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W. Schänzer
H. Geyer
A. Gotzmann
U. Mareck
(Editors)

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B.BIALAS, W.SCHÄNZER

Validation of EPO Prescan Parameters - Hct, Hb, %Reti - The Influence of Physical long-term Exercise, Long Distance Flying and the Diurnal and Annual Variation of these Parameters

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B.Bialas, W.Schänzer

Validation of EPO prescan parameters

- Hct, Hb, %Reti -

The influence of physical long-term exercise, long distance flying and the diurnal and annuale variation of these parameters

Institute of Biochemistry, German Sport University Cologne, Germany

Introduction

In endurance sports, such as cycling, marathon or cross country skiing, performance relies on an adequate oxygen supply of heart and skeletal muscles. Therefore, the rate of maximal oxygen uptake is an important determinant of aerobic capacity.

Erythropoietin (EPO), a naturally occurring hormone secreted by the kidney, stimulates the bone marrow and increases red blood cells production. This leads to an increase in red blood cell mass, hemoglobin and hematocrit. Since 1988 recombinant human erythropoietin (rhEPO) is commercially available. It stimulates the proliferation and maturation of erythroid progenitor cells and thereby increases the number of red blood cells in the identical fashion as human EPO. The use of rhEPO is officially prohibited by the International Olympic Committee (IOC) since 1989. In order to limit the risks in taking EPO, the Union Cycliste Internationale (UCI) and the Fédération Internationale de Ski (FIS) introduced a hematocrit (hct) and hemoglobin (Hb) test before competition from 1997. The cut-off for hct in males is set to 50%, in females 47% (UCI). The FIS imposed the cut-off for Hb (males 17.5 g/dl, females 16.0 g/dl). Since the winter games 2002 in Salt Lake City the IOC took in addition to hct and Hb the percentage amount of reticulocytes (%reti) into account. The cut-off was set to 2% for both gender.

The present study examined the parameters hct, Hb and %reti under the influence of different physiological parameters.

Design and Methods

The data presented describe the counting of Hb, hct and %reti in 238 (1095 blood samples) apparently healthy well trained and recreational athletes of both sexes (127 males/111 females). Their ages varied from 18 to 50 years. The volunteers participated either on a cycling race, a marathon, a long distance fly or were examined during a diurnal and annual variation study. Before the start of this study approval was obtained from the ethics committee of the German Sport University Cologne and all subjects gave their informed written consent before participation in this study.

The parameters were counted with the automated cytoanalyser Advia 120, BAYER DIAGNOSTICS, Germany.

Statistical analysis

The statistical analysis was performed using the Statistical Package for Social Science (SPSS for Windows, Version 10.0) and Microsoft Excel 2000. The parameters derived included calculations of mean ± standard deviation (SD). For comparisons between groups a Student's *t*-Test was performed. Statistical significance was considered at $P<0.05$. It was also determined how frequently the Hb-, hct- or %reti-values were over the cut-off of UCI, FIS and IOC.

Results

The results of Hb, hct and %reti are shown in table 1-5 and figure 1-11. All mean values were under the cut-off of the UCI, FIS and IOC and in between the normal reference ranges for the hematological parameters [7-19]. Mean values of hemoglobin and hematocrit of all volunteers were 14.6 g/dl (range 10.6 – 18.5 g/dl) and 43.6% (range 32.8 – 53.9%), respectively. The mean percentage of reticulocytes was 1.4% the range was from 0.3% to 3.3%. It is noticeable that the male hct mean ± SD values of the cycling race and the marathon were 46.8 ± 3.1 % (tab.1) respectively 48.0 ± 2.9 % (tab. 2). Further the parameter %reti showed a remarkable value of 1.6 ± 0.4% during the annual variation study (tab.5).

The absolute number of samples above the hb, hct and %reti cut-off are shown in table 6.

A hemoglobin value above 17.5 g/dl was observed in 27/585 (4.6%) male athletes and 6/510 (1.2%) females had a Hb over 16.0 g/dl.

A hct value over 50% was registered 31/585 (5.3%) in male and a hct value over 47% was observed 16/510 (3.1%) in female athletes.

The %reti cut-off (2%) was exceeded 35/585 times (5.9%) in male and 42/510 times (8.2%) in female.

In the group of female it is noticeable that no cut-off were exceeded under the influence of an long distance fly (hypoxie) (table 6). In the group of men, one subject (2.9%) showed an high hct value above the cut-off of 50%.

