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Guilherme Bresciani<sup>a</sup>, María J. Cuevas<sup>a</sup>, Nuria Garatachea<sup>a</sup>, Olga Molinero<sup>a</sup>, Mar Almar<sup>a</sup>, José A. De Paz<sup>a</sup>, Sara Márquez<sup>a</sup> & Javier González-Gallego<sup>a</sup>

<sup>a</sup> Institute of Biomedicine (IBIOMED), University of León, León, Spain

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ORIGINAL ARTICLE

## Monitoring biological and psychological measures throughout an entire season in male handball players

GUILHERME BRESCIANI, MARÍA J. CUEVAS, NURIA GARATACHEA, OLGA MOLINERO, MAR ALMAR, JOSÉ A. DE PAZ, SARA MÁRQUEZ, & JAVIER GONZÁLEZ-GALLEGO

*Institute of Biomedicine (IBIOMED), University of León, León, Spain*

### Abstract

The aim of this study was to monitor biological markers of inflammation and oxidative stress, mood states, and recovery-stress states throughout an entire season in male handball players. Fourteen handball players (age  $20.1 \pm 2.5$  years) with a regular training and competitive background in handball ( $11.0 \pm 3.7$  years) from the same club volunteered to participate. All participants completed 40 weeks of training. The training load was increased progressively throughout the season. Blood samples were collected and questionnaires were administered during preparatory, competitive, and recovery periods. Blood C-reactive protein and oxidized glutathione (GSSG) concentrations increased during periods of high load, while the reduced/oxidized glutathione ratio (GSH/GSSG) decreased. These changes were accompanied by a significant increase in total leukocyte count. Positive correlations were found between C-reactive protein, GSSG, GSH/GSSG ratio, and training load. No changes were observed in the Total Mood Disturbance score of the Profile of Mood States (POMS). However, scores on some Recovery-Stress Questionnaire for Athletes subscales, such as Injury, Physical Recovery, and Being in Shape, correlated with training load. Findings indicate that during periods of high training load, handball players developed a low grade of inflammation and oxidative state. Results support the usefulness of monitoring psychological and biological markers of inflammation, oxidative stress, and training load during season.

**Keywords:** *Handball, glutathione, C-reactive protein, stress-recovery, mood states*

### Introduction

Inherent in all training programmes is the application of the progressive overload principle, which implies working beyond a comfortable level to maximize athletic ability. When adequate recovery is allowed, there is an adaptation and physical performance improves. However, the balance between positive and negative adaptation, obtained through strenuous physical training, is sometimes difficult to maintain and requires careful monitoring and management. Indeed, a mild trauma could occur when there is an imbalance between training loads and the regenerative process. If this situation continues, a more severe and chronic tissue trauma could occur, possibly leading to overtraining syndrome (Kuipers, 1998). There is a large body of research on the prevalence and symptoms of the

overtraining syndrome in endurance and strength athletes (Halson & Jeukendrup, 2004). Although a few studies have examined fatigue and recovery in team sports (Elloumi et al., 2005; Maso, Lac, Michaux, & Robert, 2003), the monitoring of biochemical and psychological variables in endurance athletes has received less attention (Hoffman, Kang, Ratamess, & Faigenbaum, 2005).

To date, no single biological parameter has been identified as a reliable marker of overtraining. However, diffuse, low-grade trauma and an inflammatory response have been reported with high-intensity training loads (Margonis et al., 2007). In addition, changes in C-reactive protein and creatine kinase are associated with exercise-induced muscle damage. Creatine kinase activity mirrors the mechanical-muscular strain of the training and reacts to the intensity and volume of exercise (Urhausen &

Correspondence: J. González-Gallego, Institute of Biomedicine, University of León, Campus Universitario, 24071 León, Spain. E-mail: jgonga@unileon.es

Kindermann, 2002). C-reactive protein is a sensitive marker for inflammation, which increases in response to intense, prolonged or unaccustomed exercise (Margeli et al., 2005; Peters, Robson, Kleinveldt, Naicker, & Jogessar, 2004). The inflammatory response associated with muscle trauma also includes muscle infiltration by immune cells and neutrophil activation, with a secretion of myeloperoxidase whose magnitude depends strongly on exercise intensity (Bury & Pirnay, 1995). Moreover, it has been shown that overtraining results in a significant response of oxidative stress markers, such as concentrations of reduced (GSH) and oxidized (GSSG) glutathione (Margonis et al., 2007).

