

PREDICTION OF SUBJECTIVELY RATED PERFORMANCE CAPACITY IN ELITE MALE ENDURANCE-, INTERVAL- AND RESISTANCE EXERCISE ATHLETES

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Abstract

Introduction: Overreaching is marked by a deteriorated performance without a clear aetiology. Until now, no definite set of parameters identifying overreaching is known.

Aim of the study: To develop a multivariate factorial model derived from single measurements of Hb, Ht, red blood cell count (RBC), ferritin, cortisol (C), cortisol-binding protein (CBG), testosterone (T), sex hormone-binding protein (SHBG), free C (FC) and free T (FT), that is capable of classifying male athletes with or without a performance drop.

Methods: From 78 healthy elite male athletes (mean age: 25.4 yrs, range 17–34 yrs), who were involved in different endurance, interval and high intensity sports, venous blood was sampled under resting conditions for measurements of Hb, Ht, RBC, ferritin, C, CBG, T, SHBG, FC and FT (cross-sectional design). In addition, the athletes were asked to rate their performance capacity (PC), training work load, injury free status, and feelings of general well being during the week preceding blood sampling. Misfits of the model, defined by serious over- or underestimations of the data, were used to predict a low PC.

Results: Three independent factors could describe the O₂ transport capacity and anabolic/catabolic activity. These factors explained 84% of the total variance in all parameters. Overestimation of SHBG levels and underestimation of Ht levels coincided with low PC ratings ($R^2 = 0.48$). Inclusion of Hb to the regression model increased R^2 to 0.55.

Conclusions: The internal consistency of the selected parameters from a composite group of athletes was sufficient to construct a three-factorial model. This model could be used to classify elite male athletes with or without a performance drop. The model should be tested in prospective studies on its predictive power of overreaching-related performance decrements.

Keywords: performance, overreaching, anabolic/catabolic balance, oxygen transport capacity, multivariate analysis

Introduction

Studies in athletes with the aim of finding diagnostic parameters of overreaching/overtraining have suffered from poor statistical power and evidence. This is mainly caused by the relatively small number of athletes with overtraining, the fact that signs and symptoms of overreaching/overtraining vary considerably between individuals and definitions of overreaching/overtraining have changed over time (1). Moreover, regarding the potential markers of overreaching/overtraining, Halson and Jeukendrup (2) concluded that nearly every marker was based on small studies that have induced overreaching but not overtraining. In practical sense, this implies that sports physicians and scientists are mainly dealing with markers of overreaching rather than markers of overtraining. These markers consist of a wide range of biochemical, haematological, psychological and hormonal parameters (3,4–22). In almost all cases, associations with overreaching/overtraining were based on univariate regression or anecdotal evidence (3,

9,10,18,20,21,23). Because relatively large inter-individual variations in many overreaching/overtraining-related parameters have been found (4,5,7,10,11,17,19–23), it is not likely that such an approach will lead to strong predictive markers.

Assuming that overreaching is potentially a pre-stage or reversible first stage of overtraining (1,2,9,24), and sports physicians are dealing mainly with athletes suffering from overreaching, our major concern as scientists should be the state of overreaching. As Dal Monte (25) stated: “Failure on the part of athletes and coaches to recognise overreaching can lead, as a direct consequence of a poor competitive performance, to an inappropriate increase in the training loads, which, rather than sustaining the intended performance improvement can actually result in the onset of overtraining”. Various authors have used a training or exercise continuum, ranging from under- to overtraining, to explain how training load is associated with overreaching/overtraining and as a result, to deteriorated performances (1). Using this con-

tinuum, a recent consensus statement on the diagnosis of overtraining syndrome has defined functional (FO) and a non-functional phase (NFO) in overreaching (26), both preceding the state of overtraining (OTS). Furthermore, the statement contains a myriad of possible markers of OTS, based on the principles that absolute values of these markers or changes in the processes associated with these markers might identify OTS in athletes. Compared to OTS, NFO is characterised by a shorter duration of the performance decrement and less severe symptoms. This implies, that identifying athletes at high-risk for overreaching (NFO) in time, may prevent a further deterioration into OTS.

