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Early hemorheologic aspects of overtraining in elite athletes

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Abstract. A standardized questionnaire has been proposed by the French consensus group on overtraining of the *Société Française de Médecine du Sport* (SFMS) and allows the calculation of a 'score' that may help to quantify the early clinical symptoms of the overtraining syndrome in sportsmen submitted to a heavy training program. We investigated a possible relationship between this score and blood rheology in 36 male elite sportsmen (national level in football, volleyball and karate; age: 17–33 yr) who underwent a standardized check-up including biological measurements and an exercise-test. The overtraining score ranged between 0 and 21 items and was correlated with blood viscosity ($r = 0.413$, $p < 0.02$). This correlation was explained by a correlation of this score with plasma viscosity ($r = 0.512$, $p < 0.01$) and hematocrit ($r = 0.387$, $p < 0.05$). When subjects with a high score (>6) were compared to subjects with a lower score they appeared to have a higher blood viscosity at native (but not corrected) hematocrit (3.18 ± 0.01 vs. 2.89 ± 0.05 mPa.s, $p < 0.02$), explained by higher values in both plasma viscosity (1.39 ± 0.02 vs. 1.31 ± 0.02 mPa.s, $p < 0.01$) and hematocrit (42.8 ± 0.45 vs. 41.1 ± 0.44, $p < 0.05$). By contrast, there was no difference in RBC deformability and aggregation. Overtrained subjects have also lower levels of zinc (0.72 ± 0.024 vs. 0.84 ± 0.023 mg/l, $p < 0.01$), ferritin (55.1 ± 7.3 vs. 92.3 ± 9.4 ng/ml), and IGF-binding protein 3 (3.4 ± 0.22 vs. 4.52 ± 0.4 ng/ml). Neither zinc nor ferritin status were likely to explain the rheologic alterations since disturbances in zinc or iron are rather associated with abnormalities in erythrocyte deformability or aggregability. Therefore, the early signs of overtraining in elite sportsmen are associated with a hemorheologic pattern that suggests some degree of reversal of the 'autohemodilution' associated with fitness in athletes.

Keywords: Blood viscosity, plasma viscosity, exercise, overtraining, hemorheology, IGFBP3, serum zinc, ferritin

1. Introduction

The overtraining syndrome in athletes is characterized by an impairment in performance despite intensive training. Presumably, this syndrome results from training-induced neuroendocrine and metabolic disturbances [1]. However, the clinical picture is highly variable and the diagnostic criteria for this syndrome remain controversial [1]. Recently, the French consensus group on overtraining of the *Société Française de Médecine du Sport* (SFMS) proposed a standardized questionnaire of early clinical symptoms of this elusive syndrome, allowing the calculation of a 'score' that may help to classify on a clinical basis sportsmen submitted to a heavy training program [2,3]. This score appears to be correlated with markers of muscular damage (creatine kinase, myosin) or neuroendocrine dysfunction (somatotropic axis), but also with some hematological markers like ferritin [4,5]. Since an improvement of fitness is associated with a decrease in blood viscosity [6], we investigated a possible relationship between this score and blood rheology.

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Table 1
Clinical characteristics of the 36 subjects of the study and comparison between the two subgroups of 18 subjects divided on the basis of their overtraining score

	Overtraining score		
	Overall	<6	>6
Number of subjects	36	18	18
Age (yr)	22.3 ± 0.9	23.9 ± 1.2	20.7 ± 1.2
Weight (kg)	79.4 ± 1.4	77.9 ± 1.8	80.9 ± 2.2
Height (cm)	183.3 ± 1.3	180.9 ± 1.2	185.7 ± 2.2
Body mass index (kg/m^2)	23.6 ± 0.3	23.7 ± 0.3	23.2 ± 0.5

2. Subjects and methods

Subjects used in this study were 36 male elite sportsmen (national level in football, volleyball and karate) submitted daily to a physical training program. Their characteristics are shown on Table 1. They underwent a standardized submaximal exercise session on cycloergometer over 25 min. Pedal speed was kept constant at 60 rpm by the subjects. Physical working capacity W_{170} was calculated as the work in watts that subjects were able to perform at a heart rate of 170 b.min^{-1} [7]. Body composition was assessed with a multifrequency bioelectrical impedancemeter Dietosystem Human IM Scan that uses low intensity (100–800 μA) at the following frequencies: 1, 5, 10, 50, and 100 kHz [8]. Analysis was performed with the software Master 1.0 that gives the choice among 25 published equations for body composition calculations.

