Signs of Overload After an Intensified Training

Authors

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Abstract

This study investigated effects of a 9-week intensified aerobic training and 3-weeks of recovery on signs of overload in 9 healthy active young males. Blood and saliva samples were collected and psychological questionnaires were administered during baseline (T1), intermediate load (T2), maximal load (T3), and recovery (T4) periods. Maximal oxygen uptake increased and blood lactate concentration decreased in T3, while running time in a 3000m track field test was significantly shorter. No significant changes were found in hematocrit, haemoglobin concentration, white blood cell count, lactate dehydrogenase, transaminases, interleukin-6, tumour necrosis factor- α , myeloperoxidase and markers of oxida-

tive stress in plasma, or salivary cortisol and testosterone. Increases in different negative affect scales and in the total mood disturbance score of the Profile of Mood States were observed during T3. Scores in the stress scales of the Recovery-Stress Questionnaire for Athletes and in the State Anxiety Scale of the State-Trait Anxiety Inventory also showed significant increases during T3. The lack of effects in biomarkers together with the changes observed in psychological assessment indicates that an intensified training can produce psychological disturbances prone to early overreaching development. Additionally, it seems that psychological parameters are sensitive markers to detect stress produced by load increases.

Introduction

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Dr. Javier González-Gallego University of León Institute of Biomedicine Campus Vegazana s/n 24071 León Spain Tel.: + 34/987/291 258 Fax: + 34/987/291 267 jgonga@unileon.es Intensive aerobic training can produce beneficial effects on aerobic performance, running economy, prevention of bone and muscle loss, immune system, or the plasmatic concentration of growth factors [3, 33]. However, training volumes below what can be considered optimal do not result in the desired adaptation, whereas training volumes above the optimum may lead to an undesired condition usually referred to as *overtraining syndrome* [24]. In competitive sports hard training is essential to improve performance. So, one should be able to not just train hard, but also to recover after training [18]. Unfortunately, there is relatively little research on valid and practical tools to monitor the recovery and stress states.

There are also evidences which suggest that exercise may induce alterations in different organic systems. Thus, it is well known that long-duration, high-intensity exercise is associated with immunosuppression, a higher susceptibility to infection [34], microinjuries and a local inflammatory reaction in the musculature causing changes in serum markers of inflammation [5,26]. Overload training can also induce oxidative stress, which is also related to inflammation, leading to an impaired antioxidant defence and a lack of anticipated adaptations to training, as well as a distortion of the redox balance [40]. It is also well known that exercise alters hormonal responses and concentrations. Actually, the free testosterone to cortisol (fTCR) ratio has been suggested as a potential endocrine biomarker to monitor the training status of an athlete [41].

Increased exercise stress is not only manifested in physiological and biochemical changes, but it is often presented in conjunction with psychological alterations. The symptoms of overtraining include depressed mood, general apathy, decreased self-esteem, emotional instability, disturbed sleep and others [11]. It has been suggested that examination of the athlete's performance and mood state at different periods during a competitive season is necessary for a better understanding of performance outcomes [9]. A symptom of a disturbed stress-regeneration caused by exercise increased demands is a worsened mood state, as measured by the Profile of Mood States (POMS) [31]. In recent years it has also been indicated that the avoidance of overtraining and the achievement of optimal performance can be only realized when athletes are able to recover and optimally balance training stress and subsequent recovery [21]. The complex effects of stress and recovery may be measured through the Recovery-Stress Questionnaire for Athletes (RESTQ-Sport), which assesses the frequency of experienced stressors and regeneration related activities [32]. The RESTQ-Sport has been shown to be a valuable tool for monitoring the reactions to changes in training load [12,22], the prevention of illness, and the optimization of training and recovery programs [6,7].

To understand which mechanisms are behind exercise performance is essential in order to be able to give sport coaches scientific feedbacks so as to facilitate a better background for training outcomes and to determine the point at which training becomes maladaptive [6]. Therefore, in this study a longitudinal design was used to determine if a 9-week intensified aerobic training can induce signs of overload. Functional, biochemical, haematological, hormonal and psychological measurements were performed in order to obtain a wide-ranging effect of such type of training.

