

ORIGINAL ARTICLE

Effects of exercise on soluble transferrin receptor and other variables of the iron status

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Background: Soluble transferrin receptor (sTfr) is a new marker of iron status and erythropoietic activity. It has been included in multivariable blood testing models for the detection of performance enhancing erythropoietin misuse in sport.

Objective: To evaluate the effect of different types and volumes of physical activity on sTfr concentration, variables of iron status (ferritin, transferrin, iron, and protein), and haematological indices.

Methods: Thirty nine subjects were divided into three groups: 1, untrained ($n = 12$); 2, moderately trained ($n = 14$); 3, highly trained ($n = 13$, seven men, six women). Groups 1 and 2 carried out two exercise tests: an incremental running test until exhaustion (test A) and a 45 minute constant speed running test at 70% VO_{2MAX} (test B). Group 3 performed three days (women) or four days (men) of prolonged aerobic cycling exercise. The above variables together with haemoglobin and packed cell volume were analysed in venous blood samples before and after exercise. Changes in blood and plasma volume were estimated.

Results: sTfr levels were slightly increased in trained and untrained subjects immediately after test A. Test B and aerobic exercise had no significant effect on sTfr. Ferritin levels were increased after the laboratory tests for trained and untrained subjects and after prolonged aerobic exercise in male cyclists. Transferrin was increased significantly in trained and untrained subjects after both laboratory tests, but remained unchanged after prolonged exercise. Plasma and blood volumes were decreased after the laboratory tests but increased after aerobic exercise. No differences in the variables were observed between trained and untrained subjects with respect to response to exercise.

Conclusion: The changes in sTfr and the variables of iron status can be mainly attributed to exercise induced changes in volume. Taking these limitations into account, sTfr can be recommended as a marker of iron deficiency in athletes.

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Iron depletion is a well known trace mineral disorder of training athletes. The prevalence of inadequate iron balance in male athletes has been reported to be as high as 10% and can reach up to 20% in female athletes.¹⁻⁴ Iron plays a key role in a number of cellular processes such as DNA synthesis and electron transport. Furthermore, it is an essential component of haemoglobin, the oxygen carrying protein in the blood. Therefore iron depletion can affect physical performance.⁵⁻⁶

Several indicators are used to evaluate iron status. Serum levels of iron and transferrin, the iron transport protein, do not reflect body iron stores and show considerable within day and day to day variability.⁷ In addition, these variables are influenced by a number of factors such as food intake and acute and chronic diseases.³⁻⁹ Ferritin is the most commonly used indicator of body iron stores: low ferritin levels indicate exhausted iron reserves. However, normal or high ferritin levels do not guarantee adequate iron stores. Ferritin is an acute phase protein and may therefore vary in certain conditions without changes in iron storage; infection, inflammation, disorders of the liver, malignancies, and other conditions can cause increases in serum ferritin levels and mask potential iron depletion.^{3-8, 10-11} Exercise is known to influence variables of iron metabolism to a great extent. It has been shown that physical activity is accompanied by inflammation-like reactions.¹¹ These reactions induce the acute phase response, which can cause persistently increased ferritin levels (~20%) for several days, making assessment of the iron stores difficult if not impossible.

Since its first description in 1963 and the introduction of clinical tests,¹²⁻¹³ extensive research has shown that the soluble transferrin receptor (sTfr) is of great value in diagnosing iron deficiency.¹⁰⁻¹⁴⁻¹⁶ The cell membrane bound transferrin receptor