Negative psychological changes can also appear when athletes are exposed to excessive exercise stress. These changes have been traditionally measured through the Profile of Mood States (POMS) questionnaire (McNair, Lorr, & Droppleman, 1971), which has shown that mood disturbances increase in parallel with training loads (Filaire, Bernain, Sagnol, & Lac, 2001). However, more recent research suggests that the complex effects of stress and recovery can be better measured through the Recovery-Stress Questionnaire for Athletes (RESTQ-Sport) (González-Boto, Salguero, Tuero, Kellmann, & Márquez, 2008b; Kellmann & Kallus, 2001).

In addition to the technical and tactical skills required, handball involves actions that place a lot of strain on different muscles (Gorostiaga, Granados, Ibáñez, & Izquierdo, 2005). Gorostiaga and colleagues (Gorostiaga, Izquierdo, Iturrealde, Ruesta, & Ibáñez, 1999) have studied overreaching in adolescent handball players and Ronglan and colleagues (Ronglan, Raastad, & Borgesen, 2006) have focused on neuromuscular fatigue and recovery during handball camps and international tournaments. However, studies that have investigated effects over an entire season in handball players have not analysed aspects related to stress and recovery (Gorostiaga, Granados, Ibáñez, González-Badillo, & Izquierdo, 2006).

Therefore, the main purpose of this study was to monitor biological markers of inflammation and oxidative stress, mood states, and recovery-stress states throughout an entire season in male handball players. We hypothesized that biological markers of adaptation should improve during a handball season, while other markers related to inflammation and recovery stress should not change if the physical demands during the season are adequate.

## Methods

### *Participants*

Fourteen male handball players from the same club (age  $20.1 \pm 2.5$  years) with a regular training and

competitive background in handball ( $11.0 \pm 3.7$  years) volunteered to participate in the study. All participants competed in a national level handball league competition. Players had a physical examination and were cleared of any medical disorders that might limit their participation. The participants were informed in detail about the experimental procedures and the possible risks 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 then obtained from the players.

### *Testing schedule*

The 40-week season consisted of two preparatory periods (weeks 1–5 and weeks 20–22), two competitive periods (weeks 6–19 and weeks 23–33), and one recovery period (weeks 34–40). During the season, the participants were tested on five occasions: the first test session (T1) was performed 4 days before the beginning of the first preparatory period (PP1). The second test session (T2) was performed at the end of the first preparatory period. The third (T3) and the fourth (T4) test sessions were performed at the end of the first and second competitive periods (CP1 and CP2), respectively, of the National First Division League. The fifth test session (T5) was performed after the 7-week recovery period (RP) (Figure 1).

The participants were assessed on the same day between 09:00 and 11:00 h. A 24-h delay after each training session was followed to avoid major sample bias. Firstly, participants were asked to complete the Recovery-Stress Questionnaire for Athletes and the Profile of Mood States questionnaire. Once they had completed the psychological questionnaires, and while they were resting, blood samples were collected from an antecubital vein.

### *Training and competition data analysis*

The intensity of individual training sessions was estimated using a modified rating of perceived exertion (RPE) scale (Foster et al., 2001). Training load was calculated by multiplying the training session intensity by the duration of the training session. In addition, the intensity of each match was also estimated using a modified RPE scale. The match load was calculated by multiplying the match intensity by the amount of time each player participated in the match (Foster et al., 2001). Intensity estimates were obtained within 30 min of completing the training session or match (Gabbett, 2004).

