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Original Article

Renin-angiotensin system in stress response and the effect of chronic exercise in healthy volunteers

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Abstract

Aim: Stress is a multidisciplinary field of research. Renin-angiotensin system (RAS) components have been shown to be involved in stress. Exercise also can be considered as a stress factor. The aim of this study was to investigate the RAS components and effect of chronic exercise on stress response in a prospective design.

Material and Methods: Twenty healthy volunteers were recruited to the study. Trier psychosocial stress test was used to simulate psychosocial stress. Blood pressure, heart rate, plasma renin activity and angiotensin II, salivary cortisol were measured. Subjective stress perception is assessed.

Results: Blood pressure, plasma renin activity (PRA), Angiotensin II (Ang II) increased with the stress test. There was no statistically significant increase in salivary cortisol and heart rate levels with Trier social stress test (TSST) both before and after exercise. Self-ratings of the exercise adherence ratio were high as 72%.

Conclusion: Monitoring for a longer duration of time after stress can better explain stress response and RAS.

Keywords: Exercise; pharmacology; renin-angiotensin system; stress

INTRODUCTION

Claude Bernard described "milieu interior" at the end of the 19th century and pointed the organism's ability to maintain a constant fluid despite an external environment. Cannon coined the term "homeostasis" at the beginning of the 20th century, later Selye redefined the term "stress" and popularized the concept (1). Stress is a multidisciplinary field of research. Although the stress response varies according to the stressor, the major endocrine response includes the hypothalamohypophyseal axis (HPA) (2), adrenomedullary hormonal system and sympathetic noradrenergic system. Renin-angiotensin system (RAS) components have also been shown to play a role in regulating the response to stress stimulation (3). Angiotensin II (Ang II), the active principle of the RAS is shown to play a role in stress in experimental studies. Various factors such as cold, pain and public performance can evoke stress response (1). Also, exercise can be considered as a stress factor, because physical exercise affects many parameters such as cortisol and RAS (4). Studies have shown that regular physical exercise alters the response to psychosocial stress (5). It has been shown that regular exercise causes differences in stress response. The stress response can be evaluated with different methods. Trier social stress test (TSST) is a protocol to evaluate stress response in laboratory conditions (6). The aim of this prospective study was to investigate the RAS components in stress response and evaluate the effect of chronic exercise.

MATERIAL and METHODS

This study received IRB approval (764 - 03/19/2008) and was supported by the Istanbul University Scientific Research Projects (No. 3024). Healthy volunteers aged between 25 – 60 years who could not exercise regularly in the previous 3 months were included in the study.

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Exclusion criteria were diagnosis of the psychiatric, cardiovascular system, or musculoskeletal disorders as well as intake of medication known to alter the stress response and RAS. Participants are recruited to the study with personal contact. Healthy volunteers were evaluated with cardiopulmonary and metabolic parameters. Bruce protocol is used to determine the heart rate at the anaerobic threshold. It was calculated with V-slope method from Breath by breath analysis. Volunteers were trained to measure heart rate and advised to exercise for eight months at least three times a week between the recommended heart rate intervals. Eight months later, cardiopulmonary and metabolic parameters were reevaluated. Participants were asked to self-evaluate their adherence to the exercise program by scoring themselves between 0-100. Trier social stress test (TSST) was used to model psychosocial stress (6). The test is composed of preparation, speech in front of the jury and mental arithmetic stress test. Blood pressure and heart rate, plasma renin activity (PRA), Ang II and salivary cortisol were measured. Subjective stress was assessed with perceived stress test and Visual Analog Scale. Saliva samples for cortisol were taken with a cotton roll. Saliva was isolated by centrifugation and measured by electrochemiluminescence immunoassay method on the same day. Blood was transferred to cooled ethylenediaminetetraacetic acid tubes for PRA and Ang II measurements. Plasma was separated at +4°C by refrigerated centrifuge and stored at -40°C. Ang II kit was studied with Radioimmunoassay method (Biosource, Human Angiotensin II RIA kit®; Cat No: KIPERB320, Belgium). Results were measured with gamma counter. Blood pressure and pulse rates were measured with a calibrated blood pressure monitor (Omron 705 IT). Compliance of the volunteers was evaluated with a form in which the exercise weres evaluated subjectively. The questions included: What are the strengths of exercise if any? Has the increase in physical activity affected your daily life? and If so, can you describe how it affected it?. The reasons for inability to exercise were evaluated with an open-ended question.

