance between the activity of the sympathetic and parasympathetic responses. With regards to the heart, the ANS interferes with cardiac frequency and contractility. In addition to increasing or decreasing heart rate, the autonomic system also hinders the regularity between heart beats. This variation in the intervals between one heart beat and another is referred to as the heart rate variability (HRV) (10,11).

HRV has emerged as a simple, inexpensive, reliable and non-invasive measure of autonomic impulses. It represents one of the most promising quantitative markers of autonomic balance (10). Power spectral analysis of RR interval variability has been widely applied to assess cardiac autonomic modulation (12).

Consequently, since there is now an elevation in levels of stress in the population due to social, personal and professional strains these are simple markers that can securely measure these stresses – cortisol levels and HRV. The objective of this study was to verify the association between salivary cortisol and autonomic cardiac regulation. It is speculated that there is a relationship between the two measures since cortisol is the primary effector hormone of the stress response system.

## METHODS

### Study population

We evaluated a total of 50 healthy subjects (25 men) aged 18 to 40 years old. All subjects were informed about the study's procedures and objectives and, after agreeing, confidentially signed an informed consent form. The median salivary cortisol level was calculated (0.24 ug/dl) and the subjects were divided into two groups: G1 - cortisol below 0.24 ug/dl (n=25) and G2 - cortisol above 0.24 ug/dl (n=25). The research was approved by the Research Ethics Committee in Research (Number 993.350).

### Non-inclusion Criteria

We excluded smokers, subjects with cardiorespiratory, metabolic or neurological disorders that prevented the subjects from performing the procedures, in addition to treatment with medications that influence cardiac autonomic regulation. Also, women at 10–15 days and 20–25 days after the first day of the menstrual cycle and pregnant women so as to circumvent influence of the luteal and follicular phase of the menstrual cycle.

### Experimental Protocols

Data collection was performed in a laboratory with the temperature between 21 °C and 25 °C, humidity amid 50 % and 60 % and time fixed between 8:00 am and 12:00 am to evade circadian cycle effects. Volunteers were instructed not to ingest alcoholic beverages, caffeine or other ANS stimulants in the 24 hours before the evaluation and to remain silent during the data collection. The following information was registered: age [years], gender [masculine/feminine], weight [kg], height [meters] and body mass index (BMI) [kg/m<sup>2</sup>].

### Cortisol Levels Measurement

The subjects were instructed to avoid eating and drinking one hour before collecting the saliva and washed their oral cavity with water. The Salivette® tubes were stored under refrigeration until material analysis.

The collection was performed through the Salivette® tube, consisting of a plastic tube containing a cotton roller of high absorption and kept in the oral cavity for about three minutes and then placed in the holder inside the plastic tube collector. Later, after collection of all samples the Salivette® tube was centrifuged for 20 minutes at 2800 rpm and at a temperature of 4 °C. During centrifugation, the saliva passed from the cylindrical shape of the swab through the cavity at the bottom of the suspended tube to the clean centrifuge tube. Secretion and suspended particles were located at the conical tip of the tube, to allow easy decanting of the clarified saliva.

Following centrifugation, saliva was transferred to a capped eppendorff tube and remained in a freezer at -20 °C until the time of the assay. During the transportation phase, the material was chilled by the use of dry ice, Styrofoam and thermal bag. The samples were processed using the electro-chemiluminescence method.

### Blood pressure measurement

The blood pressure was measured using the auscultatory method, via an aneroid sphygmomanometer (Tycos®) and stethoscope (Sprague®). The values of systolic blood pressure and diastolic blood pressure were also recorded. The values of systolic blood pressure of at most 140 mmHg and diastolic of 90 mmHg were considered to avoid a potential effect of arterial hypertension on the results.

### HRV analysis

During RR intervals recording the subjects remained 20 minutes at seated rest under spontaneous breathing. We used a 10 minutes for stabilization of autonomic balance.

HRV analysis was performed according to directives from the Task Force publication <sup>(13)</sup>. HRV was recorded using the Polar RS800cx chest strap measuring device (Polar Electro Oy, Kempele, Finland) which was validated to capture beat-to-beat heart rate (HR) <sup>(14)</sup>. Particulars of HRV analysis were specified in the cited studies <sup>(15)</sup>. HRV analysis included geometric, time and frequency domain indices which were computed by Kubios HRV® analysis software.

