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[Articles]

SALIVARY TESTOSTERONE AND CORTISOL
IN PROFESSIONAL ATHLETES:
CORRELATION WITH BEHAVIOR
IN SPECIFIC ACHIEVEMENT SITUATIONS*

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Summary. Aim: This study is focused on the monitoring and subsequent evaluation of changes in selected hormone parameters (cortisol, testosterone, testosterone/cortisol ratio (T/C)) in professional athletes during top level national league play-offs.

Methods: Testosterone and cortisol levels were measured and their relative production was evaluated in specific match situations: before the pre-match meeting, after the pre-match meeting, before the match, during breaks, during the match (team winning), during the match (team losing), and after the match. The relationship between the two hormones was examined using Pearson's correlation analysis and hormone levels were compared as paired values using parametric assays.

Results: The findings suggest it is not always possible to consider testosterone and cortisol as hormones whose production is mutually exclusive or that their mutual psychophysiological effect is inhibited.

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Conclusions: It seems that although the level of one hormone is dominant, the production of the other hormone remains relatively high, not decreasing. This means that in situations associated with a threat to social evaluation (team meetings during breaks, losing a match) and when an uncontrolled situation without active management (break, interruption of the match) changes into a fully controlled situation (match), the levels of both hormones increase simultaneously - cortisol in response to the fear of defeat and testosterone as a stimulant of the fight response, which can manifest in aggressive behavior.

Key words: Hormone responses in exercise, Stress, athletes, nAchievement

Introduction

For all animals, including humans, situations in which living organisms are exposed to danger are considered to be fundamental external stressors that affect the type of reaction or behaviour necessary to maintain the existence of the individual. In situations of extreme danger, non-specific and complex hormonal, neuronal and behavioural responses are activated. Stressors (in this case, life-threatening situations) activate the hypothalamic-pituitary-adrenal axis (HPA), leading to increased production of cortisol, which has an impact on what type of reaction is engaged in to respond to the life-threatening situation (Sapolsky, 2000).

Modern man does not often find himself in situations where his life is endangered by tangible threats but is increasingly exposed to threats mediated by the environment in which he lives. A typical example of a mediated threat is a social-evaluative threat. Research on the negative effects of negative social evaluation and the magnitude of the response has revealed interesting results. Dickenson and Kemeny (2004) found that social-evaluative stress, measured by the Trier social stress test (TSST), induced significant cortisol production.

The effects of stress responses to social evaluation have been relatively well documented (Stanton and Schultheiss, 2007; Wirth, Welsh, and Schultheiss, 2006), therefore subsequent studies focused on evaluating HPA responses to situations in which the individual is confronted with a specific challenge and a situation in which there is no threat of immediate social feedback. Dickenson and Kemeny (2004) concluded that achievement that is not socially rated contains uncontrolled elements that impact the magnitude of the stress response. A study by Agrigoriaei et al. (2013) provided evidence confirming the assumption that, in addition to social evaluation, there are also other potential factors that have an impact on the stress experience. In this study, participants exhibited high cortisol responses during simulated situations in which they faced challenges they could not influence, control or direct themselves.

Based on these findings, the relationship between social environment and the stress response has been interpreted from the perspective of achievement motivation. McClelland (1989, 1992) demonstrated that people who had an increased need to achieve success were also distinguished by the fact that the actual work meant more to them than the remuneration obtained. Their achievement alone was considered

sufficient reward, and the remuneration itself, such as wage, served as a form of feedback. This feedback was then used as an external criterion for assessing the complexity of subsequent tasks. Personal achievement, therefore, was their main focus, while people with a low need for achievement success tended to focus on the reactions of their social surroundings. In conclusion, in people with a high need for achievement (nAch), negative social evaluation did not trigger the HPA axis.

Most research on social stressors and nAch shows that individuals with high nAch respond to difficulties and challenges differently than individuals with low nAch (cf. Pang, 2010; Schultheiss and Brunstein, 2005, 2010). Individuals with higher nAch were more likely to choose more challenging goals (i.e., goals with a moderate chance of success) and negative achievement feedback tended to support increasing efforts to achieving goals (cf. Brunstein and Maier, 2005; McClelland, 1992).