Thus, the goal of this study is to find patterns of markers that discriminate athletes with a performance decrease associated with the NFO state from athletes without any performance decrease.

Haematological and hormonal parameters of elite male athletes who visited our hospital for a regular health screening, were used to study patterns of potential markers. The patterns were defined by means of a multi factorial model, basically describing the relationship between the parameters in healthy athletes. Then we hypothesized that serious statistical misfits of the model—thus not adequately representing the original values of these parameters - indicated the existence of deviating regulatory mechanisms in these athletes. Assuming that such misfits were not symptomatic to any random error of the model, the model was tested by its potency to predict a reduced performance capacity. Regarding the mixture of sport activities of our athletes, a single test to objectively assess performance capacity could not be applied. Therefore, we quantified this performance capacity by means of a subjective rating, based on its validity in monitoring exercise tolerance.

Material and methods

Subjects

We analysed a database of 78 healthy male elite athletes (mean age: 25.4 yrs, range 17-34 yrs; mean height: 178 cm, range 165-197cm; mean body weight: 77 kg, range 60-95 kg), who visited our hospital for the first time. The athletes came to our hospital for various reasons, such as monitoring baseline levels before the competition season, seasonal evaluation, participation in research programs, or occasionally previous injury-related complaints. Athletes with prevalent injuries were excluded from the study. The athletes were involved in different high performance sports at national and international levels. Forty-four athletes (56%) participated in endurance and long distance sports, mainly track and field, ice speed skating and cycling. Sixteen athletes (21%) participated in sports activities with a duration between 2 and 6 min, such as middle-distance track and field, ice skating and cycling. Twelve athletes (15%) were active in short distance track and field and

short time power sports, such as sprinting and weight lifting. Finally, six athletes (8%) participated in interval sports, such as tennis or badminton. All subjects signed an informed consent before inclusion, and institutional review board approval was obtained for this study.

Blood sampling

Under resting conditions, venous blood was collected into K₃EDTA tubes between 8.00-9.00 hr a.m. after an overnight fast. At the previous day subjects refrained from intensive training. As part of our clinical evaluation protocol a selected number of haematological and hormonal parameters were measured. Blood for hormone measurements was immediately centrifuged (3000 RPM, 20 min at 4°C) and stored at -20°C until assayed within 4 wk. Hemoglobin (Hb) was measured by HiCN-method, hematocrit (Ht) by micromethod, red blood cell count (RBC) by coulter counter, and ferritin by immunometric assay. Serum cortisol (C) was measured by fluorescence polarization assay, cortisol-binding protein (CBG) and testosterone (T) by radio immuno assay (RIA), and sex hormone-binding protein (SHBG) by immunoradiometric assay (IRMA). Albumin was determined by immunochemical method to calculate free cortisol (FC) and free testosterone (FT) according to a mass action formulation model. FC was calculated from total C, albumin, CBG, and the association constants of the protein-cortisol complex, and FT according to Vermeulen et al. (27). The intra- and inter-assay coefficients of variation for all analyses were less than 10%.

Ratings

Subjects were asked to rate performance capacity (PC), training workload (TL), injury free status (IS) and feelings of general well being (WB) on a five-point scale (Likert technique (28)) during the week preceding the blood sampling. Low scores were related to problems and high scores to optimum conditions. For example, the lowest score (= 1) for PC referred to a very low performance (i.e., “not at all in a good shape”) and the highest score (= 5) to an optimum performance (i.e., “in a very good shape”).

Statistical analysis

Statistical analyses were carried out using SPSS for Windows Release 11.5. Mean values $\pm$ SD for blood parameters were calculated. Normality of distribution was examined using Kolmogorov-Smirnov statistics and a Lilliefors test. We used a Principal Component Factor Analysis with Varimax Rotation to extract independent factors from these parameters (29). The number of independent factors was based on eigenvalues larger than 1. To assess how well the model fitted the data, we required a minimum goodness-of-fit (R^2) of 70%, independent of the number of factors. Factor

