# BIOCHEMICAL, HORMONAL AND PSYCHOLOGICAL MONITORING OF EIGHT WEEKS ENDURANCE RUNNING TRAINING PROGRAM IN FEMALE RUNNERS

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#### Abstract:

Athletes improve performance when an optimal balance between training stress and recovery exists. If out of proportion, overtraining syndrome (OTS) can ensue. Currently several biomarkers are used to detect OTS, but none meets all prespecified criteria for definite diagnosis. The purpose of the study was to investigate the effects of eight week endurance running training program on biochemical, hormonal, and psychological parameters in female runners. Seventeen runners were recruited for the physical training that consisted of two three-week progressive overload periods, each followed by a week taper period, and concluded with a 10 or 21km competitive run. Blood sampling was conducted at six time-points during the eight-week training program: Baseline (Baseline), after the first and second three-week training loads (Load1, Load2), after each taper week (Taper1 and Taper2), and post-study (Recovery). At each testing, the subjects were asked to complete the Recovery-Stress Questionnaire for Athletes (RESTQ). At the completion of the program significant improvement of physical performance was observed (VO2max +4.3%; p=.03). At Recovery the cortisol values significantly decreased compared to Baseline (p=.002) and to Taper2 (p=.008). In RESTQ we found that the recovery subscales sum was significantly lower at the recovery period when compared to the second taper period, and self-efficiency increased at Taper2. We also found significantly higher selfregulation during resting periods when compared to baseline. The main finding of the current study indicates that the performed training was well-balanced between stress and recovery periods, resulting in a positive training effect. It seems that psychological parameters are more sensitive markers than biochemical and hormonal one for the detection of changes in stress/recovery. The RESTQ may provide a practical tool for recognizing OTS in its early stages.

Key words: training status, RESTQ, biochemistry, overreaching, stress, recovery

#### Introduction

The obvious goal of an athletic training is to enhance performance and is only achievable when the balance between training stress and recovery is optimal (Gleeson, 2002; Halson & Jeukendrup, 2004; Meeusen, et al., 2006; Urhausen & Kindermann, 2002). Monitoring of the recovery-stress state and performance may be warranted to avoid imbalance between stress and recovery, and thus to prevent non-functional overreaching (NFOR) or overtraining syndrome (OTS) (Meeusen, et al., 2006, Kellman & Kallus, 2001). Survey studies assessing entire athletic careers indicate that 60% of elite runners experienced at least one previous episode of NFOR/OTS (Morgan, Connor, & Sparling, 1987). It is well known that acute and chronic exercise elicits changes in the levels of physiological and hematological, biochemical, hormonal, and immunological markers. Currently, several markers are used for detection of OTS, but none of them meets all criteria to make it reliable (Meeusen, et al., 2006). Long-duration, high-intensity exercise is associated with microinjuries and a local inflammatory reaction in the musculature causing changes in the serum markers of inflammation (Bresciani, et al., 2011; Smith, 2000). It is also well known that exercise alters hormonal response, such as testosterone and cortisol. The ratio between both hormones has been used to indicate the balance between the anabolic and catabolic activity (Papacosta & Nassis, 2011; Adlercreutz, et al., 1986). Furthermore, both hormones could also be considered as an indicator of changes in state anxiety status and its relationship to performance (Gatti & De Palo, 2011).

In addition to selected biochemical indices, psychological changes have also been used to monitor the state of an athlete during different training periods. The authors (Coutts, Wallace, & Slattery, 2007) found that psychological indicators, combined with the athletes' success are better indicators of overtraining than biochemical and physiological indicators. They found that monitoring stress and recovery processes in swimmers were well monitored with the use of a psychological questionnaire RESTQ (González-Boto, Salguero, Tuero, González-Gallego, & Márquez, 2008). Stress and recovery processes in female speed skaters combine to result in overtraining not only due the amount of practice, but also due to increased levels of general stress, combined with low levels of recovery processes (Nederhof, Zwerver, Brink, Meeusen, & Lemmink, 2008). One important finding was that stress and recovery during practice must be balanced (Garatachea, et al., 2011). Recent research found no changes in laboratory tests of bodily fluids in football players when they compared their intense practice periods with the rest periods (Meister, et al., 2013). Coutts and Reaburn (2008) however, found that blood and urine chemical analysis were extremely variable and that they are only able to indicate general stress indicators. Some authors found that there was less overtraining in professional football players when they were supervised by a professional leader with the use of situation-appropriate leadership styles (Faude, Kellmann, Ammann, Schnittker, & Meyer, 2011). Authors believe that hormonal indicators should not be used as the only early predictors of overtraining, but should be combined with psychological signs (Bresciani, et al., 2011).

At this time it is not yet clear which mechanism eventually leads to the OTS and a definitive diagnostic tool for OTS is not available (Meeusen, et al., 2006). However, the combination of biochemical, hormonal, and psychological responses appears promising. In light of the gaps in the existing literature, we aimed at evaluating the influence of eightweek endurance running training program on biochemical, hormonal, and psychological parameters in female runners. The other aim was to compare these parameters obtained in the conditions of stress with those obtained in recovery periods of training.

