

# Influence of chronic training workload on the hematological profile: a pilot study in sedentary people, amateur and professional cyclists

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**Summary.** *Background and aim:* The assessment of hematological profile requires to identify possible sources of biological variability, including exercise-related variations. This study was hence aimed to evaluate hematological profile variations in amateur and professional athletes, and establish their possible dependence on cumulative training volume. *Materials and Methods:* The study population consisted of 59 sedentary male subjects, 78 amateur and 80 professional male cyclists, in whom a large number of hematological variables were measured at rest. *Results:* Red blood cell (RBC) count and hemoglobin were decreased in the two athlete cohorts compared to sedentary subjects, but did not differ between amateur and professional cyclists. Hematocrit was gradually and significantly decreased in parallel with cumulative training volume. Amateur cyclists displayed higher mean corpuscular volume (MCV) and lower mean corpuscular hemoglobin concentration (MCHC) values than sedentary subjects and professional cyclists, whilst mean corpuscular hemoglobin (MCH) was higher in professional cyclists. The reticulocyte count and soluble transferrin receptor (sTFR) values were similar across all groups. Serum ferritin was higher in professional cyclists than in the other two groups, whilst transferrin gradually decreased from sedentary group to the two cohorts of amateur and professional cyclists. In univariate analysis, cumulative training volume was inversely associated with age, body mass index (BMI), RBC count, hematocrit, hemoglobin and transferrin, whilst a positive association was found with ferritin. In multivariate analysis, BMI, RBC count and ferritin remained significantly associated with training volume. *Conclusions:* These results show that the volume of endurance training may affect some hematological variables. ([www.actabiomedica.it](http://www.actabiomedica.it))

**Key words:** red blood cells, hemoglobin, anemia, athletes, exercise intensity

## Introduction

Laboratory testing in general, and more specifically the assessment of the hematological profile in recreational and competitive athletes, is justified by a number of practical reasons, some of which are typically similar to those of the general population (e.g., for preventing, diagnosing and monitoring common pathologies) (1), whilst others appear highly sport-specific, such as for diagnosing a kaleidoscope of sports

injuries (2), for optimizing or maximizing athletic performance (3), for preventing or early diagnosing overtraining syndrome (4), as well as for screening athletes who may be engaged in unfair or illicit practices (i.e., blood doping) (5). This last aspect is especially relevant, whereby the validated and currently exploited anti-doping approach is based on the so-called Athlete Biological Passport (ABP), encompassing the measurement of a number of hematological variables such as red blood cell (RBC) and reticulocyte counts, hema-

tocrit, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) (6). These parameters are also essential part of the diagnostic workout of the so-called sport anemia, which can be due to a large number of parapsychological factors (i.e., "pseudoanemia", mechanical and exercise-induced hemolysis, menstruation) or pathological causes (e.g., genetic defects, blood loss, nutritional deficiencies, among others) (7). The accurate assessment of the individual hematological profile thus requires the identification of the many potential sources of biological variation, which may be responsible for extra-analytical variation of various hematological variables and ultimately contribute to reducing the overall diagnostic efficiency of diagnostic testing (8). Therefore, the aim of the present study was to explore the variation of the hematological profile in populations of amateur and professional athletes and establish their possible dependence on cumulative endurance training volume.

## Materials and Methods

The initial study population consisted of 60 sedentary and ostensibly healthy male blood donors consecutively visited in the local service of transfusion medicine before blood donation, 80 male amateur cyclists belonging to three different local amateur teams and 80 male professional cyclists belonging to four separate WorldTour Teams. One sedentary subject and two amateur cyclists ought to be immediately excluded from the study for fever (1 sedentary subject and 1 amateur cyclist) and strong muscular pain (1 amateur cyclist). Blood samples were collected early in the morning (at the same hour, between 7 and 9 AM), after overnight fasting, and under the same conditions (temperature and humidity) by a single experienced phlebotomist, into evacuated blood tubes (Becton-Dickinson, Oxford, UK) containing either clot activator or dipotassium ethylenediaminetetraacetic acid (K<sub>2</sub>EDTA), which is the reference additive for hematological testing. Both amateur and professional athletes reached our center for scheduled quarterly laboratory analyses in the middle of their competitive season (i.e., July), and had rested for at least 24 h since the

last competition or training session in order to avoid acute bias due to recent exercise (9). Demographical data were collected at laboratory admission, by means of a standardized questionnaire.

