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Biological and psychological monitoring of training status during an entire season in top kayakers

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Aim. **The aim of this study was to analyze changes in selected biological and psychological variables in a group of top level kayakers along a 42-week training season.**

Methods. **Eight top junior sprint kayakers (age=16.8±2.1) (5 men and 3 women) with international competitive experience participated in the research. During the 42-wk season the subjects were tested in three occasions: (T1) in the second week of the general training period, (T2) at the beginning of the specific training period, (T3) at the beginning of the competitive training period. Firstly, subjects were asked to complete the Recovery-Stress Questionnaire for Athletes (RESTQ-Sport) and the Profile of Mood States (POMS) questionnaires, and Borg´s rate of perceived exertion scale (RPE). Immediately after, blood samples were collected and white blood cells, creatine kinase (CK), C-reactive protein (CRP), myeloperoxidase protein levels (MPO) and glutathione status were determined. ANOVA with repeated measures was used to determine the differences between tests.**

Results. **From the hematological and biochemical measures only total leukocytes changed significantly, increasing at T3 when compared to T1. There were no differences along the entire season in both RESTQ-Sport and POMS scores or indices. Concerning performance, the group improved their maximal strength (+17.4% in bench-press 1RM) and their specific-distance time (+9.8%). The main finding of the present study was that training was well-balanced between stress and recovery because while specific performance increased, signs of overtraining were not found.**

Conclusion. **Training monitoring in athletes should be performed in a multilevel approach using measurements of performance as well as biological or psychological parameters.**

KEY WORDS: Glutathione - C-reactive protein - Heat stress disorders - Mood disorders.

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Conflicts of interest.—None.

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It is well accepted by coaches and sport scientists that to maximize physiological adaptations and to avoid overtraining, proper control of training variables, including intensity, duration and frequency of exercise is needed. However, most elite athletes commit themselves to training programs that push them to the very limit of their physical capacity, thereby frequently entering a state typically referred to as "overreached".[1] Optimal performance is only achievable if athletes are able to recover after competition and optimally balance training stress and adequate recovery.[2] In this line, coaches should manage changes in training volume/intensity and restitution in daily practice that include a temporary short-term fatigue and exertion followed by recovery leading to a long-term performance enhancement.[3]

When the training process states an imbalanced situation between training loads and regenerative process, a mild trauma could develop into a more chronic, severe form of tissue trauma, eventually leading to an overtraining syndrome which is char-

acterized by a decrease in performance, accompanied by physiological, biochemical, immunological, and psychological symptoms.[4] No single biological parameter has been identified until now as a reliable marker of overtraining, but diffuse, low-grade trauma and an inflammatory response related to changes in the functional status of immune cells have been reported.[5] Changes in C-reactive protein (CRP) and creatine kinase (CK) are associated with exercise-induced muscle damage. The CK activity mirrors the mechanical-muscular strain of the training in the preceding days and reacts to the intensity and volume of exercise,[1] while CRP is a sensitive marker for inflammation regardless of etiology and increases in response to intense exercise [6] or in response to unaccustomed exercise.[7] The inflammatory response associated to muscle trauma also includes muscle infiltration by immune cells and neutrophil activation, with a secretion of myeloperoxidase (MPO) whose magnitude depends strongly on exercise intensity.[8] Exercise-induced overtraining has also been shown to elicit a significant response of oxidative stress markers, such as levels of reduced (GSH) and oxidized (GSSG) glutathione, as a consequence of neutrophil and macrophage generation of reactive oxygen species.[5]

In addition to selected biochemical indices, psychological changes have also been used to monitor the state of an athlete during different training periods. These changes have been traditionally measured through the Profile of Mood States (POMS) questionnaire. It has been shown that mood disturbances increase in parallel to training loads,[9] and that individual POMS scores may be used to modulate training.[10] However, more recent research supports that the complex effects of stress and recovery can be better measured through the Recovery-Stress Questionnaire for Athletes (RESTQ-Sport).[11, 12] Moreover, it has been demonstrated in endurance athletes that this last questionnaire aids in the detection of overreaching in its early stages, even when biochemical and physiological measures do not.[13]