## **Methods**

#### **Participants**

Seventeen healthy active female recreational endurance runners volunteered to participate in the present study. Prior to the inclusion, all the participants regularly had 3-4 regular running sessions per week. Physical characteristics of the participants are shown in Table 2. The study protocol was approved by the National Ethical Committee of Slovenia. All the participants gave written informed consent to participate in the study.

## Study design

The study was completed during an intensified training phase focusing on the competitive 10 km or 21 km runs in the International Ljubljana Marathon, October 2008. Before the training process began, all the runners completed a two-week run-in period of low-intensity physical training to ensure their familiarity with the experimental procedures and to not reaching a state of fatigue during a practice.

The physical training program consisted of two, three-week progressive overload periods (training loads: Load1 and Load2) each followed by a week of taper period (Taper1 and Taper2). After the second taper week, the runners participated in the race,

Tests	Baseline	Week 1	Week 2	Week 3 (Load1)	Week 4 (Taper1)	Week 5	Week 6	Week 7 (Load2)	Week 8 (Taper2)	Week 9-10 (Recovery)
Incremental test to determine VO <sub>2</sub> max	Х									х
2400-m time trial (Cooper test)	Х									х
Anthropometry	Х									х
Race (10 or 21 km)									Х	
Blood and saliva collection	х			х	Х			х	х	Х
Pshycological testing	х			Х	Х			Х	Х	х

Table 1. Summary of performance, physiological, laboratory, and psychological tests completed during the investigation

and after a week post competition (Recovery), we again measured their physiological status. Table 1 shows the testing schedule for the study.

# **Running training**

The participants had three or four training sessions per week in the increased load training phases consisting of one or two interval training sessions (one at 88–95% maximum heart rate [HRmax], the second up to 100% HRmax), and two easy runs (at 70–87% HRmax) of 6–8 km and 12–18 km. The training volume was matched with the anticipated race distance. We set each runner's training protocol to match the same training stimulus based on the runner's HRmax determined on the baseline incremental testing to exhaustion. All training sessions were supervised by at least one qualified athletics coach and one member of the research group.

# **Experimental procedures**

Anthropometric measurements were made at baseline and recovery. Body weight (kg) and height (cm) were measured to the nearest 0.1 kg and 0.5 cm, respectively, then body mass index (BMI) was calculated. Body fat percentage (%) and lean body mass (kg) were assessed using the skinfold technique. Skinfold thicknesses at biceps, triceps, and subscapular were measured with GPM skinfold callipers (Siber Hegner & Co. Ltd., Zurich, Switzerland) with a precision of 0.2 mm.

All participants completed incremental treadmill tests till exhaustion at the baseline and recovery phases. All the runners had previous experience of treadmill running and testing. After a 6-minute warm-up, an incremental protocol on a calibrated treadmill (Technogym, UK), with a 2% incline, was applied. The starting speed was 3 km/h with speed increments of 2 km/h every 2 minutes. The runners walked the first stage and then ran until volitional exhaustion. The last half or full stage that the subject could sustain (for either 1 min or 2 min) was defined as that individual's maximal speed. Maximum oxygen consumption (VO<sub>2</sub>max) was assessed using a Cosmed K4b2 (Rome, Italy) spirometry system. Heart rate (HR) was recorded continuously during the test using telemetric heart monitors (Polar Electro, Oulu, Finland) and data was stored up a computer.

Each participant wore an HR monitor (Group I, Polar RS800sd; Group C, Polar RS400sd, Finland) during the process to record physiological data and control training load, without warm-up and cooldown intervals, but noting the recovery interval on interval training days. Weekly exercise scores were calculated from the training loads using a method based on the HR zone training point system ( $\Sigma$  HR; Edwards, 1996). The length of time (in min) within various HR-based zones was computed from the HR monitor, multiplied by the value of the zone, and summed to derive  $\sum$  HR zone training points. The HR zones were based on <70% (zone 1=1), 71– 79% (2), 80–87% (3), 88–93% (4) and >94% (5) of the previously established HRmax values for that mode of exercise in a particular individual.

# **Psychological assessment**

Psychometric instrument we used was the RESTQ-76 Sport questionnaire. It can be used to assess an individual's recovery-stress state to determine if the subjects become overreached or overtrained (Kellman & Kallus, 2001). Consisting of 12 basic scales, with seven additional sport specific-scales, the questionnaire uses a self-report approach to evaluate physical, subjective, behavioural, and social aspects of stress and recovery. Within each scale, the subject must respond to four specific items. The items are then rated according to their frequency on the Likert-like scale ranging from 0 (never) to 6 (always), which indicate how often the subject has participated in various activities during the last three days. Total stress was calculated as the sum of the scores of the subscales representing stress ( $\sum 10$  stress subscales), and the total recovery was calculated as the sum of the scores of the subscales representing recovery ( $\sum 9$  recovery subscales). A general indicator of the recovery-stress balance was calculated as the total stress score minus the total recovery score. The subjects was asked to complete the RESTQ-76 Sport questionnaire six times during the testing period, 30 min before blood sampling. A high mean score in the stress-associated activity represents intense subjective strain, whereas a high mean score in the recovery-oriented scale represents adequate recovery. The test-retest variability of the RESTQ-76 Sport has previously been reported as a relatively high (r=.51-.76) (Kellman & Kallus, 2001).