For the purpose of our survey, Schultheiss et al. (2014) conducted important research in which they monitored the attenuating effects of achievement motivation on an organism's cortisol response. It was noted that achievement motivation had a dampening effect on the cortisol response only after a dominance challenge or tasks with social stressors, but not in situations where tasks were under minimal social control. It was concluded that the cortisol response in the face of a challenge can be reduced by achievement motivation. Schultheiss et al. (2014) further tested the hypothesis that individuals with high nAch, who had learned to connect tasks with the joy of having completed them, had a lower stress response to challenging tasks than individuals with low nAch who were not used to connecting challenging tasks with the joy of having completed them. The results of the study provided evidence that higher levels of nAch predicted a weaker stress response and a change in cortisol levels in certain situations.

An interesting claim from the research on HPA axis activation and achievement motivation has been that high nAch is generally not associated with lower cortisol responses. Rather, studies seem to support the hypothesis that the stress-reducing role of nAch is limited, in particular to difficult tasks and to the associated hope of completing them and not to situations that are without any social evaluation. This observation is consistent with Schultheiss and Brunstein (2005), who explained the development of nAch as being related to task difficulty and the subsequent joy of having mastered the task and the ability to convert negative feedback to a positive challenge and not a threat. This is consistent with the concept of nAch as the capacity to positively regulate the degree of perceived difficulty (Baumann and Scheffer, 2010; Kuhl, 2001; McClelland, 1989). For this reason, nAch is considered to be an automatic regulator of emotional responses, the development of which begins when the individual is first confronted with reasonably demanding tasks.

Our view of achievement as an emotionally regulated construct is supported not only by exploratory endocrine data (quotes) but also by experimental studies in which high nAch positively influenced responses to demanding tasks (Engeser and Rheinberg, 2008; Reeve et al., 1987), and where there was improved achievement in response to demanding situations in high nAchievement individuals (Brunstein and

Hoyer, 2002; Brunsten and Maier, 2005). Additional supporting data can be found in studies that have documented increased emotional well-being and lower levels of decreased mood and depression in high nACh individuals (McAdams and Valliant, 1982; Orlofsky, 1978). These data could be interpreted in the context of the above-mentioned results, which show that individuals with high nACh deal better with challenging situations and tasks than individuals with lower nACh.

Thus, the question arises as to how an individual responds to completing a challenging task and how this response is reflected in the activity of the HPA axis. An interesting indicator is the circulating levels of testosterone. Like cortisol, the role of testosterone in the stress response has been associated with the type of social situation the individual is exposed to, i.e., the magnitude of the threat, which then influences the dynamic of the response (Choong et al., 2008; Ko et al., 2014; Edwards, 2010). However, testosterone is produced during coping behaviour and influences the dominance and positive completion of tasks, thus eliminating the negative effects of a threat. On the other hand, the endocrine system is sensitive to physiological irritation, such as pain or physical exertion (Susoliakova et al., 2014; Arruda et al., 2014), which are related to trigger mechanisms that assess stimuli as threat or social challenge. This fact complicates decisions as to whether reactions are actually psychological or physiological. The relationship between testosterone and cortisol has been monitored in relation to a variety of conditions, such as work-related stress (Susoliakova et al., 2014; Serrano et al., 2014) or stress connected to physical activity and a sports match for amateur or professional athletes. The influence of the match or competition results on the levels of these two hormones is of interest, as well as changes in their levels on sport achievement, since cortisol and testosterone affect the ability to cope with stressful situations in different ways, although both affect motivation for further achievement. Older studies have demonstrated the relationship between pre-game nervousness and elevated cortisol levels. Postgame levels of cortisol were affected by the competition results, i.e., winners had significantly lower cortisol levels than losers (Michailidis, 2014). The opposite phenomenon has also been studied, i.e., whether high cortisol levels affect sport achievement. Increased concentrations induced by stress from solving a numerical task led to decreased achievement in competition (Lautenbach et al., 2014). Testosterone levels have also been studied in relation to team members and to the environment in which the match took place.