In the marathon and cycling group the hct values of both gender and the hb values of female are significant higher than in the other groups (fig.11).

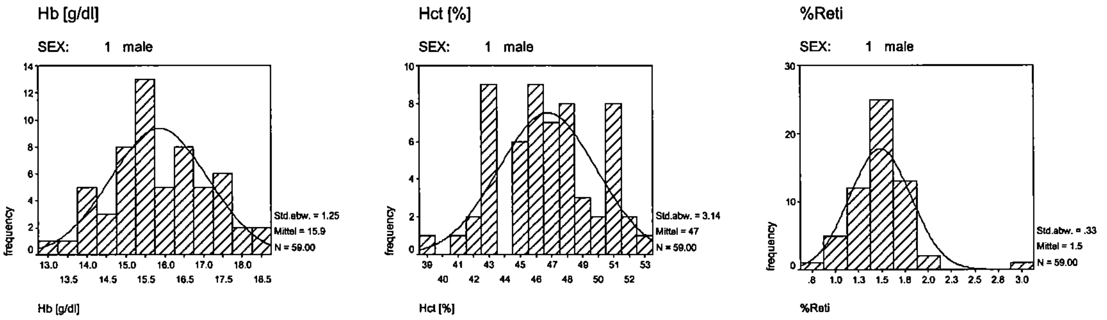

Fig. 1: Distribution of Hb [g/dl], Hct [%] and %reti in males under the influence of a cycling race

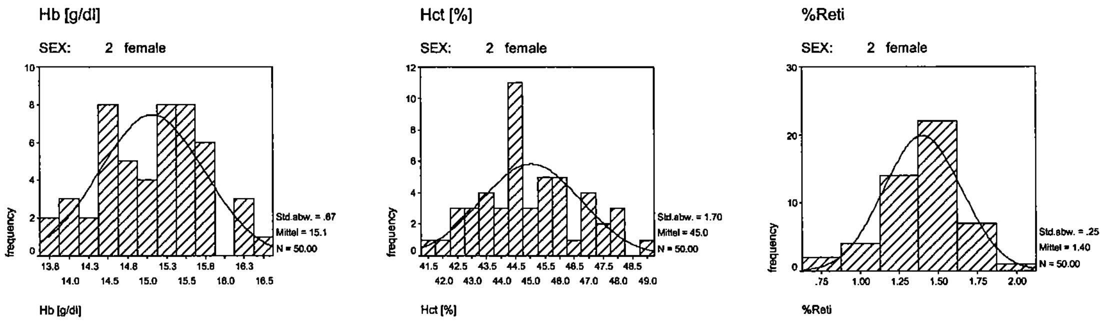

Fig. 2: Distribution of Hb [g/dl], Hct [%] and %reti in females under the influence of a cycling race

Sex = male		Hb [g/dl]	Hct [%]	%Reti
N		59	59	59
Mean		15.9	46.8	1.5
Median		15.7	47.0	1.5
Standard deviation		1.3	3.1	.3
variance		1.58	9.8	.11
Minimum		13.0	39.4	.8
Maximum		18.5	52.6	3.1
percentile	25	15.1	44.5	1.3
	50	15.7	47.0	1.5
	75	16.8	49.2	1.7
	97.5	18.4	52.3	2.6

Sex = female		Hb [g/dl]	Hct [%]	%Reti
N		50	50	50
Mean		15.1	45.0	1.4
Median		15.2	44.7	1.4
Standard deviation		.7	1.7	.3
variance		.45	2.9	6.265E-02
Minimum		13.7	41.6	.8
Maximum		16.4	49.0	1.9
percentile	25	14.6	43.8	1.2
	50	15.26	44.7	1.4
	75	15.56	46.17	1.6
	97.5	16.4	48.8	1.9

Tab. 1: Statistics of Hb [g/dl], Hct [%] and %reti in males and females under the influence of a cycling race