## Laboratory tests

After an overnight fast, blood and saliva samples were taken between 7 and 8 a.m. to avoid variations in circadian rhythms. All the participants were asked to refrain from drinking coffee, tea, chocolate, or cola drinks, and they were asked to avoid alcohol for the preceding 24 hours. Blood samples were taken while the subject was in a seated position. Saliva samples were collected in polypropylene tubes using a plastic straw. EDTA blood samples were used for the red and white blood cells count and the analysis of Hb and hematocrit (Ht) in an Advia 2010 analyser (Siemens Healthcare, Erlangen, Germany). For analyses of urea, creatine kinase (CK), C-reactive protein (CRP), and IL-6, blood samples were collected without additives; after the centrifugation, the serum was stored at -20 °C until analyses. Urea and CK were measured spectrophotometrically in an Advia 1800 analyser (Siemens Healthcare, Erlangen, Germany).

The CRP was measured using a chemiluminescent immunometric high-sensitivity assay with a detection limit of 0.3 mg/L (Immulite analyzer; Siemens Healthcare, Erlangen, Germany). Salivary cortisol and serum IL-6 were measured by electrochemiluminescence assay (Cobas e411 analyzer, Roche Diagnostics, Mannheim, Germany), the limit of detection was 0.5 nmol/L for cortisol and 2 ng/L for IL-6. For the measurement of salivary testosterone, competitive ELISA assay with the limit of detection 6 pmol/L was used (Demeditec GmbH, Kiel, Germany).

#### **Statistical analysis**

The R language for statistical computing (R Development Core Team, R 2.14.2) was used for all analyses. For each time (training period) and each physiological and psychological indicator means and standard deviations were computed. The sizes of differences between means were evaluated using Cohen's d. The effect of time, controlling for training load per week, and participants was estimated with linear mixed-effects model using *lme* function of *nlme* library. The simple (each time against time 0) and repeated (each time against the previous time) contrasts were computed using *estimable* function of a *g-models* library. An alpha level of .05 was chosen to present statistical significance.

#### Results

Table 2 shows the anthropometric assessment. We did not observe any significant differences in body composition between Baseline and Recovery. At the completion of the programme a significant improvement of physical performance was observed: the average increase in VO<sub>2</sub>max from Baseline to Recovery was +4.3% and the times during the Cooper test were shorter by -5.4% (p=.03 and p<.001, respectively).

The mean result for the 21km race was  $1:48:55\pm(12:02)$  and for the 10 km run was  $53:07\pm(7:12)$ . Figure 1 presents the mean weekly training load in distance (km) and as exercise scores ( $\Sigma$  HR zone training point).

Table 3 shows the laboratory parameters. The testosterone concentration tended to decrease after the both load phases and was the lowest at the Recovery phase. The greatest value of cortisol was measured before the investigation (Baseline) and two days before the competition (Taper2). After the resting period (Recovery) the values significantly decreased compared to Baseline and to Taper2. The lowest non-significantly testosterone/cortisol ratio was in Load 2. Urea levels significantly increased in Load1 compared to Baseline. No significant changes were found for high-sensitivity CRP (hsCRP) and creatine kinase (CK). IL-6 levels at

Table 2. Physical characteristics (Mean±SD) of participants prior to training and post study in the recovery phase

Time point	Age (years)	Weight (kg)	Height (cm)	BMI	Lean mass (kg)	Fat (%)	VO <sub>2max</sub> (mL/kg/min)	2400-m time trial (s)
Baseline	33.2±5.0	60.2±7.8	168.2±6.7	21.3±2.8	49.8±5.0	19.3±3.0	46.5±4.5	679±83
Recovery		59.7±7.4		21.2±2.6	49.2±4.4	19.0	*48.4±5.4	*642±82

\* p<.05 vs. baseline

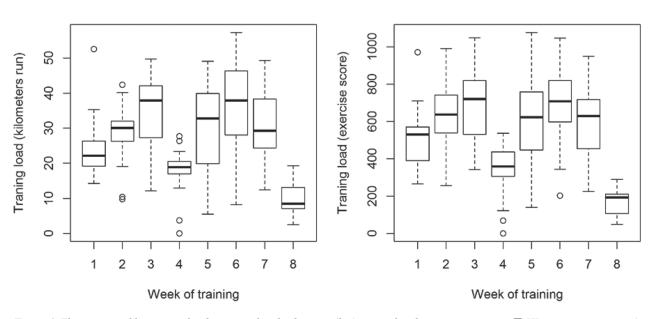


Figure 1. The mean weekly training load expressed in the distance (km) covered and as exercise scores ( $\sum$  HR zone training point).