Whole blood samples were analyzed immediately after collection, using the hematological analyzer Siemens Advia 120 (Siemens Healthcare Diagnostics, Tarrytown NY, USA). The analytical performance of this instrument has been described elsewhere (10). Serum ferritin was analyzed with an automated chemiluminescent immunoassay (sandwich principle) on DiaSorin Liaison (DiaSorin, Saluggia, Italy) (11), soluble transferrin receptor (sTFR) was measured with an immunonephelometric technique on Siemens BN II System (Siemens Healthineers, Erlangen, Germany) (12), whilst transferrin was assayed with an immunoturbidimetric assay on Roche Cobas (Roche Diagnostics, Basel, Switzerland) (13).

Test results were expressed as median and interquartile range (IQR), and the differences between groups were analyzed with Mann-Whitney U test. The significance of the association between training regimen and hematological parameters was tested with both univariate (Spearman's correlation) and multivariate (multivariate linear regression) analyses. Statistical analysis was performed using Analyse-it (Analyse-it Software Ltd, Leeds, UK). All subjects provided informed consent for being enrolled in this investigation. The study has been approved by the competent Institutional Review board of the University of Verona ("Identification of variation of main hematological parameters in athletes populations"; <http://www.dnbm.univr.it/?ent=progetto&id=744&lang=en>) and was carried out in accordance with the Helsinki Declaration of 1975.

## Results

The final study population consisted of 59 sedentary male subjects, 78 amateur and 80 professional male cyclists, whose demographic characteristics are summarized in table 1. Professional cyclists were slightly older than sedentary subjects, whilst the age did not differ between professional and amateur cyclists. The value of body mass index (BMI) gradually

**Table 1.** Demographic characteristics and laboratory data of the study population. Results are presented as median and interquartile range (IQR)

Parameter	Sedentary subjects Values	Amateur cyclists		Professional cyclists		
		Values	p vs sedentary	Values	p vs sedentary	p vs amateur
N	59	78		80		
Age (years)	30 (12)	20 (21)	0.217	27 (5)	0.003	0.121
Body mass index (km/m <sup>2</sup> )	23.7 (4.4)	21.4 (2.3)	<0.001	21.0 (1.7)	<0.001	0.030
Training (km/year)	0 (0)	15500 (10000)	<0.001	32000 (3500)	<0.001	<0.001
Red blood cells (x10 <sup>12</sup> /L)	5.22 (0.47)	4.97 (0.41)	<0.001	4.95 (0.49)	<0.001	0.242
Reticulocytes (x10 <sup>9</sup> /L)	54 (30)	51 (26)	0.105	63 (39)	0.458	0.144
Hematocrit	0.472 (0.040)	0.461 (0.032)	0.003	0.455 (0.037)	<0.001	0.017
Hemoglobin (g/L)	158 (13)	151 (12)	<0.001	150 (11)	<0.001	0.371
MCV (fL)	91.2 (4.3)	93.2 (4.8)	0.004	91.5 (6.4)	0.064	0.062
MCH (pg)	30.1 (1.6)	30.4 (1.8)	0.091	30.7 (1.4)	0.008	0.133
MCHC (pg/L)	33.2 (1.6)	32.5 (1.3)	0.005	33.3 (1.6)	0.222	<0.001
Ferritin (ng/mL)	93 (79)	118 (113)	0.118	265 (190)	<0.001	<0.001
sTFR (mg/L)	1.20 (0.38)	1.19 (0.35)	0.468	1.20 (0.34)	0.195	0.187
Transferrin (g/L)	2.34 (0.43)	2.19 (0.38)	0.032	2.11 (0.41)	<0.001	0.003

MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin content; sTFR, soluble transferrin receptor

and significantly decreased from sedentary subjects to amateur and then professional cyclists, whereas the cumulative training volume displayed an opposite trend.