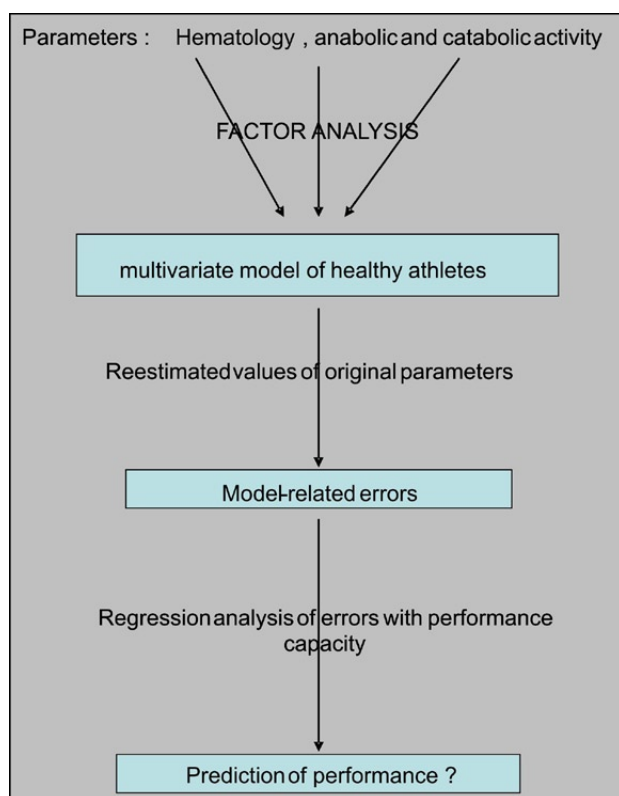


Figure 1. A step-by-step description of the data analysis

loadings of parameters represent the magnitude of the correlations between relevant parameters and the extracted factors. Each factor was represented by factor scores, indicating the impact of each factor on an individual athlete. These scores were used to reproduce the original blood levels by a multivariate linear regression technique. The residuals ($X_{\text{original}} - X_{\text{predicted by factor scores}}$) were used to identify athletes with an extreme misfit of the model, defined by cases in which residuals represented one of the 5 largest over- or underestimations by the model. As a result, for each original parameter ten (2x5) athletes could be marked as “deviated”. Under- and overestimation were then saved into binary variables, with “deviated” athletes labeled as ‘1’ and others as ‘0’. Finally, univariate regression and analysis

of variance, as well as multivariate regression were used to test the link with PC. Statistical significance was defined as $P \leq 0.05$. A step-by-step description of the data analysis is shown in figure 1.

Results

Univariate analysis of blood- and performance-related parameters

The values of the parameters (table 1) showed a normal distribution. A correlation matrix between these parameters is shown in table 2. Within three distinct sets of parameters strong correlations were found 1) Hb, Ht, RBC; 2) T, FT, SHBG; and 3) C, FC. CBG did not correlate with any parameter.

Table 3 shows a correlation matrix of the performance-related parameters obtained using a Likert scale. Regarding these relationships, a decreased performance capacity will appear in a number of cases in athletes with previous injury-related complaints, loss of well being, or a reduction in training volume and intensity.

Table 1. Obtained values of blood-related parameters from a database of 78 male elite athletes

	Mean $\pm$ SD
Hb (mmol/l)	9.4 $\pm$ 0.6
Ht (l/l)	0.45 $\pm$ 0.03
RBC ($\times 10^{12}$ /l)	5.0 $\pm$ 0.4
Ferritin (ng/ml)	127 $\pm$ 77
C (μ mol/l)	0.39 $\pm$ 0.12
CBG (mg/l)	47.0 $\pm$ 10.4
FC (nmol/l)	20.7 $\pm$ 10.5
T (nmol/l)	23.6 $\pm$ 7.4
SHBG (nmol/l)	38.0 $\pm$ 12.7
FT (pmol/l)	510 $\pm$ 154

Hb=hemoglobin; Ht= hematocrit; RBC= red blood cell count; C= cortisol; CBG=cortisol-binding protein; FC= free Cortisol; T= testosterone; SHBG= sex hormone-binding protein; FT= free Testosterone

Table 2. Correlation matrix (Pearson's correlation coefficient R) of blood-related parameters in male athletes (N=78)