However, different metabolic and hormonal parameters as well as psychometric scales may have different time courses in response to the periods of heavy training and following recovery.[14] This suggests that training monitoring in athletes should be performed in a multi-level approach using measurements of performance as well as biological and psychological indices. This study was undertaken to analyze changes in selected biological and psychological variables in a group of top level kayakers across a 42-week training season. it was hypothesized that biological and psychological markers of overtraining should not be significantly modified if the demands of physical conditioning and competition are adequate during the preparatory period and are maintained during the in-season to provide adequate stimuli.

Materials and methods

Study population

Eight top junior sprint kayakers (age=16.8±2.1 yrs) (5 men and 3 women) with international competitive level in 500 m and 1 000 m flatwater voluntereed to participate in the study. All the subjects were members of the *Federación de Castilla y León de Piragüismo*, with a competitive background in kayaking of 3.4 ± 1.1 yrs. Before commencing the study kayakers had a physical examination by the club physician and were cleared of any medical disorders that might limit their participation in the investigation. The subjects and coach were informed about the experimental procedures and the possible risk and benefits of the study which was approved by the Ethics Committee of the University of León and carried out according to the Declaration of Helsinki. Written informed consent was obtained from players. The subjects did not take any medications that would have an impact on the results of the study.

Testing schedule

The 42-wk season lasted from November 2008 to August 2009 and consisted of a general training period (from weeks 1 to 18), a specific training period (from weeks 19 to 32) and a competitive period (from weeks 32 to 42). During the season the subjects were tested on three occasions: the first testing session (T1. November 12) was performed on the second week of the general training period. The second testing session (T2. March 12) was performed at the beginning of the specific training period. The third (T3. June 17) testing session was performed at the beginning of the competitive training period. The

TABLE I.—*Objective quantification of training load, expressed as kilometres of training per week (km/wk) and percentage of different training speeds, during the week before the testing session of subjects at selected training phases.*

Testing session	Training speed (%)					Training kilometers (km/h)
	1	2	3	4	5	
T1	100	—	—	—	—	58
T2	30	17.2	17.1	35.7	—	70
T3	33.3	29.8	36.9		—	84

peak performance level was expected to occur in August, during the National Championship.

All of the kayakers were assessed on the same day between 9:00 to 11:00 a.m. 24 h after last training session (in order to avoid the last training bout effect). and the tests were always performed in the same order in the testing session. Firstly, subjects were asked to complete the RESTQ-Sport and the POMS questionnaires. In addition to this subjects filled out Borg´s 15 category RPE. Immediately after, blood samples were collected in a rest position from the antecubital vein in order to analyze the different biomarkers selected. Then, subjects performed the maximal dynamic force test on bench-press (1RM).

Training and competition data analysis

An objective quantification of training load describing the previous week´s workouts was performed weekly, at the same time as each testing session. The objective training load was classified on the basis of training kilometers per week and training speed (Table I). The following scale was used for training speed: 1=low intensity "jogging"; 2=mean speed for long distance racing (>60 min), 3=threshold speed (mean speed during a 30-min race); 4=mean speed during a 4-min race; 5=maximal speed during a 30-s race.[15] All sessions were supervised by team coaches. Diets or lifestyles were not controlled during the course of the season.

Blood collection

Venous blood samples (12 mL) were taken using EDTA as an anticoagulant. Immediately after extraction, blood samples (0.5 mL) were treated, at 4 ºC. either with 0.5 mL icecold perchloric acid (PCA) (12%), containing 40 mM NEM and 2 mM bathophenanthrolinedisulfonic acid for oxidised glutathione (GSSG) assay or 0.5 mL icecold trichloroacetic acid (TCA) (30%), containing 2 mM EDTA for reduced glutathione (GSH) assay, and mixed thoroughly. Samples were centrifuged at 15 000 g for 5 min at 4 ºC and the acidic supernatants were used for derivatization or spectrophotometric determination of GSH;[16] 3 mL of whole blood were centrifuged immediately after sampling (1 500 g 10 min 4 ºC) and plasma aliquots were stored at -80 ºC until further determination of creatine kinase (CK) and C reactive protein.