According to these biosocial hypotheses, it is possible to conclude that testosterone increases during threat management, rises after victory and falls after failure, and influences dominance-related behaviour (Carre et al., 2006). In addition, testosterone levels correlate with hypotheses regarding nACh (e.g., testosterone rises during social task management), which is why testosterone testing was included in our research.

Our research has allowed us to contribute to the discussions about the relationship between stress responses (among which may be triggered also aggressive reactions) and achievement motivation in situations where athletes find themselves under pressure of social-evaluative threat.

The research was designed in such a way as to allow test to the following hypotheses:

- H1: Testosterone levels would increase significantly during competition when the team was winning (or leading) and after the final victory.
- H2: Cortisol levels would increase significantly during competition when the team was losing and after the final defeat.
- H3: Cortisol and testosterone levels would fluctuate significantly after the pre-game meeting and before the actual competition.
- H4: Hormonal production of testosterone and cortisol are negatively related.

Materials and methods

Participants

Research was conducted with two players of the highest-level hockey league in the Czech Republic, two players of the highest-level basketball league, and two players of the highest-level volleyball league. All persons gave their informed consent prior to their inclusion in the study. Samples were taken during two play-offs on home ground. To maintain consistency, samples were always taken in the same or similar situations during a competitive match. The selection of players was random, and samples were taken according to predefined rules. Given that samples were taken during competition, it was necessary to train assistant trainers in how to take samples. The authors of this study did not take samples themselves to minimise disrupting the team regimen during competition, which could have affected the results of the investigation. All human studies have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Since there are many factors affecting cortisol and testosterone levels in the body, it is not sufficient to study the effects of sports competition before and after the game. Hormone levels have been shown to vary according to physical exertion, e.g., cortisol increases during endurance training and sudden stress. However, changes due to psychological stress are likely to have greater impact and are reflected as changes in hormone levels during competition when physical exertion is approximately constant. Another factor that influences cortisol levels is time of day. Cortisol levels are associated with circadian rhythms, with the highest cortisol levels occurring in the morning (Karlamangla et al., 2013). This effect was eliminated from our study because all matches were played at approximately the same time (start time 17:10–18:20 at the latest) when cortisol levels were more or less constant.

Changes to testosterone levels may occur as a response to grouping together with other team members (Walsh and Kitchens, 2015; Michailidis, 2014). Another factor that increases testosterone in athletes may be the site where the match takes place. It has been shown that hockey players have significantly higher levels of testosterone

when playing on home ground than on their opponent's ice. This may be connected to greater self-confidence and aggression associated with knowledge of the location but also due to nervousness caused by home fans (Arruda et al., 2014; Carre et al., 2006). All matches observed in this study were played on home ground, and thus, this effect should be constant. The results showed that hormone levels changed during competition and must therefore be affected by factors that remain constant during the match but are other than those mentioned above.

Biological samples were taken in pre-determined situations that were the same for all teams. Saliva samples were taken as follows: players rinsed their mouths with clean water and then spat a saliva sample into a clean test tube. Test tubes were frozen as quickly as possible and stored at -20°C . During hockey games, samples were taken during team changeovers. During basketball and volleyball games, samples were taken during team changeovers or when the game was interrupted. Samples were taken as many times as possible to increase the possibility of detecting differences in steroid hormone levels to draw relevant conclusions. Sample collection took place in the following situations:

1. Before the pre-game meeting.
2. After the pre-game meeting.
3. Before the match.
4. Pauses, breaks.
5. During the match – team losing.
6. During the match – team winning.
7. End of the match.

Method and data analysis

Analysis of the biological material proceeded as follows. Before analysis, saliva was thawed, centrifuged ($13\,000 \times g$, 10 minutes) and 0.5 ml was collected in a clean test tube. Subsequently, 10 μl of methyl testosterone solution ($c = 25 \text{ ng/ml}$) was added as an internal standard and 0.5 ml water. After stirring, 2 ml of the solution was extracted, and 1.5 ml of diethyl ether was added. The organic layers were placed in a clean test tube, evaporated, and the residue dissolved in 100 μl methanol (20% (v/v)).

