Regular physical activity may influence stress hormone cortisol in Wistar rats

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Published online: January 31, 2020
Accepted for publication: January 08, 2020
DOI:10.7752/jpes.2020.01018

Abstract
Engaging in regular physical activity is one of the best ways to improve overall health. Physical activity can produce differences in neural and endocrine systems. However, it is challenging to promote regular physical activity in a population that is predominantly sedentary. Therefore, exercise protocols are used to rehabilitate patients with chronic musculoskeletal conditions, cardiorespiratory and mood disorders. The aim of this study was to observe differences in hormonal status after regular aerobic physical activity for 1-7 d. This in vivo experiment analyzes the impact of physical stress on the level of cortisol. A total of 20 Wistar rats were divided into 2 groups of 10 animals per group (i.e., the-control group, and the group of rats that was subjected to the forced swim test for 7 d. On the 1st and 7th day of the experiment, blood was taken from animals in order to determine the level of cortisol using the Mouse/Rat Cortisol ELISA protocol. Our results showed that by inducing physical stress for 1-7 d on the rats, their cortisol levels decreased. The results showed that regular aerobic activity for 7 d (i.e., 60-90 min of swimming) had a positive impact on lowering the levels of stress hormone cortisol.

Key Words: cortisol, aerobic activity, physical stress, rats.

Introduction
Cortisol is the main endogenous glucocorticoid (GC). This steroid hormone is produced and secreted in the adrenal fasciculate zone of the adrenal cortex (Delbende, et al., 1992; Pauli, Souza, Rogatto, Gomes, & Luciano, 2006). The GC hormones-cortisol in humans is produced owing to stressful experiences (Radahmadi M., Alaei, Sharifi, & Hosseini, 2015a); in addition, corticosterone is involved in the regulation of stress responses in rodents (Gong, et al., 2015). Cortisol can be activated by psychological stress, physical stress (Buynitsky & Mostofsky, 2009), and physiological stressors (Hackeny, 2006).

Cortisol is essential in physiological processes throughout the body; when cortisol is released it can be taken up by various tissues (e.g., skeletal muscle, adipose tissue, and the liver) to promote the braking down of proteins in the skeletal muscles into aminoacids and triglycerides into adipose tissue to be hydrolyzed into free fatty acids and glycerol (Viru & Viru, 2004; Hackeny, 2006; Patton & Thibodeau, 2014; McMurray & Hackney, 2000).

Physical activity is defined as any physical movement that is produced by skeletal muscles that requires energy consumption. Physical inactivity is identified as a leading risk factor for global mortality and cause approximately 2 million deaths globally. Regular physical activity of moderate intensity (e.g., walking, cycling, or participating in sports) has considerable benefits for health. For example, regular physical activity can reduce the risk of cardiovascular disease, diabetes, colon and breast cancer, and depression (Con, Hafdahl, & Brown, 2009; Gillison, Skevington, Sato, Standage, & Evangelidou, 2009; van Praag, 2009; Volpon, et al., 2015). Because physical activity can affect the level of cortisol in serum, the purpose of our study was to determine the effect of swimming on the level of cortisol in rats.

Materials and methods
Experimental animals
All experimental procedures were performed in accordance with the Manual for Care and Use of Laboratory Animals, approved by the Macedonian Center for Bioethics. The protocols were approved by the
Ethics Committee for Animals at the University “St. Cyril and Methodius” Skopje, according to the recommendations for biomedical research involving animals, issued by the Council of International Organizations for Medical Sciences. Anesthesia was applied in accordance with the standards provided in the EU Directive, Directive 86/609 / EEC. During the study, the animals were exposed to standard food and water regime available ad libitum and stayed in a room under a constant light regime of 12 hours (06:00 to 18:00) light and 12 hours dark at the temperature of 26°C. A total of 20 female, white laboratory rats from the Wistar strain (total number of rats n = 64) were used that were aged 4.5 months and had a body mass of 220 ± 20 g. The experiment lasted for 8 d.

This was a preliminary experiment. In this study we will present only preliminary cortisol results. As mentioned above, 20 rats were used and, divided into 2 groups of 10 animals per group. The first group was the control group, and the second group of rats was the group that was forced to swim.

On the 1st and 7th day of the experiment, blood was taken from the tail of the rat by incision, to determine the level of cortisol, adrenaline, and serotonin. A total of 1.5 mL of blood was collected. The 1.5 mL blood sample was allowed to clot for 30 min at room temperature. The serum was separated by centrifugation at 3000 rpm (rotations per minute) for 15 min. After centrifugation, 0.75 mL of the serum was collected and subsequently stored at -20˚C. To measure the concentration of cortisol, the Mouse/Rat Cortisol ELISA (SIG-ALD SE120082) protocol was used. Physical stress was caused to the rats by placing them in a water barrel with the water temperature of 34 ± 2°C. The rats swam every day for (60-90 min).