	Baseline	Load1	Taper1	Load2	Taper2	Recovery
Testosterone (pmol/L)	55.59±69.62	39.82±49.65	61.59±68.03	31.53±33.44	55.88±49.21	27.29±45.80
Cortisol (nmol/L)	20.09±11.18	13.92±16.06	13.87±10.86	16.07±7.22	19.47±11.52	9.82±6.44*#
T/C ratio (x 10^-3)	3.26±3.65	6.84±16.18	7.03±13.96	1.95±1.86	6.39±12.84	5.19±11.28
Leukocytes (10^9/L)	6.02±1.26	6.79±1.26	6.52±1.90	7.06±1.99	6.31±1.27	6.27±1.26
RBC (10^12/L)	4.35±0.19	4.23±0.22	4.29±0.26	4.14±0.26	4.35±0.30	4.27±0.23
Hb (g/L)	133.6±7.49	128.2±6.25	133.2±8.54	127.3±6.20	132.9±7.32	131.2±6.53
Ht (%)	0.39±0.02	0.38±0.02	0.39±0.02	0.38±0.02	0.39±0.02	0.39±0.02
Neutrophils (%)	53.88±6.43	57.50±6.48	59.46±5.89*	61.23±6.75	55.60±7.12	58.51±8.63*
Lymphocytes (%)	34.92±7.50	31.29±6.12	30.68±4.72	28.15±5.64	32.75±6.29	30.75±7.43*
Monocytes (%)	8.12±2.51	8.48±0.99	7.64±1.95	8.05±2.79	8.41±1.59	7.47±1.41
Urea (mmol/L)	4.35±1.13	5.40±0.91*#	4.36±1.04#	4.89±0.87	4.51±1.22	4.57±0.88
CK (µkat/L)	1.69±0.72	3.68±2.06	1.46±0.50	2.93±1.27	1.47±0.42	5.52±14.06
CRP (mg/L)	1.17±1.23	1.26±1.23	1.08±2.06	1.34±1.20	1.20±2.13	3.31±9.63

Table 2 Laboratory parameters	during the outing	tugining protocol
Table 3. Laboratory parameters	uuring ine entire	ir aining proiocoi

\* p<.05 vs. baseline; # p<.05 vs. previous (immediately preceding) time

Table 4. Means±SD of RESTQ 76 Sport subscales of stress and recovery

	Baseline	Load1	Taper1	Load2	Taper2	Recovery
General Stress	0.57±0.87	1.01±1.16	0.94±1.19	1.16±1.21	0.51±0.53	0.54±0.59
Emotional Stress	1.59±0.49	1.71±0.83	1.38±0.86	1.74±1.04	1.57±0.62	1.18±0.65
Social Stress	1.04±0.76	1.51±1.00	1.13±0.94	1.32±1.01	0.99±0.69	0.87±0.75
Conflict, Pressure	2.15±0.99	2.09±0.82	1.99±1.03	2.19±1.12	2.16±0.91	1.76±0.82
Fatigue	1.38±0.85	2.00±0.98	1.72±0.96	2.12±1.44	1.82±0.89	1.29±0.74
Lack of Energy	1.24±0.76	1.19±0.85	1.26±0.94	1.25±0.88	1.00±0.45	1.04±0.71
Somatic Complaints	0.87±0.47	1.43±1.03	1.01±0.75	1.51±1.23	0.88±0.69	0.76±0.89
Success	2.85±1.03	2.78±0.85	2.76±0.98	3.07±0.85	3.13±1.02	3.21±1.09
Social Recovery	3.53±1.22	2.66±1.27	3.12±1.46	3.03±1.35	3.09±1.17	3.29±1.39
Somatic Relaxation	3.76±0.65	2.96±1.07	3.24±1.36	3.32±1.32	3.40±1.03	3.66±1.21
General Well-Being	3.94±1.03	3.29±1.23	3.56±1.52	3.63±1.26	3.65±0.99	3.67±1.30
Sleep Quality	4.54±0.94	3.82±1.27	4.69±0.87	3.76±1.35	4.24±1.06	4.24±1.39
Disturbed Breaks	0.85±0.61	1.06±0.75	0.79±0.62	1.26±1.13	0.76±0.66	0.54±0.55
Emotional Exhaustion	0.60±0.55	0.96±0.92	0.93±0.96	1.21±1.38	0.65±0.79	0.53±0.80
Fitness/Injury	1.31±0.79	1.62±1.12	1.20±1.07	2.06±1.80	1.46±1.52	1.00±1.00
Being in Shape	3.60±0.86	3.03±1.31	3.50±1.38	2.97±1.47	3.88±1.14	3.72±1.13
Personal Accomplishment	2.91±0.94	3.10±1.04	2.61±1.48	2.91±0.74	3.35±0.93	2.90±1.15
Self-Efficacy	3.22±0.84	3.21±0.94	2.79±1.47	3.06±1.37	3.78±1.06*	3.37±1.06
Self-Regulation	2.29±1.07	2.50±1.05	1.84±1.24#	2.72±0.91#	3.19±1.22*	2.03±1.05#
Recovery Subscale scores	29.88±6.35	26.67±7.18	26.28±9.28	28.85±7.33	30.87±7.31	27.52±7.83#
Stress Subscale scores	12.56±5.86	16.19±9.51	14.49±10.04	16.03±8.34	12.81±7.33	11.69±8.52
Recovery-Stress Subscales	18.93±8.28	12.83±13.50	16.32±13.75	10.91±15.99	19.77±10.67	19.37±10.20

\* p<.05 vs. baseline; # p<.05 vs. previous (immediately preceding) time

all-time points were below the detectable plasma concentration of 2 ng/l.