Testosterone and cortisol levels in the samples were determined using high-performance liquid chromatography and mass detection using the Agilent 1290 HPLC Infinity and Agilent 6460C Triple Quadrupole with electrospray ionisation and an Agilent Zorbax RRHD Eclipse Plus Phenyl-Hexyl chromatographic column ($2.1 \times 100 \text{ mm}$; $1.8 \mu\text{m}$) (Agilent Technologies). Gradient elution of the mobile phases of 1 mM ammonium fluoride and acetonitrile/methanol (75/25 (v/v)) was used. Ionisation for mass detection was in positive mode.

Each sample was prepared and analysed twice. A total of 203 samples were processed. However, some were devalued, resulting in a statistical analysis of

188 samples (57 samples from hockey players, 90 samples from basketball players, 51 samples from volleyball players).

Statistical analysis

Data were evaluated using descriptive and test statistics. Fluctuations in testosterone and cortisol hormone levels were evaluated using correlation analysis (Table 1). For Students's paired t-tests, hormone values were assigned individually to each player, and the paired values were then analysed in the selected sports match situations (Tables 2 and 3).

Table 1. Correlation Analysis of Testosterone and Cortisol Hormone Levels

Factors (different phases of the game)	Hockey match	Volleyball match	Basketball match
B – after the meeting	-.923	-.997	-.447
C – before the match	.629	.488	.324
A – before the meeting	-.176	-.730	-.164
D – pauses, <u>breaks</u>	.097	-.030	.524
E – during the match – team <u>losing</u>	-.100	.416	.364
F – during the match – team <u>winning</u>	-.268	.049	-.556
G – end of the match	-.163	-.974	-.306

Note. Factor loadings greater than .30 are **in bold**.

Results

Tables 1 and 2 and the individual descriptive analyses values show that the baseline levels of both testosterone and cortisol were significantly lower before the match than during the match. During the match, we observed very significant variations in cortisol hormone levels, whereas testosterone appeared relatively stable; when determining the level of testosterone during various phases of the match, there were no significant, dramatic or frequent fluctuations in its level, except in situations where players experienced success during or after a match, i.e., they won.

After the pre-game meeting but before the match, a gradual and significant increase in cortisol levels was recorded in response to pre-match excitement at the meeting before the match (information about opponents, game strategy profiling, specific challenges for specific players, etc.) and the upcoming match. The samples from after the pre-game meeting contained the highest recorded levels of cortisol

Table 2. Differences in hormone levels in the different phases of the match – summary

Hormone	Phases of the match	Test <i>t</i>
TES	<u>A</u> – B	2.352*
TES	A – <u>C</u>	1.86>*
COR	A – <u>D</u>	2.6*
COR	A – <u>E</u>	2.485*
COR	B – <u>D</u>	3.532**
TES	B – <u>C</u>	2.067*
TES	B – <u>E</u>	2.618*
TES	B – <u>F</u>	3.153**
TES	C – <u>F</u>	1.871>*
COR	<u>C</u> – G	2.216*
COR	<u>D</u> – G	3.802**

Note. TES – Testosterone, COR – Cortisol; A – before the meeting, B – after the meeting, C – before the match, D – pauses, breaks, E – during the match – team losing, F – during the match – team winning, G – end of the match; Underlined letter means higher value; α .1>*; α .05*; α .01**

Table 3. The differences in hormone levels in the different phases of the match.

Hockey match			Volleyball match			Basketball match	
Hormone Levels	<i>p</i>	<i>t</i>	Hormone Levels	<i>p</i>	<i>t</i>	Hormone Levels	<i>p</i>
COR: A – <u>D</u>	.031*	3.853	COR: A – <u>C</u>	.04*	4.818	TES: A – <u>D</u>	.036*
COR: B – <u>D</u>	.037*	3.608	TES: B – <u>C</u>	.04*	4.846	COR: A – <u>E</u>	.028*
COR: <u>D</u> – E	.022*	2.673	TES: B – <u>D</u>	.04*	4.843	COR: B – <u>D</u>	.042*
COR: <u>D</u> – F	.04*	2.357				COR: B – <u>E</u>	.003**
COR: <u>D</u> – G	.002**	5.657				COR: E – <u>F</u>	.036*
						COR: <u>F</u> – G	.001**