The Polar transmitter detects all heart beats through electrical signals in the left ventricle muscle and the data recorded transmit the signal to the computer with an infrared approach through a wireless technology. The software enabled the visualization of HR and the extraction of a cardiac period (RR interval) file in "txt" format.

Digital filtering was undertaken to remove artifacts. The filtering process method in the software included an algorithm that calculates median and moving average based filtering methods to detect errors. The algorithm calculated several more matching 'candidates' to replace the identified errors. Before elaborating the preview curve, which presents the optional corrections, the algorithm verifies the difference between consecutive accepted RR intervals and makes the series of corrected values (typically 2-4 intervals) to follow identical differential coefficient. The error correction algorithm precisely preserves the total time of the recording, i.e. the sum of RR intervals is exactly the same as the elapsed time. The minimum protection zone used was six beats per minute ([http://support.polar.com/en/support/tips/How\\_R-R\\_Data\\_is\\_Filtered](http://support.polar.com/en/support/tips/How_R-R_Data_is_Filtered)).

Following digital filtering supplemented with manual filtering for the elimination of artifacts, 256 stable RR intervals were necessary for the data analysis. Only series with more than 95 % sinus rhythm was included in the study (13).

The time domain analysis was performed in terms of SDNN (standard deviation of normal-to-normal RR intervals), pNN50 (percentage of adjacent RR intervals with a difference of duration greater than 50 ms) and RMSSD (root-mean square of differences between adjacent normal RR intervals in a time interval) (Table 1) (13).

To obtain the spectral indexes for HRV analysis in the frequency domain, the frequency recordings underwent mathematical processing, generating a tachogram that expressed the variation of RR intervals as a function of time. The tachogram contained a signal apparently periodic that varies in time and was processed by the mathematical Fast Fourier Transform algorithms (13).

Low frequency (LF- ranging between 0.04 and 0.15 Hz) and high frequency (HF- ranging from 0.15 to 0.4 Hz) spectral components were appointed in normalized units (n.u.). The ratio between these components in absolute values (LF/HF) represents the relative value of each spectral component in relation to the total potential minus the very low frequency (VLF) components (13).

The triangular index (RRtri) was calculated from the construction of a density histogram of RR intervals, which contains the horizontal axis of all possible RR intervals measured on a discrete scale with 7.8125 ms boxes (1/128 seconds) and on the vertical axis, the frequency with which each occurred. The union of points of the histogram columns forms a triangle-like shape. The RRtri was obtained by dividing the number of RR intervals used to construct the histogram by their modal frequency (i.e., the RR interval that most frequently appeared on RR).

The triangular interpolation (TINN) consists of the measure of the base of a triangle. The method of least squares is used to determine the triangle. The RRtri and the TINN express the overall variability of the RR intervals (14).

The Poincaré plot is a map of points in Cartesian coordinates that is constructed from the values of the RR intervals. Each point is represented on the x-axis by the previous normal RR interval and on the y-axis by the following RR interval.

For the quantitative analysis of the plot, an ellipse was fitted to the points of the chart, with the center determined by the average RR interval. The standard deviation of the instantaneous variability of the beat-to-beat heart rate (SD1) indices were calculated to measure the standard deviation of the distances of the points from the diagonal  $y=x$ , and the standard deviation of long-term continuous RR interval variability (SD2) measures the standard deviation of the distances of points from the line  $y=-x+RRm$ , where  $RRm$  is the average RR interval. The SD1 is an index of the instantaneous recording of the variability of beat-to-beat and represents the parasympathetic activity, whereas the SD2 index represents the long-term HRV and reflects the overall variability (14).

For calculation of the linear indices we used the HRV Analysis software (Kubios HRV v.1.1 for Windows, Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland).

### Statistical analysis

Sample size calculation was based on a previous publication (16), which considered the at rest rMSSD index. The size of the difference was assumed for 11 ms, considering a standard deviation of 16.2 ms, with alpha risk of 5 % and beta risk of 80 %. So, a minimum of 18 subjects were specified.

