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Original Article

Serial measurements of cortisol, creatine kinase, and TNF-α levels in elite basketball athletes during a training season

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Abstract
Objectives: The aim of this study was to explore the impact of different training interventions on cortisol and inflammatory cytokines in elite athletes. **Materials and Methods:** The current study consisted of male, healthy, elite, and professional basketball athletes aged 18-34 years old from two elite teams from Greece and Turkey. The current experimental procedure involved measurements of serum cortisol, tumor necrosis factor A (TNF-α), and creatine kinase (CK) levels as well as lymphocytes and neutrophils. The current study consisted of three different blood sample collection time points: before initiation, mid-season, and at the end of the training season. Statistical analysis was carried out using GraphPad Prism 9.2. Two-way Analyses of Variance (ANOVA) with repeated measures were performed for all the parameters. The P value was set at <0.05. **Results**: The Turkish elite athletes showed a 3.857-fold increase in TNFα in all measurements as well as 58% and 34% increases for the second and third measurements of cortisol compared to the Greek athletes. There was no statistically significant difference between the groups regarding serum CK, lymphocytes, and neutrophils. In addition, there was a trend of an increase in serum CK. However, it did not reach statistical significance.
Conclusion: The current study shed light on the impact of different training regiments on cortisol and inflammatory cytokines. It was clearly highlighted how small training changes between countries can influence several biochemical markers. Our results highlight the crucial role of training design for cortisol and TNF-α levels in elite athletes during an entire training season.
Key words: **TNF-α, cortisol, creatine kinase, basketball athletes**

Introduction

Exercise training regimens play a very crucial role in improving metabolic and cardiovascular health as well as for maintaining a healthy immune system (Apostolopoulos *et al*., 2014; Beavers *et al*., 2010).
The exercise-associated activation of the immune system can lead to a modified concentration of proinflammatory cytokines. The frequency and intensity of these exercise training stimuli can have a considerable impact on these adaptive mechanisms and modify the proinflammatory cytokines accordingly (Gokhale *et al*., 2007).

One of the main inflammatory cytokines with very controversial results in the literature regarding exercise adaptations is Tumor Necrosis Factor alpha (TNF-α). TNF-α is a protein that is mainly produced by activated macrophages, T lymphocytes, and natural killer (NK) cells. Exercise can result in different modifications of TNF-a. A quite pronounced increase in TNF-α levels has been shown in endurance elite athletes (Bernecker *et al*., 2013).

However, several controversial results in the literature have not resulted in a clear picture of the role of different types of exercise on specific cytokines (McMahon *et al.,* 2019; Smart *et al*., 2011)
Cortisol is a glucocorticoid hormone that is secreted via the adrenal cortex. It is generated as a response to several physical, physiological, and psychological stressors (Wittert 1996, Hackney 2006, Hill 2008). Exercise is considered one of the main stressors that can modify circulating serum cortisol levels (Davies & Few 1973, Hill 2008). In addition, moderate to high-intensity exercise can lead to skeletal muscle damage, resulting in elevation of serum CK. CK is a protein that is is widely recognized as a biomarker of muscle damage, and increased levels can indicate prevalence of muscle disease, myocardial infraction, or mitochondrial myopathies (Brancaccio et al. 2007)

Collectively, the main literature findings have shown that moderate to high-intensity exercise can lead to modified circulating cortisol levels (Battaglini, 2008), TNF-α (Ulven 2015), and serum creatine kinase (Stelzer, 2015). Even though many studies have investigated the acute and chronic effects of exercise, there is no clear picture regarding the different training interventions and their impact on these parameters; thus, we explored this in this study.

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Materials and Methods

Participants

The experimental population for the study was comprised of healthy male, elite basketball players from Greek and Turkish teams. The inclusion criteria for the current study included good health and no prevalence of any chronic pathological conditions or fever episodes for at least 4 weeks before each blood sampling. In addition, the participants were not allowed to use any medication or nutrition supplements for at least two weeks before each of the measurements (confirmed by dietary recall). In addition, the athletes participating were advised to follow a healthy lifestyle with stable dietary conditions during the entire training season. The current experimental procedure consisted of 31 participants—elite athletes competing in the Euro league. In total, 15 athletes competed in a Greek Basketball team, while the other group of 16 athletes competed in a Turkish basketball team. Before initiation of the measurements, both verbal and written informed consents were obtained from all the participants. Furthermore, all the basketball athletes went through routine clinical examinations, including echocardiogram (ECG), blood pressure, and heart rate measurements, chest X-rays, etc.