Materials and Methods

Participants

9 healthy active young males participated in the study (22.3±1.4yr), which was approved by the Ethics Committee of the University of León and carried out according to the Declaration of Helsinki and Ethical Standards in Sport and Exercise Science Research [17]. Written informed consent was then obtained from participants, who were not training regularly before the beginning of the study.

Experimental design

The training protocol consisted of an intensive aerobic training based on outside running followed by a 3-week recovery period. The training frequency was 3 times a week, with an initial duration of the sessions of 40, 30 and 40 min respectively, increasing weekly training volume by 5 min for each session. Training intensity was controlled by training impulse (TRIMPS) [2] and participants were fully encouraged to not slow down the intensity. A global positioning system device (GPS) was also used for training monitoring (Garmin Edge 305, New Zealand). Heart rate (HR) was monitored with a short-range telemetry system monitor (Polar Team System, Polar Electro Oy, Kempele, Finland).

Moreover, participants had to perform several physical and psychological tests at 4 different time periods during the training schedule: baseline (T1), intermediate load (T2), maximal load (T3), and recovery (T4). During periods T1 and T4 subjects did not train. During periods T3 and T4 measurements were performed 24h after the last training session to avoid major sample bias.

Testing protocols

The participants filled in the psychological questionnaires and a blood sample was obtained at the laboratory. Venous blood and saliva samples were collected while participants were resting. Blood samples were centrifuged in order to obtain the plasma. Plasma aliquots and saliva samples were stored at -80 °C for

further analysis. Next, anthropometric data were obtained according to the American College of Sports Medicine. After the blood sampling and the anthropometric assessment, a maximal oxygen uptake test was performed in order to access training adaptation. Participants walked on a motorized treadmill (Cosmos, Pulsar, Germany) for 3 min at 6 km.h⁻¹. Speed was then increased by 1 km.h⁻¹ every minute until exhaustion. Achievement of VO₂max was considered as the attainment of at least one of the following criteria: (a) participant's volitional exhaustion, (b) a plateau in VO₂, or (c) a HR±10 beats.min⁻¹ of age-predicted maximal HR. Expired gases were collected and analyzed using a breath-by-breath automated gas analysis system (CPX plus, Medical Graphics, St. Paul, MN, USA). Heart rate was monitored and a capillary blood sample was obtained from the earlobe of each participant 3 min after the end of the test, and blood lactate concentration measurements were performed using a miniphotometer (Miniphotometer Plus LP20, Dr. Lange, Germany). Perceived exertion was verified every time the speed was increased using the Borg scale of 20 items [4].

Finally, a period of 48 h after the maximal oxygen uptake test was respected before participants performed the track field test. The test was performed individually and consisted of running 3000 m as fast as possible on a track of Olympic measures. HR data and wind speed was controlled using an anemometer (Anemo Cup Anemometer, BSRIA Instrument Solutions, UK). A chronometer was used to control lap and total times. Participants were verbally encouraged to run as fast as possible on each of the 4 track trial tests during the training schedule.

Haematological, Biochemical and Hormonal Analysis Hematocrit (Hct), haemoglobin concentration (Hb), and total and differential counts of white blood cells (neutrophils, lymphocytes, monocytes, basophils and eosinophils) were determined using an automated haematology analyzer (Coulter Juniors JS, Coulter Electronics, Delkenheim, Germany).

Creatinine, lactate dehydrogenase (LDH), alanine amitranferase (ALT), aspartate aminotranferase (AST), bilirubin direct, albumin, and uric acid were determined in plasma by a Cobas Integra 400 analyzer (Hoffmann-La Roche, Basel, Switzerland).

Myeloperoxidase (MPO) protein levels and cytokines IL-6 and TNF- α were measured in plasma using an enzyme immune assay (OxisResearchTM, BioCheck, Inc MPO-EIA, USA and Human IL-6 and TNF- α /TNFSF1A immunoassays, Quantikine[®] HS, R&D Systems, USA, respectively). Malondialdehyde (MDA) was assayed by Western blot analysis using an appropriate antibody [19]. Protein carbonyls content was measured using a commercial kit (Oxyblot[™] protein oxidation kit, Millipore, USA).