In our study, stress responses were also evaluated with cold stress in which cold stress responses were recorded with Electroencephalography, although this data is not reported in this article.

Statistical Package for the Social Sciences (SPSS version 15.0) program was used for data analysis. Wilcoxon or Friedman test was used depending on whether two or more dependent variables. The analysis was performed with Analysis of Variance (ANOVA). The graphics were drawn with the help of a special program (GraphPad Prism 8.1.0. version).

RESULTS

A total of 20 volunteers (9 females and 11 males) were included in the study. Four people whose initial stress responses were evaluated could not complete the study because they could not follow the exercise program or were recently prescribed beta-blocker or oral contraceptives. Five female and nine male volunteers completed the study. Self-rating of the compliance with the recommended exercise program was found to be 72% ± 3.45%. After the exercise program, the average weight of volunteers decreased from 73.4 kg to 71.8 kg, resting heart rate decreased from 82 to 78, while the maximum heart rate increased from 174 to 180. Changes in these values did not reach statistical significance. The anaerobic threshold value increased significantly from 149.8 to 153.6 beats per minute ($p < 0.05$).

Compliance of the volunteers was evaluated with a form in which the exercise was evaluated subjectively. Some sample answers to the two questions are shown in the table (Table 1). Lack of time was one of the most frequent causes, while the lack of time and laziness were the main answers. Environmental conditions were also among the reason that made exercise difficult. In response to the question"Has the increase in physical activity affected your daily life?" 25% of volunteers responded as "not much", while 75% answered "yes".

Table 1. Sample of open-ended questions and answers are given in the table

Sample of Questions	Some examples of Responses
What are the conditions that make it difficult for you to exercise?	Difficulty in gaining new habits
	Environmental conditions
	Lack of time
	Lack of safe space
	Laziness
	Life goals
	Sports fascism
	Work intensity
Can you describe how exercise affected your life?	I became a more positive thinker
	I have gained a more fit appearance
	I have muscle pain
	I lost weight
	I was physically strengthened
	I'm less tired, I'm getting tired late
	I'm walking faster
	My concentration increased
	My point of view has changed
	My self-confidence has increased

TSST and blood pressure measurements

Before chronic exercise, systolic blood pressure measured at the onset of TSST significantly decreased after 30 minutes of rest ($p < 0.05$) (Figure 1). After 30 minutes of rest, blood pressure was accepted as basal blood pressure. Preparation of stress, speech and mental arithmetic stress increased systolic blood pressure significantly when compared with basal systolic blood pressure ($p < 0.05$). After a total of 20 minutes exposure to stress blood pressure was significantly higher when compared with basal values. Poststress measurements at 60 and 70 minutes returned to basal blood pressures.

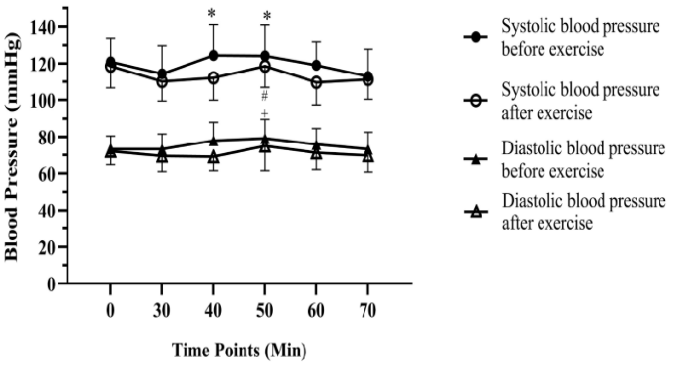

Figure 1. Blood pressure measurements with Trier psychosocial stress test (TSST). Anticipation stress is applied between 30th-40th minutes and talk/mental arithmetic test between 40th and 50th Min.

After chronic exercise, the first systolic blood pressure decreased significantly at 30 minutes post-rest ($p < 0.05$). Preparation stress did not increase blood pressure. After mental arithmetic stress, systolic blood pressure increased significantly compared to basal systolic blood pressure ($p < 0.05$). Poststress systolic blood pressure values at 60 and 70 minutes significantly decreased ($p < 0.05$) when compared to 50 minute value and was comparible with basal blood pressure measurements. Systolic blood pressure values increased significantly with stress test ($p < 0.05$). Basal systolic blood pressures decreased 11 mmHg after long-term exercise ($p < 0.05$).