Means and standard deviations were computed with normal Gaussian distribution of the data evaluated through the Shapiro-Wilk goodness-of-fit test ( $z$  value  $> 1.0$ ). To equate variables between groups we applied non-paired Student t-test for parametric distributions and Mann-Whitney for nonparametric distributions. Differences were considered significant when the probability of a Type I error was less than 5 % ( $p < 0.05$ ).

To assess the magnitude of differences between groups we computed the effect size using Cohen's  $d$  for significant differences. Large effect magnitude was considered for values  $\geq 0.9$ , medium for values between 0.5 and 0.9 and small for values  $\leq 0.5$  (17).

## RESULTS

Table 1 illustrates the mean and standard deviation of the variables of age, height, mass and BMI of the volunteers.

**Table 1.** Age, height, weight and BMI of the volunteers.

Variables	G1 (n=25)	G2 (n=25)	p value	Cohen's d
Age (years)	28.00 ± 6.97	29.48 ± 6.82	0.4518	0.21
Height (m)	1.58 ± 0.08	1.62 ± 0.08	0.0722	0.50
Weight (kg)	62.64 ± 11.10	66.55 ± 11.07	0.2188	0.35
BMI (kg/m <sup>2</sup> )	25.79 ± 6.31	25.18 ± 4.21	0.9073	0.11

**Legend:** Mean ± Standard-deviation; G1: cortisol below 0.24 ug/dl; G2: cortisol above 0.24 ug/dl; m: meters; kg: kilograms; BMI: height and body mass index; kg/m<sup>2</sup>: kilograms per square meter.

Table 2 illustrates the mean and standard deviation of systolic (SBP) and diastolic blood pressure (DBP), heart rate (HR), RR intervals, geometric, time and frequency domain indices of HRV. As predicted, cortisol levels were significantly higher in the G2, however, we found no significant difference regarding SBP, DBP and HRV between groups.

**Table 2.** Mean and standard deviation of salivary cortisol levels, SBP, DBP, HR, RR intervals, time and frequency domain of HRV.

Variables	G1 (n=25)	G2 (n=25)	p value	Cohen's d
Cortisol (ug/dl)	0.14 ± 0.05	0.37 ± 0.13	<0.0001	2.33
SBP (mmHg)	112.80 ± 12.42	120.80 ± 18.69	0.1539	0.50
DBP (mmHg)	76.40 ± 8.10	80.40 ± 12.74	0.3512	0.37
HR (bpm)	90.04 ± 17.99	83.29 ± 17.09	0.2003	0.38
RR (ms)	689.62 ± 122.52	748.68 ± 136.29	0.1137	0.45
SDNN (ms)	39.92 ± 16.57	44.60 ± 15.17	0.3038	0.29
rMSSD (ms)	27.53 ± 14.59	34.45 ± 20.21	0.2072	0.39
NN50 (ms)	17.24 ± 36.39	15.10 ± 16.51	0.1174	0.07
pNN50 (ms)	10.56 ± 12.90	15.86 ± 18.49	0.2101	0.33
RRtri (ms)	8.03 ± 4.83	9.64 ± 3.89	0.1743	0.36
TINN (ms)	156.60 ± 64.23	174.00 ± 67.22	0.3541	0.26
SD1 (ms)	19.59 ± 10.38	24.49 ± 14.35	0.2072	0.39
SD2 (ms)	52.18 ± 22.96	57.25 ± 18.93	0.3988	0.24
LF (ms <sup>2</sup> )	524.96 ± 481.42	663.31 ± 530.92	0.9381	0.27
LF (nu)	61.63 ± 19.02	60.41 ± 18.40	0.8193	0.06
HF (ms <sup>2</sup> )	339.45 ± 424.65	529.68 ± 570.74	0.1138	0.37
HF (nu)	38.16 ± 18.93	39.34 ± 18.33	0.8244	0.06
LF/HF	2.95 ± 3.70	2.38 ± 2.50	1.000	0.18