2.1. Hemorheological measurements

Blood samples for hemorheological measurements (7 ml) were drawn with potassium EDTA as the anticoagulant in a vacuum tube (Vacutainer). Viscometric measurements were done at high shear rate (1000 s^{-1}) with a falling ball viscometer (MT 90 Medicatest, F-86280 Saint Benoit) [9,10]. Accuracy of the measurements was regularly controlled with the Carrimed Rheometer 'CS' (purchased from Rhéo, 91120 Palaiseau, France) [11]. The coefficient of variation of this method ranged between 0.6 and 0.8% [12]. With this device we measured apparent viscosity of whole blood at native hematocrit, plasma viscosity, and blood viscosity at corrected hematocrit (0.45) according to the equation of Quemada [13]. Dintenfass' 'Tk' index of erythrocyte rigidity was calculated [14]. RBC aggregation was assessed with the Myrenne aggregometer [15] which gives two indices of RBC aggregation: 'M' (aggregation during stasis after shearing at 600 s^{-1}) and 'M1' (facilitated aggregation at low shear rate after shearing at 600 s^{-1}). The hematocrit/viscosity (h/η) ratio, an index of oxygen supply to tissues, was calculated according to Chien [16] and Stoltz [17], with hematocrit (as percentage) divided by viscosity at high shear rate determined as described above.

The AFFIBIO (previously SEFAM) aggregometer was used for a more precise assessment of RBC aggregation. This device is based upon the experiments of Mills [18,19] on cell disaggregation behavior in shear flow. This device measures the changes in backscattered light which are observed when sheared RBC suspensions are abruptly brought to a full stop. The decrease in the optical signal reflects the formation of RBC aggregates [20,21]. Some parameters are derived from the curve of light intensity as a function of time. The aggregation time is the reciprocal of the initial slope (calculated between 0.5 and 2 sec after the shear has stopped). The aggregation index at 10 sec is a measurement of the

extent of erythrocyte aggregation and is the relative surface area above the curve calculated over the first 10 sec. This device measures also disaggregation thresholds, by submitting blood to a succession of shear rates from 600 s^{-1} to 7 s^{-1}. The total disaggregation threshold is the shear rate below which the backscattered light intensity starts to decrease, indicating that the shear stress applied to aggregates is no longer sufficient for allowing complete dispersion of RBC aggregates. The partial disaggregation shear rate is defined as the shear rate corresponding to the intersection point of the two asymptotes drawn from the extremes (maximum and minimum shear rate).

Lactate was assayed with a kit from DuPont specially adapted to the DuPont de Nemours automatic clinical analizer. This assay was based on NADH production by rabbit lactate dehydrogenase. Coefficients of variation range beween 0.7 and 5.6%.

Serum Somatomedin C/IGF-I was assayed with the INCSTAR IGF-I RIA (from INCSTAR Corporation, Stillwater, MN 55082–0285 USA, purchased from Sorin Biomedica France SA). This is a double antibody desequilibrium assay which includes an ODS-silica extraction procedure from serum samples. After the extraction procedure the RIA is performed employing addition of sample and rabbit anti-IGF-I, followed by a 2 hr incubation at 2–8°C. Iodine-125 IGF-I is then added followed by a second incubation for 20 hr at 2–8°C. Pre-precipitated carrier, second antibody and polyethylene glycol are added in a single step. The assay is centrifuged after the 2 hr second antibody incubation at 2–8°C. Detection limit is 2 nmol/l. This assay does not cross-react (<1%) with IGF-II, hGH, FGF, TGF, PDGF. Within assay CVs range between 9.1–10.1%, between-assay CVs range between 10.3–15.2%.