White blood cells, hematocrit, and hemoglobin concentration

Hto, Hb, and total and differential counts of white blood cells were determined using an automated haematology analyzer (Coulter Juniors Js. Coulter Electronics, Delkenheim, Germany). Differential analyses of lymphocytes, monocytes, and neutrophils were conducted automatically.

Creatine kinase

CK activity was determined in 0.5 mL of plasma by a Cobas Integra 400 analyzer (Hoffmann-La Roche, Basel, Switzerland).

C-reactive protein

For Western blot analysis plasma proteins were derivatized with dinitrophenylhydrazine. Protein concentration was determinated according to the Lowry *et al*. (1951). Samples containing 10 µg of protein were separated by SDS-polyacrylamide gel electrophoresis (9% acrylamide) and transferred to PVDF membranes. Non-specific binding was blocked by preincubation of the PVDF membranes in PBS containing 5% bovine serum albumin for 1

h. The membranes were then incubated overnight at 4 ºC with appropriate antibody. Antibody against C reactive protein (25 kDa) was purchased from Abcam. Bound primary antibody was detected using a peroxidase conjugated secondary antibody (DAKO) by chemiluminiscence using the ECL kit (Amersham). The density of the specific bands was quantitated with an imaging densitometer. The blots were stripped in 6.25 mM Tris, pH 6.7, 2% SDS and 100 mM mercaptoethanol at 50 ºC for 15 min and probed again for anti-beta-actin antibodies (Sigma) (42 kDa) to verify equal protein loading in each lane.[17]

Myeloperoxidase protein levels

MPO protein levels were measured in plasma by using an enzyme immune assay (OxisResearch™, BioCheck, Inc MPO-EIA) according to the manufacturer's instructions.

Glutathione status

Reduced glutathione determination was performed by a modification of the glutathione S-transferase (GST) assay described by Brigelius *et al*.[18] The following reaction mixture was added into a cuvette: 825 µL of 0.5 M potassium phosphate buffer, pH 7, containing 1mM EDTA. 25 µL of the acidic supernatant of the sample and 10 mL of chlorodinitrobenzene solution (2 mg/mL of ethanol) recording the absorbance at 340 nm as a baseline. The glutathione S-transferase solution was prepared by dissolving 500 U/mL of phosphate buffer. Then, 10 µL of dialyzed glutathione S-transferase were added and absorbance was recorded at 340nm until the end point of the reaction (E=9.6/mM/cm).[19]

For oxidised glutathione analysis, blood samples were derived by the following procedure: 50 µL an internal standard solution (1 mM γ-glutamylglutamate prepared in 0.3% PCA) was added to 500 µL of acidic supernatant. Ten µL of a pH indicator solution (1 mM m-cresol purple) were also added and samples were neutralized up to pH 8-8.5 with 2 M potassium hydroxide containing 0.3 M 3-(N-morpholino) propanesulfonic acid (MOPS) to prevent excessive alkalinization. Then, samples were centrifuged at 15 000 g for 5 min and 50 µL of 1% 1-fluoro-2.4-dinitrobenzene, dissolved in ethanol, were added to an aliquot of 25 µL of each supernatant. After derivatization, samples were stored in darkness at -20 ºC until injection. Samples processed were dissolved in 50 µL of 80% methanol and, 25 µL was injected into high-performance liquid chromatography (HPLC) system. A Spherisorb-NH_2 column (Waters 5 µm 0.46 x 25 cm) was used. The flow rate was 1 mL/min during the gradient. The mobile phase and the gradient were the same as those described previously by Viña *et al*.[16] Solvent A was 80% methanol, and solvent B was 0.5 M sodium acetate in 64% methanol. After injection of the derivatized sample, the mobile phase was held at 80% A, 20% B for 5 min followed by a 10 min linear gradient up to 1% A, 99% B. Then, the mobile phase was held at 99% B until GSSG eluted.