Baseline diastolic blood pressure before exercise and after 30 minutes' rest was comparable with initial measurement. Mean diastolic BP did not increase with preparation stress but increased with speech and mental arithmetic stress test ($p < 0.05$).

After chronic exercise, basal diastolic blood pressure with 30 minutes rest was lower than the initial measurement ($p < 0.05$) Diastolic blood pressure did not increase with the stress of preparation, speech and mental arithmetic. The effect of chronic exercise on diastolic blood pressure was found to be significant ($p < 0.05$).

Anticipation stress is applied between 30th-40th minutes and talk/mental arithmetic test between 40. and 50minutes. Measurements are repeated before and after chronic exercise (mean±SEM).

TSST and Heart Rate

Before exercise, the initial mean heart rate was 80.45 ± 1.97 (mean ± SEM), 8.45 ± 1.93 after 30 minutes resting and 80.33 ± 2.94 30 minutes after preparation stress. After the speech and mental arithmetic stress heart rate was measured as 81.78 ± 3.68, 77.94 ± 2.64 at the 60th minute and 77.89 ± 2.46 at the 70th minute.

After chronic exercise, the mean heart rate was 81.50 ± 3.83 (mean ± SEM) initially. After 30 minutes rest it was 81.29±3.38 and after preparation stress, it was measured as 81.00 ± 4.42. After the speech and mental arithmetic stress, the heart rate was 79.14 ± 3.50. Post stress measurements were 79.07 ± 2.98 at 60th minute and 78 ± 3.16 at the 70th minute, Heart rate measurements did not change with stress exposure and chronic exercise ($p > 0.05$).

TSST and cortisol

Initial salivary cortisol level was 0.13 ± 0.03 µg / dL after rest, 0.14 ± 0.03 µg / dL after preparation stress, 0.20 ± 0.04 µg / dL after speech and mental arithmetic stresses. Repeated measurements with ten minute intervals were 0.21 ± 0.06 µg / dL and 0.20 ± 0.06 µg / dL, respectively. Basal salivary cortisol levels after exercise were 0.22 ± 0.05µg / dL, 0.21 ± 0.06 µg / dL after 10 minutes of preparation stress, 0.25 ± 0.05 µg / after speech and mental arithmetic stress in front of the committee. Cortisol was measured as 0.29 ± 0.06 µg / dL and 0.19 ± 0.04 µg / dL after repeated tests. There was no statistically significant increase in salivary cortisol levels with TSST both before and after exercise ($p > 0.05$). There was no significant difference in salivary cortisol between the values before and after exercise ($p > 0.05$)

PRA

Baseline PRA measurements were determined as 1.35 ± 0.33 ng / ml / hour (mean ± SEM) before exercise (Figure 2a). After TSST test, it increased to 2.06 ± 0.39 ng / ml / h (mean ± SEM) ($p < 0.05$). After rest, the PRA level decreased to 1.71 ± 0.36 ng / ml / h (mean ± SEM) at 60 minutes compared with the 50th minute value ($p < 0.05$). The 60th minute PRA value was significantly higher ($p < 0.05$) when compared to the baseline 30th minute PRA value.

Basal PRA measurements after exercise were 0.72 ± 0.21 ng / ml / h (mean ± SEM). After the stress test, it increased to 1.49 ± 0.40 ng / ml / hour (mean ± SEM; $p < 0.05$). After rest, the PRA level at the 60th minute was 1.71 ± 0.37 ng / ml / h (mean ± SEM). Elevation was not significant when compared with the 50th minute value ($p > 0.05$), whereas the 60th minute PRA value was higher when compared with the baseline PRA value ($p < 0.05$). PRA levels in three blood samples taken during TSST did not differ between before exercise and after exercise ($p > 0.05$).

Angiotensin II

In healthy volunteers, basal Ang II was measured 30 minutes after intravenous cannulation. Prestress, basal, 30th minute Ang II level was 11.38 ± 1.7 pmol / l (mean ± SEM) (Figure 2b). After stress test they were mesasured

as 18.75 ± 3.35 pmol / l (mean ± SEM). The increase was statistically significant (p <0.05). After ten minutes of rest, the Ang II level was 17.13 ± 4.18 pmol / l (mean ± SEM). Basal, prestress Ang II after exercise was 12.52 ± 3.9 pmol / l (mean ± SEM), 12.44 ± 2.18 pmol / l after stress test (mean ± SEM).

