Pre- and Post Exercise Changes of Salivary Cortisol as a Response to Heavily Training among Students of Physical Education College in Karbala University

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Abstract
Background: Cortisol, the principal glucocorticoid in humans, plays a major role in metabolism and immune function. A cute exercise induces a change in plasma cortisol concentrations, which is dependent on the type of exercise. Several studies have investigated the effect of both acute and chronic resistance exercise on adrenocortical function.

Objective: This study was designed to determine the level of salivary cortisol as a stress related hormone during pre- and post- exercise in college student- athletes.

Materials and Methods: 10 males subjects (college student- athletes) were measured for height, weight, the general features of the participants are: Mean ±SD Age (year) 22 ± 2.79 Weight (Kg) 70.5±8.46, Height (Cm) 175.34±7.12, Three milliliters of un-stimulated total saliva was collected via passive drooling, at the beginning of each testing session (without stimulation, by spitting directly into a plastic tube), 5 min before, 5 min after the end of the match.

Results: The results showed a significant increase the salivary cortisol level between post and pre- exercise and there was a strong association between increase salivary cortisol concentration and heavier exercise.

Conclusions: During the course of a competitive season collegiate soccer players are exposed to a number of physical and psychological stressors from practice, conditioning, and competition. The ability of players to recover following such activities can ultimately affect the ability of the performance for ensuring physical activity.

Key words: Cortisol , Saliva, Football

الخلاصة
المقدمة
Cortisol، principal glucocorticoid in humans، plays a major role in metabolism and immune function. A cute exercise induces a change in plasma cortisol concentrations، which is dependent on the type of exercise. Several studies have investigated the effect of both acute and chronic resistance exercise on adrenocortical function.

الهدف من الدراسة: وجدت هذه الدراسة تحديد مستوى cortisol في اللعاب كمؤشر لنداء خلال فترة قبل وبعد إجراء التمرين لدى الرياضيين من طلبة الكلية.

المواصفات والطرق العملية: اخذت عينات لعاب لعشرة دعاة (10) من الرياضيين (طلبة الكلية) حيث قام الالتحام والوزن والعلماء العامة الأخرى من حيث سلامته الصحية. يوجد الإحصائيات الجسدية لهؤلاء المشاركين. وكان معدل العمر 27.9 ± 22 والوزن 70.5 ± 8.4 وطول 175.34 ± 7.12 من المعدل تم جمعه بالطريقة العادية وبدون عمله اثرا أو تحفيز في أثوابه. وقطر يدقاق قبل 5 دقائق بعد إتمام التمرين.

النتائج: وجدت النتائج ارتفاع واضح في مستوى cortisol في اللعاب مابين قبل وبعد أداء التمرين. وكذلك وجدت علاقة قوية بين تركيز cortisol وشد التمرين.
Introduction

Cortisol, the principal glucocorticoid in humans, plays a major role in metabolism and immune function. A cute exercise induces a change in plasma cortisol concentrations, which is dependent on the type of exercise. Several studies have investigated the effect of both acute and chronic resistance exercise on adrenocortical function. However, there appear to be no studies that have measured salivary cortisol responses to different intensities of resistance exercise. Salivary measures of cortisol have been shown to be a valid and reliable reflection of serum cortisol [1,2,3]. Salivary cortisol may actually provide a better measure than serum cortisol of the stress response as it more accurately measures the amount of unbound cortisol compared to serum measures [4]. There is also evidence that suggests fitter individuals show increased cortisol responses compared to less trained individuals [5].

Recently, researchers studying stress and its physiological impact have focused on saliva borne stress biomarkers because saliva sampling is rapid, non-invasive, and thus permits frequent sampling. The physiologic basis of salivary biomarkers to study stress is based on the control of sympathetic activity. The main response to stress is via activation of the hypothalamic-pituitary-adrenocortical and sympatho-adrenal axis and this can be represented by the concentrations of related hormones in saliva [6]. Competition is well known to bring about a stress response, even prior to beginning the sporting event. The physical stress of the actual event itself is likely to compound the stress response, which can be observed; cortisol is one of such stress biomarker. Numerous studies have measured the salivary cortisol changes in response to sporting competition [7]. Increase of salivary cortisol after football training and competition has been observed, and is higher in the beginners in contrast to amateur players [8].