	Hb	Ht	RBC	FERRITIN	C	CBG	FC	T	SHBG	FT
Hb		.91**	.80**	.09	.03	-.12	.08	.17	-.17	.28*
Ht			.84**	.06	.05	-.02	.07	.12	-.18	.22
RBC				.00	.02	-.08	.05	.21	-.12	.29*
FERRITIN					-.07	.12	-.11	.25*	.18	.23*
C						.11	.88**	-.01	.07	-.13
CBG							-.28*	-.18	-.08	-.18
FC								.07	.05	.10
T									.51**	.88**
SHBG										.07

** significant at $P < 0.01$ (2-tailed)

* significant at $P < 0.05$ (2-tailed)

For abbreviations, see legends table 1

Table 3. *Correlation matrix* (Pearson's correlation coefficient R) of feelings of well being, injury free status, training workload, and performance capacity in male elite athletes (N=78)*

	Well Being	Injury Free Status	Training workload	Performance Capacity
Well Being		.53	.35	.55
Injury Free Status			.54	.56
Training workload				.44

*all correlations are significant at $P < 0.001$, except for the correlation between Well Being and Training workload at $P < 0.01$

Multivariate factorial analysis

Using all parameters from table 2 the Principal Component Factor Analysis extracted a maximum of three independent factors, representing O_2 transport capacity in blood, anabolic activity and catabolic activity. Ferritin and CBG levels did not fit in this three-factorial model very well, and the model did not reach the minimum required goodness-of-fit of 70%. After removal of ferritin and CBG levels from the analysis, a new three-factorial model could explain 84% of the total variance. The first factor, covering O_2 transport capacity, explained 37% of the variance (eigenvalue=3). The second factor, covering anabolic activity, explained another 24% of the variance (eigenvalue=1.9), whereas the last factor, related to catabolic activity, explained the remaining 23% of the variance (eigenvalue=1.8).

Cumulative variances for parameters explained by the three factors (communalities) are shown in the last column of table 4. Factor loadings of parameters are shown in the middle three columns of table 4. Apart from the moderate loading of SHBG on the anabolic activity-related factor, factor loadings were high (≥ 0.82). For example, a factor loading of 0.91 for RBC indicated that the O_2 transport capacity-related factor explained 83% ($= 0.91^2$) of the variance in RBC. Because the cumulative explained variance in RBC was 84%, the remaining anabolic and catabolic activ-

ity-related factors could only contribute 1% to the variance of RBC (84-83%).

Prediction of PC

Univariate

From all haematological and hormonal parameters used in the study, only Hb levels showed a significant positive Pearson's correlation with PC ($R=0.24$; $P < 0.05$). A non-parametric Spearman rank correlation revealed small but significant positive correlations of Hb and SHBG levels with PC ($R=0.24$; $P < 0.05$ and $R=0.23$; $P < 0.05$). An analysis of variance on these parameters showed mean differences between PC groups on T and FT levels, with highest values in both the lowest and highest PC group.

Such an analysis also showed that a model-overestimation of SHBG levels ($R = -.43$, $F = 16.9$; $P < 0.001$), as well as a model-underestimation of FT levels ($R = -0.29$, $F = 6.9$; $P < 0.01$) were significantly related to PC. Therefore, if the factorial model either predicted higher levels of SHBG or lower levels of FT than actually found, a lower than average PC was found. Additionally, four athletes with underestimated FT levels also belonged to a group of five athletes with overestimated SHBG levels ($\chi^2 = 48.2$; $DF = 1$; $P < 0.001$). In latter group, the average FT-level (734 pmol/l) was about 48% higher than that in the athletes without overestimated SHBG levels ($t = -3.6$; $P < 0.001$, Mann-Whitney test: $U = 53.0$; $P < 0.05$).

Table 4. *Factor loadings of relevant parameters, and cumulative variance explained by three factors for all parameters separately (N=78)*

	O_2 transport capacity	Anabolic activity	Catabolic activity	Cumulative variance (%)*
Ht	.96			92
Hb	.95			90
RBC	.91			84
T		.99		99
FT		.82		77
SHBG		.62		45
C			.97	94
FC			.97	94

For abbreviations, see legends table 1.