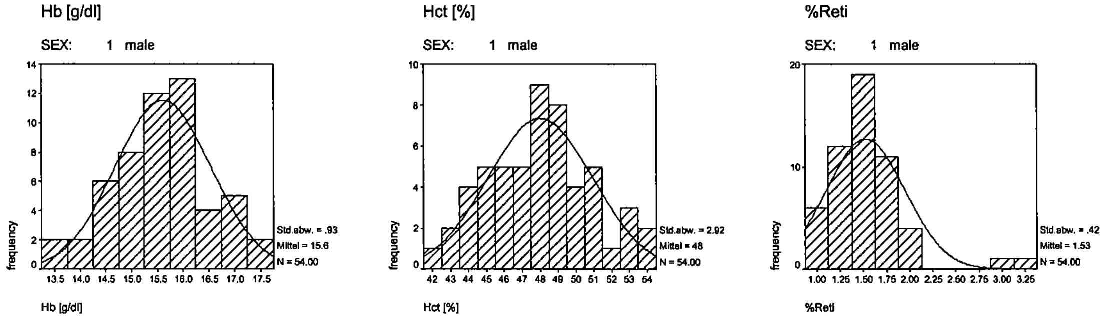

Fig. 3: Distribution of Hb [g/dl], Hct [%] and %reti in males under the influence of a marathon race

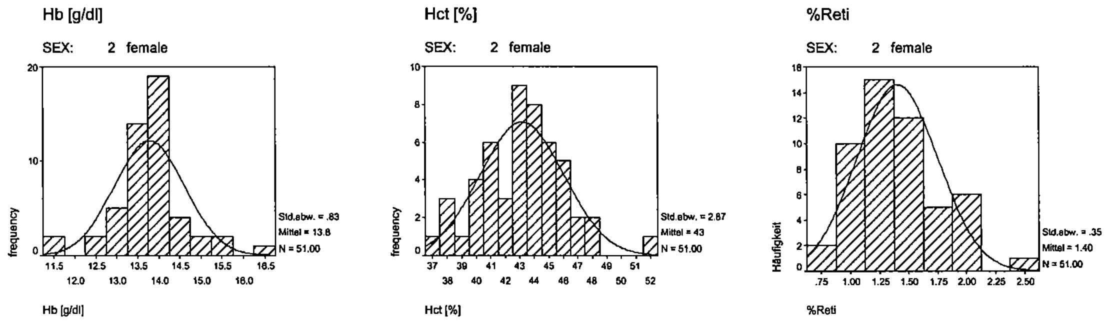

Fig. 4: Distribution of Hb [g/dl], Hct [%] and %reti in females under the influence of a marathon race

Sex = male		Hb [g/dl]	Hct [%]	%Reti
N		54	54	54
Mean		15.6	48.0	1.5
Median		15.7	47.9	1.5
Standard deviation		.932	2.9	.423
variance		.869	8.6	.18
Minimum		13.4	41.6	.9
Maximum		17.5	53.9	3.3
percentile	25	14.99	45.9	1.28
	50	15.79	47.9	1.5
	75	16.29	49.8	1.75
	97.5	17.5	53.9	3.23

Sex = female		Hb [g/dl]	Hct [%]	%Reti
N		51	51	51
Mean		13.8	43.2	1.42
Median		13.8	43.2	1.32
Standard deviation		.83	2.87	.35
variance		.693	8.3	.121
Minimum		11.5	37.3	.7
Maximum		16.3	51.5	2.4
percentile	25	13.4	41.1	1.25
	50	13.8	43.2	1.35
	75	14.2	45.2	1.65
	97.5	16.0	50.5	2.285

Tab. 2: Statistics of Hb [g/dl], Hct [%] and %reti in males and females under the influence of a marathon race

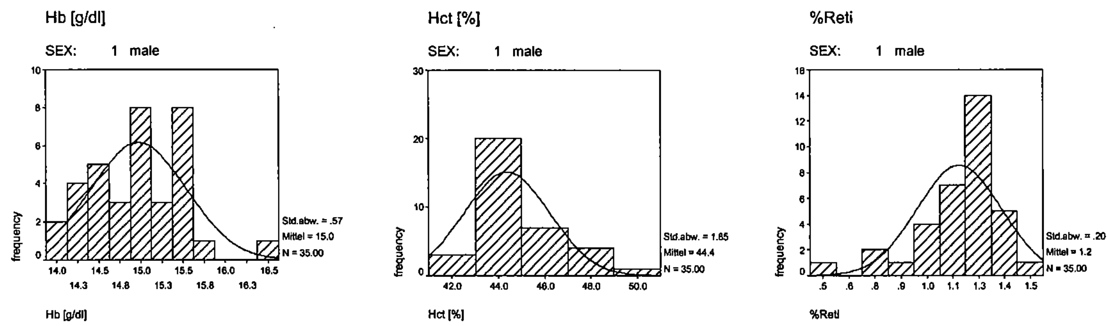

Fig. 5: Distribution of Hb [g/dl], Hct [%] and %reti in males under the influence of a long distance fly