*Haematological and biochemical analyses*

Haematocrit, haemoglobin concentration, mean corpuscular haemoglobin concentration (MCHC), and total and differential counts of white blood cells (neutrophils, lymphocytes, monocytes, basophils, and eosinophils) were determined using an automated haematology analyser (Coulter Juniors Js. Coulter Electronics, Delkenheim, Germany). Creatine kinase activity was determined in 0.5 ml of plasma by a Cobas Integra 400 analyser (Hoffmann-La Roche, Basel, Switzerland). Western blot analysis of C-reactive protein was carried out after separation by sodium dodecyl sulphate-polyacrylamide gel electrophoresis, transfer to polyvinylidene difluoride membranes, and incubation with appropriate antibody (García-López et al., 2007). Myeloperoxidase concentrations were also measured in plasma by using an enzyme immune assay (OxisResearch™, BioCheck, Inc., MPO-EIA). Reduced glutathione determination was performed by a modification of the glutathione *S*-transferase assay described by Brigelius and colleagues (Brigelius, Muckel, Akerboom, & Sies, 1983). For oxidized glutathione analysis, blood samples were analysed by high-performance liquid chromatography as described previously (Viña, Sastre, Asensi, & Packer, 1995).

*Questionnaires*

POMS (McNair et al., 1971) is a 65-item questionnaire that includes five negative affect scales – Fatigue, Depression, Tension, Anger, Confusion – and one positive affect scale – Vigour. The Total Mood Disturbance score is calculated by summation of the negative scales and subtraction of the positive scale. RESTQ-Sport (Kellmann & Kallus, 2001) consists of 76 items that indicate how often the respondent participated in various activities during the past 3 days and nights. The questionnaire includes 12 scales that assess various stressing agents of a general nature and general recovery activities and seven additional sports-specific scales. 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 total stress score minus the total recovery score. The Spanish versions of the POMS and the RESTQ-Sport have been previously demonstrated to be valid and reliable (González-Boto et al., 2008b; González-Boto, Salguero, Tuero, & Márquez, 2009).

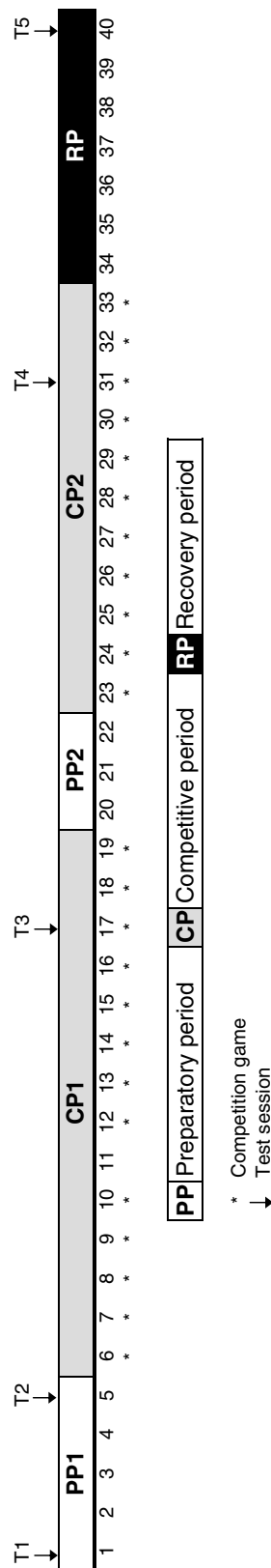


Figure 1. Schedule of testing periods and games for the elite male handball season. Preparatory period (white), competitive period (grey), recovery period (black). \*Competition game. ↓ T1 to T5: test sessions 1–5.

*Statistical analysis*

A one-way repeated-measures analysis of variance was applied to determine the differences between haemoglobin, haematocrit, mean corpuscular haemoglobin concentration, creatine kinase, C-reactive protein, GSH, GSSG, GSH/GSSG, myeloperoxidase, and training load. For POMS and RESTQ-Sport scales, a one-way repeated-measures multivariate analysis of variance was used. Sphericity was checked using the Mauchly sphericity test. *Post-hoc* multiple comparisons were used to identify the location of pair-wise significant differences between training phases corrected using the Bonferroni adjustment. An alpha level of 0.05 was chosen *a priori* to represent statistical significance. Correlations between changes in training load and psychological and biological variables were assessed by means of Pearson product-moment correlations. The Statistical Package for Social Sciences v.17 (SPSS, Inc., Chicago, IL) was used for all analyses.