Profile of mood states

The POMS [20] is a 65-item adjective checklist-type questionnaire with responses made on a Likert type scale with values ranging from 0 (never) to 5 (always). The measure includes five negative effect scales: fatigue, depression, tension, anger, confusion, and a positive effect scale: vigour scale. The total mood disturbance score (TMD) is calculated by summation of the negative scales and substraction of the positive scale. Validity and reliability of the Spanish version have been previously reported.[21]

Recovery-stress questionnaire for athletes (RESTQ-SPORT)

The RESTQ-Sport is a questionnaire consisting of 76 items.[12] A Likert-type scale with values ranging from 0 (never) to 6 (always), indicates how often the respondent participated in various activities during the past three days and nights. The measure includes twelve scales which assess various stressing agents of a general nature and general recovery activities during the day-to-day life. To go into more details of stress and recovery in sports, seven additional sports-specific scales were developed which investigate aspects complementary to stress that are derived from the area of sport and assess specific recovery activities derived from the sport context. The scores of stress-related scales were summed and divided by the number of scales to obtain a total stress score. The same procedure was used for the recovery-oriented scales, resulting in a total recovery score. A general indicator of the recovery-stress balance was calculated as the to-

TABLE II.—*Biological markers (mean±SD) measured in kayakers during a 42-wk training.*

	T1	T2	T3
Erythrocytes (cells x 10^9 l^{-1})	4.7±0.3	4.7±0.5	4.8±0.4
Hb (g dL^{-1})	14.6±0.9	14.2±1.2	14.2±1.0
Hto (%)	42.4±2.1	40.8±3.4	42.5±2.7
Total leukocytes (cells x 10^9 L^{-1})	7,3±0.9	8.1±1.6	9.6±1.3 *
Neutrophils (%)	57.2±8.2	57.6±7.9	59.6±9.0
Lymphocytes (%)	33.8±6.5	32.7±7.5	32.1±7.9
Monocytes (%)	6.6±1.7	6.8±1.5	6.3±0.9
Basophils (%)	0.4±0.1	0.4±0.1	0.5±0.2
Éosinophiles (%)	2.1±1.3	1.9±1.0	1.5±0.8
GSH (µM)	776.6±96.1	623.5±141.1	679.7±148.5
GSSG (µM)	21.2±10.1	16.9±5.0	14.5±6.1
GSH/GSSG	43.1±18.9	39.7±14.4	58.4±25.9
MPO (µg L^{-1})	6.4±2.7	7.6±2.0	7.4±1.7
CRP (%)	100.0±0.0	159.4±40.2	148.2±35.1
CK (U L^{-1})	178.4±73.4	285.3±241.8	189.6±73.7

Hb: hemoglobin; Hto: hematocrit; GSH: reduced glutathione; GSSG: oxidized glutathione; MPO: myeloperoxidase; CRP: C-reactive protein; CK: creatin kinase. T1 to T3: test sessions. * Significantly different from T1 (P<0.05).

tal stress score minus the total recovery score.[11] The Spanish version of the RESTQ-Sport has been previously demonstrated by factor analysis and Crombach alphas to be a valid and reliable instrument.[22]

Ratings of perceived exertion

RPE were obtained using the 15-category Borg RPE scale.[23] The scale was explained before the exercise. Participants were asked "How hard do you feel the sport training was?" during each testing session while they were sitting. Subjects had to give ratings corresponding to their sensations during the last week training period.

Bench press maximal strength measurement

1RM bench press was assessed using a previously established protocol.[24] After a light warm up on the bench press using a Smith Machine (Telju, Toledo, Spain), subjects attempted to lift a progressively increasing load, allowing three minutes of resting periods between attempts. 1RM value was obtained using as few attempts as possible (five attempts at maximum).