**Legend:** ms: milliseconds; bpm: beat per minute; mmHg: millimeters of mercury; ug/dl: micrograms per deciliter; nu: normalized unit; ms<sup>2</sup>: absolute unity; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; SDNN: standard deviation of all normal RR intervals; pNN50: percentage of adjacent RR intervals lasting difference greater than 50 milliseconds; rMSSD: square root of the mean square of differences between adjacent normal RR intervals; LF: low frequency; HF: high frequency; LF/HF: ratio low frequency/high frequency; TINN: triangular interpolation of NN interval histogram; RRtri: triangular index; SD1: standard deviation of the instantaneous variability of the beat-to-beat heart rate; SD2: standard deviation of long-term continuous RR interval variability.

We also preformed correlation analysis between cortisol levels and resting HRV in the 50 subjects. There was no significant correlation between the variables (Table 3). Considering that we found no significant correlation between variables it was superfluous to perform a linear regression.

**Table 3.** Correlation of cortisol with SBP, DBP, HR, RR intervals, time and frequency domain of HRV.

Variables	R	p value
SAP (mmHg)	0.080	0.460
DAP (mmHg)	0.120	0.270
HR (bpm)	0.10	0.360
RR (ms)	-0.10	0.380
SDNN (ms)	0.070	0.510
rMSSD (ms)	0.050	0.660
pNN50 (ms)	0.020	0.820
RRtri (ms)	0.160	0.170
TINN (ms)	0.080	0.470
SD1 (ms)	0.050	0.650
SD2 (ms)	0.070	0.500
LF (ms <sup>2</sup> )	0.010	0.880
LF (nu)	0.003	0.970
HF (ms <sup>2</sup> )	-0.010	0.870
HF (nu)	-0.004	0.960
LF/HF	-0.100	0.380

**Legend:** ms: milliseconds; bpm: beat per minute; mmHg: millimeters of mercury; nu: normalized unit; ms<sup>2</sup>: absolute unity; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; SDNN: standard deviation of all normal RR intervals; pNN50: percentage of adjacent RR intervals lasting difference greater than 50 milliseconds; rMSSD: square root of the mean square of differences between adjacent normal RR intervals; LF: low frequency; HF: high frequency; LF/HF: ratio low frequency/high frequency; TINN: triangular interpolation of NN interval histogram; RRtri: triangular index; SD1: standard deviation of the instantaneous variability of the beat-to-beat heart rate; SD2: standard deviation of long-term continuous RR interval variability.



## DISCUSSION

Considering that the ANS and Hypothalamic-Pituitary-Adrenal axis is physically connected and synchronized (18), which is demonstrated in physiologically stressful situations (19), we sought to evaluate this association in healthy subjects. Nevertheless, we failed to find a connection between salivary cortisol levels and resting HRV.

In order to extend the results, we decided to split healthy subjects into two equal groups according to median baseline salivary cortisol levels (higher or lower than 0.24 ug/dl). There was a significant change between the cortisol value of the two groups ( $p < 0.0001$ ). However, it was detected that the cardiac autonomic modulation continued without significant alteration.

The ANS and the Hypothalamic-Hypophysis Adrenal (HHA) axis control important physiological functions of the human body. Fluctuations of the function in these two systems are associated with metabolic disorders and dysregulation of the cardiovascular system, with altered autonomic cardiac function (20,21). Evaluation of the ANS function through HRV and the use of salivary biomarkers such as salivary cortisol to assess the purpose of the ANS and HHA axis can help identify patterns associated with the stage of evolution of some diseases, beyond the severity and effectiveness of treatment (21,22).

There is an absence of notable scientific literature highlighting the influence of cortisol levels, performing as a stressor factor, and autonomic cardiac modulation. Most of the studies in the relevant research literature evaluate some motor/psychological and/or pathology to detect an association between psychological stress and the ANS. Clays et al. (23) is a study that correlated the perception of psychological stress in an occupational environment with the parasympathetic modulation of 653 healthy male workers, illustrating that the higher this perception, the lower the parasympathetic modulation.