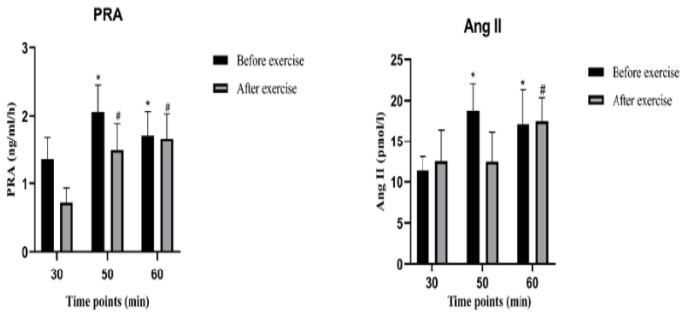

Figure 2. a., Plasma Renin Activity (PRA) (mean±SEM). Measurements are repeated before and after chronic exercise. (*, p <0.05 vs 30th min before exercise and #, p<0.05 vs 30th min after exercise levels).
b., Angiotensin II (Ang II) (mean±SEM). Anticipation stress is applied between 30th-40th minutes and talk/mental arithmetic test between 40th and 50th min. Measurements are repeated before and after chronic exercise. (*, p <0.05 vs 30th min before exercise and #, p<0.05 vs 30th min after exercise levels)

Subjective stress assessments

Visual Analog Scale

TSST test evaluation with visual analog score values did not change between the first and second stress test and chronic exercise. Difficulty, stress, uncontrollability, unpredictability parameters are comparable before and after exercise (p> 0.05).

Perceived stress test

Perception of the previous month was evaluated with the perceived stress test. There was no difference in before and after exercise measurements.

DISCUSSION

TSST test is a frequently used test to induce stress in laboratory conditions. In this study stress response to TSST was found to be an increase in blood pressure, PRA and AngII. Salivary cortisol showed a tendency to increase but not statistically significant. This could be due to inadequate stressors. The stress test is repeated as it is described. But there may be interlaboratory factors including communication approach to participants. Even though volunteers graded the stressfulness of the intervention, cortisol responses did not increase significantly. Cortisol level is known to be affected by many other factors such as fasting, circadian rhythm, etc. We considered fasting as a stressor, and fasting is not obligated in the TSST protocol, therefore we did not require fasting before TSST. Breakfast content might have interfered with cortisol levels. All TSST sessions are performed between 9-12 am to avoid the effect of the circadian rhythm. The initial salivary cortisol level in this study was 0.13 µg / dL, this result is quite similar to mean salivary cortisol levels of 0.12 µg / dL determined with the same electrochemimmunassay method (7) but lower than the studies with TSST in the literature (8, 6). In our study, both genders are included. Genetics, age and gender is reported to be modifiers of stress response (9). Even we have performed the TSST in the same phase of the menstruation cycle to decrease the interindividual differences it might lead to a lesser degree of cortisol response. Liu et al. (10) reported in their meta-analysis their findings suggest that sex differences in stress response might result from variations in methodologies. Wiemers et al. (11) explores a friendly version of TSST and showed that the friendly version did not exert cortisol response. Our study is not the friendly version and their friendly TSST cortisol response showed tendency for a decrease in contrary to our salivary cortisol levels. Increase in blood pressure also supports an evoke in stress response. RAS was evaluated with TSST in a recent study by Gideon et al. (12) PRA was found to be elevated. Our study is in concordance with their findings and moreover it is shown that one of the important mediator in RAS, Ang II is also increased with social stress. RAS may be a predictor for stress response even when saliva cortisol increase is not prominent. In preclinical studies RAS inhibition is shown to inhibit excess central nervous system stimulation and may avoid stress-induced cardiovascular burden (13).