Salivary cortisol concentrations were analyzed using a competitive solid phase with the commercial kit Coat-A-Count C adapted to salivary levels (DPC, Siemens Medical Solutions Diagnostics). Salivary testosterone concentrations were analyzed by a competitive chemiluminescence immunoassay (Diagnostics Biochem Canada Inc.).

Psychological assessment

The Profile of Mood State (POMS) [28] is a 65-item questionnaire which provides a method of assessing transient, fluctuating mood states. It includes 5 negative affect scales: fatigue, depression, tension, anger, confusion and a positive affect scale: vigour scale. Subjects are given a score for each of the 6 mood states. The total mood disturbance score (TMD) is calculated by summation of the negative scales and subtraction of the positive

scale. The Recovery-Stress Questionnaire for Athletes (RESTQ-Sport) [23] is a questionnaire consisting of 76 items which indicate how often the respondent participated in various activities during the past 3 days and nights. The measure includes 12 scales which assess various stressing agents of a general nature and general recovery activities and 7 additional sports-specific scales, with 4 questions per scale and a warm-up question. The scores of the stress-related scales were summed up 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. Scores were also obtained for general stress, sport-specific stress, general recovery and sport-specific recovery. The Spanish versions of the POMS and the RESTQ-Sport have been previously demonstrated to be valid and reliable instruments [13,14]. Participants also filled in the Spanish version [38] of the 20 items State-Trait Anxiety Inventory State-Anxiety scale (STAI-S), which measures the temporary condition of state anxiety [37].

Statistical analysis

ANOVA with repeated measures was used to determine the differences between Hb, Hct, creatinine, MDA, protein carbonyls, MPO, TMD and training load. In the rest of the data (i.e., POMS and RESTQ-Sport) MANOVA main effects were analyzed. Sphericity was checked using the Mauchly sphericity test. Post-hoc multiple comparisons were used to identify the location of the pair-wise significant differences between training phases corrected using the Bonferroni adjustment. An alpha level of 0.05 was chosen to represent statistical significance. The Statistical Package for Social Sciences (SPSS, Ins, Chicago, I), Version 17, was used for all analyses. Training impulses were calculated using a spreadsheet (Microsoft Office Excel 2007, Microsoft Corporation, USA).

Results

• **Table 1** shows the anthropometric assessment and participants' characteristics. No significant changes were found in the different time periods of the training protocol for weight or body fat. Maximal oxygen uptake increased significantly during the period T3 when compared to T1 (P=0.019). Maximal HR and blood lactate decreased significantly in T3 compared to the T1 (P=0.039; P=0.016, respectively). Concerning track trial test, the time performance in the test showed a decrease during the T3 period compared to T1 (P=0.033).

• Fig. 1 illustrates the work load registered in each of the 9 weeks. Training load (TRIMPS) increased progressively during the 9-week intensified training protocol.

• **Table 2** presents the haematological parameters, which showed no differences during the different periods of the training program.

• **Table 3** shows that biochemical data did not significantly differ among the various training periods.

• **Table 4** presents the hormonal data obtained during the study. Testosterone concentration tended to decrease and that of cortisol to increase, resulting in progressive non significant lower values for the free testosterone to cortisol ratio (fTCR).

• **Table 5** shows POMS scales, STAI-S and RESTQ-Sport scales measured during the entire training protocol. Several scores for the different scales tended to increase in T3 when compared to T1, reaching significance in the tension (*P*=0.024), fatigue

(P=0.016) and confusion (P=0.021) scales, and in the total mood (P=0.037) of the POMS, in the STAI-S score (P=0.005), and in the general stress (P=0.008), sport specific stress (P=0.011), and total stress (P=0.008) scales of the RESTQ-Sport.

