SHORT REPORT

Day to day variability in the transferrin receptor/ferritin index in female athletes

R Stupnicki, J Malczewska, K Milde, A C Hackney

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Objective: To determine the within subject (day to day) variability for variables reflecting iron status (concentrations of ferritin and soluble transferrin receptor (sTfR), and the sTfR/logFerr index) in female athletes during a training camp, and in female control subjects.
Results: The error for ferritin concentration was nearly twice as high in athletes as in controls, that for sTfR concentration was identical in the two groups, and that for the sTfR/logFerr index was about 50% higher in athletes than in controls.
Conclusions: The within subject, day to day error for the sTfR/logFerr index computed from data recorded for untrained subjects cannot serve as a reference value for training athletes. When the sTfR/logFerr index is used to monitor iron stores in athletes, an error value of 0.20 should be used, because determination of the index after a few days of rest may not be feasible.

The soluble transferrin receptor (sTfR), especially in the form of the ratio to the log concentration of ferritin (sTfR/logFerr), has been widely used as a measure of latent iron deficiency.[1–3] Although several papers discuss various sources of variability in iron status indices, such as ferritin or sTfR alone,[1 4–8] no such data have been found for sTfR/logFerr. Thus, the aim of this study was to determine the within subject (day to day) variability for variables reflecting iron status (concentrations of ferritin and soluble transferrin receptor (sTfR), and the sTfR/logFerr index) in female athletes during a training camp, and in female control subjects. This is especially important in sport, because ferritin levels are known to be affected by physical loads applied to athletes.

MATERIAL AND METHODS

Subjects

Data from 10 elite female judoists participating in a training camp, in whom concentrations of ferritin and sTfR were determined for 10 consecutive days,[8] were used. Six healthy female subjects (age, 26–28 years; body mass, 50–59 kg), who did not engage in sport, gave their informed consent to participate in the study and served as untrained controls.

Analytical methods

Blood for determining morphological indices and for biochemical assays was sampled from earlobes in the morning (0700–0800), after overnight fasting, for 10 (athletes) or four (controls) consecutive days. Plasma sTfR and ferritin concentrations were assayed with commercial immunoenzymatic kits (Orion Diagnostica, Espoo, Finland and Boehringer-Mannheim, Mannheim, Germany respectively). All assays (except of haematological indices) were carried out in duplicate, the results being expressed as means. The within assay errors were within the limits stated by the manufacturers of the assay kits. To minimise the assay error, all samples from the same subject were assayed on the same day.

Table 1 Mean (SD) values for ferritin concentration (Ferr; µg/l), soluble transferrin receptor concentration (sTfR; mg/l), and sTfR/logFerr index in female judoists and untrained control subjects

Subject No	Ferr	sTfR	sTfR/logFerr	CV
Female judoists (10 days)				
1	71.5	1.67	0.90 (0.08)	9
2	55.2	3.09	1.78 (0.12)	7
3	76.8	2.64	1.41 (0.14)	10
4	87.0	3.59	1.89 (0.34)	18
5	105.6	2.32	1.15 (0.11)	9
6	53.9	2.01	1.18 (0.19)	16
7	64.3	2.52	1.40 (0.12)	9
8	77.3	3.67	1.96 (0.18)	9
9	27.5	2.76	1.98 (0.38)	19
10	64.5	2.18	1.22 (0.16)	13
Female controls (4 days)				
1	26.8	1.66	1.18 (0.18)	16
2	31.5	0.76	0.51 (0.09)	18
3	22.8	2.15	1.59 (0.16)	11
4	32.7	0.86	0.57 (0.03)	4
5	19.4	2.07	1.61 (0.16)	10
6	22.2	1.42	1.06 (0.04)	4

Ferritin and sTfR concentrations are geometric means.
CV, Coefficient of variability for the sTfR/logFerr index.

Data processing

The distributions of ferritin and sTFR concentrations were log normal, so the values were subjected to logarithmic transformation before data processing. Residual (within subject) standard deviations were computed from two way analysis of variance for three variables: logarithm of ferritin concentration (logFerr), logarithm of soluble transferrin receptor concentration (logsTfR), and the sTfR/logFerr ratio.

RESULTS

Table 1 gives the mean results for the three variables studied, and table 2 the results of statistical analysis for control subjects and athletes. Subjects in both groups were significantly differentiated with regard to all three variables. As seen in table 2, no significant between day differences were found in the control group for all three variables ($F<1$). Therefore, the sums of squares (S_{xx}) for "Days" were combined with those for the "Remainder", thus increasing the number of degrees of freedom for the residual error to 18. In the athletic group, significant differences were found between consecutive days for logFerr and logsTfR values ($F = 2.38$ and 2.50 respectively;

Abbreviations: sTfR, soluble transferrin receptor; logFerr, log ferritin concentration

$p<0.05$), but not for the sTfR/logFerr index ($F = 1.16$). Standard deviations obtained from the residual sums of squares were 1.6-fold and 2.1-fold higher in the athletic group than in the control group for the sTfR/logFerr index and log ferritin respectively ($p<0.001$). The average within subject (day to day) error (SD) for ferritin concentrations amounted to $1.216^{\pm1}$—that is, 21.6%—for the control group, and to as much as $1.46^{\pm1}$—that is, 46%—for the athletic group. These errors for sTfR were similar in the two groups ($1.088^{\pm1}$ and $1.086^{\pm1}$ respectively), and for the sTfR/logFerr index they were ±0.129 and ±0.203 respectively.