Experimental procedure

The experimental procedure included blood sampling at three different time points, conducted in a 12-hour fasted state. The three time points were at the beginning of the training season, mid-way, and at the completion of the annual training season, and 20 mL of blood was collected each time from each participant. The sample was placed in special tubes, which accelerated coagulation, and it was left to coagulate at room temperature and was subsequently centrifuged at 3500 rpm for 5 min for (Eppendorf, UK) separation of the serum. The serum was stored at −20°C before measurement using automatic analyzers {(Mindray-BS-200 / China), [CLIA (Vidas, Roche, Basel, Switzerland)]}.

Statistical analysis

Graphpad Prism (9.2) was used for all analyses. The values are presented via the utilization of means ± SEM. For comparison of the repeated measurements in the groups, two-way ANOVA for repeated measures was used. The P-value was set at $p<0.05$ to determine the statistically significant results.

Results

The results of the current study are presented as means ± SEM.

There were no significant differences in serum CK levels between the groups (Figure 1). In addition, even though there was an increasing trend at the second measurement mid-season, it did not reach statistical significance.

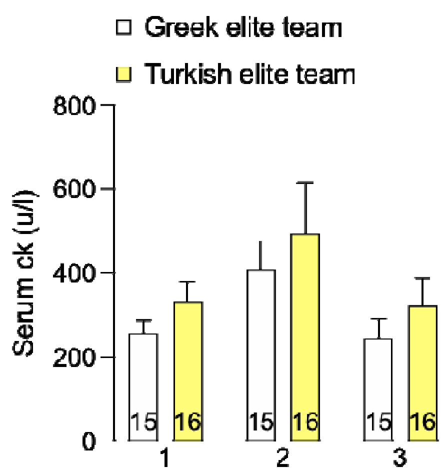

Figure 1. Serum CK in elite athletes from Greece or Turkey at the start (measurement 1), mid-season (measurement 2), and at the end (measurement 3) of the training season. Results are presented as means ± SEM, n=15–16 per group.

One of the most important results highlighted the importance of training stimuli and their role on cortisol levels. More specifically, the Turkish elite athletes showed 58% and 34% increased cortisol levels at the second measurements mid-season and at the third measurements at the end of the season, respectively, compared to the Greek basketball team.

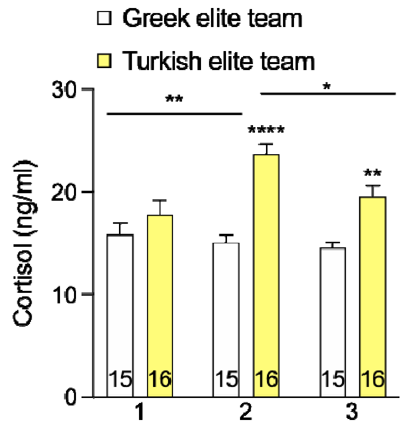

Figure 2. Serum circulating cortisol levels in elite athletes from Greece or Turkey at the start (measurement 1), mid-season (measurement 2), and at the end (measurement 3) of the training season. Results are presented as means ± SEM, n=15–16 per group.

In addition, the Turkish elite athletes showed a 3.857-fold increase in TNFα levels in all measurements compared to the Greek athletes.

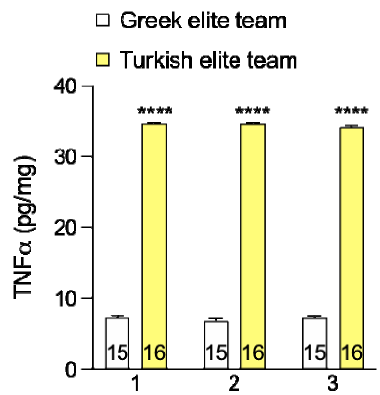

Figure 3. TNFα levels in elite athletes from Greece or Turkey at the start (measurement 1), mid-season (measurement 2), and at the end (measurement 3) of the training season. Results are presented as means ± SEM, n=15–16 per group.

In terms of the number of lymphocytes or neutrophils, the measurements showed no differences between the groups or among the different measurements.

A **B**

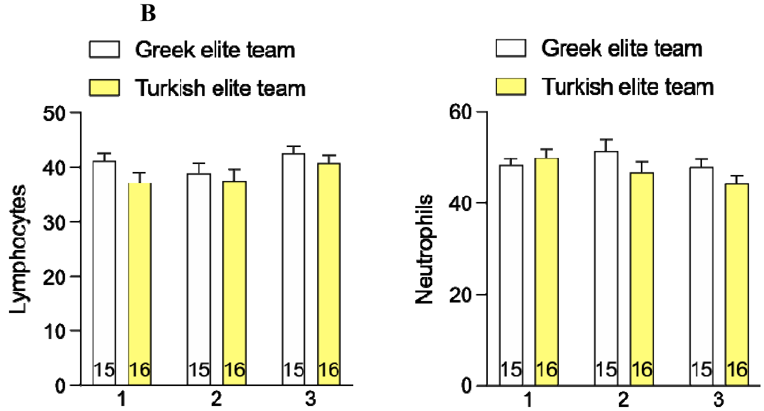

Figure 4. (A) Lymphocytes and (B) neutrophils in elite athletes from Greece or Turkey at the start (measurement 1), mid-season (measurement 2), and at the end (measurement 3) of the training season. Results are presented as means ± SEM, n=15–16 per group.

