**DYNAMICS OF TRAINING DISTRESS, PERFORMANCE, AND EXCRETION OF CORTISOL AND CORTISONE IN URINE DURING SIX WEEKS OF TRAINING IN ELITE SWIMMERS**

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**Introduction**

Elite swimmers perform large amounts of training at high intensity to improve their competition results. In order to avoid the development of overtraining syndrome, they have to maintain an optimal balance between training intensity and recovery [1].

Studies on urinary cortisol/cortisone ratio (C/Cn ratio) overnight [5] and C/Cn ratio for 24 h [1] have been conducted to monitor the effect of the training load on elite swimmers. The hypothalamic-pituitary-adrenal axis (HPA axis) plays the main role in the adaptation for endurance training and for the response to intensive exercises. Repeated exercises bring adaptation of the HPA axis with decreased sensitivity of tissues to glucocorticoids [6] and reduced glucocorticoids receptor levels [3]. Urine sampling methods, as opposed to plasma, are applied to assess HPA axis responses when athletes perform large amounts of training and participation in competitions, without any disturbances to their training conditions. These methods are non-invasive, non-stressful, reliable, and practical [1]. Plasma cortisol levels are modulated by variations of their binding protein (CBG) and bear a low level of correlation with the rate of cortisol production, unless differences in CBG are corrected, whereas urinary free cortisol concentrations are independent of CBG concentrations and thus, closely reflect the production of 24-h free active plasma cortisol [2].

Different physiological variables have been investigated as a potential marker of training distress, which include metabolic, cardiovascular, immunological, neuromuscular, and endocrinological measures [8]. However, due to their high intra-individual variability, those variables have shown only weak relationships with training distress [7, 8]. On the other hand, psychological and behavioural measures have been observed to have stronger relationships. Additionally, they are efficient, inexpensive and non-invasive [16].

Grove et al. published findings from three...
experimental studies which estimated the short-term training distress and performance readiness in swimmers [7]. The authors confirmed that reductions in performance capabilities which are induced by the training workload were associated with parallel increase in the score of the Training Distress Scale (TDS). TDS is a 19-item questionnaire which measures generated distress symptoms previously identified by Fry et al. [4]. The link between TDS scores and swimming performance was evident from the results of the swimmers with low levels of distress symptoms 2 weeks before a major competition, who swam better during the event than those who reported higher levels of distress symptoms [7].

Aim

The aim of this study was to record the dynamics of training distress, performance, and excretion of cortisol and cortisone in urine during six weeks of training in elite swimmers.

Materials and Methods

Participants

Twenty-four elite swimmers (10 women and 14 men) from the national swimming team of Bulgaria took part in this study. They had an average age of 18.7 ± 3.78 years.

Design of the study

The athletes were tested over a 6-week period starting 2 weeks after the National Team Championship and finishing 2 weeks before the National Individual Championship (both in a 50 m swimming pool). The degrees of training distress and urine concentration of cortisol and cortisone were evaluated every two weeks. In total, the measurements were taken four times: on the 1-st (T1), 14-th (T2), 28-th (T3) and 42-nd days (T4) on Monday mornings. Additionally, anthropometric measurements were conducted at T1 and T4.

Training programme

The study was conducted in the summer season (3rd macrocycle) stretching from April to July, in which the training sessions in a 50 m swimming pool were performed. The reported six-week period was part of the general preparation phase, and it started right after the 2 weeks of recovery phase following the main competition. The swimmers trained twice per day from Monday to Friday, performing a total of 10 training sessions a week. During this period, the training process of the swimmers is associated with a gradually increased training volume, starting from 40 km for the first week and finishing at 65 km for the last. The main goals for this training phase are to improve aerobic capacity and to maintain anaerobic power and speed. The training has to produce central circulatory and respiratory adaptations which improve the delivery of oxygen and nutrients in the muscles [14], and as a result of that, an increased VO₂max is expected. The training programme of the participants in this study, including training volume (km) and training zones (% of all volume), is presented in Table 1.

<table>
<thead>
<tr>
<th>Training programme of the elite swimmers</th>
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<td>Training zones/Volume (km)</td>
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<td>I. Basic Endurance HR 120-150</td>
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<td>II. Threshold Endurance HR 150-180</td>
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<td>III. High performance endurance HR &gt;180</td>
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<td>IV. Anaerobic: Lactate tolerance + Lactate production + Race pace training</td>
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<td>V. Sprint</td>
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<td>VI. Recovery HR &lt; 120</td>
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Additionally, first-morning urine samples were taken to determine the concentration of cortisol and cortisone. The participants, with only 3 exceptions, noted that this was their only urine for the whole night. The competitors always had 60 hours of recovery (from Friday night to Monday morning) before the samples were taken.