mediates endocytotic transfer of iron from the transferrin protein into erythroid cells. The surface density of this receptor is regulated by the iron stores of the cell and the intracellular iron turnover. The transferrin receptor is a direct indicator of functional iron and starts to increase when iron stores are depleted or iron turnover is stimulated. It thereby reflects the iron stores of the body and, together with other haematological variables such as reticulocytes, the rate of erythropoiesis. A small fraction of these proteins appears in soluble form in the blood and can be measured. Its metabolism is closely related to transferrin, and, although only few data are available, it has been hypothesised that sTfr elimination from the circulation is closely related to transferrin degradation.¹⁷ In contrast with ferritin levels, sTfr levels are not affected by inflammatory reactions or other diseases and can therefore be used for diagnosing iron deficiency even under such conditions.¹⁴⁻¹⁸ Furthermore, as sTfr is a sensitive marker of stimulated erythropoiesis, it has been included in several models for the indirect detection of the performance enhancing misuse of recombinant human erythropoietin (rhEPO) by athletes.¹⁹⁻²²

Few data exist on the effect of physical exercise on sTfr levels. This may be important in the assessment of iron status in training athletes and evaluation of stimulated erythropoiesis. Therefore, the aim of this study was to evaluate the effect of different types of endurance exercise on sTfr and other variables of iron status in endurance trained athletes and untrained volunteers.

Abbreviations: sTfr, soluble transferrin receptor; rhEPO, recombinant human erythropoietin; VO_{2MAX} , maximal oxygen uptake

Table 1 Characteristics of the groups studied

	Age (years)	Height (cm)	Weight (kg)	VO ₂ MAX
Group 1 (n=12)	31.6 (7.6)	179.9 (6.7)	75.6 (8.2)	48.5 (5.1)
Group 2 (n=14)	32.5 (7.5)	180.5 (7.6)	72.5 (6.0)	60.7 (3.2)
Group 3				
Men (n=7)	19.6 (0.5)	176.4 (5.8)	69.0 (7.4)	64.2 (1.6)
Women (n=6)	21.3 (3.0)	162.0 (7.5)	55.1 (5.9)	61.3 (6.0)

Values are mean (SD). VO₂MAX is expressed as ml/min/kg body weight.
Groups: 1, untrained; 2, moderately trained; 3, highly trained.

Table 2 Training schedule (km/day) for cyclists in group 3

	Day 1	Day 2	Day 3	Day 4
Men (n=7)	100	120	150	180
Women (n=6)	135	180	200	-

SUBJECTS AND METHODS

Subjects

Thirty nine subjects (33 men, six women) gave their written informed consent. Twelve did not practise any sport on a regular basis and formed group 1 (untrained). Fifteen were leisure time triathletes with weekly training averaging nine hours (group 2, moderately trained). Thirteen (seven men, six women) were members of the German national cycling team with an average training load of 21 000 km/year; they formed group 3 (highly trained). Before entering the study, every subject had a medical examination; there were no pathological findings. No iron supplementation was used by any of the participants for three months before and during the study. Table 1 shows the characteristics of the different groups.

Testing

Groups 1 and 2 performed two different laboratory tests: A, incremental treadmill running test (Woodway treadmill, XELG 2; Woodway, Weil am Rhein, Germany), inclination 2°, start at 8 km/h, + 2 km/h increment every three minutes until volitional exhaustion; B, 45 minutes running test at constant speed, at 70% of the individual maximal oxygen uptake (VO₂MAX) reached during test A.

The tests were performed after the subjects had been familiarised with treadmill running. No food was allowed for three hours before the test. The interval between the tests was three to five days. The subjects were instructed to refrain from strenuous physical exercise for two days before each test.

Subjects in group 3 performed three days (female athletes) or four days (male athletes) of aerobic exercise of increasing duration on a bicycle (50–70% VO₂MAX). For group 3, VO₂MAX was determined two weeks before the study during an incremental cycling test until volitional exhaustion (initial workload 80 W, increased by 20 W every three minutes). Training intensity was controlled through heart rate monitors ("Pacer" model; Polar Electro, Oy, Finland). Table 2 shows the training schedule. Training was performed on road cycles on tarred mainly flat roads without major climbs during an early season training phase, when aerobic capacity of the riders was not fully developed and peak form not achieved. Fluid and food intake during training sessions was not restricted for subjects in group 3.