* R^2 explained by the three-factorial model

Table 5. Regression model of subjective performance capacity (PC) without (top) and with inclusion of Hb (bottom)

Parameter	Regression coefficient	Significance
Constant	3.1	$P<0.001$
Overestimated SHBG*	-2.2	$P<0.001$
Overestimated Ht*	1.1	$P<0.05$
Constant	-1.4	non-significant
Overestimated SHBG*	-2.2	$P<0.001$
Overestimated Ht*	1.2	$P<0.01$
Hb**	0.5	$P<0.01$

* Overestimated SHBG and Ht as binary parameters: 1 = overestimated, 0 = non-overestimated

**Hb in mmol/l

Multivariate

The upper panel of table 5 showed that PC was associated with a combination of an overestimation of both SHBG and Ht levels. The proportion of the explained variation (R^2) was 0.48 ($F=11.5$; $P<0.001$). After inclusion of the original Hb levels (in mmol/l) in the regression model, the proportion of the explained variance increased to 0.55 ($F=10.6$; $P<0.001$, lower panel of table 5). Based on these results, the following regression equation could be obtained, in which the constant (-1.4) was no longer significant:

$$\text{Performance Capacity}^1 = -1.6 - (2.2 \times \text{overestimation SHBG}) + (1.2 \times \text{overestimation Ht}) + 0.5 \times \text{Hb}$$

Discussion

In this study in a composite group of elite male athletes, data of haematological and hormonal parameters were considered suitable for developing a three factorial model that could adequately describe deviating parameters. We hypothesize that athletes with an extreme misfitting to the model may represent a group with deviating regulatory mechanisms. This study has shown that such a misfitting can be used to classify elite male athletes with or without a subjectively rated performance drop; hence supporting the concept that subjectively rated underperformance does seem to have a real physiological background. Because a reduced performance capacity is a key symptom in overreaching, this result may help to develop a toolkit that identifies athletes at a high-risk of overreaching.

Regarding the mixture of sport activities of our athletes, we have chosen to quantify their PC by rating.

Kenntä & Hassmén (32) postulated that 'a physical effort is best conceptualized as a complex psychobiological construct'. Such a construct validates the use of ratings, such as the rating of perceived exertion (RPE), as a valuable and reliable indicator in monitoring an individual's exercise tolerance (33). The observed as-

sociations between the scores of the rated parameters (see table 3) showed that a reduced PC may coincide with an impact on the athlete's well being and may not always represent a 'minor issue'. This supports the construct validity of the scores, and the concept that underperformance is related to more problems than purely physical overload only. Unfortunately, because of the retrospective character of the data, it was impossible to describe the nature of the reduced performance as e.g. injury- or disease-related, or simply due to voluntary undertraining. Of course, future studies have to address further the validity and reliability of the PC-scores by comparing those scores with more objective data, as obtained by field or laboratory tests.

Three-factorial model and PC

The data used in the factorial analysis can be considered as representative for elite male athletes involved in various endurance, interval and high intensity sports, as the average values of the selected parameters were consistent with data obtained from the literature (1,4-6,8,9,13,14,18,19,21-26,30,31).

The three-factorial model is very robust. The internal consistency of the selected parameters was more than sufficient to construct this model, with factors explaining 84% of the total variance in all parameters. However, regarding the anabolic activity-related factor, SHBG data fitted only moderately. This was as expected because FT levels were not correlated with SHBG levels, although T levels correlated with SHBG levels (table 2). This suggests that the underlying construct of this factor did not cover the typical function of SHBG, being binding of T and controlling FT. Similar conclusions could be drawn for the data of CBG. As CBG data did not fit into the model, the catabolic activity-related factor only represented C and FC levels. Therefore, both factors seem to point at constructs that describe net hormone levels that are based on the balance be-

¹ Rating of performance capacity: 1 = a very low performance (i.e., "not at all in a good shape") and 5 = an optimum performance (i.e., "in a very good shape"). Overestimated SHBG and Ht as binary parameters: 1 = overestimated, 0 = non-overestimated.