The results of hematological testing are also summarized in table 1. The values of the RBC count were considerably decreased in both groups of athletes compared to the sedentary group, but did not differ between amateur and professional cyclists. The hemoglobin values exhibited an identical trend. Unlike these findings, the hematocrit value was found to be gradually and significantly decreased in parallel with cumulative training volume (Table 1). Amateur cyclists displayed higher values of MCV and lower values of MCHC than both sedentary subjects and professional cyclists, whilst MCH was higher in professional cyclists than in the two other cohorts. The values of reticulocyte count and sTFR were instead similar across all groups. Serum ferritin values were higher in professional cyclists than in the other two groups. Finally, transferrin values displayed a gradually decreasing trend, from the sedentary group to the two cohorts of amateur and professional cyclists.

In univariate analysis, cumulative training volume (km/year) was found to be inversely associated with age, BMI, RBC count, hematocrit, hemoglobin and transferrin, whilst a positive association was found

with ferritin (Table 2). In multivariate analysis, BMI, RBC count and ferritin remained significantly associated with training regimen (Table 2). A multivariable function incorporating these three parameters was capable to predict cumulative training volume with 60% accuracy. A highly significant correlation was finally observed in the entire study population between RBC count and hematocrit ( $r=0.74$ ; 95% CI, 0.68 to 0.80;  $p<0.001$ ), hemoglobin ( $r=0.81$ ; 95% CI, 0.76 to 0.85;  $p<0.001$ ), MCV ( $r=-0.51$ ; 95% CI, -0.60 to -0.40;  $p<0.001$ ), MCH ( $r=-0.48$ ; 95% CI, -0.58 to -0.37;  $p<0.001$ ), sTFR ( $r=0.16$ ; 95% CI, 0.03 to 0.29;  $p=0.020$ ) and transferrin ( $r=0.16$ ; 95% CI, 0.03 to 0.29;  $p=0.015$ ), but not between RBC count and MCHC ( $r=0.10$ ; 95% CI, -0.03 to 0.23;  $p=0.138$ ) or ferritin ( $r=-0.13$ ; 95% CI, -0.26 to 0.01;  $p=0.056$ ). A highly significant inverse association was also found between ferritin and transferrin ( $r=-0.36$ ; 95% CI, -0.47 to -0.24;  $p<0.001$ ), but not between ferritin and sTFR ( $r=-0.09$ ; 95% CI, -0.22 to 0.05;  $p=0.210$ ).

## Discussion

It is now undeniable that diagnostic testing is an essential component of sports medicine, since labo-

**Table 2.** Univariate and multivariate analysis between training regimen (km/years) and hematological parameters

Parameter	Univariate analysis	Multivariate analysis
Age (years)	-0.19 (95% CI, -0.32 to -0.06); p=0.005	0.709
Body mass index (km/m <sup>2</sup> )	-0.42 (95% CI, -0.52 to -0.30); p<0.001	<0.001
Red blood cells (x10 <sup>12</sup> /L)	-0.24 (95% CI, -0.36 to -0.11), p<0.001	0.010
Reticulocytes (x10 <sup>9</sup> /L)	0.09 (95% CI, -0.05 to 0.22); p=0.201	-
Hematocrit	-0.27 (95% CI, -0.39 to -0.15), p<0.001	0.407
Hemoglobin (g/L)	-0.22 (95% CI, -0.34 to -0.09); p=0.001	0.174
MCV (fL)	0.00 (95% CI, -0.13 to 0.13); p=0.986	-
MCH (pg)	0.10 (95% CI, -0.04 to 0.23); p=0.151	-
MCHC (pg/L)	0.09 (95% CI, -0.04 to 0.22); p=0.176	-
Ferritin (ng/mL)	0.58 (95% CI, 0.48 to 0.66); p<0.001	<0.001
sTFR (mg/L)	0.07 (95% CI, -0.06 to 0.20); p=0.306	-
Transferrin (g/L)	-0.29 (95% CI, -0.41 to -0.17); p<0.001	0.097

MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin content; sTFR, soluble transferrin receptor

ratory test results have a substantial impact on diagnostic reasoning, clinical decision making, returning to play after sports injuries or overtraining, as well as for screening the possible use of blood doping (2). To be really effective, however, laboratory diagnostics requires an accurate standardization of all the different parts of the total testing process, thus including the identification of potential exercise-induced variation of blood parameters, either between sedentary people and physically active subjects, but also between professional and recreational athletes (14).