**Results***Physical training*

Characteristics of the physical training during the season are presented in Table I. Training load was significantly higher in the second competitive period (CP2) compared with any training load period ( $P < 0.05$ ) (Table I). Training load correlated with GSH/GSSG ( $R^2 = 0.63$ ,  $P < 0.05$ ), GSSG ( $R^2 = 0.65$ ,  $P < 0.05$ ), and C-reactive protein expression ( $R^2 = 0.58$ ,  $P < 0.05$ ). There was also a correlation with some REST-Q subscales, such as Injury ( $R^2 = 0.70$ ,  $P < 0.05$ ), Physical Recovery ( $R^2 = 0.69$ ,  $P < 0.05$ ), and Being in Shape ( $R^2 = 0.93$ ,  $P < 0.05$ ).

*Haematological and biochemical measures*

Erythrocyte counts and haematocrit increased significantly at T5 compared with T1 (Table II). Although haemoglobin concentration did not change significantly during the training season, mean corpuscular haemoglobin concentration decreased

significantly at T5 (Table II). These changes were accompanied by an increase in total leukocytes at T4 and T5. Percentage of neutrophils increased significantly at T4 compared with T1 (Table II). Creatine kinase did not change during the training season. No statistically significant changes were observed in GSH concentration during the season. However, GSSG concentration increased significantly at T3 and T4 compared with T1. The GSH/GSSG ratio was significantly reduced both at T3 and T4 (Table II). C-reactive protein expression changed in parallel, with significant increases at T3 and T4. However, myeloperoxidase concentrations was unchanged throughout the season (Table II).

*Psychological measures*

After analysing the six subscales of the POMS separately, only Anger appeared to increase significantly at T3 and Vigour at T4. However, the Total Mood Disturbance score was not significantly affected by the training season (Table III). Table IV shows scores on the different scales of the RESTQ-Sport for the five measures taken. The stress subscale Social Stress increased significantly at T4, while Injury score decreased significantly at T5. On the other hand, the recovery subscale Being in Shape increased significantly at T4, while a significant decrease was observed for Self-regulation score at T5. However, no significant changes were observed for Total Stress, Total Recovery or Recovery-Stress State indexes throughout the season.

**Discussion**

In this study, various haematological, biochemical, and psychological variables were measured throughout an entire handball season to identify changes with training load. The investigation demonstrated that there was an increase in erythrocyte count and haematocrit, suggesting a possible physiological adaptation. In fact, erythrocyte volume expansion usually occurs slowly over many weeks to months in response to exercise. Trauma induced by high training loads has been also reported to include

Table I. Characteristics of physical training throughout an entire season (mean  $\pm$  sd)

	PP1	CP1	PP2	CP2	RP
Duration (weeks)	5	14	3	11	7
Total training sessions	24	74	12	55	21
Mean sessions per week	4.8	5.3	4.0	5.0	3.0
Number of training games	8	0	0	0	4
Number of competitive games	0	13	0	11	0
Training load (arbitrary units)	1509 $\pm$ 285*#	2244 $\pm$ 273#	1911 $\pm$ 200#	2712 $\pm$ 292	1901 $\pm$ 400#

Note: PP = preparatory period; CP = competitive period; RP = recovery period.

\*Significantly different from CP1 ( $P < 0.05$ ). #Significantly different from CP2 ( $P < 0.05$ ).