Statistical Analyses
The data were analyzed using the statistical package SPSS 22.0 software for Windows (SPSS Inc., Chicago, IL, USA). Basic descriptive statistics were computed. The data normality distribution was determined using the skewness, kurtosis, and Kolmogorov Smirnov methods. Differences in variables were analyzed by one-way repeated-measures ANOVA.

Results
On the basis of the absorbers obtained by measuring the prepared samples from the serum analyses, a series of data was obtained that was statistically processed and appropriately graphically presented.

Using the one-factor analysis of the variance of repeated measurements, from the overview of arithmetic means and the level of statistical significance, it was determined that there was no statistically significant differences determined between the control group and the group that swam for 7 d. The results in table 1 and chart 1 show a slight decrease in cortisol levels in the group of rats that swam for 7 d relative to that in the control group.

Table 1: Statistical data on cortisol levels among the groups

<table>
<thead>
<tr>
<th>CORTISOL</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>SD</th>
<th>s.e.</th>
<th>Skew</th>
<th>Kurtos</th>
<th>K-S</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>0.43</td>
<td>0.34</td>
<td>0.53</td>
<td>0.06</td>
<td>0.02</td>
<td>-0.04</td>
<td>-0.13</td>
<td>p &gt; .20</td>
</tr>
<tr>
<td>7 D SWIM</td>
<td>0.39</td>
<td>0.35</td>
<td>0.42</td>
<td>0.02</td>
<td>0.01</td>
<td>-1.37</td>
<td>3.04</td>
<td>p &gt; .20</td>
</tr>
</tbody>
</table>

Chart 1: Comparison of cortisol level among the groups
Discussion
The purpose of our study was to determine the effect of forced swimming for 7 d on the level of cortisol in rats. There is a strong evidence that acute and chronic exercise is essential in the neuroendocrine system. Neuroendocrine response can be changed by exercise which can reduce the hormonal stress response (Hackeny, 2006).

The analysis of cortisol levels by the ELISA technique showed that in the group of control animals group had, a higher cortisol level than the group of animals analyzed on the 7th day of swimming. The blood taken on the 7th day of swimming had the lowest level of cortisol in serum, which proved that physical stress positively affected the level of stress hormone cortisol.

According to recent studies, there are some contradictory results on the cortisol levels after the forced swim test. According to (Jameel, et al., 2014), Wistar male rats exposed to the forced swim test for 15 d, showed an increased level of cortisol. (Smitha & Mukkadan, 2014) have reported that after the forced swim test for 2 h for, 7 d, Wistar male rats showed a significant increase in the cortisol level compared to that of the control group. Another study have reported that after 4 w of moderate exercise, 45 min of treadmill running produced a significant positive increase in cortisol level (Alghadir, Gabr, & Aly, 2015). In addition, a single running test for 2 h increased cortisol levels (Viru, Hackeny, Valja, Karelson, Janson, & Viru, 2001; Jiang, Kawashima, Iwasaki, Uchida, Sugimoto, & Itoi, 2004; Okutsu, Suzuki, Ishijima, Peake, & Higuchi, 2008), similar to acute stress (Soria, Gonzalez-Haro, Anson, & Lopez-Colon, 2015). The above mentioned data showed that 2 h of aerobic physical activity for 7 d produced a significant increase in cortisol levels in Wistar rats. However, our results contradict these data, thought the work methodology was approximately the same. The only important difference is the duration of activity per day, which was 60-90 min in our experiment. Thus, we can conclude that regular aerobic exercise for 60-90 min is sufficiently effective in regulating the level of cortisol in the body.

The contradictory finds on the levels of cortisol after physical activity may be due to the duration and intensity of the applied exercises. According to (Hill, Zack, Battaglini, Viru, Viru, & Hackney, 2008), the levels of cortisol were lower when a low intensity exercise at 40% of VO2 max for 30 min was applied. However, divergent findings form other studies have been reported. Specifically, 60 min of exercise at 45, 60, and 75% of VO2 max produced higher cortisol levels (Jacks, Sowash, Anning, McGloughlin, & Andres, 2002; Duclos, Corcuff, Rashedi, Fougere, & Manier, 1997). These contradictions may be due to several factors such as, the levels of training (Rimmele, Zellweger, Marti, Seiler, Mohiyeddini, & Ehlert, 2007), duration of exercise, timing of when the blood samples were collected, as well as gender of subjects. On the basis of evidence in previous studies and our results, we can state that consistent exercising at submaximal level can reduce the hormonal stress response.

Conclusion
The results obtained during the experimental study allow to conclude that physical activity (e.g., swimming) positively affects cortisol levels. Therefore, swimming can be used as a non-pharmacological strategy to improve the level of cortisol in the body.

Acknowledgment
We would like to thank the Faculty of Natural Sciences and Mathematics, Institute of Biology, Skopje, North Macedonia for helping to conduct the experiment.

Conflict of interests
No conflict of interests

Reference