Note. TES – Testosterone, COR – Cortisol; A – before the meeting, B – after the meeting, C – before the match, D – pauses, breaks, E – during the match – team losing, F – during the match – team winning, G – end of the match; Underlined letter means higher value; α .1>*; α .05*; α .01**.

before the match. Testosterone levels decreased significantly compared to cortisol, but before the match, the levels of both hormones rose significantly, and this level of testosterone remained stable during the game, except when the team won (see Table 3). These findings are consistent with the assumption in hypothesis H3: cortisol and testosterone levels will change significantly after the meeting and before the game itself, which we accept. Before the match, focus on achievement (rising testosterone) and social assessment (fear of failure, reactions of others/environment) might be important motivational factors. Given pre-competition hormonal stress responses, players may have a typically high need for successful achievement and a similarly high need to avoid failure.

Cortisol and testosterone levels fluctuated based on the contextual situation during the match. Interestingly, cortisol levels rose to their maximum (compared to other situations) during breaks between each third/quarter of the game but were significantly reduced during the game, regardless of whether players were winning or losing, and the role of cortisol was taken over by rising testosterone. This conclusion supports the theory behind the psychophysiological effects of cortisol and testosterone. During breaks between each third/quarter of the match (i.e., periods of rest and relaxation), cortisol emerges as a physiological response to a number of psychosocial stress factors. In particular, trainer feedback, match situation, supportive behaviour from the trainer, or proposed game strategy for the next phase of the match, which can be perceived as situations with a specific challenge but also as situations that cannot be influenced or controlled by the player at the time (see Agrigoriaei, 2013). The production of testosterone did not change significantly during breaks and corresponded to pre-match levels.

A significant increase in cortisol occurred even in situations where players were losing; there was a similar (although more gradual) rise in testosterone. A high level of cortisol in the losing phase suggested that players evaluated match loss as a situation with negative feedback that activated within them a fear-related motivation, i.e., fear of failure and participation in failure. However, a slightly higher level of testosterone correlated with physical excitement and increased concentration on achievement. When players were winning, cortisol levels decreased significantly and remained stable at levels measured after the meeting but before the match, while the dominant hormone in these situations was testosterone. Increased testosterone was expressed along with increased physical achievement, both with increased concentration on achievement and with increased critical thinking, which positively affected player achievement, e.g., more accurate passes or more creative and effective solutions to game situations.

At the end of the match, cortisol and testosterone levels reflected the progress and result of the match. If players took the lead during the match or achieved final victory, testosterone levels increased significantly and cortisol levels decreased proportionally after the end of the match. When players were losing during the match or lost the entire match, cortisol levels increased significantly after the end of that match, while testosterone levels remained high in losing during a match.

The above findings are in line with the following hypotheses that were accepted: H1: testosterone levels rose significantly during gameplay in situations where the team was winning (leading), and after final victory and the end of the match, and H2: cortisol levels rose significantly during gameplay when the team was losing and when the team lost the entire match. In contrast, we rejected H4, which was that testosterone and cortisol production are in a negative relationship of dependence based on the contradictory effects of testosterone and cortisol. Having monitored the development of both hormone levels, it was not always possible to consider testosterone and cortisol as hormones that mutually eliminate, suppress or inhibit a psychophysiological state. Table 1 shows that before the match, there were high levels of both hormones, or rather, their levels increased significantly compared to previous measurements. Similarly, in periods of rest (match breaks) or while losing (during or after the match), there were no significant opposing, antagonistic tendencies. However, in situations where the production of one hormone was dominant, the production of the other hormone remained relatively high and did not fall. This means that in situations associated with the threat of social evaluation (team meetings during match breaks, losing during the game), as well as in situations that change from those impossible to control or manage actively (match breaks, game interruptions) to those controlled fully (gameplay), the levels of both hormones rise together – cortisol as a fear response to failure, and testosterone as a stimulant to fight.