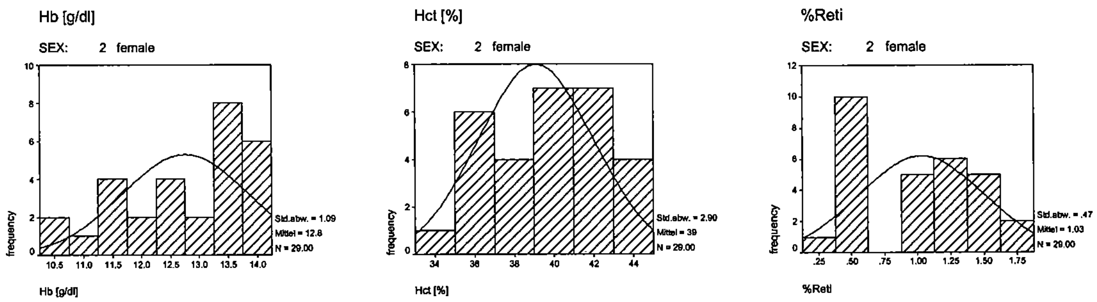

Fig. 6: Distribution of Hb [g/dl], Hct [%] and %reti in females under the influence of a long distance fly

Sex = male		Hb [g/dl]	Hct [%]	%Reti
N		35	35	35
Mean		14.9	44.4	1.2
Median		15.0	44.0	1.2
Standard deviation		.57	1.9	.20
variance		.32	3.4	4.134E-02
Minimum		14.0	41.0	.5
Maximum		16.5	50.0	1.5
percentile	25	14.5	43.0	1.1
	50	15.0	44.0	1.2
	75	15.5	45.0	1.3
	97.5	16.5	50.0	1.5

Sex = female		Hb [g/dl]	Hct [%]	%Reti
N		29	29	29
Mean		12.8	39.1	1.0
Median		13.200	40.000	1.1
Standard deviation		1.089	2.895	.47
variance		1.19	8.38	.22
Minimum		10.7	34.0	.3
Maximum		14.2	43.0	1.8
percentile	25	11.7	36.5	.55
	50	13.2	40.0	1.1
	75	13.5	41.5	1.4
	97.5	14.2	43.0	1.8

Tab. 3: Statistics of Hb [g/dl], Hct [%] and %reti in males and females under the influence of a long distance fly