tween production and degradation, rather than the dynamic status of hormones with feedback mechanisms. The interaction of an overestimated SHBG with high T and FT values in the prediction of PC posed the question how androgen levels respond to training and overtraining. Fry et al. (34) studied a group of weight-trained athletes during an overtraining period and compared them with controls. They found no significant drop in FT after an overtraining period. They also concluded that SHBG levels simply followed the changes in T levels, implying that FT and SHBG levels were not related to each other. This is a similar result as we obtained from our factorial model based on a cross-sectional sample. However, because of the highly non-linear relationship between SHBG and FT (35), absence of a significant correlation between SHBG and FT could be expected.

Reviews also confirmed the lack of proof for decreased androgen levels in overreaching or overtraining (1,2). Recently, a prospective study with middle-aged to older men following a 12-month aerobic exercise program again did not show any change in androgens (36). However, a recent cross-sectional study by Maimoun et al. (37) showed that endurance athletes had reduced T levels compared to controls. Another prospective study by Safarinejad et al. (38) showed similar results. Finally, a prospective study on the effects of aerobic training by Grandys et al. (39) revealed that improved local muscle endurance after a 5-week training period coincided with increased T, decreased SHBG, and of course, increased FT levels. But in spite of a drop in average SHBG levels, the highest increases in T and FT levels were achieved in athletes who had the highest SHBG levels. They reported no linear relation between performance and T or FT levels. In our study, we also did not find a linear correlation between performance and T or FT levels, but indeed a correlation between performance and SHBG levels.

This intriguing similarity between the results of the intervention study of Grandys et al. (39) and our cross-sectional study triggered us to verify the reason for a lack of a relationship between performance and T or FT levels in our data. One should keep in mind that the study of Grandys et al. (39) used an objective performance index, whereas our study used a subjective one. In our study a highly overestimated SHBG-level coincided with the lowest PC rating. SHBG-levels in the overestimated group were significantly lower, and showed a narrower range compared to the non-overestimated group (mean SHBG: 27.2 versus 38.7 nmol/l; range: 21-33 versus 14-71 nmol/l, respectively). Although average T levels were not significantly different between the groups, the overestimated group again showed a narrower range in T than the non-overestimated SHBG group (range: 19-36 versus 10-42 nmol/l, respectively). However, FT levels were 1.5 times higher in the overestimated group

($F=13$ $P<0.01$), and the range was also smaller than in the non-overestimated group. Such a finding makes it tempting to suggest that in the SHBG-overestimated group with poorly rated performances, FT levels were highest of all. But such an interpretation is not correct, because FT levels in the non-overestimated group with a maximal performance rating (PC=5) were equally high. A similar pattern was found in T. Therefore, the average T and FT levels showed a type of U-shaped curve with performance, and no linear relationship. This may also explain why Glandys et al. (39) only reported a positive correlation between SHBG levels and performance, but not between T or FT levels and performance.

Our results suggest that there was simply less SHBG available to bind T in the overestimated SHBG group with a poor performance rating. This caused relatively high FT levels. In the non-overestimated SHBG group with the highest PC values, absolute levels of T were highest. However, these athletes also had more SHBG to bind T compared to athletes in the overestimated SHBG group. This resulted in marginally lower FT levels in the non-overestimated SHBG group compared to FT levels in the overestimated SHBG group with the lowest performance rating.

There maybe several reasons why androgen studies in athletes have revealed more or less conflicting results. In our study, both low and high performance athletes showed similarly high FT levels, but different T/SHBG ratios. More importantly, a direct comparison of androgen levels between different studies is very difficult, because methods to obtain T and SHBG, as well as methods to measure or calculate FT vary substantially between studies (35,40).

Multivariate regression analysis revealed that an underestimated Ht (*low predicted values of Ht compared to the actual levels*) was linked with a relatively low PC. The blood viscosity is relatively high in those cases, which may limit the blood flow to the muscles. Monitoring of Ht has been mentioned as an important factor in early detection of overtraining in elite sportsmen (5). It should be noted that both RBC and Hb levels were not significantly different in the overestimated and non-overestimated Ht-group. The inclusion of Hb in the regression model as a positive predictor of PC is in line with the experience that low levels of Hb, indicating a low binding capacity for O₂, may contribute to a low PC.