Table 4 presents results of RESTQ 76 Sport subscales. Stress subscales results show a pattern that could have been expected according to the determined load and taper periods. Means of general, emotional and social stress and the amount of somatic complaints subscales, for example, were all higher in the load periods of training and the sleep quality and the means of feeling of being in shape were higher at the taper periods, when practice was a somewhat less demanding. It is also interesting that the means of feeling of fitness was higher at the load periods. When we look at the sum of stress subscales, we can see more stress at the load periods, whereas at the taper periods somewhat higher results were obtained in the summation of recovery subscales. The mean of the recovery subscales summation was a significantly lower at the recovery period when compared to the second taper period. Other significant differences were few, however. On average, the participants felt significantly more self-efficient at the second taper period when compared to baseline. Also, the participants felt that they could regulate their behaviour more efficiently at the second resting period when compared to baseline. Self-regulation decreased at the first taper period and increased after the second taper period, when they were fully rested.

## **Discussion and conclusions**

We assessed the effect of eight-week endurance running training on the biochemical, hormonal, and psychological parameters in recreational female runners. Only a few relevant alterations in biochemical and hormonal parameters and RESTQ-76 Sport scales data were observed. The main finding of the current study indicates that the performed training was well-balanced between stress and recovery periods resulting in a positive training effect. The changes in biochemical and hormonal parameters did not relate to the onset of OTS. The results of RESTQ-76 show that self-regulation and the sum of recovery scales are the most evident indicators of psychological changes within the training process.

The impairment in the VO<sub>2</sub>max and Cooper test is the result of proper training adaptation as previously reported (Bresciani, et al., 2011; Coutts, Reaburn, Piva, & Rowsell, 2007; Coutts, Wallace, et al., 2007). Also, all participants reached their best result in the 21 km or 10 km runs, which could support the positive physiological adaptation to intensive aerobic training. However, the inappropriate recovery/taper phase or detraining may lower performance outcomes and caused a decrease in VO<sub>2</sub>max (Mujika, et al., 2002; Schmikli, Brink, de Vries, & Backx, 2011).

The cytokine hypothesis of overtraining proposes that the repetitive trauma of musculoskeletal system resulting in inflammation is the predominant cause of OTS (Smith, 2000). With no change in IL-6 and hsCRP, we can confirm no presence of an exercise-induced inflammation as reported in previous research (Bresciani, et al., 2011; Meister, et al., 2013). In this study, there was a significant increase in serum urea during the Load1 training period suggesting an enhanced muscle protein catabolism and reduced protein synthesis. However, the mean serum urea levels were within clinical normal range 1,7-8,3 mmol/L and athletic female normal range 4-6 mmol/L (Mujika, et al., 2002), suggesting a normal response to increased physical training, as reported previously (Coutts & Reaburn, 2008; Coutts, Wallace, et al., 2007; Meister, et al., 2013).

With regard to training and in comparison with other studies (Kraemer, et al., 2004; Maso, Lac, Filaire, Michaux, & Robert, 2004) we expected an increase of saliva cortisol during the intensive period of training, but the greatest value was measured before the investigation (Baseline) and two days before the competition (Taper 2). This could be explained by the relation to the state anxiety intensity before the investigation and competition, as previously reported (Filaire, Alix, Ferrand, & Verger, 2009; Hanton, Thomas, & Maynard, 2004). On the other hand, some studies investigating longterm training did not verify the alteration of C levels (Bresciani, et al., 2011; Coutts & Reaburn, 2008; Flynn, et al., 1994). In this study, the testosterone (T) concentration tended to decrease after both load phases with no statistically significant differences over the training period, and were in accordance with previous reports (Bresciani, et al., 2011; Coutts, Wallace, et al., 2007). On the other hand, the significantly reduced T concentration after few weeks of the training program implementation were observed in the intensity group (Coutts, Reaburn, et al., 2007) and in previous research (Filaire, et al., 2009; Flynn, et al., 1994; Hartmann & Mester, 2000). However, a decrease in the testosterone/cortisol (T/C) ratio of >30% or more may be a more useful indicator to indicate the anabolic/catabolic balance in response to training (Adlercreutz, et al., 1986), still some results from literature have not supported it (Filaire, Bernain, Sagnol, & Lac, 2001; Gatti & De Palo, 2011; Santhiago, Da Silva, Papoti, & Gobatto, 2011). In the present study, the T/C ratio decrease after the second training load (Load2) by more than 30% might be considered as an index of fatigue since a low T/C ratio at Baseline might be linked to the generated stress before the investigation. Measured cortisol in saliva can successfully provide a reference for blood levels, while salivary testosterone may not be a reliable indicator of the anabolic response to exercise in females and should be interpreted with caution as well as the T/C ratio (Gatti & De Palo, 2011; Papacosta & Nassis, 2011). In fact, on the basis of the data presented, the awakening cortisol response is probably more related to a psychological stressor than to physiological adaptation to exercise.