Almost any types of stress, whether physical or nervous, causing immediate and significant increase in Adrenocorticotrophic Hormone (ACTH) by the anterior pituitary gland followed by a large increase in cortisol and adrenal cortex secretion up to 20 times within a few minutes. Cortisol often doesn’t release active and major proteins such as muscle contraction and neuron cells proteins, unless nearly all other proteins are released. This preferential effect of cortisol on the release of unstable proteins may provide the amino acids to make the crucial, necessary materials for the required cells [9]. Studies have shown that intense physical activities lead to reducing anxiety and depression and increasing self-confidence and self-esteem. We can say that exercising increases our sense of psychological well-being and thus will have a positive impact on our mental health. On the other hand, competitive sports, for example when a person loses the game or doesn’t play as he expected may also cause anxiety, depression and aggressiveness. Besides, improving athletic performance is relevant to psychological factors including anxiety, concentration, confidence, motivation, mental preparation and the like issues. A key point in improving athletic performance is that the mind affects the body. So our feeling will have a profound effect on our physical performance. In competitive sports, the competitors are often of similar skill level and the only difference is in their mental preparation [10]. Current knowledge suggests that heavy acute and/or chronic exercise is associated With an increased risk of URTIs. Some studies have suggested that the incidence of Infections, which are often thought to be a marker of early stages of overtraining Syndrome, were related to...
excursions above individually identifiable thresholds of Training strain [11]. The purpose of this study was to investigate the stress - Induced alterations in immune function and stress hormones in college students and College student-athletes.

**Materials and Methods:**

**Subjects:**

Potential subjects (10 males) were initially invited to an information session where the goals and procedures of the research were explained in detail. All the players were agreed to participate in this study and provided signed informed consent. At the time of study, none of these participants was under medication, had a history of behavioral, or sleep disorders, absence of any skeletal, muscle, cardiovascular, or endocrine limitations a history of a resistance - training program of at least two sessions per week prior to participation in this study. All subjects were measured for height, weight, the general features of the participants are: Mean ±SD Age (year) 22 ± 2.79 Weight (Kg) 70.5±8.46, Height (cm) 175.34±7.12.

**The football official competition:**

The competition included a football match officially recognized by the Regional Amateur League, and was conducted according to the International Football Federation regulations. The match began at 2 PM, ended at 3:50 PM, and included a 15 period between halves.

**Methods:**

Three milliliters of un-stimulated total saliva was collected via passive drooling, at the beginning of each testing session (without stimulation, by spitting directly into a plastic tube), 5 minutes before, 5 min after the end of the match. The participants drank 200 ml water to guard against hydration-related dry mouth 30 min before the first sampling. After collection, the samples immediately were kept in the ice and within 2 hours frozen at -20 °C. Subjects were asked to avoid drinking caffeine 24 hours before participation in the study and also not to eat 2 h before sampling. They were banned of undertaking any physical activity 48 h before the first sampling. On the day of analysis the selected frozen serum samples to be analyzed were thawed and brought up to room temperature. All samples were analyzed in duplicate using a commercial ELISA (DRG Diagnostics, Germany) according to manufacturer’s instructions. Appropriate volume (20 μl) of standards, control and samples were dispensed into appropriate wells (in duplicate) followed by addition of 200 μl of enzyme conjugate (anti-cortisol antiserum conjugated to horseradish peroxidise) as competition for binding site. After mixing and incubation (60 minutes), the unbound conjugate was washed off by rinsing the wells three times with provided wash solution. After washing, 100 μl of substrate solution (Tetramethylbezidine; TMB) was added to wells before incubation for 15 minutes. The colorimetric enzymatic reaction was then stopped via addition of 100 μl of stop solution 0.5-M HzS04 (Sulphuric acid). Wells were then read at 450 ηm within 10 minutes of stopping the reaction. The lower and upper limit for detection in this assay was 0-800 ηg/ml (sensitivity 2.5 ηg/ml).

**Statistical Analysis:**

Means and standard deviations were calculated for all variables of interest. One-way analyses of variance with repeated measures and Student t- test with Bonferroni corrections for multiple comparisons were used to determine significant differences. To determine the correlation among variables, Pearson correlation coefficients were used. Before these analyses were performed, the frequency distributions were tested for normally using the Kolmogrof-Smirnov test. The level of significance was set at P< 0.005. All analyses were run by SPSS for Windows.