Discussion

To elucidate the impact of training design and parameters on several biochemical markers, we carried out serial measurements of creatine kinase, cortisol, TNF-α levels as well as lymphocytes and neutrophils during an entire annual training season. The current study included measurements from players from two elite basketball teams (Euroleague level) from Greece and Turkey. This was the first study to conduct these types of serial measurements and comparisons between the two countries during an entire training season.

The impacts of acute and chronic exercise on creatine kinase, cortisol, TNF-α levels as well as on lymphocytes and neutrophils have been mentioned in several scientific papers showing many different and contradictory results (Araujo *et al.,* 2019; Kilic *et al.*, 2019; Estrela *et al.*, 2017; Lira *et al.*, 2015; Nieman *et al.*, 2005). It is likely that the role of different training stimuli in different settings plays a major role at some of these parameters, including TNF-α and cortisol. This specific field of research has not been investigated in depth, and results have been contradictory; this is the reason why we explored this topic in the current study.

First, serum CK is widely recognized as a representative biomarker indicating skeletal muscle damage and recovery (Nikolaidis *et al.*, 2003). It has been primarily shown to increase after strenuous exercise and stay elevated for days when there is no adequate recovery or in the prevalence of any skeletal muscle pathological condition, myocardial infraction, or mitochondrial myopathy (Brancaccio *et al.,* 2007; Baird et *al.*, 2012; Koutedakis *et al.*, 1993). Overall, the serum CK levels of both groups were increased compared to non-athlete normal individuals. However, for both groups in the current study that were comprised of elite athletes, there was no statistically significant differences between the groups. There is a strong trend for increased serum CK levels at mid-season, most likely because of the increased training load and training intensities. However, because both groups were from the same level, it was very hard to obtain significant differences from such subtle differences. In addition, the variability of the results in the second measurement may be a reason for the lack of statistical significance. An increase of sample number in each group could potentially lead to statistically significant differences in the serum CK levels, especially mid-season. This is the reason why a greater participant number is recommended for future studies.

Second, circulating cortisol levels have been shown to be either increased (Peters *et al.,* 2001; Lee *et al.,* 2015) or reduced (Rist & Pearce, 2019) in elite athletes. The increase or reduction of this particular hormone can be attributed to chronic stress because of continuous games and leagues during the training season (Lee *et al.,* 2015; Rist & Pearce, 2019). However, the precise regulation of this hormone is not yet clear. In the current study, the cortisol levels were greatly increased for athletes that were on the Tuskish team. This was most likely due to the chronic stress, the increased number of games and leagues during the season, as well the different training design, organization, and volume.

Furthermore, physical exercise training leads to several exercise adaptations and benefits via modifying the immune system and metabolic health. One of the main cytokines that plays a crucial role during acute and chronic exercise is TNF-α. TNF-α is released as a part of an inflammatory response to exercise stimuli (Abedelmalek et al., 2013; Andersson *et al.,* 2010; Gokhale et al., 2007). Its levels depend on several exercise parameters. However, very high or very low levels of TNF-α have been associated with oxidative stress (Simioni *et al.,* 2018). In the current study, the athletes from the Turkish team showed an approximate 3.9-fold increase in TNF-α, which is indicative of potential stress and oxidative stress, likely due to overtraining and other associated parameters. Because this finding is descriptive, more research is required to identify the specific mechanisms linking exercise training regimes in elite athletes and TNF-α responses.

Regarding the immune cell count (lymphocytes and neutrophils) as a response to exercise stimuli in elite training regimes, it has been previously shown that the cell count may be lower in athletes participating in selected endurance-associated sports (Horn *et al.,* 2010). However, many parameters can influence this, including the time of measurement post-exercise and exercise intensity. In our current study, there was no difference in the cell counts between the groups. This can be attributed to the fact that all athletes were at the same performance level. In addition, basketball includes endurance bouts of exercise, but it also has other components. This is likely to have influenced the result. More research is required to shed light on this.

Conclusions

The impact of different training regimens and different training designs between elite basketball teams in different countries has not been addressed adequately in the literature. This is the first study that attempted to explore this to shed light on the different training adaptations and how these are addressed in different countries. The measured parameters in this study can be a basis for future research and screening during serial measurements in elite basketball teams to prevent chronic stress and fatigue and to optimize the performance and well-being of athletes.

Conflicts of interest - The authors declare that they have no competing interests.

Ethical approval:

All procedures performed in our studies involving human participants were in accordance with the ethical standards of the institutional (National and Kapodistrian University of Athens Ethics Committee) and national research committee with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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