TABLE III.—*POMS subscales (mean ± SD) and total mood disturbance (TMD) measured in kayakers during a 42-wk training season.*

	T1	T2	T3
Tension	7.4±4.1	9.1±6.1	10±5.7
Depression	8.8±6.2	9.0±6.9	12.7±11.9
Anger	11.5±5.4	13.0±6.3	17.7±8.3
Vigour	19.8±6.4	19.4±6.4	19.6±7.7
Fatigue	9.1±3.9	10.9±5.5	10.1±5.5
Confusion	2.9±2.6	4.0±3.6	6. 0±4.7
TMD	119.9±21.2	126.6±26.0	137±34.5

T1 to T3: test sessions.

Statistical analysis

Standard statistical methods were used to calculate the mean and standard deviations (SD). ANOVA with repeated measures was used to determine the differences between tests. When a significant *F* value was achieved, appropriate Bonferroni *post hoc* tests procedures were used to locate the difference between means. Pearson product-moment correlation coefficients (r) were used to determine association between RESTQ-Sport, POMS, RPE and biochemical variables. The P<0.05 criterion was used to establish statistical significance.

Results

As shown in Table II, data obtained indicate that erythrocyte count, Hto and Hb did not change significantly during the season. When leukocyte formula was measured, only total leukocytes changed significantly, increasing at T3 when compared to T1 (Table II).

CK did not change along the entire canoeing season. Blood reduced and oxidized glutathione were measured as markers of oxidative stress. Neither in both GSH and GSSG nor in the GSH/GSSG-ratio statistically significant changes occurred. CRP and MPO concentrations did not change throughout the season as compared with T1 (Table II).

The POMS subscales are given in Table III. After analyzing the six subscales of the POMS separately, none of the subscales changed. In this line, TMD score was not significantly affected by the training season.

Table IV shows scores in the different scales and

TABLE IV.—*RESTQ-Sport subscales scores and indices of stress and recovery (mean±SD) measured in kayakers during a 42-wk training season.*

	T1	T2	T3
Stress subscales			
General stress	0.9±0.9	1.3±1.3	1.8±1.6
Emotional stress	1.0±0.8	1.8±0.8	1.9±1.2
Social Stress	0.9±0.8	1.2±0.8	1.8±1.3
Conflicts/pressure	1.5±1.0	2.7±0.6	1.9±1.4
Fatigue	1.5±0.8	2.3±1.2	2.4±1.1
Lack of energy	1.4±0.5	2.4±0.9	2.2±1.3
Physical complaints	1.0±0.7	1.8±1.1	1.8±1.3
Disturbed breaks	1.5±0.8	1.5±1.1	1.9±1.3
Emocional exhaustion	0.3±0.4	0.8±0.8	1.2±1.3
Injury	2.0±1.2	2.9±1.3	1.8±1.3
Recovery subscales			
Success	3.2±1.4	2.9±0.8	2.9±1.0
Social recovery	4.4±0.7	4.3±0.8	3.6±0.8
Physical recovery	3.2±0.9	3.1±0.8	3.2±1.3
General wellbeing	4.7±1.1	4.4±1.0	3.9±1.5
Sleep quality	3.1±1.1	2.6±1.0	2.9±1.0
Being in shape	3.6±1.5	3.6±1.3	3.5±1.2
Personal accomplishment	3.3±1.3	3.1±0.7	3.0±1.0
Self-efficacy	3.8±1.7	3.4±1.5	3.6±1.3
Self-regulation	4.0±2.2	3.7±1.6	3.1±1.8
Indexes			
Total stress	1.2±0.5	1.8±0.8	1.8±1.2
Total recovery	3.7±1.1	3.5±0.9	3.3±1.0
Recovery-stress state	2.5±1.3	1.6±1.4	1.5±1.7

T1 to T3: test sessions.

indices of the RESTQ-Sport. No significant changes along season were observed in any scale or calculated index (Table IV).

RPE increased progressively (T1: 13.5±1.9; T2: 13.9±1.1; T3: 14.3±2) but did not show statistically differences among the three testing periods.

Concerning performance, the experimental group improved their maximal strength (+17.4% in bench-press 1RM) and their specific-distance time (+9.8%).