Serum IGF binding protein-1 was assayed with the DSL ACTIVE IGFBP-1 coated tube immunoradiometric assay kit (from Diagnostic system laboratories Inc., P.O. Box 57946, Webster, TX 77598, USA, purchased from Chiron Diagnostics BP109, 95613 Cergy Pontoise, France, SA). This is a two site immunoradiometric assay (IRMA) in which the analyte to be measured is "sandwiched" between two antibodies. The first antibody is immobilized to the inside wall of the tubes. The other antibody is radiolabelled for detection. The analyte present in the patient samples, standards and controls is bound by both of the antibodies to form a 'sandwich' complex. Unbound materials are removed by decanting and washing tubes. Detection limit is 0.01 ng/ml. Within assay CVs range between 3.4–6%, between-assay CVs range between 1–3.5%. No cross reactivity with IGFBP-2, 3 and 4 has been dectected.

Serum IGF-binding protein-3 was assayed with the DSL IGFBP-3 radioimmunoassay kit (from Diagnostic system laboratories Inc., P.O. Box 57946, Webster, TX 77598, USA, purchased from Chiron Diagnostics BP109, 95613 Cergy Pontoise, France, SA). This is a classical radioimmunoassay where there is competition between a radioactive and a non-radioactive antigen for a fixed number of antibody binding sites. The separation of free and bound antigen is achieved by using a double antibody system. Detection limit is 0.01 ng/ml. Within assay CVs range between 3.4–6%, between-assay CVs range between 1–3.5%. No cross reactivity with IGFBP-2, 3 and 4 has been dectected.

Serum zinc was measured by flame atomic spectrophotometry. The lowest limit of sensitivity of this method is 0.0125 mg/l. Its coefficient of variation is 7.2% ($n = 9$).

Study protocol

The check-up included clinical examination and body composition evaluation by bioelectrical impedancemetry. The psychological scale for overtraining proposed by the consensus group on overtraining of the French Society of Sports Medicine was used [2,3]. This scale consists of a list of 53 items selected among the reported clinical manifestations of the syndrome and that have to be quoted 'yes' or 'no' by the subject. The total of the positive items (quoted 'yes') is used as a 'score' in this study [5]. Isometric strength was measured with home-made devices which are designed to assess handgrip strength

Table 2
Comparison of some biological parameters between the two groups

	Overtraining score	
	<6	>6
Creatine kinase (UI/l)	415.93 ± 106.4	444.27 ± 74.74
Ammonia (μg/l)	83.76 ± 5.3	77.93 ± 4.93
Fibrinogen (g/l)	2.4 ± 0.09	2.47 ± 0.1
Serum zinc (mg/l)	0.84 ± 0.023	0.72 ± 0.024***
IGF1 (nmol/l)	32.83 ± 2.47	40.28 ± 2.99
IGFBP1	12.41 ± 4.71	21.96 ± 4.62
IGFBP3 (ng/ml)	4.52 ± 0.4	3.4 ± 0.22*
IGF1/IGFBP3 (ng/ml)	6.76 ± 0.8	11.42 ± 1.11***

*$p < 0.05$; ***$p < 0.01$.

and tight adductors isometric strength. Baseline samples for the measurement of zinc and various hormones (see below) were drawn. Subjects were given a standardized breakfast (50 g bread; 15 g butter; 200 g milk with chocolate; 10 g sucrose). This breakfast was used for assessing glucose tolerance [23] and to standardize the exercise session. At the 120th min a standardized exercise test was performed as indicated above [24].

2.2. *Statistics*

Results are presented as mean ± the SE of the mean. A value of $p < 0.05$ was considered as significant. Comparisons were made with nonparametric tests [25]. Correlations were tested by least square fitting for linear, exponential, logarithmic and power relationships.

3. Results

The overtraining score ranged between 0 and 21 items and was correlated with blood viscosity (Fig. 1: $r = 0.413$, $p < 0.02$). This correlation was explained by a correlation of this score with plasma viscosity (Fig. 2: $r = 0.512$, $p < 0.01$) and hematocrit (Fig. 3: $r = 0.387$, $p < 0.05$).

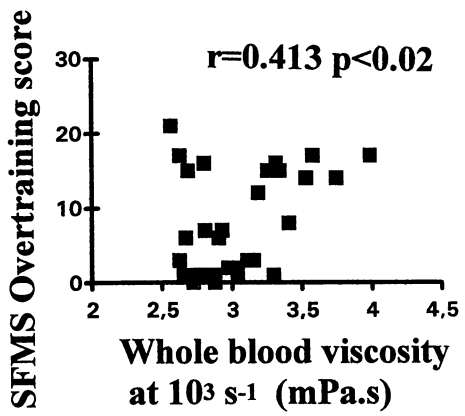

Fig. 1. Correlation between the overtraining score and blood viscosity ($r = 0.413$, $p < 0.02$).