During test A and the incremental cycling test, VO₂ was measured using a Jaeger open circuit system (Jaeger GmbH, Höchberg, Germany). VO₂MAX was determined as the highest

VO₂ measurement reached for 30 seconds. From these data, the treadmill speed for test B (speed at 70% VO₂MAX) was calculated, and heart rate limits corresponding to 50% and 70% VO₂MAX for group 3 were determined by direct interpolation from the simultaneously registered heart rate curve.

Analysis

Immediately before and 20–30 minutes after the different types of exercise, blood was withdrawn, with the subject in a supine position, from an antecubital vein into a vacutainer system (Sarstedt S-Monovette, 10 ml; Sarstedt, Nürnberg, Germany). The following variables were then analysed in the serum samples: sTfr (kit: Dade Behring N-Latex sTfr analyser, Dade Behring BNA 1000; Dade Behring, Marburg, Germany), ferritin, transferrin, iron, and protein (kits: Roche/Hitachi Modular analyser; Roche Diagnostics, Mannheim, Germany). According to the manufacturer of the sTfr analysing system, the intra-assay coefficient of variation is 1.4–2.1%, and the interassay coefficient of variation 0.8–1.2%. The sensitivity of the test depends on the standard used to calibrate the system. The test is specific for sTfr.

Haemoglobin and packed cell volume were measured using an automated cell counter (Serono-Baker Diagnostics, Allentown, Pennsylvania, USA; model 9000 Diff) within three hours of sampling.

To investigate further the effect of haemodilution or haemoconcentration on the measured variables, plasma, blood and red cell volume were estimated using the formulae of Dill and Costill²³; initial BV (BV_a) was set at 100:

$$BV_b = BV_a (Hb_a/Hb_b)$$

$$PV = BV - RCV$$

$$RCV = BV (PCV)$$

where BV is blood volume, PV is plasma volume, RCV is red cell volume, suffix a is the variable before exercise, suffix b is the variable after exercise, Hb is haemoglobin, and PCV is packed cell volume.

$$\text{Percentage change in BV} = 100 \times ((BV_b - BV_a)/BV_a)$$

$$\text{Percentage change in RCV} = 100 \times ((RCV_b - RCV_a)/RCV_a)$$

$$\text{Percentage change in PV} = 100 \times ((PV_b - PV_a)/PV_a)$$

Statistical analysis was performed using SPSS 10.0 software. Descriptive data are presented as means (SD). To determine normal distribution of the analysed data, Kolmogorov-Smirnov testing was performed. Non-parametric testing (Wilcoxon) was used to determine significance of changes within the different groups, and the Mann-Whitney test for independent sample calculated group differences for groups 1 and 2. Compared with analysis of variance, this test reduces the degrees of freedom, but may show better significance. p<0.05 was considered to be significant.

RESULTS

Table 3 gives the results for groups 1 and 2, and table 4 shows the results for group 3. For all but one subject, the variables were within the normal range suggested by the manufacturers of the assay kits. One subject (group 2) showed low ferritin values with high sTfr.

Haemoglobin and packed cell volume

Haemoglobin and packed cell volume showed significant increases immediately after physical exercise in all groups,

Table 3 Iron status, haematological variables, and changes in vascular volumes in untrained (group 1) and trained (group 2) subjects before and after laboratory exercise tests