Discussion

This study showed changes in selected biological and psychological parameters of top level kayakers across an entire season. The results are important and unique due to the top level of the kayakers, the very high demands of physical strength and endurance discipline as well as the scarcity of this type of studies in the literature. The main finding of the present study was that the training performed by the subjects performed training was well-balanced between stress and recovery because while specific performance increased, signs of overtraining were not found.

Although in recent years there have been advances in relation to knowledge of the pathogenic mechanisms of overtraining, there is still a large gap in respect of overtraining indicators and the availability of methods for an early diagnosis of overreaching.[25] Different studies have suggested that the use of various hematological, immunological, biochemical, or hormonal, together with psychological measures may allow to distinguish between acute training-related fatigue and overreaching,[26] but most research has been unable to establish a relationship between training loads and the use of those markers for the diagnosis of overreaching.

Concerning biological variables, significant differences were only found in total leukocytes. Our results suggest a mobilization of leukocytes in response to periods of high training load (Table I) at T3, but this finding is not confirmed by an increment in CRP expression. Trauma induced by high training loads has been reported to include leucocytosis,[27, 28] and increases in CRP plasma levels.[29] Given that there was no change in CRP, we could not confirm the presence of an exercise-induced sustained systemic inflammatory response. Moreover, we did not observe significant changes in MPO plasma concentration, GSH, GSSG and CK. Therefore, we postulate that the lack of changes in all assessed biological variables, except for total leukocytes, suggest that the training load was well-balanced between stress and recovery.

To minimize faking, the kayakers were instructed to be honest to themselves and assured that the results of the psychological tests were their personal property and would not be given to the coach unless they so desired. In this line, to minimize the influence of external stressors on changes in training load, the results of each test was individually discussed between athletes and a person trained in the use and analyses of psychological tests.

Different studies have indicated that POMS could be a valid tool to detect overreaching or overtraining.[9, 10] Raglin *et al*.[30] found increment on TMD scores of 84 women vying for a position on a collegiate freshman rowing team during the training season. Berglund and Safstrom [10] found changes in TMD along season in their studied group of world-class canoeists; TMD increased during hard training and decreased in tapering period. The authors concluded that the monitoring of psychological mood disturbances is useful in reducing the risk of staleness in canoeists undergoing hard training. On the contrary, in our study, repeated measures ANOVA revealed that TMD and scores in the different POMS subscales were not changed along the entire season. Thus, titration of the training stimulus on the basis of POMS scores did not suggest development of signs of overtraining or overreaching during the training season.

The RESTQ-Sport for athletes has been used to assess the subjective stress and recovery during training cycles for major competitions in German [4, 31] and Estonian [26, 32] rowers. These studies have found significant alterations of somatic components of stress (Lack of Energy, Somatic Complaints, Fitness/Injury) and recovery factors (Fitness/Being in Shape) over time that mirrored the training load sessions; but the kayakers sample of our study did not show significant differences in either the stress and recovery subscales or the calculated indices. This absence of changes occurred in biological selected variables too, except total leukocytes. In contrast with our data, Jurimae *et al*.[26] found changes in specific stress and recovery scales of the RESTQ-Sport for athletes and changes in stress hormone values. So they concluded that their results indicated a state of heavy training stress. According to the present results, the kayakers were undergoing a well-balanced training between recovery and stress phases. It has recently been made clear that high levels of stress do not necessarily imply getting into situations of overtraining if parallel appropriate strategies for recuperation are put in place.[33]

Conclusions

In conclusion, elite athletes and their coaches are constantly faced with the task of balancing training and recovery when striving for optimal performance. By incorporating frequent measurements of such parameters as recovery-stress, mood, perceived exertion, and biological variables, and monitoring them closely during periods of heavy training, the onset of overreaching could be prevented. Data in the present study indicate that following the above suggested approach training appeared to be well-balanced between stress and recovery in the participating kayakers, because while specific performance increased, signs of overtraining were not found. Training monitoring in athletes should be performed in a multi-level approach using measurements of performance as well as biological or psychological parameters.

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