In summary, a relatively low PC was predicted if 1) FT levels were high because SHBG levels were relatively low, 2) lower than actual levels of Ht were predicted by the model, and 3) low Hb levels were found.

Conclusions

A multi-factorial model in healthy male elite athletes that describes the regulatory systems of O₂ transport in blood, the anabolic and catabolic activity,

appears to provide sufficient information to detect those athletes who might not be 'in good shape' according to their misfit to the model. The strongest misfits, associated with parameters of O₂ transport and anabolic activity, were capable of predicting a subjectively rated low performance capacity. However, the cross-sectional design of this study demands that the predictive power of this multivariate model approach must also be studied prospectively, before its application can be helpful for the early detection of overreaching. Finally, additional parameters, such as insulin or IGF-1, must be explored to improve the potency of the factorial model further.

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References

1. Armstrong L, VanHeest J. The unknown mechanism of the overtraining syndrome: clues from depression and psychoneuroimmunology. *Sports Med* 2002; 32: 185-209.
2. Halson SL, Jeukendrup AE. Does overtraining exist? An analysis of overreaching and overtraining research. *Sports Med* 2004; 34: 967-81.
3. Kuipers H, Keizer HA. Overtraining in elite athletes – review and directions for the future. *Sports Med* 1988; 6: 79-92.
4. Adlercreutz H, Harkonen M, Kuoppasalmi K, et al. Effect of training on plasma anabolic and catabolic steroid hormones and their response during physical exercise. *Int J Sports Med* 1986; 7 (Suppl): 27-8.
5. Aïssa Benhaddad A, Bouix D, Khaled S, et al. Early hemorheologic aspects of overtraining in elite athletes. *Clin Hemorheol Microcirc* 1999; 20: 117-25.
6. Barron JL, Noakes TD, Levy W, et al. Hypothalamic dysfunction in overtrained athletes. *J Clin Endocrinol Metab* 1985; 60: 803-6.
7. Dickson DN, Wilkinson RL, Noakes TD. Effects of ultramarathon training and racing on haematological parameters and serum ferritin levels in well-trained athletes. *Int J Sports Med* 1982; 3: 111-7.
8. Fellman N. Hormonal and plasma volume alterations following endurance exercise. *Sports Med* 1992; 13: 37-49.
9. Fry RW, Morton AR, Keast D. Overtraining in athletes – an update. *Sports Med* 1991; 12: 32-65.
10. Fry RW, Lawrence SR, Morton AR, et al. Monitoring training stress in endurance sports using biological parameters. *Clin J Sports Med* 1993; 3: 6-13.
11. Gastmann U, Petersen KG, Bocker J, et al. Monitoring intensive endurance training at moderate energetic demands using resting laboratory markers failed to recognize an early overtraining stage. *J Sports Med Phys Fitness* 1998; 38: 188-93.
12. Keast D, Arstein D, Harper W, et al. Depression of plasma glutamine concentration after exercise stress and its possible influence on the immune system. *Med J Aust* 1995; 162: 15-8.
13. O'Connor PJ, Morgan WP, Raglin JS, et al. Mood state and salivary cortisol levels following overtraining in female swimmers. *Psychoneuroendocrinol* 1989; 14: 303-10.
14. Snyder AC, Jeukendrup AE, Hasselink MKC, et al. A physiological/psychological indicator of overreaching during intensive training. *Int J Sports Med* 1993; 14: 9-32.
15. Tegelman R, Johansson C, Hemmingsson P, et al. Endogenous anabolic and catabolic steroid hormones in male and female athletes during off season. *Int J Sports Med* 1990; 10: 103-6.
16. Urhausen A, Kullmer T, Kindermann W. A 7-week follow-up study of the behaviour of testosterone and cortisol during the competition period in rowers. *Eur J Appl Physiol* 1987; 56: 528-33.