This claim is well supported by the results of psychological testing and somewhat coincides with certain previous research (Urhausen & Kindermann, 2002), where was found that biological pa-

rameters did not show signs of overtraining quickly, as well as with those (Coutts, Reaburn, et al., 2007) indicating that psychological predictors, combined with success, are better indicators of overtraining than biological ones. We found that when our participants were resting, in the taper phase of the training, they found themselves to be more efficient, which usually means that they felt that they had more energy, more will to work, and that they felt like they had more potential for achieving results. We also found that our participants had lower self-regulation at Taper 1 and Recovery period, and higher at Load2. The finding seems a somewhat unusual, but it can be easily explained by the pace and rhythm of practice. When they had more practice, the participants had a more steady rhythm of practice; they felt like their time was being fulfilled, whereas when the practice was easier, they felt "empty" – like they were wasting their time, which was what we also heard them saying.

This just indicates that the RESTQ is a very sensitive instrument for detecting changes in the person's state of mind, which was already confirmed earlier (Garatachea, et al., 2011). It does behave in accordance with expectations - stress subscales are higher at load, recovery scales are higher in taper periods, general, emotional and social stress scales are higher at load periods, there are more somatic complaints when the stress (i.e. load) is higher and sleep quality is better in rest (taper) periods. The same happens with stress and recovery subscales - stress scales are higher at load periods of training, recovery subscales are higher at taper periods. These differences are not significant, yet our sample of participants was quite small and the changes were consistent. This confirms that we are dealing with a useful instrument, which shows consistent patterns and can be used to identify early signs of overtraining well before any physiological changes occur. Moreover, this happened in our study even without our participants were overtrained. It is, therefore, safe to assume that if it came to that, we could detect overtraining earlier with the RESTQ than with biological or physiological changes.

Our finding, which was interesting, was that the sum of recovery subscales was lower at the recovery period, when compared to Taper2. Stress sometimes occurs with a somewhat delayed effect; namely, we often function well under immediate stress and feel its effects only afterwards (Kajtna, Štukovnik, & Dolenc Grošelj, 2011). Similarly, increased emotional stress and mood swings occur only after intense workouts, not during them (Bresciani, et al., 2011). So this probably happened in our case – the participants felt that they were tired only when they had "time" to notice it – in the recovery period.

We are aware of potential limitations of the study. Firstly, a sample of 17 participants may not be sufficient enough to show potentially relevant changes in selected markers over time, partially due to the variability associated with statistical issues rather than true physiological and psychological effects. Secondly, very low concentration of testosterone (T) in females, in particular in saliva, suggests that the measurement of saliva dehydroepiandrosterone (sDEHA) could serve as a substitute for T measurement in studies on training response in females, as well as sDHEA/sCortisol ratio (Gatti & De Palo, 2011). Lastly, our results may not be applicable to men and should also be confirmed in elite athletes.

We can conclude that the RESTQ may be a useful instrument for detecting early signs of OTS, since it appears that psychological changes occur prior to biochemical and hormonal ones. The instrument could thus be used for rapid information about the signs of OTS on a regular basis even by coaches, which could help them to prevent the progression from functional overreaching (FO) to NFO/OTS on time, rather than waiting for physiological changes to occur. It could help also a more efficient application of rest; perhaps the main thing to look for is the balance between stress and recovery subscales, always somewhat in favour of recovery subscales (Garatachea, et al., 2011). Moreover, these findings gain importance because they were obtained in recreational female runners, the population to which medical access and laboratory expenses are limited. However, the instrument does not provide a final diagnosis of OTS (Meeusen, et al., 2006).