Table II. Biological markers measured in handball players throughout an entire season (mean  $\pm$  sd)

	T1	T2	T3	T4	T5
Erythrocytes (cells $\times 10^{12} \cdot l^{-1}$ )	4.9 $\pm$ 0.3	5.0 $\pm$ 0.3	5.0 $\pm$ 0.3	5.1 $\pm$ 0.3	5.3 $\pm$ 0.2*
Haemoglobin (g $\cdot dl^{-1}$ )	15.0 $\pm$ 0.8	15.3 $\pm$ 0.8	15.1 $\pm$ 1.0	15.2 $\pm$ 0.7	15.1 $\pm$ 1.1
Haematocrit (%)	42.5 $\pm$ 1.6	44.7 $\pm$ 2.7	44.3 $\pm$ 2.1	44.1 $\pm$ 2.3	45.8 $\pm$ 1.6*
MCHC (g $dl^{-1}$ )	34.62 $\pm$ 0.53	34.34 $\pm$ 0.5	33.9 $\pm$ 0.7	34.6 $\pm$ 0.4	33.9 $\pm$ 0.5*
Total leukocytes (cells $\times 10^9 \cdot l^{-1}$ )	6.1 $\pm$ 0.7	6.6 $\pm$ 0.9	6.9 $\pm$ 1.2	7.6 $\pm$ 0.8*	8.2 $\pm$ 1.3*
Neutrophils (%)	56.6 $\pm$ 9.3	54.2 $\pm$ 7.2	50.4 $\pm$ 7.5	62.3 $\pm$ 8.6*	57.9 $\pm$ 7.4
Neutrophils (cells $\times 10^9 \cdot l^{-1}$ )	4.6 $\pm$ 0.3	3.9 $\pm$ 0.7	4.0 $\pm$ 0.7	5.5 $\pm$ 2.7*	4.5 $\pm$ 1.5
Lymphocytes (%)	31.6 $\pm$ 8.5	34.4 $\pm$ 6.1	38.8 $\pm$ 6.9	27.8 $\pm$ 6.8	30.9 $\pm$ 6.7
Lymphocytes (cells $\times 10^9 \cdot l^{-1}$ )	1.9 $\pm$ 0.5	2.1 $\pm$ 0.4	2.4 $\pm$ 0.5	2.0 $\pm$ 0.3	2.0 $\pm$ 0.9
Monocytes (%)	8.5 $\pm$ 1.6	8.1 $\pm$ 1.3	8.0 $\pm$ 1.0	7.4 $\pm$ 1.9	8.7 $\pm$ 2.2
Monocytes (cells $\times 10^9 \cdot l^{-1}$ )	0.6 $\pm$ 0.2	0.5 $\pm$ 0.1	0.6 $\pm$ 0.1	0.5 $\pm$ 0.16	0.7 $\pm$ 0.2
Basophils (%)	0.5 $\pm$ 0.2	0.5 $\pm$ 0.1	0.5 $\pm$ 0.1	0.4 $\pm$ 0.1	0.4 $\pm$ 0.2
Basophils (cells $\times 10^9 \cdot l^{-1}$ )	0.0 $\pm$ 0.1	0.0 $\pm$ 0.1	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.1 $\pm$ 0.1
Eosinophils (%)	2.8 $\pm$ 1.3	2.8 $\pm$ 1.8	2.3 $\pm$ 0.9	2.1 $\pm$ 1.4	2.1 $\pm$ 1.2
Eosinophils (cells $\times 10^9 \cdot l^{-1}$ )	0.17 $\pm$ 0.05	0.2 $\pm$ 0.1	0.2 $\pm$ 0.05	0.1 $\pm$ 0.05	0.2 $\pm$ 0.1
GSH ( $\mu mol \cdot l^{-1}$ )	539.7 $\pm$ 163.1	730.5 $\pm$ 159.9	609.2 $\pm$ 163.7	639.6 $\pm$ 168.2	696.5 $\pm$ 193.3
GSSG ( $\mu mol \cdot l^{-1}$ )	11.7 $\pm$ 1.4	17.3 $\pm$ 5.6	21.6 $\pm$ 5.0*	38.6 $\pm$ 14.9*	19.6 $\pm$ 10.4
GSH/GSSG ratio	49.0 $\pm$ 15.8	48.9 $\pm$ 21.3	28.9 $\pm$ 10.2*	18.8 $\pm$ 9.9*	41.4 $\pm$ 28.7
Myeloperoxidase ( $\mu g \cdot l^{-1}$ )	4.6 $\pm$ 2.7	3.7 $\pm$ 2.0	3.7 $\pm$ 1.5	2.9 $\pm$ 1.9	5.0 $\pm$ 2.4
C-reactive protein (arbitrary units)	1.0 $\pm$ 0.1	1.2 $\pm$ 0.3	1.7 $\pm$ 0.2*	1.4 $\pm$ 0.2*	0.8 $\pm$ 0.1
Creatine kinase (U $\cdot l^{-1}$ )	176.7 $\pm$ 44.5	172.1 $\pm$ 107.1	209.6 $\pm$ 71.9	198.6 $\pm$ 155.3	177.44 $\pm$ 152.4