The results of this study clearly show that the hematological profile is profoundly influenced by an active lifestyle and, more specifically, by the volume of endurance training. This is clearly reflected by the demonstration that the values of many hematological parameters (RBC count, hematocrit, hemoglobin, MCV, MCHC, and transferrin) were consistently different between sedentary subjects and people engaged in recreational endurance sports such as amateur cycling (Table 1). On the other hand, some of these parameters (hematocrit, MCHC, ferritin, and transferrin) were also different between amateur and professional cyclist (Table 1). Interesting evidence emerged from the correlation analysis with cumulative training volume, whereby significant associations were found between distance covered per year and RBC count, hematocrit, hemoglobin, ferritin and transferrin (Table 2).

The significant correlation between the first three parameters (RBC count, hematocrit, hemoglobin) is not really surprising since a well-known direct relationship exists between cumulative physical workload and plasma volume. In a seminal article published nearly 20 years ago, Heinicke et al measured plasma volume and total hemoglobin mass in untrained individuals and in different cohorts of endurance athletes (15). Notably, the plasma volume was found to be increased from 3.76 L in untrained individuals to 4.46 L (+18.6%) in young elite cyclists, up to 4.48 L in professional cyclists (+19.1%). Nearly identical results were then published by Schmidt and Prommer (16), who showed that the plasma volume increased in parallel, almost linearly, with training workload, from 0.49 mL/kg in subjects with normal levels of physical activity, to 0.54 mL/kg (+10%) in those with moderate levels of physical activity, 0.59 mL/kg (+20%) in those with high levels of physical activity, up to 0.63 mL/kg (+29%) in those with elite levels of physical activity. More recently, Zelenkova et al published an interesting study showing that both blood volume (7.71 vs 7.09 L) and erythrocyte mass (1.01 vs 0.96 kg) were significantly higher in male competitive cyclists compared to untrained subjects, but the relative expansion of the blood volume was ultimately higher than that of the erythrocyte mass (+9% vs +5%) (17). This concept that the total erythrocyte mass also increases significantly in response to chronic training workloads is

well-known, though has also been clearly established that this expansion is usually inferior to that of plasma volume (i.e., 1-3% lower), so that the final balance is a decrease of absolute RBC count, hematocrit value and hemoglobin concentration as a result of a dilution effect (18). To support this previous evidence, the results of our study also show that a highly significant relationship exists between training intensity and decrease of RBC, hematocrit and hemoglobin values. As predictable, the associations between cumulative training volume and hemoglobin or hematocrit are mostly dependent on the decrease of RBC count, as revealed by the results of our multivariate analysis in which the significance of both hematocrit and hemoglobin was lost (Table 2).

Interesting findings also emerged from the analysis of ferritin, sTFR, and transferrin. The increased ferritin values observed in our population of professional cyclists is not surprising and confirms previously published data by our (19) and other groups (20, 21). This is an obvious consequence of the major awareness that professional cyclists have on the effectiveness of iron supplementation for supporting erythropoiesis and thereby maintaining the hemoglobin levels to levels compatible with competitive activity, as well as of their easier access to nutritional supplements (22). Rather understandably, the gradual increased ferritin value in parallel with the cumulative training volume is reflected by an almost overlapping decrease of transferrin concentration ( $p < 0.001$ ), which is clearly dependent on the larger amount of iron stores displayed by both categories of athletes, as well as by the possible influence of exercise-induced intravascular hemolysis, which is also frequent in cycling (23). The high overlap of sTFR values among the cohorts of subjects included in this study is also simply explainable. The concentration of this parameter conventionally increases in conditions of iron deficiency (24), which are absolutely rare in blood donors and recreational or professional athletes, as attested by the normal transferrin values observed in our study population.

In conclusion, the results of this pilot study in sedentary people, recreational and professional cyclists demonstrate that many parameters of the hematological profile may be substantially modified by the cumulative volume of endurance exercise, and these changes

shall hence be straightforwardly acknowledged while interpreting athletes' data for clinical, training or anti-doping purposes.

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