	Test A			Test B			Normal range
	Before	After	Sign.	Before	After	Sign.	
Group 1 (untrained; n=12)							
sTfr (mg/l)	2.3 (0.4)	2.5 (0.5)	*	2.3 (0.4)	2.3 (0.4)		0.83–2.76
Ferritin (ng/ml)	107 (64)	116 (68)	*†	94.5 (48)	95 (50)	†	12–200
Transferrin (mg/dl)	239 (22)	262 (21)	*†	238 (20)	245 (24)	*†	200–400
Iron (µg/dl)	121 (25)	126 (29)		92 (22)	99 (25)	*	50–180
Protein (g/dl)	8.0 (0.4)	8.7 (0.4)	*	7.9 (0.3)	8.2 (0.3)	*	6.5–8
Haemoglobin (g/dl)	15.1 (0.8)	16.2 (0.9)	*	14.9 (1.2)	15.4 (0.9)	*	12–18
PCV (%)	46.2 (2.9)	49.4 (2.3)	*	45.7 (3)	46.9 (2.2)	*	37–54
BV (% change)		-6.8 (2.8)	*		-3.2 (1.6)	*	
PV (% change)		-12.3 (5.3)	*		-5.4 (2.9)	*	
RCV (% change)		-0.3 (0.1)			-0.7 (0.1)		
Group 2 (trained; n=14)							
sTfr (mg/l)	2.5 (0.8)	2.7 (0.9)	*	2.5 (0.72)	2.5 (0.7)		0.83–2.76
Ferritin (ng/ml)	36 (27)	38 (27)	*†	33 (25)	35 (25)	*†	12–200
Transferrin (mg/dl)	285 (41)	316 (45)	*†	286 (36)	297 (29)	*†	200–400
Iron (µg/dl)	112 (42)	124 (47)	*	91 (33)	92 (31)		50–180
Protein (g/dl)	7.8 (0.4)	8.6 (0.3)	*	7.8 (0.5)	8.3 (0.4)	*	6.5–8
Haemoglobin (g/dl)	14.7 (0.5)	15.6 (0.8)	*	14.7 (0.6)	15.1 (0.8)	*	12–18
PCV (%)	45.3 (2.1)	47.7 (3)	*	44.9 (2.6)	46.2 (3)	*	37–54
BV (% change)		-5.8 (2.0)	*		-2.6 (1.1)	*	
PV (% change)		-9.9 (3.2)	*		-4.9 (1.9)	*	
RCV (% change)		-0.8 (0.2)			0.1 (0.0)		

Values are mean (SD).

*Significant difference (Sign.) from pre-test value; †significantly different from group 1 (exercise related changes between trained and untrained). Normal ranges as suggested by the manufacturers of the assay kits.

sTfr, soluble transferrin receptor; PCV, packed cell volume; BV, blood volume; PV, plasma volume; RCV, red cell volume.

regardless of the type of exercise. In group 3, the values obtained before exercise decreased over the testing period.

Vascular volumes

Plasma volume and blood volume were decreased after test A and B in groups 1 and 2. The changes were more pronounced

after the all out incremental test (test A). After prolonged aerobic cycling, blood volume and plasma volume were increased. RCV was mainly unaffected by exercise.

sTfr

sTfr was increased significantly ($p < 0.05$) after test A in groups 1 and 2, but had not changed after test B and after

Table 4 Iron status, haematological variables, and changes in vascular volumes in trained subjects (group 3) during three days (women) and four days (men) of aerobic cycling exercise