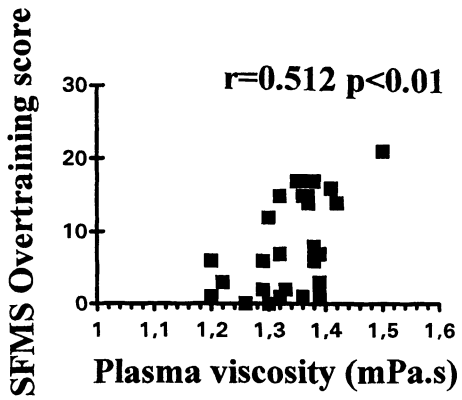

Fig. 2. Correlation between the overtraining score and plasma viscosity ($r = 0.512$, $p < 0.01$).

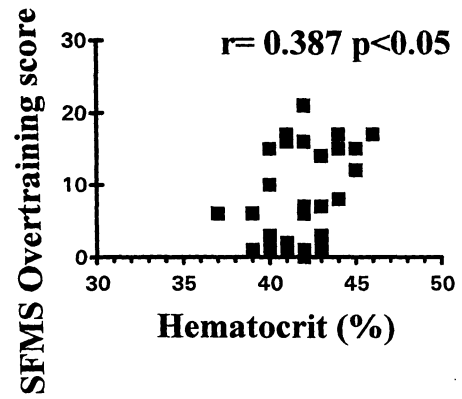

Fig. 3. Correlation between the overtraining score and and hematocrit ($r = 0.387$, $p < 0.05$).

Subjects can be divided into two subgroups on the basis of their overtraining score, assuming as previously reported [4,5] that a score higher than 6 is strongly suggestive of an excess exercise load while a score lower than 6 has no specific meaning. Comparison of the two subgroups that include in each case 18 subjects shows (Table 1) that they have the same body mass index and a quite similar age, weight and height. Biological parameters (Table 2) exhibit some differences with a lower serum zinc ($p < 0.01$) and IGFBP3 ($p < 0.05$) and a higher ratio between IGF1 and IGFBP3 ($p < 0.05$) in the group with a high overtraining score. Table 3 focuses on the comparison of hemorheologic parameters. The group with the high overtraining score exhibits higher values of whole blood viscosity (=10%, $p < 0.02$). This difference was explained by an increase in the following determinants of viscosity: plasma viscosity (+6%, $p < 0.02$) and hematocrit (+4%, $p < 0.01$). Therefore, the hematocrit/viscosity ratio is lower ($p < 0.01$). By contrast, there was no difference in RBC deformability and aggregation (neither with Myrenne nor SEFAM devices). In addition the group with a high overtraining score has also a lower ferritin ($p < 0.01$).

The score of overtraining was also negatively correlated with zinc ($r = -0.475$, $p < 0.05$) and ferritin ($r = -0.370$, $p < 0.05$), both parameters being correlated to each other ($r = 0.550$, $p < 0.01$).

Comparisons of body composition and ergometric responses to the exercise-test show no significant difference (Table 4). However, there is a non-significant tendency to a lower percentage of water in the fat free mass in subjects with a high score.

Table 3
Comparison of hemorheologic parameters between the two groups

	Overtraining score	
	<6	>6
Number of subjects	$n = 18$	$n = 18$
Ferritin (ng/ml)	92.3 ± 9.4	55.1 ± 7.3***
Hematocrit (%)	41.1 ± 0.4	42.8 ± 0.4***
h/η mPa^{-1}.s	14.2 ± 0.2	13.7 ± 0.4***
Blood viscosity ηb (mPa.s)	2.89 ± 0.05	3.18 ± 0.1**
ηb at corrected hct 45%	3.15 ± 0.04	3.31 ± 0.1
Plasma viscosity ηp (mPa.s)	1.31 ± 0.02	1.39 ± 0.02**
Erythrocyte rigidity 'Tk'	0.65 ± 0.01	0.64 ± 0.02
Erythrocyte aggregation 'M'	3.8 ± 0.5	4.8 ± 0.9
Erythrocyte aggregation 'M1'	8.6 ± 0.9	9.2 ± 1.05
Aggregation kinetics 'TA'	2.99 ± 0.3	2.71 ± 0.2
Aggregation kinetics 'S10'	21.4 ± 1.2	22.1 ± 1.2
Aggregation kinetics 'S60'	40.02 ± 1.06	40.72 ± 1.3
Disaggregation γS (s^{-1})	92.5 ± 8.7	78.4 ± 7.7
Disaggregation γD (s^{-1})	45.1 ± 1.2	44.7 ± 2.2