Discussion

In the current study VO_{2max} increased significantly during the period of maximal load when compared to the baseline values. Gormley et al. [15] also found an increase in VO_{2max} after a training period of similar intensity with match-aged participants, concluding that high intensity training is more effective than a moderate-intensity program in order to improve the VO_{2max} of young participants. Confirming previous data, detraining caused a decrease in the VO_{2max} measurements [29, 30].

Mujika et al. found higher values for lactate concentration during an intensified training period [30]. However, the decrease in blood lactate levels observed in our study suggests a proper training adaptation to the load imposed during the training, assuring that participants were more able to cope with the acid load produced during the test protocol. Changes in the time to perform the track field test appear to confirm the physiological adaptation to training. Therefore the data presented here support the beneficial effects of the training program on the functional measurements performed. In fact, Swain and Franklin [39] stated that vigorous-intensity exercise confers greater cardioprotective health benefits than moderate-intensity exercise. Moreover, clinical trials have found that higher-intensity exercise results in greater reductions in resting blood pressure than lower intensity exercise [1,20].

Haematological parameters did not show differences over the training period. Previous studies which focused on intensive training also failed to show changes in the immune cells

 $\label{eq:stable} \begin{array}{l} \mbox{Table 1} & \mbox{Anthropometric and training data (mean \pm SD) obtained during the entire training protocol. \end{array}$

	T1	T2	Т3	T4
weight (kg)	78.9±7.4	77.2±7.8	74.8±6.8	78.0±8.3
body fat (%)	11.9±5.2	9.9±5.3	7.9±5.1	11.8 ± 5.0
VO ₂ max (ml/kg/min ⁻¹)	45.2±2.3	49.3±7.2	63.6±6.6*	44.9±2.5
HRmax (bpm)	191±5	189±11	183±7*	188±7
blood lactate (mmol/l)	11.5±1.4	11.0±2.6	8.3±2.1*	11.1±1.5
track trial test (s)	785 ± 44	787±59	751±49*	793±44

T1 to T4: test sessions. * Significantly different from T1 (P<0.05)



Fig. 1 Training load increment along 9 weeks of training (mean ± SD). TRIMPS: training impulses; T1: baseline; T2: intermediate load; T3: maximal load; T4: recovery.

[▼]

Table 2	Haematologial parameters			
(mean ± SD) analyzed during the				
entire training protocol.				

	T1	T2	Т3	T4
erythrocytes (cells × 10 ¹² l ⁻¹)	4.9 ± 0.3	4.8±0.2	4.9±0.3	5.1±0.2
Hb (g dl ⁻¹)	15.2±0.8	14.9 ± 0.4	15.3±0.6	15.7±0.7
Hct (%)	43.7±2.3	43.1±1.7	43.6±1.6	45.8±2.0
total leukocytes (cells × 10 ⁹ l ⁻¹)	6.0 ± 1.5	6.4±2.5	5.8±1.5	6.2±1.9
neutrophils (%)	53.5±9.8	54.3±10.8	55.0±10.6	49.7±10.0
neutrophils (cells × 10 ⁹ l ⁻¹)	3.2 ± 1.0	3.6±2.3	3.2±1.2	3.2±1.4
lymphocytes (%)	35.5±9.2	33.9±9.9	34.2±9.5	37.1±6.9
lymphocytes (cells × 10 ⁹ l ⁻¹)	2.1±0.7	2.0 ± 0.4	1.9 ± 0.7	2.2±0.7
monocytes (%)	8.1±1.4	8.6±1.2	7.8±1.2	9.6±3.0
monocytes (cells × 10 ⁹ l ⁻¹)	0.5 ± 0.1	0.5 ± 0.2	0.4 ± 0.0	0.5 ± 0.2
basophils (%)	0.3 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.4±0.2
basophils (cells × 10 ⁹ l ⁻¹)	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
eosinophils (%)	2.4±1.0	2.6±1.1	2.3 ± 1.0	2.9±2.7
eosinophils (cells $\times 10^9 l^{-1}$)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.3