17. Uusitalo ALT, Huttunen P, Hanin Y, et al. Hormonal responses to endurance training and overtraining in female athletes. *Clin J Sports Med* 1998; 8: 178-86.
18. Vervoorn C. Neuro-endocrine aspects of exercise and training (Thesis). ISBN 90-393-0051-8. Utrecht: University Utrecht, 1992.
19. Platen P. Overtraining and the endocrine system. Part 2: Review of scientific studies. *Eur J Sport Sci* 2002; 2: 1-7.
20. Baumert M, Brechtel L, Lock J, et al. Heart rate variability, blood pressure variability, and baroreflex sensitivity in overtrained athletes. *Clin J Sport Med* 2006; 16: 412-7.
21. Coutts AJ, Wallace LK, Slattery KM. Monitoring changes in performance, physiology, biochemistry, and psychology during overreaching and recovery in triathletes. *Int J Sports Med* 2007; 28: 125-34.
22. Hartmann U, Mester J. Training and overtraining markers in selected sport events. *Med Sci Sports Exerc* 2000; 32: 209-15.
23. Vervoorn C, Vermulst LJM, Boelens-Quist AM, et al. Seasonal changes in performance and free testosterone: cortisol ratio of elite female rowers. *Eur J Appl Physiol* 1992; 64: 14-21.
24. Kuipers H. Training and overtraining: an introduction. *Med Sci Sports Exerc* 1998; 30: 1137-9.
25. Dal Monte A. Fatigue and sport. *Funct Neurol* 2002; 17: 7-10.
26. Meeusen R, Duclos M, Gleeson, M, et al. Prevention, diagnosis and treatment of the Overtraining Syndrome. *Eur J Sport Sci* 2006; 6: 1-14.
27. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab* 1999; 84: 3666-72.
28. Likert R. A technique for the measurement of attitudes. *Arch Psychol* 1932; 140: 1-55.
29. McDonald RP. Factor analysis and related methods. Hillsdale, New Jersey: Lawrence Erlbaum Associated Publisher, 1985: 198-201.
30. Fry AC, Kraemer W. Resistance exercise overtraining and overreaching. Neuroendocrine responses. *Sports Med* 1997; 23: 106-29.
31. Kreider RB, Fry AC, O'Toole ML (eds). Overtraining in sport. Champaign (IL): Human Kinetics, 1998.
32. Kenntä G, Hassmén P. Overtraining and recovery. A conceptual model. *Sports Med* 1998; 26: 1-16.
33. Noble BJ, Borg GAV, Jacobs I, et al. A category-ratio perceived exertion scale: relationship to blood and muscle lactates and heart rate. *Med Sci Sports Exerc* 1983; 15: 523-8.
34. Fry AC, Kraemer J, Ramsey LT. Pituitary-adrenal-gonadal responses to high-intensity resistance exercise training. *J Appl Physiol* 1998; 85: 2352-9.
35. Ly LP, Handelsman DJ. Empirical estimation of free testosterone from testosterone and sex hormone-binding globulin immunoassays. *Eur J Endocrinol* 2005; 152: 471-8.
36. Hawkins VN, Foster-Schubert K, Chubak J, et al. Effect of exercise on serum sex hormones in men: a 12-month randomized clinical trial. *Med Sci Sports Exerc* 2008; 40: 223-33.
37. Maïmoun L, Lumbroso S, Manetta J, et al. Testosterone is significantly reduced in endurance athletes without impact on bone mineral density. *Horm Res* 2003; 59: 285-92.
38. Safarinejad MR, Kolahi AA, Azma K. The effects of intensive, long-term treadmill running on reproductive hormones, hypothalamus-pituitary-testis axis, and semen quality: a randomized controlled study. *J Endocrinol* 2009; 200: 259-71.

39. Grandys M, Majerczak J, Duda K, et al. The effect of endurance training on muscle strength in young, healthy men in relation to hormonal status. *J Phys Pharm* 2008; 59 (s7): 89-103.
40. de Ronde W, van der Schouw YT, Pols HAP, et al. Calculation of bioavailable and free testosterone in men: a comparison of 5 published algorithms. *Clin Chem* 2006; 52: 1777-84.

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