Note: MCHC = mean corpuscular haemoglobin concentration, GSH = reduced glutathione, GSSG = oxidized glutathione. T1–T5 = test sessions 1–5.

\*Significantly different from T1 ( $P < 0.05$ ).

muscle infiltration by immune cells and leukocytosis (Pedersen & Hoffman-Goetz, 2000). Total leukocyte count was in the physiological range ( $< 10 \text{ cells} \times 10^9 \cdot l^{-1}$ ); however, a significant increase was detected at T4. This increase in total leukocytes was not suppressed during the recovery period of low training load. These results may indicate that leukocytes are mobilized in response to exercise-induced muscle damage, and confirm previous findings in which an increase in leukocytes was not suppressed following a regeneration period of low-volume training (Margonis et al., 2007). In the present research, plasma C-reactive protein also increased significantly during periods of high load (T3 and T4), returning to low values during the recovery period (T5). These data confirm previous research showing increases in C-reactive protein during periods of intense resistance training (Fatouros et al., 2006). Although the physiological

relevance of the small change in C-reactive protein was probably minimal, the increase observed during periods of high training load may suggest tissue damage and inflammation.

Plasma myeloperoxidase concentration did not change during this study; however, neutrophils were altered. There is evidence that myeloperoxidase concentration is not correlated with the modification of neutrophil count, which, considering that the degranulation leads to an increase of the plasma concentration of neutrophil enzyme markers (Bury & Pirnay, 1995), suggests that neutrophil degranulation is independent of their mobilization. Thus, myeloperoxidase secretion does not only depend on neutrophilia but may also reflect a modification of the “functional status” of neutrophils. In fact, neutrophils are mobilized during exercise and remain in circulation for approximately 9 days until break down, and repeated exercise may result in

Table III. POMS subscales scores measured in handball players throughout an entire season (mean  $\pm$  sd)

	T1	T2	T3	T4	T5
Tension	5.6 $\pm$ 6.4	7.1 $\pm$ 6.4	6.6 $\pm$ 13.8	4.4 $\pm$ 4.5	5.4 $\pm$ 4.7
Depression	7.8 $\pm$ 9.5	7.9 $\pm$ 10.0	13.9 $\pm$ 12.0	7.6 $\pm$ 8.8	8.9 $\pm$ 7.6
Anger	8.7 $\pm$ 6.9	11.6 $\pm$ 10.7	14.1 $\pm$ 10.1*	11.0 $\pm$ 7.6	13.6 $\pm$ 10.5
Vigour	19.1 $\pm$ 3.8	18.9 $\pm$ 5.3	19.1 $\pm$ 5.1	20.6 $\pm$ 4.4*	18.5 $\pm$ 4.1
Fatigue	13.9 $\pm$ 4.0	8.0 $\pm$ 4.7	9.6 $\pm$ 6.6	7.4 $\pm$ 5.8	7.2 $\pm$ 4.7
Confusion	2.4 $\pm$ 4.7	3.0 $\pm$ 5.1	3.9 $\pm$ 5.2	1.8 $\pm$ 5.1	2.5 $\pm$ 3.0
Total Mood Disturbance	119.0 $\pm$ 27.3	118.7 $\pm$ 33.7	129.0 $\pm$ 42.9	111.6 $\pm$ 28.7	119.2 $\pm$ 29.1

Note: T1–T5 = test sessions 1–5.