**Chemicals and reagents**

Formic acid (HPLC grade) was obtained from Merck (Darmstadt, Germany). Acetonitrile, methanol and tert-butyl methyl ether (HPLC grade), K₂CO₃ and KHCO₃ puriss p.a. were purchased from Sigma-Aldrich (Steinheim, Germany). Cortisol and cortisone were from LGC (Luckenwalde, Germany). D₃-Methyltestosterone was from RIKILT (Wageningen, Netherlands).

**Sample preparation**

Two millilitres by volume of urine were mixed with 0.5 mL carbonate buffer pH 9.6 (K₂CO₃/KHCO₃ 20/20, w/w) and shaken. After the addition of 20 μL internal standard (ISTD, d₃-methyltestosterone, 200 ng/mL), the urine sample was extracted by 3.0 mL of t-butyl-methyl ether for 20 min and centrifuged at 5000 rpm for 10 min. The aqueous phase was frozen and the organic layer was isolated in another tube and evaporated to dryness at 55 °C under a stream of nitrogen. The residue was reconstituted in 200 μL of CH₃OH/0.2% formic acid, H₂O (10:90).

A five-point calibration curve and two quality controls (QC) in water (Table 2) were used for the quantification of samples.

**Instrumentation**

LC-MS-MS analysis was performed on an Agilent Technologies UHPLC 1290 Infinity with 6460 Triple Quad mass spectrometer.

A reversed-phase ZORBAX Eclipse Plus C18 Rapid Resolution HD column (2.1 × 50 mm 1.8 Micron) was used for the analyses. The mobile phase was composed of 0.2% formic acid, H₂O as solvent A and acetonitrile as solvent B. A gradient (Table 3) was run at 0.4 mL/min and the injected volume was 10 μL.

MS/MS detection was performed in multiple reaction monitoring (MRM) mode and electrospray ionization (ESI) in the positive ion mode. Acquisition parameters were summarised in Table 4.

The obtained sample concentrations were adjusted to a urine specific gravity (SG) of 1.020 based on the following equation:

\[
\text{Conccorrected} = \text{Concmeasured} \times \frac{(1.020 - 1)}{(\text{SG} - 1)}
\]

**Statistical analyses**

Statistical analysis was performed using the GraphPad Prism 5.0 software. The significance of differences of anthropometric mean values was evaluated by paired Student’s t-test. Kolmo-
gorov-Smirnov test of normality was performed for TDS, Cortisol (C), Cortisone (Cn) and C/Cn ratio. The differences between the obtained values for each measurement were evaluated by the non-parametric Friedman’s test for repeated measures with Dunnn post hoc test for the significance of differences between each pair. The data is presented as Mean ± Standard Error (SE) in graphics and as Mean ± Standard Deviation (SD) in the text.

**Results**
The anthropometric parameters which were measured in T1 and T4 are presented in Fig. 1. There were no statistically significant differences in body composition, both in males and in females.

The analysis of the responses from the Training Distress Scale (TDS) showed a good internal consistency for the composite measure, with an average of Cronbach’s alpha of 0.871 across administrations. The TDS score dynamics are presented in Fig. 2. The TDS score at T4 (6.92 ± 7.15) was significantly lower than that at T1 (14.96 ± 10.63), as well as that at T2 (15.21 ± 12.44), T1 14.96 ± 10.63; T2 15.21 ± 12.44; T3 11.54 ± 7.91; T4 6.92 ± 7.15.

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Fig. 1. Anthropometric parameters of female (Left) and male (Right) swimmers at T1 and T4

Fig. 2. Dynamics of TDS score for the elite swimmers across T1 to T4:

* p < 0.05 vs T4; *** – p < 0.001 vs T4
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Fig. 3. Dynamics of cortisol and cortisone urine concentration across T1 to T4:
* p < 0.05 vs T1

Fig. 4. Dynamics of the cortisol/cortisone ratio across T1 to T4

Fig. 5. Dynamics of the sum of cortisol and cortisone urine concentration across T1 to T4:
* p < 0.05 vs T1
The dynamics of cortisol and cortisone urine concentration are presented in Fig. 3. The concentration of cortisol at T1 (82.7 ± 62.8) was significantly higher than those at T3 (35.9 ± 47.7) and T4 (35.0 ± 24.2). The concentration of cortisone did not show any significant differences at any of the times tested (T1 to T4).