	Day 1		Day 2		Day 3		Day 4	
	Before	After	Before	After	Before	After	Before	After
Men (n=7)								
sTfr (mg/l)	1.39 (0.1)	1.39 (0.2)	1.31 (0.2)	1.37 (0.2)†	1.32 (0.2)	1.36 (0.2)	1.3 (0.1)	1.4 (0.2)
Ferritin (ng/ml)	83 (40)	87 (39)	67 (30)	75 (34)	77 (36)	87 (38)†	87 (38)	94 (44)
Transferrin (mg/dl)	219 (32)	232 (43)	206 (38)	219 (46)	207 (44)	233 (35)†	211 (37)	212 (33)
Iron (µg/dl)	104 (31)	110 (22)	132 (51)	108 (49)†	136 (48)	101 (49)†	131 (43)*	150 (57)
Protein (g/dl)	7.9 (0.3)	8.2 (0.4)†	7.3 (0.7)	7.5 (0.8)	7.3 (1)	8.2 (0.6)†	7.6 (0.6)	8 (0.6)†
Haemoglobin (g/dl)	14.2 (0.5)	14.8 (0.6)†	14.1 (0.9)	14.4 (0.8)	13.5 (0.7)*	14.9 (0.5)†	13.4 (0.8)*	14 (0.7)†
PCV (%)	43.7 (2.2)	45.2 (2.6)†	43.1 (3.2)	44.3 (2.8)	41.8 (2.1)*	45.4 (1.9)†	41.4 (2.0)*	43.2 (1.8)†
BV (% change)		-4.0 (1.2)†	0.7 (0.3)	-2.1 (0.8)	5.2 (1.9)*	-9.4 (3.2)†	6 (1.3)*	-4.2 (1.0)†
PV (% change)		-6.6 (2.1)†	1.8 (0.5)	-4.2 (1.2)	8.7 (3.1)*	-15.0 (5.3)†	10.3 (3.1)*	-7.2 (1.9)†
RCV (% change)		-0.8 (0.1)	-0.6 (0.1)	0.6 (0.1)	0.6 (0.1)	-1.6 (0.4)	0.4 (0.2)	-0.1 (0.0)
Women (n=6)								
sTfr (mg/l)	1.52 (0.3)	1.47 (0.2)	1.5 (0.3)	1.6 (0.4)	1.6 (0.3)	1.6 (0.3)		
Ferritin (ng/ml)	50 (27)	50 (7)	50 (29)	52 (29)	45 (26)	47 (28)		
Transferrin (mg/dl)	271 (36)	282 (40)	262 (36)	288 (33)	276 (20)	296 (24)		
Iron (µg/dl)	129 (25)	84 (31)	115 (43)	128 (16)	106 (26)	120 (27)		
Protein (g/dl)	7.9 (0.3)	8.1 (0.7)	7.9 (0.6)	8.5 (0.4)†	8 (0.4)	8.4 (0.6)†		
Haemoglobin (g/dl)	13.7 (0.6)	14.1 (0.8)	13.4 (0.9)	14.5 (0.7)†	12.8 (0.6)*	13.4 (0.8)†		
PCV (%)	42.1 (2.7)	43.2 (2.4)	40.9 (2.9)	44.1 (2.1)†	38.1 (2.6)*	40.8 (2.9)†		
BV (% change)		-2.8 (1.2)	2.2 (0.6)	-7.6 (2.1)†	7 (2.9)*	-4.8 (1.6)†		
PV (% change)		-4.7 (1.8)	4.4 (1.5)	-12.6 (3.2)†	14.4 (5.2)*	-8.6 (2.9)†		
RCV (% change)		-0.3 (0.1)	-0.6 (0.1)	-0.4 (0.1)	-3 (1.3)	2.2 (0.1)		

Values are mean (SD). Differences for resting values of BV, PV and RCV from the initial values (day 1) are calculated for every day (column "before"); exercise related changes are calculated for every day from the respective pre-exercise data (column "after").

*Significant difference from day 1 (same sampling time); †significant difference from same day pre-exercise value.

sTfr, soluble transferrin receptor; PCV, packed cell volume; BV, blood volume; PV, plasma volume; RCV, red cell volume.

prolonged aerobic exercise in group 3. No difference was found between the different groups.

Ferritin and transferrin

Ferritin had increased significantly after test A in untrained subjects and after both tests in trained subjects. Transferrin had increased significantly in trained and untrained subjects after both tests, but remained unchanged after several days of cycling exercise (group 3). Untrained subjects (group 1) had significantly higher ferritin and lower transferrin levels than trained athletes (group 2). Female athletes had lower ferritin but higher transferrin levels than male athletes (group 3).

Iron

Iron had increased after laboratory testing in groups 1 and 2, but the changes were only significant in group 1 (test B) and group 2 (test A). It changed significantly after prolonged exercise in male cyclists. No group difference was found.