#### References

- Adlercreutz, H., Härkönen, M., Kuoppasalmi, K., Näveri, H., Huhtaniemi, I., Tikkanen, H., ...Karvonen, J. (1986). Effect of training on plasma anabolic and catabolic steroid hormones and their response during physical exercise. International Journal of Sports Medicine, 1, 27–28.
- Bresciani, G., Cuevas, M.J., Molinero, O., Almar, M., Suay, F., Salvador, A., ... Gonzalez-Gallego, J. (2011). Signs of overload after an intensified training. *International Journal of Sports Medicine*, 32, 338–343. doi:10.1055/s-0031-1271764
- Coutts, A.J., & Reaburn, P. (2008). Monitoring changes in rugby league players' perceived stress and recovery during intensified training. *Perceptual and Motor Skills*, 106, 904–916. doi:http://dx.doi.org/10.2466/PMS.106.3.904-916
- Coutts, A.J., Reaburn, P., Piva, T.J., & Rowsell, G.J. (2007). Monitoring for overreaching in rugby league players. *European Journal of Applied Physiology*, 99, 313–324. doi:10.1007/s00421-006-0345-z
- Coutts, A.J., Wallace, L.K., & Slattery, K.M. (2007). Monitoring changes in performance, physiology, biochemistry, and psychology during overreaching and recovery in triathletes. *International Journal of Sports Medicine*, 28, 125–134. doi:10.1055/s-2006-924146
- Edwards, S. (1996). Heart Zone Training. Hoolbrook: Adams Media Corporation.
- Faude, O., Kellmann, M., Ammann, T., Schnittker, R., & Meyer, T. (2011). Seasonal changes in stress indicators in high level football. *International Journal of Sports Medicine*, *32*, 259–265. doi:10.1055/s-0030-1269894
- Filaire, E., Alix, D., Ferrand, C., & Verger, M. (2009). Psychophysiological stress in tennis players during the first single match of a tournament. *Psychoneuroendocrinology*, *34*, 150–157. doi:10.1016/j.psyneuen.2008.08.022
- Filaire, E., Bernain, X., Sagnol, M., & Lac, G. (2001). Preliminary results on mood state, salivary testosterone: Cortisol ratio and team performance in a professional soccer team. *European Journal of Applied Physiology*, 86, 179– 184. doi:10.1007/s004210100512
- Flynn, M.G., Pizza, F.X., Boone, J.B., Andres, F.F., Michaud, T.A., & Rodriguez-Zayas, J.R. (1994). Indices of training stress during competitive running and swimming seasons. *International Journal of Sports Medicine*, 15, 21–26. doi:10.1055/s-2007-1021014
- Garatachea, N., García-López, D., José Cuevas, M., Almar, M., Molinero, O., Márquez, S., & González-Gallego, J. (2011). Biological and psychological monitoring of training status during an entire season in top kayakers. *The Journal of Sports Medicine and Physical Fitness*, 51, 339–346.
- Gatti, R., & De Palo, E.F. (2011). An update: Salivary hormones and physical exercise. *Scandinavian Journal of Medicine & Science in Sports*, *21*, 157–169. doi:10.1111/j.1600-0838.2010.01252.x
- Gleeson, M. (2002). Biochemical and immunological markers of overtraining. *Journal of Sports Science and Medicine*, *1*, 31–41.
- González-Boto, R., Salguero, A., Tuero, C., González-Gallego, J., & Márquez, S. (2008). Monitoring the effects of training load changes on stress and recovery in swimmers. *Journal of Physiology and Biochemistry*, 64, 19–26.
- Halson, S.L., & Jeukendrup, A.E. (2004). Does overtraining exist? An analysis of overreaching and overtraining research. *Sports Medicine*, *34*, 967–981. doi:10.2165/00007256-200434140-00003
- Hanton, S., Thomas, O., & Maynard, I. (2004). Competitive anxiety responses in the week leading up to competition: The role of intensity, direction and frequency dimensions. *Psychology of Sport and Exercise*, 5, 169–181. doi:10.1016/S1469-0292(02)00042-0
- Hartmann, U., & Mester, J. (2000). Training and overtraining markers in selected sport events. Medicine and Science in Sports and Exercise, 32, 209–215.
- Kajtna, T., Štukovnik, V., & Dolenc Grošelj, L. (2011). Effect of acute sleep deprivation on concentration and mood states with a controlled effect of experienced stress. Zdravniški Vestnik, 80, 354–361.
- Kellmann, M., & Kallus, K. (2001). Recovery-stress Questionnaire for Athletes. Champaign, IL: Human Kinetics.
- Kraemer, W.J., French, D.N., Paxton, N.J., Häkkinen, K., Volek, J.S., Sebastianelli, W.J., ... Knuttgen, H.G. (2004). Changes in exercise performance and hormonal concentrations over a big ten soccer season in starters and nonstarters. *Journal of Strength and Conditioning Research*, 18, 121–128. doi:10.1519/1533-4287(2004)018<0121:CI EPAH>2.0.CO;2
- Maso, F., Lac, G., Filaire, E., Michaux, O., & Robert, A. (2004). Salivary testosterone and cortisol in rugby players: Correlation with psychological overtraining items. *British Journal of Sports Medicine*, 38, 260–263. doi:10.1136/ bjsm.2002.000254
- Meeusen, R., Duclos, M., Gleeson, M., Rietjens, G., Steinacker, J., & Urhausen, A. (2006). Prevention, diagnosis and treatment of the Overtraining Syndrome. *European Journal of Sport Science*, 6 (1), 1 - 14. doi:10.1080/17461390600617717
- Meister, S., Faude, O., Ammann, T., Schnittker, R., & Meyer, T. (2013). Indicators for high physical strain and overload in elite football players. *Scandinavian Journal of Medicine & Science in Sports*, 23, 156–163. doi:10.1111/j.1600-0838.2011.01354.x
- Morgan, W.P., O'Connor, P.J., & Sparling, P.B. (1987). Psychological characterization of the elite female distance runner. *International Journal of Sports Medicine*, 8 (2), 124–131.