Studies show that low HRV, indicating a sympathetic predominance, is related to specific stress patterns and, consequently, to higher values of cortisol. Chida & Steptoe (24) performed a systematic review with meta-analysis to identify cortisol responses with psychosocial factors. The authors (24) found that cortisol increase after awake was positively associated with stress at work and overall stress of life and negatively with fatigue, burnout, and exhaustion. Therefore, the authors (24) concluded that different psychosocial factors are associated with an increased or decreased cortisol response.

Some studies have examined the relationship between HRV and cortisol. High levels of cortisol were associated with low HF and LF values, but showed no change in HF/LF in a cross-sectional study<sup>(25)</sup>. However, as in the results of the present study, results were also published that showed no association between HRV and cortisol (26,27).

In addition to cross-sectional studies, there are studies in the literature that provoke stress reactions to evaluate the association of HRV and cortisol. Sgoifo et al. (28) investigated the relationship between autonomic cardiac, endocrine and behavioral responses to real-life stress episodes. This authors (28) exposed 30 college students to two stressful social challenges and found that individuals who had higher levels of sympathetic predominance also had increases in cortisol levels. Therefore, the results suggest that there is a narrow relationship between the degree of physiological excitability and the style of behavioral adaptation to social stressors. It is worth noting that in the present study no intervention was performed that could cause stress in the individual, and therefore, this fact may explain, at least in parts, the absence of correlation between HRV and cortisol.

Cortisol is recognized as a "stress hormone" formed by the glucocorticoid that is released by the adrenal glands when the Hypothalamic-Pituitary Adrenal (HPA) axis is activated by psychological stress (29). Cortisol is vital for establishing homeostasis after exposure to a stressor (29). According to Castro and Moreira (30), cortisol circulates in the bloodstream connected to the transporter proteins, transcortin, the corticoid transporter protein (CBG), and albumin. Only 5 % to 10 % is in the free form; the biologically active element of the hormone.

Nevertheless, measurement of cortisol levels in saliva is gaining extensive acceptance as a diagnostic technique. The salivary cortisol test has been applied for HPA axis function, under

various cognitive conditions and in the presence of mental stress and anxiety (31). Previously, when an individual is exposed to the stressful situation, the neuroendocrine systems are activated and secrete substances such as adrenaline and cortisol, promoting physiological reactions in response to stress (32).

Regarding the effects of mental stress, anxiety and depression on the modulation of the ANS, Langewitz et al. (33) evaluated the effect of psychological stress on the ANS by means of HRV, where decreases in some HRV variables were observed. According to Morino et al. (34) HRV spectral analysis is beneficial in assessing psychological tension and anxiety.

Statistically there was no relationship between changes in cortisol and cardiac autonomic regulation. Yet, when these parameters were analyzed in detail, it was perceived that some of them responded with a greater influence in relation to others, for instance, HF indices in absolute units, NN50 and RR with p value of approximately 0.11. Another statistically trivial change is the SBP where in the group with cortisol levels above the median, values are greater in relation to the group with cortisol levels below the median.

These results allow us to reflect why these changes in salivary cortisol levels did not significantly influence the cardiac autonomic control process. A potential theory for such a discovery would be that the ANS suffers considerable influence of the hormone cortisol only in situations where more intense stress occurs to the organism, as pathological conditions, psychological tests and the performance of strenuous physical exercises.

The present study contributes to the scarce literature that addresses the relationship between HRV and cortisol. The strength of the study is the standardization of HRV measurements with well-established criteria and filtering processing that ensures study quality. However, some limitations of the study should be highlighted. Concerning the methodological aspects, the studied age range was wide (18 to 40 years), yet, prioritizing the exclusion criteria, we sought normative standards in relation to the selection sample. Additionally, it should be recognized that the sample consisted of both males and females and individuals classified as overweight according to the World Health Organization.

It is worth highlighting that this current study is significant because it illustrates discontinuities between the association of cortisol quantities and cardiac autonomic modulation in relation to the primary effector response of the system that directs psychological stress in situations where there is no influence of a stressor factor.

## CONCLUSION

Heart rate variability autonomic control was unchanged in healthy subjects with physiological distribution of salivary cortisol levels. There was no association between normal salivary cortisol and resting autonomic regulation of heart rate.

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