Serum protein

Serum protein had increased immediately after exercise in all groups. No group difference for the increase was found. Prolonged aerobic exercise (group 3) produced no significant change in serum protein concentration.

The reported changes in the measured variables were not corrected for changes in plasma volume.

DISCUSSION

The aim of this study was to determine the effect of different types of exercise on variables of iron metabolism, particularly sTfR. Two different types of exercise (running and cycling) and three different exercise volumes were studied.

Haemoglobin and packed cell volume

These variables responded to physical stress mostly as described in the literature.²⁴⁻²⁶ Immediately after exercise, both variables increase as the result of exercise induced haemoconcentration. As a reaction to several days of aerobic exercise, plasma volume increases and haemoglobin and packed cell volume decrease on a long term basis.

Vascular volumes

The estimated changes in plasma and blood volume are responses to exercise: immediately after exercise, plasma and blood volumes are decreased as the result of fluid loss through sweat and respiration, filtration to the extravascular space following increased arterial pressure and muscle contraction during exercise, and increased tissue oncotic pressure gradient mediated by accumulated metabolites such as lactate.²⁷ Increased oncotic pressure caused by metabolites and considerable pressure influences from muscles and arterial blood may be the reasons for the more pronounced decreases in plasma and blood volume after test A, in which intensity was greater than in test B. After several days of aerobic exercise, resting plasma volume increases to counter the above mechanisms, thereby improving adaptation of the body to physical exercise.

Ferritin

At present, iron status is mainly assessed by measuring serum ferritin levels, but it has been shown that this variable is affected by infection, inflammation, and other diseases as well as food intake.^{3, 8, 10, 11} In addition, ferritin increases appreciably after physical exercise.²⁸⁻³⁰ The mechanism of this reaction is as follows: exercise induces (a) an inflammatory-like reaction in the reticuloendothelial system with increased ferritin synthesis and (b) cell membrane damage in ferritin storage tissues, such as the liver, with subsequent release of ferritin into the serum.³⁰ Additional ferritin may result from mild, exercise induced haemolysis.³¹ Our data support previous reports of a ferritin increase immediately after exercise. In our study, the

type (cycling/running) and duration (short/long test, aerobic) of the exercise seem to have no effect on the extent of ferritin increase, as it did not differ significantly between the analysed groups. This and the estimated changes in plasma volume indicate that the increase in ferritin observed immediately after exercise is mainly due to haemoconcentration (as suggested by the changes in serum protein) and only to a small extent to cell destruction and inflammatory-like reactions: different types and durations of exercise (running/cycling, aerobic/anaerobic) may cause different degrees of damage. However, an acute phase reaction with increased ferritin or transferrin would begin after a delay and may therefore not have been detected in our investigation, in which sampling took place shortly after exercise. After long term exercise (group 3), the diluting effect of the expanded plasma volume on the one hand and the exercise induced acute phase reaction with an expected increase in ferritin on the other may result in mainly unchanged levels of this variable, as observed.

Transferrin and protein

Transferrin showed the response to exercise usually reported for this variable: a mild increase.²⁹ As for ferritin, it can be attributed to haemoconcentration, which occurs during and immediately after exercise. Serum protein concentration, which was determined to show changes due to haemoconcentration or haemodilution, increased, probably by the same mechanism.

The differences in ferritin and transferrin levels between untrained (group 1) and trained (group 2) subjects are in accordance with other publications: several authors have reported decreased ferritin combined with increased transferrin in trained athletes compared with untrained persons.^{32, 33} The reason why we found that ferritin was higher and transferrin lower in highly trained cyclists than in moderately trained athletes may be that several of the moderately trained athletes were triathletes with a certain amount of running in their training schedule. It has been shown that runners have significantly lower ferritin concentrations than other athletes because of increased iron turnover and cell destruction during running.³⁴