Discussion

The main objective of the study was to determine whether there was a correlation between testosterone and cortisol in different situations during sports achievement and whether this relationship also occurred in situations in which players were at physical rest and exposed to social pressures, e.g., during pre-game meetings or match breaks. Our study focused on whether the levels of circulating cortisol and testosterone changed depending on the type of situation in which the athlete found himself. A negative relationship between circulating cortisol and testosterone in winning situations was expected. We were also interested in how players would react to social pressure during pre-game meetings and how testosterone levels would change at times of rest and during a match.

The study showed that in certain situations, there was a positive, not negative, relationship between cortisol and testosterone levels. Negative fluctuations did occur, and in particular, they occurred in situations after pre-game meetings or after the final victory. This fact could be especially interesting for trainers who lead these meetings. In situations of relative physical rest, the body responded as if stressed and in this case to social situations. Certain levels of hormonal activation before the match were expected, but the levels of cortisol in these situations were among the highest. In such cases, there could be disproportionate stress on the athlete before the game, which could lead to unnecessary energy loss. A positive relationship between

cortisol and testosterone was observed in situation C (before the match). At this moment, with rising stress, there was also growing fervour and desire for victory. Athletes were ready to engage, and the body was excited, which corresponded to the above-mentioned HPA axis theory.

A closer look at the results of the analysis in terms of the different sport sectors examined reveals several interesting indicators. During hockey games, the level of cortisol changed during the match, in particular in situations of relative rest, i.e., before the match and during match breaks, regardless of whether the team was winning or losing. Testosterone levels rose especially before a match, while during the match or after the match, there were no significant fluctuations in testosterone levels. Hockey is a very fast contact sport, and players are under constant pressure to maintain concentration and keep up the fast pace of the game. Given that the observed matches were held towards the end of the season, extreme social pressure from the trainer, team owner and fans should be taken into account. Players might react more stressfully at this time than during other parts of the season. These reactions could affect their achievement. According to Schultheiss et al. (2014), trainers could use this finding to prepare athletes psychologically and emotionally, especially in the area of nAch. That is, to work with them in such a way as to reduce social threat and negative social evaluation in case of failure. If athletes do not combine achievement with potential failure or potential threat but learn to perform for the joy of overcoming the challenge at hand, they will have a lower stress response to challenging games than individuals who have learned to connect achievement with potential failure or potential threat. In this respect, these findings also confirmed the results of our study, especially in situations during the pre-match meetings when cortisol levels increased and could have dampened achievement.

Conclusions

The results of the study suggest (1) that testosterone, which is produced during dominance-related behaviour and influences dominance and positive management of challenges, increased before matches. Thus, in these situations, testosterone suppresses the cortisol response, and the individual is goal-oriented and ready to “fight”, to “attack”, which can also lead to aggressive behavior.

It is worth mentioning research by Carney & Cuddy & Yap Cuddy (2010) in which it was confirmed that posture and certain gestures increase testosterone levels and reduce cortisol responses. Carney and Cuddy and Yap (2010) stated that by simply changing posture, the individual sets his or her psychological and physiological systems to withstand difficult and stressful situations and perhaps even improves confidence and achievement in situations of potential threat or potential negative social evaluation.

Our study showed (2) that potentially problematic situations were pre-match meetings when testosterone levels dropped significantly and cortisol levels increased.

Although testosterone levels increased in pre-matched situations, cortisol levels increased also; hence, there was also an increase in perceived stress.

This study aimed to map the physiological reactions of athletes to different situations and connect these changes with possible behavioural responses to discover how to develop nAch athletes and dampen responses arising from HPA axis activation. This potential benefit is particularly important for trainers and coaches who prepare athletes psychologically and have an immediate impact on the development of an athlete's achievement, especially athletes with lower nAch who are potentially more susceptible to fear of negative social evaluation in case of failure.

Our study has limitations that should be mentioned. First of all, it is worth noting the small size of the study group. Including a larger number of athletes in the study would provide a more solid empirical basis for generalizing the conclusions drawn so far. However, they indicate interesting relationships and outline a further direction of searching for an explanation of athletes' behavior during their careers.

Translated by Authors

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