The dynamics of cortisol/cortisone ratio (C/Cn ratio) are presented in Fig. 4, and that of the sum of cortisol and cortisone urine concentration is presented in Fig. 5. The C/Cn ratio did not show any significant differences at any of the times tested (T1 to T4), while the sum of cortisol and cortisone urine concentration was significantly lower in T3 and T4 vs T1.

Discussion
The body composition data of the elite swimmers during the investigated period remained the same, which suggested that the nutritional status of the participants was unchanged. As a side observation, a bigger difference between the height and the arm span, more visible in men (about 7 cm), was registered in this study. Similar findings were reported for swimmers in the literature [12].

The adapted Training Distress Scale (TDS) has been shown to be an easy and appropriate instrument which can be used in everyday coaching practice, and as a tool for longitudinal studies. The results from our study showed that the TDS scores decreased during the investigated period (Fig. 2), despite the training volume increasing by 5 km per week (Table 1). This was also backed up by the reduced concentration of cortisol in T3 and T4 vs T1.

In our study, in almost all cases the morning urine was the all-night urine, and the concentration of the hormones measured was adjusted to its density. Thus, the effect of a volume of urine was considerably reduced, and the measured cortisol and cortisone concentrations reflected the total amount of hormones released overnight.

Conclusions
The significantly reduced cortisol concentration in urine in T4, as well as the sum of the concentrations of the cortisol and cortisone, are in line with the reduction of the TDS score in T4. The lower concentration of the stress hormone, cortisol, in the urine, and the lack of subjective symptoms of training distress led to good physical and mental recovery of the athletes after 60 hours of rest.

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ДИНАМИКА ТРЕНИРОВОЧНОГО ДИСТРЕССА, ПРОИЗВОДИТЕЛЬНОСТИ И УРОВНЯ КОРТИЗОЛА И КОРТИЗОНА В МОЧЕ У ЭЛИТНЫХ ПЛОВЦОВ ВО ВРЕМЯ ШЕСТИНЕДЕЛЬНОГО ТРЕНИРОВОЧНОГО ЦИКЛА

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Цель. Оптимальный баланс между интенсивностью тренировок и восстановлением особенно важен для улучшения результатов элитных пловцов. Данное исследование было нацелено на выявление динамики тренировочного дистресса, производительности и экскреции кортизола и кортизона у элитных пловцов во время шестинедельного тренировочного цикла. Материалы и методы. В исследовании приняли участие 24 спортсмена (10 женщин и 14 мужчин) национальной сборной Болгарии по плаванию, средний возраст которых составил 18,7 ± 3,78 года. Тренировочный дистресс и концентрация кортизола и кортизона в моче оценивались каждые две недели. Измерения проводились в 1-й (Т1), 14-й (Т2), 28-й (Т3) и 42-й день (Т4). Антропометрические измерения проводились в 1-й и 42-й дни. Процентное содержание жира и мышечной массы оценивалось с использованием метода измерения толщины кожной складки. Результаты. Показатель тренировочного дистресса на 42-й день (6,92 ± 7,15) был значительно ниже, чем в 1-й (14,96 ± 10,63) и 14-й днях (15,21 ± 12,44). Концентрация кортизола на 28-й день (35,9 ± 47,7) и 42-й день (35,0 ± 24,2) также была значительно ниже, чем в 1-й день измерений (82,7 ± 62,8). Концентрация кортизона не показала каких-либо существенных различий между 1-м и 42-м днем измерений, однако сумма концентраций кортизола и кортизона в моче была значительно ниже на 28-й и 42-й дни по сравнению с 1-м днем измерений. Заключение. Значительно сниженная концентрация кортизола в моче на 42-й день измерений, а также сумма концентраций кортизола и кортизона соответствовали уменьшению показателя тренировочного дистресса к 42-му дню тренировочного цикла.

Ключевые слова: кортизол, кортизон, пловцы, перетренированность, показатель тренировочного дистресса.
Динамика тренировочного дистресса, производительности и уровня кортизола…

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