\*Significantly different from T1 ( $P < 0.05$ ).

Table IV. RESTQ-Sport stress and recovery subscale scores measured in handball players throughout an entire season (mean  $\pm$  sd)

	T1	T2	T3	T4	T5
<b>Stress subscales</b>					
General stress	1.1 $\pm$ 1.1	1.1 $\pm$ 1.2	1.5 $\pm$ 1.2	1.3 $\pm$ 0.9	1.5 $\pm$ 0.9
Emotional stress	0.9 $\pm$ 0.9	1.3 $\pm$ 1.1	1.5 $\pm$ 1.2	1.4 $\pm$ 1.0	1.9 $\pm$ 1.4
Social stress	0.7 $\pm$ 0.8	1.1 $\pm$ 1.1	1.6 $\pm$ 1.3	1.8 $\pm$ 0.9*	1.5 $\pm$ 1.2
Conflicts/pressure	2.2 $\pm$ 1.2	2.4 $\pm$ 1.3	2.8 $\pm$ 1.1	2.4 $\pm$ 0.9	2.6 $\pm$ 0.5
Fatigue	2.8 $\pm$ 1.4	2.2 $\pm$ 1.3	2.7 $\pm$ 1.2	2.3 $\pm$ 1.0	2.5 $\pm$ 0.8
Lack of energy	1.6 $\pm$ 1.1	2.2 $\pm$ 1.1	1.9 $\pm$ 1.3	1.9 $\pm$ 1.2	2.1 $\pm$ 1.0
Physical complaints	1.8 $\pm$ 1.1	1.4 $\pm$ 0.7	2.1 $\pm$ 1.3	1.5 $\pm$ 0.9	1.7 $\pm$ 0.8
Disturbed breaks	2.4 $\pm$ 0.9	1.9 $\pm$ 1.1	1.3 $\pm$ 1.2	1.4 $\pm$ 0.9	1.5 $\pm$ 1.2
Emocional exhaustion	0.7 $\pm$ 0.8	1.3 $\pm$ 1.3	1.3 $\pm$ 1.5	1.3 $\pm$ 0.9	0.9 $\pm$ 0.8
Injury	3.4 $\pm$ 0.8	3.0 $\pm$ 1.2	2.7 $\pm$ 1.5	2.4 $\pm$ 0.8	1.8 $\pm$ 0.9*
<b>Recovery subscales</b>					
Success	2.7 $\pm$ 0.9	3.0 $\pm$ 1.1	3.4 $\pm$ 1.0	2.8 $\pm$ 0.7	3.3 $\pm$ 1.0
Social recovery	4.3 $\pm$ 1.1	3.6 $\pm$ 1.3	3.5 $\pm$ 0.6	4.2 $\pm$ 0.9	4.2 $\pm$ 1.1
Physical recovery	2.7 $\pm$ 1.0	3.0 $\pm$ 1.1	3.3 $\pm$ 1.0	3.4 $\pm$ 1.1	3.3 $\pm$ 0.9
General well-being	4.5 $\pm$ 1.1	4.1 $\pm$ 1.3	4.1 $\pm$ 0.9	4.5 $\pm$ 1.0	4.5 $\pm$ 1.3
Sleep quality	3.3 $\pm$ 1.1	2.9 $\pm$ 0.9	3.3 $\pm$ 0.9	3.5 $\pm$ 0.7	3.1 $\pm$ 0.8
Being in shape	2.6 $\pm$ 0.8	3.7 $\pm$ 1.9	3.8 $\pm$ 1.2	4.0 $\pm$ 0.8*	3.2 $\pm$ 0.9
Personal accomplishment	2.9 $\pm$ 0.9	3.4 $\pm$ 0.9	3.4 $\pm$ 0.8	3.3 $\pm$ 0.9	2.7 $\pm$ 1.0
Self-efficacy	3.7 $\pm$ 1.0	3.8 $\pm$ 1.0	3.7 $\pm$ 0.9	3.9 $\pm$ 1.1	3.2 $\pm$ 1.1
Self-regulation	3.6 $\pm$ 1.0	4.1 $\pm$ 1.2	4.1 $\pm$ 1.0	4.1 $\pm$ 1.0	2.1 $\pm$ 2.0*
Total stress	1.9 $\pm$ 0.6	1.9 $\pm$ 0.8	1.9 $\pm$ 1.1	1.8 $\pm$ 0.6	1.7 $\pm$ 0.7
Total recovery	3.5 $\pm$ 0.6	3.6 $\pm$ 0.8	3.6 $\pm$ 0.7	3.7 $\pm$ 0.7	3.2 $\pm$ 0.9
Recovery-Stress state	1.6 $\pm$ 0.9	1.7 $\pm$ 1.4	1.7 $\pm$ 1.4	2.0 $\pm$ 1.1	1.5 $\pm$ 1.1