Hb: haemoglobin; Hct: hematocrit, T1 to T4: test sessions

 $\label{eq:stable} \textbf{Table 3} \quad \text{Biochemical data (mean \pm SD) obtained during the entire training protocol.}$

	T1	T2	Т3	T4
LDH (U/I)	243±81	275±51	214±56	200±76
ALT (U/I)	22.9±8.9	20.4±10.6	18.5±6.4	19.0±6.0
AST (U/I)	33.7±21.2	31.9±32.3	25.9 ± 5.7	24.4±6.6
albumin (g/dl)	4.3±0.2	4.0±0.3	4.3±0.2	4.3±0.1
direct bilirubin (mg/dl)	0.1 ± 0.1	0.1 ± 0.1	0.1±0.1	0.1±0.1
creatinine (mg/dl)	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.1
uric acid (µmol/l)	356±68	314±34	334±51	362±73
IL-6 (pg/mL)	0.9 ± 0.8	1.2±3.7	0.4 ± 0.6	0.9 ± 1.2
TNF-alpha (pg/mL)	1.1±0.3	0.9 ± 0.4	1.0 ± 0.2	1.0 ± 0.4
MPO (ng/mL)	4.0 ± 2.4	4.5±3.3	4.7±3.5	4.5±1.9
-SH (nmol/mg prot)	45.6±7.8	44.4±7.1	46.5±5.3	45.6±7.9
protein carbonyls (%)	100 ± 4	99±5	98±3	100 ± 2
MDA (%)	100 ± 6	101±4	97±5	100±2

LDH: lactate dehydrogenase, ALT: alanine transaminase, AST: aspartate transaminase, IL-6: interleukine 6, TNF-alpha: tumour necrosis factor alpha, MPO: myeloperoxidase, -SH: sulfhydryl groups, MDA: malondialdehyde. T1 to T4: test sessions

 Table 4
 Hormonal data (mean ± SD) obtained during the entire training protocol.

	T1	T2	Т3	T4
testosterone (pmol/l)	45.9±17.5	43.8±10.8	40.0±9.7	39.2±14.8
cortisol (nmol/l)	3.4±1.5	3.5±1.6	3.8±1.3	4.8±2.8
fTCR	18.3±13.9	15.2±7.7	12.0±4.2	11.3±7.0

fTCR: Free Testosterone to Cortisol ratio. T1 to T4: test sessions

measurements [8, 16, 25, 36]. Along the same lines, no changes were observed for the biochemical parameters measured in this study. The cytokine hypothesis of overtraining proposes that trauma to the musculoskeletal system leading to a local inflammatory response is the initiating event in the development of overtraining [36]. Finally, inadequate recovery and a continuation of the athletes' training regimen result in the release of inflammatory mediators and the subsequent release of proinflammatory cytokines. Confirming previous research, plasma IL-6 and TNF- α concentrations remained unchanged [16]. So, although the underlying causative mechanism/s of overreaching and overtraining is still unclear, it does not appear that elevations in circulating cytokines are primarily responsible for the fatigue associated with long-duration, high-intensity exercise. Impaired antioxidant capacity and increased oxidative stress
 Table 5
 POMS scales, STAI-S, and RESTQ-Sport scales (mean ± SD) measured during the entire training protocol.

	T1	T2	Т3	T4
POMS				
tension	6.0±3.1	2.9±3.9	10.7±5.1*	3.6±3.2
depression	8.0±9.4	5.2±4.4	10.6±3.8	5.1±4.3
anger	6.0±8.1	4.9±3.9	10.8 ± 5.5	6.8 ± 4.9
vigour	18.3±6.9	18.7±6.5	13.7±6.8	21.5±2.9
fatigue	6.2±4.8	7.6±3.7	16.3±6.5*	4.0 ± 2.4
confusion	2.9±3.7	1.7±2.2	6.5±2.4*	1.1±3.3
total mood	111±27	104±13	141±16*	99±15
disturbance				
STAI-S	14.0±7.6	15.1±6.3	23.0±4.3*	15.5±5.9
RESTQ-Sport				
total stress	1.2 ± 0.7	1.5 ± 0.7	2.2±0.7*	1.1±0.7
total recovery	3.4±0.8	3.5±0.7	2.9 ± 0.6	3.9±0.7
general stress	1.4±0.6	1.4±0.5	2.3±0.5*	1.2 ± 0.6
general recovery	3.8±1.0	3.9±0.8	3.2 ± 0.7	3.6±0.9
sport-specific stress	1.0 ± 0.9	1.5 ± 1.0	2.2±1.1*	1.2 ± 0.9
sport-specific recovery	2.8±0.8	3.0±0.7	2.6±0.8	3.2±1.2