- Mujika, I., Goya, A., Ruiz, E., Grijalba, A., Santisteban, J., & Padilla, S. (2002). Physiological and performance responses to a 6-day taper in middle-distance runners: Influence of training frequency. *International Journal* of Sports Medicine, 23, 367–373. doi:10.1055/s-2002-33146
- Nederhof, E., Zwerver, J., Brink, M., Meeusen, R., & Lemmink, K. (2008). Different diagnostic tools in nonfunctional overreaching. *International Journal of Sports Medicine*, *29*, 590–597. doi:10.1055/s-2007-989264
- Papacosta, E., & Nassis, G.P. (2011). Saliva as a tool for monitoring steroid, peptide and immune markers in sport and exercise science. *Journal of Science and Medicine in Sport*. doi:10.1016/j.jsams.2011.03.004
- Santhiago, V., Da Silva, A.S.R., Papoti, M., & Gobatto, C.A. (2011). Effects of 14-week swimming training program on the psychological, hormonal, and physiological parameters of elite women athletes. *Journal of Strength and Conditioning Research*, 25, 825–832. doi:10.1519/JSC.0b013e3181c69996
- Schmikli, S.L., Brink, M.S., de Vries, W.R., & Backx, F.J.G. (2011). Can we detect non-functional overreaching in young elite soccer players and middle-long distance runners using field performance tests? *British Journal of* Sports Medicine, 45, 631–636. doi:10.1136/bjsm.2009.067462
- Smith, L.L. (2000). Cytokine hypothesis of overtraining: A physiological adaptation to excessive stress? *Medicine and* Science in Sports and Exercise, 32, 317–331. doi:10.1097/00005768-200002000-00011
- Urhausen, A., & Kindermann, W. (2002). Diagnosis of overtraining: What tools do we have? *Sports Medicine*, *32*, 95–102. doi:10.2165/00007256-200232020-00002

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# PRAĆENJE BIOKEMIJSKIH, HORMONSKIH I PSIHOLOŠKIH PARAMETARA TIJEKOM OSMOTJEDNOG TRKAČKOG PROGRAMA TRENINGA IZDRŽLJIVOSTI TRKAČICA

Sportaši unapređuju svoju sportsku izvedbu kada postoji optimalna ravnoteže između trenažnog stresa i oporavka. Ukoliko postoji nesrazmjer između navedenoga, može nastupiti sindrom pretreniranosti. Trenutačno se koristi nekoliko biomarkera za procjenu sindroma pretreniranosti, ali nijedan ne zadovoljava sve specificirane kriterije za definitivnu dijagnozu. Cilj ovog rada bio je istražiti učinke osmotjednog trkačkog programa treninga izdržljivosti na biokemijske, hormonske i psihološke parametre trkačica. Za realizaciju trenažnog programa, koji se sastojao od dva trotjedna progresivna prepokrivajuća perioda nakon kojih je slijedio period tapera, a bio je zaključen natjecateljskim trčanjem na 10 km ili 21 km, bilo je angažirano sedamnaest trkačica. Uzorci krvi su uzimani u šest vremenskih točaka tijekom osmotjednog trenažnog programa: inicijalno mjerenje (Osnovno), nakon prvog i drugog trotjednog perioda opterećenja (Opterećenje 1 i Opterećenje 2), nakon svakog perioda tapera (Taper 1 i Taper 2) te nakon programa treninga (Odmor). Tijekom svakog mjerenja ispitanice su bile zamoljene da popune Upitnik Oporavak-Stres za sportaše (RESTQ). Na kraju programa zabilježene su značajne promjene u kondicijskoj pripremljenosti (VO<sub>2max</sub> +4,3%; p=0,03). U vremenskoj točki Odmor vrijednosti kortizola su značajno smanjene u odnosu na inicijalno mjerenje (p=0,002) i Taper 2 (p=0,008). Suma varijabli subskale oporavka u RESTQ bila je značajno manja u odnosu na Taper 2 te je samoučinkovitost porasla u Taperu 2. Također smo utvrdili značajno višu samoregulaciju tijekom perioda oporavka u odnosu na inicijalno mjerenje. Glavni rezultati ovog istraživanja ukazuju na činjenicu da je realizirani program treninga bio dobro uravnotežen u smislu udjela perioda stresa i odmora, što je rezultiralo pozitivnim trenažnim učincima. Čini se da su psihološki parametri osjetljiviji markeri od biokemijskih i hormonskih za otkrivanje promjena u stresu i oporavku. Upitnik RESTQ može se koristiti kao praktično sredstvo za prepoznavanje sindroma pretreniranosti u njegovim ranim fazama.

*Ključne riječi:* trenažni status, RESTQ, biokemija, akutna pretreniranost, stres, oporavak