Take home message

The within subject, day to day error for the sTfR/logFerr index computed from data recorded in untrained subjects cannot serve as a reference value for athletes who are training hard. This conclusion was reached because of the much higher variability in the index in female elite judo athletes than controls and difficulties in assessing iron stores in these athletes under resting conditions.

DISCUSSION

Determining the within subject variability of iron metabolism indices is of practical value, because, expressed as a standard deviation, it provides a significance criterion for changes in a given variable. This enables, for example, a subject to be monitored with respect to that variable and clinically significant deviations from the normal state to be detected.

Various authors have reported the within subject variability in ferritin and sTfR concentrations. The variability was, however, reported as a within subject or day to day coefficient of variability—that is, the ratio of within subject SD to the overall mean—the values ranging from 10% to 26% for ferritin and about 10% for sTfR.[4–6] Maes *et al*[7] found a coefficient of variability for ferritin as high as about 71%, which clearly indicated a skewed distribution. As ferritin and sTfR are known to be log normally distributed,[9] applying indices based on normal distribution may give erroneous results. Moreover, the within subject coefficient of variability will depend on the magnitude of the mean in question, and the reported means vary greatly.

The approach presented here for ferritin and sTfR involved logarithmic transformation, which produced normal distributions and thus reliable error measures, which are multiplication coefficients for given values of variables.

The most important finding is a much greater (mean within subject) error of the sTfR/logFerr index values in female athletes than in the female controls (0.203 *v* 0.129; table 2). This was due to pronounced variability in the ferritin results for the athletes ($1.460^{\pm1}$), nearly twice that in controls ($1.216^{\pm1}$), because the within subject errors for log sTfR were almost identical in the two groups ($1.086^{\pm1}$ and $1.088^{\pm1}$ respectively). It should be emphasised that the sTfR/logFerr index remained stable in athletes in spite of significant day to day changes in either ferritin or sTfR. On the other hand, the day to day changes were, as expected, not significant in the control group for all variables studied ($F<1$; table 2).

A practical conclusion is that, whereas in control subjects the day to day changes in the sTfR/logFerr index may be considered significant when they exceed about 0.26—that is, twice the residual error equal to 0.129—in athletes this value would be about 0.40, which is a lot when confronted with the threshold value for that index (1.80), indicating iron deficiency.[10] This means that the within subject, day to day error for the sTfR/logFerr index computed from data recorded in untrained subjects cannot serve as a reference value for training athletes. Moreover, previous results[9] suggested that the determination of iron stores in athletes who are training hard may give unreliable results. It would be desirable to carry out the determinations after several days of rest, but the training periods are prolonged, intermissions lasting only one day. Thus, as iron stores need to be monitored under such conditions, these precautions when using the sTfR/logFerr index as a measure, should be considered.

Table 2 Two way analysis of variance and within subject SD for the variables measured in female control subjects (n=6) for four consecutive days and in athletes (n=10) for 10 consecutive days

	Control subjects				Athletes			
	S_{xx}	df	s^2	F	S_{xx}	df	s^2	F
logFerr								
Total	0.292	23			5.048	99		
Days	0.005	3	0.002	<1	0.587	9	0.065	2.38*
Subjects	0.162	5	0.032	3.90*	2.240	9	0.249	9.08***
Remainder	0.125	15	0.0083		2.221	81	0.027	
SD			±0.0911	±0.0849†			±0.164	
antilog(SD)			$1.233^{\pm1}$	$1.216^{\pm1}$†			$1.460^{\pm1}$	
logsTfR								
Total	0.757	23			1.197	99		
Days	0.001	3	0.000	<1	0.029	9	0.0032	2.50*
Subjects	0.733	5	0.147	96.1***	1.064	9	0.1182	92.2***
Remainder	0.023	15	0.0015		0.104	81	0.0013	
SD			±0.0391	±0.365†			±0.0358	
antilog(SD)			$1.094^{\pm1}$	$1.088^{\pm1}$†			$1.086^{\pm1}$	
sTfR/logFerr								
Total	4.844	23			17.21	99		
Days	0.012	3	0.004	<1	0.43	9	0.048	1.16
Subjects	4.544	5	0.909	47.3***	13.43	9	1.493	36.1***
Remainder	0.288	15	0.019		3.35	81	0.041	
SD			±0.139	±0.129†			±0.203	

sTfR, Soluble transferrin receptor; Ferr, ferritin; S_{xx}, residual sums of squares; df, degrees of freedom; s^2, S_{xx}/df; F, values of Snedecor's F function. Antilog(SD) = 10^{SD}; the expression, e.g. $1.086^{\pm1}$ means, that the interval corresponding to mean ±SD is obtained by dividing and multiplying the mean by antilog(SD). For example, for judoist No 1, that interval for sTfR is equal to $1.67 \times 1.086^{\pm1}$, or from 1.54 to 1.81.
*p<0.05; ***p<0.001; †SD and antilog(SD) computed from "Days" and "Remainder" combined (df=18).

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Authors' affiliations

R Stupnicki, K Milde, Department of Biometry, Academy of Physical Education, Warsaw, Poland
J Malczewska, Department of Nutrition Physiology, Institute of Sport, Warsaw
A C Hackney, Applied Physiology Laboratory, University of North Carolina, Chapel Hill, NC, USA

Correspondence to: Professor Stupnicki, Department of Biometry, Academy of Physical Education, Marymoncka 34, 00-968 Warsaw 45, Poland; rstupnicki@awf.edu.pl

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