sTfR

Since its clinical introduction, sTfR has been used as a reliable marker of tissue iron deficiency.^{10, 13-15} The truncated form of the tissue transferrin receptor responds rapidly to changes in cellular iron metabolism and iron stores. Unlike serum ferritin and transferrin, sTfR seems not to be appreciably influenced by acute phase responses, and can therefore be used to differentiate between true iron deficiency and iron deficiency of chronic disease. In addition, serum sTfR levels correlate closely with total erythroid precursor mass and reflect the total rate of erythropoiesis. Many disorders characterised by increased red cell turnover are associated with increased sTfR levels: megaloblastic anaemia, β thalassaemia, myelodysplastic syndrome.¹⁸ Only a few studies on the impact of physical exercise on sTfR have been conducted. It has been suggested that exercise has no effect on serum sTfR levels.³⁵ Our results show a slight increase in sTfR after exercise (which was significant after test A, but not for the other types). As for the other variables, it may be attributed to exercise induced haemoconcentration, which is (more so in test A than in test B) as visible in the more pronounced fall in plasma volume. After several days of aerobic exercise (group 3), no significant changes in sTfR were observed. We cannot explain why overall sTfR levels in highly trained cyclists tended to be lower than in moderately trained athletes. It is known that red blood cell mass and therefore erythropoietic activity is increased in endurance trained athletes.³⁶ As sTfR reflects the overall erythropoietic activity, this variable should therefore be higher in athletes than in untrained or moderately trained subjects. No reports on higher sTfR levels in athletes are available to date.

Take home message

During exercise, sTfR concentration is solely affected by intracorporal volume shifts. This new marker of tissue iron and erythropoiesis can therefore be used for the assessment of iron status and erythropoietic activity in athletes involved in training or competition.

Nevertheless, the observed levels were still well within the normal range.

These findings indicate that sTfR may be a reliable marker for the assessment of iron status in athletes, if blood sampling takes place at a sufficient interval from intense exercise and the effect of exercise induced haemoconcentration is taken into account. To illustrate this, one subject in our study (group 2) showed subnormal ferritin levels before exercise testing (7.8 ng/ml before test A; 8.2 ng/ml before test B). Physical activity almost doubled these values, thereby masking iron deficiency (after test A: 13.1 ng/ml; after test B: 14.7 ng/ml). In contrast, sTfR concentration was raised in this subject throughout the different types of exercise and showed no substantial variation (before test A: 3.7 mg/l; after test A: 3.9 mg/l; before test B: 3.7 mg/l; after test B: 3.8 mg/l).

sTfR has gained considerable importance in drug testing. International sporting federations perform random blood tests before major competitions for the indirect detection of rhEPO misuse. The international cycling federation (UCI) has introduced cut off values for packed cell volume: 50% for male athletes, 47% for female athletes. If a rider exceeds these values, he is banned from competition for 14 days. Because of the great variability in packed cell volume, these limits have become controversial.³⁷ To improve indirect detection, several multivariable models, including sTfR as a marker for increased erythropoiesis, have been developed. However, it was not clear how sTfR levels react to physical exercise. This could be of great importance in the evaluation of these tests. Malczewska *et al.*³⁵ showed that the variable did not change after several days of intense Judo exercise. However, physiological adaptations may be different in endurance athletes (cyclists) from those in Judo athletes. Our data mainly confirm the findings of the above study for different types of endurance exercise. The effect of physical exercise on sTfR concentration seems to be only slight and should not mask increased levels if a sufficient interval is allowed between exercise and blood sampling to counter the problem of exercise induced haemoconcentration. (UCI has certain regulations for blood testing: blood sampling must take place in the morning after sufficient rest and without prior exercise and food intake.) Furthermore, the increase in sTfR due to the use of rhEPO is usually large, outweighing by far the mild effect of haemoconcentration.

The limitation of this new marker is the absence of an internationally recognised reference standard of measurement and comparable units, which makes comparison of results from different assay kits difficult.³⁸

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In memoriam Pia Sundstedt.

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