Note: T1–T5 = test sessions 1–5.

\*Significantly different from T1 ( $P < 0.05$ ).

immature cells being released into the circulation from depleted bone marrow. In addition, the rapid rate of clearance of myeloperoxidase from the circulation cannot be excluded (Suzuki et al., 2003).

High-intensity exercise accompanied by an increase in neutrophils results in oxidative stress, and it is known that antioxidants may neutralize the reactive species that are produced by neutrophils (Pedersen & Hoffman-Goetz, 2000). The results of the present study show that GSSG concentration increased significantly at T3 and T4, and decreased during the recovery period. This is in line with previous studies reporting that cycling time trials also induce significant post-exercise increases in GSSG, while a tapering period does not seem to be associated with substantial changes in exercise-induced oxidative stress (Vollaard, Cooper, & Shearman, 2006). In contrast, the GSH/GSSG ratio showed lower values during the periods of higher load. In fact, the oxidative stress response was proportional to the training load, similar to the high correlation among isoprostanes and GSH/GSSG with training volume previously described in resistance training protocols (Margonis et al., 2007). Therefore, these changes in markers of oxidative stress suggest an increased generation of reactive oxygen species, which could be linked to the inflammatory response and possibly with the propagation of muscle damage.

To date, monitoring of psychological variables in response to training load has focused on sports in which large changes in training volume are part of normal training, leading to a taper at the end of the season (Jürimäe, Mäestu, Purge, Jürimäe, & Sööt, 2002). However, few studies have been conducted on changes in mood states in team sports using the POMS questionnaire (Coutts & Reaburn, 2008; Filaire et al., 2001). In this study, Total Mood Disturbance was not significantly modified across the season. This seems to confirm that the training programme did not result in psychological perturbations and suggests that the participants were psychologically well adapted to training loads. The POMS focuses mainly on the stress component, while the RESTQ-Sport (Kellmann & Kallus, 2001) measures both current perceived stress and recovery in a multidimensional way, and is thus a more sensitive marker of changes in training status. The RESTQ-Sport has been used to evaluate the effects of rapidly increased training volume on recovery-stress state and its relationship with performance and biochemical markers (Coutts, Wallace, & Slattery, 2007). It has been recently reported in competitive swimmers that the Recovery-Stress state index is significantly reduced during sessions in which there is an increase in training volumes (González-Boto, Salguero, Tuero, González-Gallego, & Márquez, 2008a). Similar to the POMS, only one report has



measured changes in stress and recovery during normal or intensified training in team sport athletes (Coutts & Reaburn, 2008). The current study is the first to use the RESTQ-Sport as a monitoring tool of psychological changes throughout a season of team sports. Although scores on some scales were significantly related to training load, no significant changes were observed in Total Stress, Total Recovery or Recovery-Stress state. This suggests that players maintained the stress-recovery balance across the entire season.

In summary, the findings indicate that handball players may develop small elevations in inflammatory and oxidative state during periods of high training load. Although there were positive correlations between biological and psychological markers, and training load, the physiological relevance is unclear and further studies should be carried out. Therefore, the present results support monitoring these markers during a season to adjust training loads and periods of recovery.

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