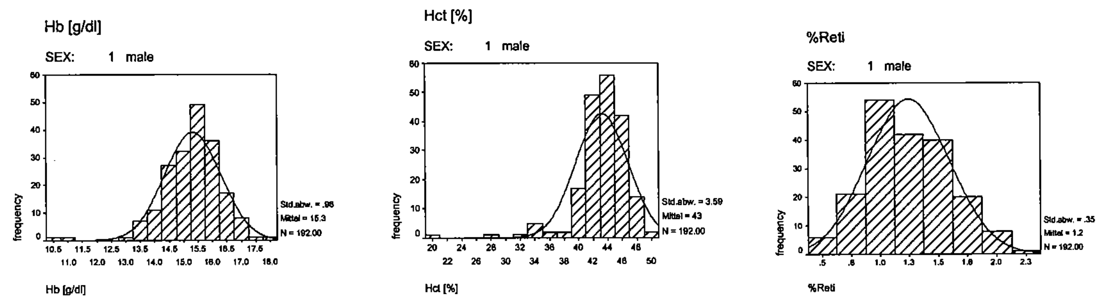

Fig. 7: Distribution of Hb [g/dl], Hct [%] and %reti in males during the day

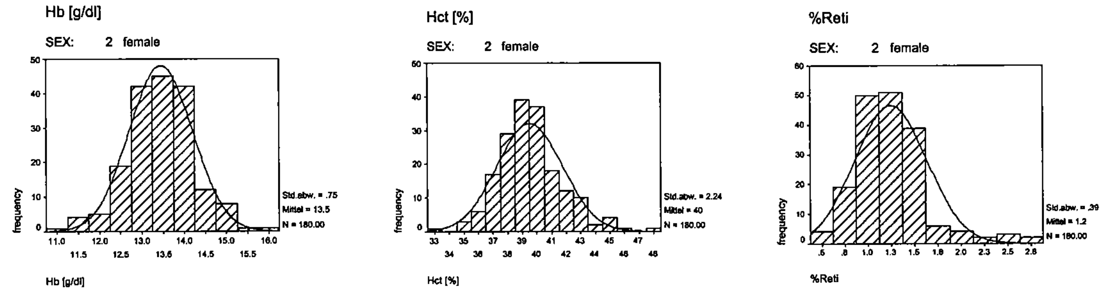

Fig. 8: Distribution of Hb [g/dl], Hct [%] and %reti in females during the day

Sex = male		Hb [g/dl]	Hct [%]	%Reti
N		192	192	192
Mean		15.3	43.2	1.3
Median		15.5	43.5	1.2
Standard deviation		.9	3.6	.4
variance		.96	12.9	.14
Minimum		10.6	19.9	.5
Maximum		17.8	50.1	2.2
percentile	25	14.7	41.9	1.04
	50	15.5	43.5	1.2
	75	15.9	45.4	1.5
	97.5	17.1	48.3	2.0

Sex = female		Hb [g/dl]	Hct [%]	%Reti
N		180	180	180
Mean		13.6	39.5	1.3
Median		13.4	39.3	1.2
Standard deviation		.8	2.2	.4
variance		.6	5.0	.2
Minimum		10.8	32.8	.5
Maximum		15.8	47.6	2.7
percentile	25	13.0	38.2	1.0
	50	13.4	39.3	1.2
	75	13.9	40.7	1.4
	97.5	14.9	45.2	2.5

Tab. 4: Statistics of Hb [g/dl], Hct [%] and %reti in males and females during the day

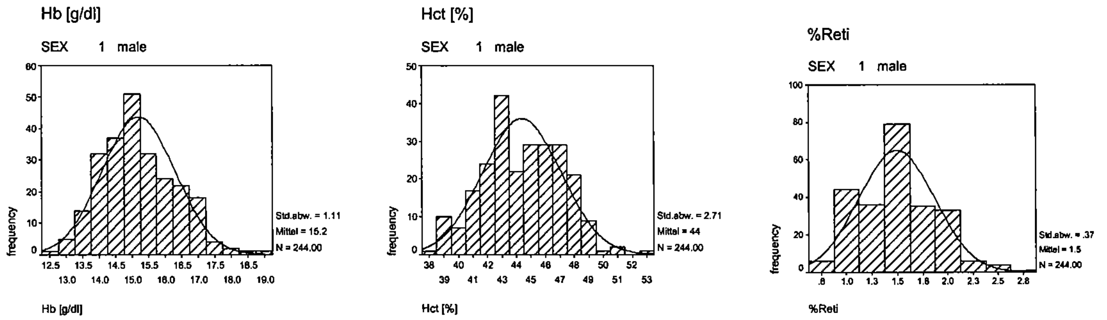

Fig. 9: Distribution of Hb [g/dl], Hct [%] and %reti in males over a year

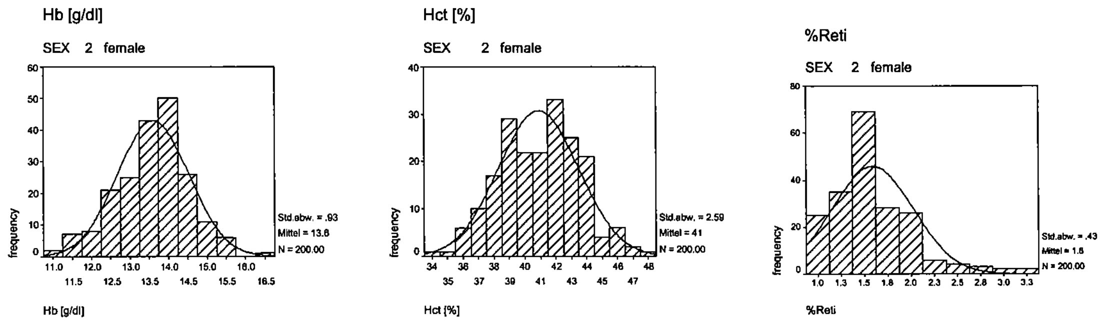

Fig. 10: Distribution of Hb [g/dl], Hct [%] and %reti in females over a year

Sex = male		Hb [g/dl]	Hct [%]	%Reti
N		244	244	244
Mean		15.2	44.4	1.5
Median		15.0	44.4	1.5
Standard deviation		1.1	2.7	.4
variance		1.2	7.3	.14
Minimum		12.6	38.4	.8
Maximum		19.1	52.6	2.8
percentile	25	14.4	42.5	1.2
	50	15.0	44.4	1.5
	75	16.0	46.6	1.7
	97.5	17.6	49.1	2.3