**$p < 0.02$; ***$p < 0.01$.

Table 4
Comparison of body composition and ergometric parameters between the two groups. No significant difference

	Overtraining score	
	<6	>6
Number of subjects	$n = 18$	$n = 18$
W_{170} (W/kg)	2.68 ± 0.15	2.70 ± 0.13
Handgrip strength (N)	598 ± 30.3	602.1 ± 50.2
% of fat	13.7 ± 0.6	13.9 ± 0.5
% water in fat free mass	70.6 ± 1.1	68.9 ± 1.3

4. Discussion

The standardized questionnaire of early signs of overtraining [2,3] has been developped by a French consensus group in order to detect early disturbances in the tolerance of intensive training. Although further work is needed to improve it, it allows the calculation of a 'score' of symptoms suggestive of overtraining that is correlated to some biological markers [3–5]. Thus, it was interesting to investigate the relationships of this 'score' under its current form with blood rheology, which is influenced by exercise and training [6]. We have divided our subjects into two subgroups on the basis of their overtraining score, assuming as previously reported [4,5] that a score higher than 6 is strongly suggestive of an excess exercise load while a score lower than 6 has no specific meaning. This cut-off value has been defined after a multivariate, multicentric study of 322 questionnaires in highly trained sportsmen [5]. This study showed that trained subjects may quote as much as 6 items without any evidence of being overtrained, while higher scores clearly reflect a training overload. The results of the current study show that high overtraining scores in elite sportsmen are associated with some significant alterations: higher hematocrit and plasma viscosity, lower insulin-like growth factor binding protein 3, lower serum zinc, lower ferritin.

The hemorheologic pattern (with significantly higher hematocrit and plasma viscosity compared to controls with a low score) requires some comments. It is well established that some degree of 'overhydration' or 'autohemodilution' is associated with fitness in athletes [6,26,27]. This process is likely to exert two beneficial hemodynamic effects: it both increases blood volume and reduces blood viscosity. Our current results show a reversal of this hemodilution in subjects with a high overtraining score. Interestingly, this pattern is not detected by bioelectrical impedance analysis of body water content, probably because it is moderate. Nevertheless, experimental studies suggest that even a moderate rise in plasma viscosity may induce a linear increase of erythrocyte resistance to flow [28].

Subjects with a high score have also an impairment in zinc and iron status, as suggested by the low serum zinc and the low plasma ferritin levels. Both abnormalities have been reported to be associated with rheologic disturbances [6,29–31]. However, the hemorheologic pattern of these alterations in mineral status is rather characterized by modifications of erythrocyte rigidity and aggregability [6,29–31] that are not found here. Thus, the hemorheologic syndrome associated with high scores on the overtraining questionnaire is not the same than that found in those trace element disturbances, although all these disorders could be frequently associated.

We also evidenced in this study a lower IGF BP3 in subjects with a high score, suggesting alterations in the GH-somatomedin function in overtraining. In the light of our preceding reports of a relationship between IGFBP3 and strength [32,33] as well as a lower IGFBP3 associated with impaired performance in zinc-deficient gymnasts [34], we think that this biological aspect of the early signs of the syndrome requires further investigations. The function of the GH-somatomedins axis (as reflected by IGFBP3 levels) is generally improved in trained individuals [35] so that a lowered IGFBP3 in overtraining might reflect a reversal of the neuroendocrine adaptation to training. However, this problem is beyond the scope of the current paper.

On the whole, this study suggests that individuals in whom the standardized questionnaire evidences early signs of overtraining are characterized by a specific hemorheologic pattern which is the opposite of the well known hyperhydration of fit athletes.

This paper was presented at the Xth European conference on Clinical Haemorheology, Lisbon, 29 June–3 July [36] and at the XVIIth congress of the French society of sports medicine (SFMS), Caen (France), 19–21 June 1997 [37].

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