**Results**

There was a significant increase in the level of salivary cortisol of (10) studied [student-athletes] immediately following the high intensity exercise session as shown in table (1), whereas, mean pre-exercise
=3.7 ± 0.55 and Mean post- exercise =7.8 ± 3.39 as shown in table (2).

**Table (1):** Values pre- and post- exercise of salivary cortisol for students samples

<table>
<thead>
<tr>
<th>Pre- exercise values (µg/dl)</th>
<th>post- exercise values (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.38</td>
<td>7.80</td>
</tr>
<tr>
<td>3.61</td>
<td>6.69</td>
</tr>
<tr>
<td>2.23</td>
<td>5.13</td>
</tr>
<tr>
<td>3.59</td>
<td>8.10</td>
</tr>
<tr>
<td>4.62</td>
<td>8.75</td>
</tr>
<tr>
<td>3.41</td>
<td>7.73</td>
</tr>
<tr>
<td>4.52</td>
<td>8.90</td>
</tr>
<tr>
<td>3.78</td>
<td>7.30</td>
</tr>
<tr>
<td>4.10</td>
<td>8.97</td>
</tr>
<tr>
<td>3.66</td>
<td>8.87</td>
</tr>
</tbody>
</table>

**Table (2):** Mean Values ± SD

<table>
<thead>
<tr>
<th>Mean Pre-exercise</th>
<th>Mean Post-exercise</th>
<th>Total</th>
<th>Pearson (P-Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.7 ± 0.55</td>
<td>7.8 ± 3.39</td>
<td>10</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

The salivary cortisol was measured before doing of sessions and after heavily exercise (high intensity exercise), Mean before the exercise = 3.7 ± 0.55, and the Mean of salivary cortisol after the heavy exercise = 7.8 ± 3.39, these results were found the significant increase in the salivary cortisol between pre- and post- exercise.

P- Value considered being statistically significant (P < 0.005).*

The results showed that P value was statistically significant in 10 (100 %) of students samples as shown in table (2).

There was a significant increase in the level of salivary cortisol immediately following the high intensity exercise (post exercise).

**Discussion**

In the present study, there was significant increase of salivary cortisol concentration between pre and post exercise in response to sport competition in an incremental fashion such that it reaches to the highest level 30 minutes after the cessation of the event.

The reason for the increase in cortisol could be explained, suggest that excitement prior to and during competition affects the physiological hypothalamic-pituitary-adrenocortical (HPA) and sympathoadrenal axis resulting in an increase of cortisol secretion, our data were agreed with [Filaire et al., 2007].
stated that the cortisol response to physical activity is dependent to the intensity and duration of activity.

Increase of cortisol following a football competition, that cortisol concentration increased even thought the game had finished is likely due to the duration and intensity of the physical activity on the cortisol concentration, however it might also be explained by a post exercise lowering of blood glucose as no food was administered after the match[15].

Salivary cortisol levels have been shown to increase following acute exercise with the response dependent on the intensity and duration of activity [Lac and Berthon, 2000; Jacks et al., 2002)]were agreed with our data and study.

[Vining and McGinley, 1987 and Lacks et al., 1993] stated that salivary cortisol provides a stress free, non-invasive procedure that avoids additional stress caused by vein puncture, salivary cortisol may also be a better measure of adrenocortical function as it represents more accurately the level of unbound cortisol. Post-exercise cortisol concentration changes seem to be affected by several mechanisms: stimulation of sympathetic nervous system, stimulation of hypothalamic-pituitary-adrenal secretion, increase of body temperature, change in blood- PH, hypoxia, lactate accumulation and mental stress [16]. [Kaciaba- Usiko et al., 1992] reported that cortisol concentration increases with increased daily exercise volume. [Ben-Aryeh et al.,1989] stated that cortisol concentration increases by continued exercises. These researches reported that physical exercise could stimulate HPA; increases body temperature, increases cortisol secretion and release of cortisol from the carrier proteins. Therefore, the high concentration of salivary cortisol accompanied with increase of saliva viscosity, is the good indicator of sympathetic nervous system activation[23].

References
11. Foster, C. Monitoring training in athletes in reference to overtraining