T1 to T4: test sessions. * Significantly different from T1 (P<0.05)

could be also associated with fatigue [35], and might be related to the overtraining syndrome [10]. Previous studies found that overtraining status was associated with an increase in resting oxidative stress, reflected in higher protein carbonyl concentrations in overtrained athletes compared to trained controls. Furthermore, responses to acute exercise-induced stress were disminished in overtrained athletes [40]. However, the intense training program here proposed had no effects on MDA, protein carbonyls, and SH groups. Moreover, the lack of significant changes in serum enzymes observed in the present research seems to indicate that the organic systems involved in the training adaptation/ regeneration cope well with the training demands of an intensified training period in the young healthy participants.

A similar trend was observed for the hormonal responses. Although cortisol tended to increase and testosterone tended to decrease, no statistically significant differences were found over the whole training season. Several studies have previously investigated the hormonal response in overtraining, but widely conflicting results have been reported and no useful quantitative criteria have been defined [42]. In fact, it is difficult to compare these studies, since there are many variables, such as the definition of training status, the diurnal variation, and the glycogen status, to consider when interpreting hormonal data. Our results, in agreement with others, suggest that hormonal markers should not be used as an early marker of overreaching in a practical training environment [8].

If functional parameters showed improvements and there were no significant modifications in biomarkers, changes in different scales of the STAI, POMS and RESTQ-Sport indicated the existence of psychological alterations and suggested a risk of overtraining. In 1999, McKenzie stated that overtrained athletes may be firstly identified by a change in psychological markers, followed by medical condition and then performance outcome [27]. Coutts et al. [8] also found changes in the RESTQ-Sport, with a significant increase in total stress and a reduction in total recovery, after an intensive training in comparison to a normal training. These authors proposed that the overtraining syndrome may be related to other factors rather than large training loads, and suggested that psychological measures, together with performance, are useful indicators of overreaching. Nevertheless, although Coutts et al. [8] registered lower performance outcomes, in the present study the performance improved at the end of the training. This discrepancy is possibly due to the fact that in the mentioned study there were 3 different types of exercises during training: swimming, cycling and running, which involves more exercise-related stress. Apart from that, Coutts et al. designed a training protocol aiming to purposely overreach the participants, while our study was focused on investigating the impact of an intensified training protocol [8]. In fact, the unfavourable pattern of stress and recovery was mainly apparent in both the general and the sport-specific stress scales. This provides an indication that the focus should be on helping athletes with subjective stress. In any case, although psychometric instruments provide useful and rapid information, they do not provide the final diagnosis that someone is overtrained, which requires under-recovery over a longer period of time and other indicators such as a chronic performance plateau that cannot be positively influenced by short amounts of rest [21].

A limitation of our research is the absence of a control group which unabled us to 'filter' out threats to experimental validity like learning and habituation effects on tests. Moreover, the number of subjects participating in the study was small. However, in spite of the possibility of type II errors development, sample size was enough to confirm that training was becoming maladaptive and to demonstrate that psychological instruments were sensitive enough to detect signs of overload. From the data obtained it became obvious that a short-period intensified training program can induce several performance improvements in a few weeks time, however, the participant's perceived effort should be taken into account in order to avoid an undesirable stress-related situation. In summary, we can conclude that psychological questionnaires may be a useful means to detect early overreaching even though when it is disguised by positive performance outcomes, and that longitudinal monitoring of changes in stress and recovery may by useful for detection of overtraining in its early stages.

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