Sex = female		Hb [g/dl]	Hct [%]	%Reti
N		200	200	200
Mean		13.6	40.9	1.6
Median		13.7	41.1	1.6
Standard deviation		.93	2.6	.5
variance		.86	6.7	.19
Minimum		11.1	33.9	.9
Maximum		16.3	47.8	3.3
percentile	25	13.0	38.9	1.3
	50	13.7	41.1	1.5
	75	14.2	42.8	1.8
	97.5	15.4	45.9	2.8

Tab. 5: Statistics of Hb [g/dl], Hct [%] and %reti in males and females over a year

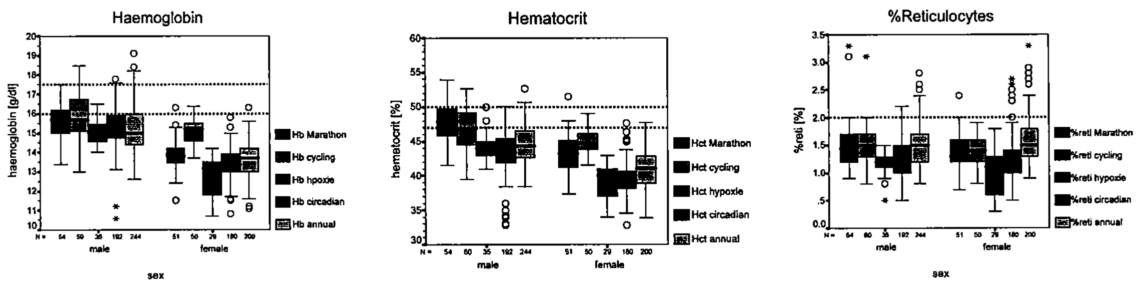

Figure 11: hemoglobin, hematocrit, %reti under the influence of different physiological parameters

male	Hb 17.5g/dl		hct 50%		%Reti 2 %		female	Hb 16.0g/dl		hct 47%		%Reti 2%	
	abs.	%	abs.	%	abs.	%		abs.	%	abs.	%	abs.	%
cycling (n=60)	8	13.3	13	21.7	2	3.3	cycling (n=50)	4	8	9	18	0	0
marathon (n=54)	3	5.6	12	22.2	3	5.6	marathon (n=51)	1	2	4	7.8	1	2
hypoxia (n=35)	0	0	1	2.9	0	0	hypoxie (n=29)	0	0	0	0	0	0
circadian (n=192)	10	5.2	1	0.5	4	2.1	circadian (n=180)	0	0	1	0.6	10	5.6
annual (n=244)	6	2.5	4	1.6	26	10.7	annual (n=200)	1	0.5	2	1	31	15.5
Total (n=585)	*27*	*4.6*	*31*	*5.3*	*35*	*5.9*	*Total (n=510)*	*6*	*1.2*	*16*	*3.1*	*42*	*8.2*

Table 6: The absolute and percentage values above the FIS, UCI and IOC cut-off

Discussion

To prevent doping with rhEPO and to protect athletes health against possible damage caused by elevated hct, Hb and %reti values the UCI, FIS and IOC introduced cut off values for these parameters. Since the introduction of the cut-offs the limits have been discussed.

The results of our investigations demonstrate that hct, Hb and %reti are no constant values (fig.11). We could further show that the range of these hematological parameters indicate variations from subject to subject (data not shown). Change of body posture, diurnal variation, dehydration, thermal stress, gender, nutritional differences, iron stores and the use of plasma expanders have been reported to influence hct and Hb-levels [4, 3, 6]. Our analysis produced 93/585 false positives in men and 64/510 in women.

In consideration of these results and to find an appropriate method to detect the rhEPO misuse we demand the introduction of a subject specific hematological passport. Each athlete should therefore participate in a regular investigation of hematological parameters. The results of the sequential determination of hemoglobin, hematocrit and percentage of reticulocytes make it possible to arrange individual reference ranges for these parameters. An individual hematological passport could explain abnormal hb, hct or %reti values determined before competition because these values could be constitutionally high. On the other hand values outside the individual reference range could lead to exclusion from competition.

The hematological passport could be an important and helpful instrument in the battle against blood doping, but over the pros and cons of an individual hematological passport we shouldn't disregard that only the direct urine method is currently able to detect an rhEPO misuse.

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