Chronic exercise duration was eight months. Self-ratings of the exercise adherence ratio were as high as 72%. After exercise period cardiopulmonary and metabolic parameters were reevaluated. Anaerobic threshold is increased supporting the effect of exercise and this value is an important marker for aerobic capacity (14). The TSST is repeated to evaluate stress response. The visual analog score that evaluates the TSST were comparable with before exercise scores. Petrowski et al. (15) also mentioned the lack of habituation effect with the repeated TSST test with 10 weeks interval. After chronic stress PRA, Ang II and systolic blood pressure increased with stress. Ang II increase was shown just after stress exposure (50th minute) before chronic exercise and at 60th minute after chronic exercise. This may be interpreted as a delayed increase in Ang II after chronic exercise. Cortisol and heart rate did not increase, similar to before exercise stress test. Diastolic blood pressure did not increase with stress exposure after chronic exercise. A meta-analysis reported that greater reactivity to mental stress predicts poor future cardiovascular disease (16). Chronic exercise blunted stress response in diastolic blood pressure which may be important to decrease the future cardiovascular risk. Giorgiades et al. (17) investigated the effect of aerobic exercise in mildly to moderately overweight patients with elevated blood pressure and observed stress induced blood pressure decreases.

CONCLUSION

Our study results showed even in healthy volunteers with no apparent weight loss, chronic exercise decreased diastolic blood pressure in stress response. Cortisol was not shown to be playing a role but Ang II may be a possible contributor in this effect. Blood sample timing after stress may not be sufficient to examine the recovery period. Further studies examining a longer duration of time after stress may better explain stress response differences.

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Competing interests: The authors declare that they have no competing interest.

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REFERENCES

1. Goldstein DS, Kopin IJ. Evolution of concepts of stress. Stress 2007;10:109-20.
2. Armando I, Volpi S, Aguilera G, et al. Angiotensin II AT1 receptor blockade prevents the hypothalamic corticotropin-releasing factor response to isolation stress. Brain Research 2007;1142:92-9.
3. Saavedra JM, Benicky J. Brain and peripheral angiotensin II play a major role in stress. Stress 2007;10:185-93.
4. Hackney AC. Stress and the neuroendocrine system: the role of exercise as a stressor and modifier of stress. Expert Review of Endocrinology & Metabolism 2006;1:783-92.
5. Gröpel P, Urner M, Pruessner JC, et al. Endurance- and resistance- trained men exhibit lower cardiovascular responses to psychosocial stress than untrained men. Front Psychol 2018;9:852.
6. Kirschbaum C, Pirke KM, Hellhammer DH. The 'Trier Social Stress Test'a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 1993;28:76-81.
7. Polat C, Duzer S, Ayyıldız H, et al. Association between anxiety, depression, and salivary cortisol levels in patients with recurrent aphthous stomatitis. Turk Arch Otorhinolaryngol 2018;56:166-9.
8. McQuaid RJ, McInnis OA, Paric A, et al. Relations between plasma oxytocin and cortisol: The stress buffering role of social support. Neurobiol Stress 2016;3:52-60.
9. Allen AP, Kennedy PJ, Dockray S, et al. The Trier Social Stress Test: Principles and practice. Neurobiol Stress 2016;6:113-26.
10. Liu JJW, Ein N, Peck K, et al. Sex differences in salivary cortisol reactivity to the Trier Social Stress Test (TSST): A metaanalysis. Psychoneuroendocrinology 2017;82:26-37.
11. Wiemers US, Schoofs D, Wolf OT. A friendly version of the trier social stress test does not activate the HPA axis in healthy men and women. Stress 2013;16:254-60.
12. Gideon A, Sauter C, Fieres J, et al. Kinetics and interrelations of the renin aldosterone response to acute psychosocial stress: a neglected stress system. J Clin Endocrinol Metab 2020;105:190.
13. Uresin Y, Erbas B, Ozek M, et al. Losartan may prevent the elevation of plasma glucose, corticosterone and catecholamine levels induced by chronic stress. JRAAS 2004;5:93-6.
14. Ghosh AK. Anaerobic threshold: its concept and role in endurance sport. Malays J Med Sci 2004;11:24-36.
15. Petrowski K, Wintermann GB, Siepmann M. Cortisol response to repeated psychosocial stress. Appl Psychophysiol Biofeedback 2012;37:103-7.
16. Chida Y, Steptoe A. Greater cardiovascular responses to laboratory mental stress are associated with poor subsequent cardiovascular risk status: a meta-analysis of prospective evidence. Hypertension 2010;55:1026-32.
17. Georgiades A, Sherwood A, Gullette EC, et al. Effects of exercise and weight loss on mental stress–induced cardiovascular responses in individuals with high blood pressure. Hypertension 2000;36:171-6.