HEMATOLOGICAL VARIABILITY ANALYSIS AFTER ROAD MARATHON VS ULTRATRAIL – PREDICTIVE FACTORS

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Abstract:

The practice of long- and medium-distance races has exponentially increased the number of participants in recent years. They are carried out under extreme conditions that cause both biochemical and anthropometric changes in athletes and, in addition, have serious risks associated, such as dehydration, thermal disorders, or hypoglycemia. Knowledge regarding alterations at hematological level after this type of effort is limited. The objectives of this *pre-post* analytical descriptive observational study were: to describe and compare hematological values in amateur runners *pre* and *post* road marathon and ultratrail races, and to compare the hematological parameters between the groups measured at two time points analyzed by means of univariate descriptive statistical analyses, intra-group *pre-post* comparisons (Wilcoxon Signed-Rank Test), and comparisons between the groups (Mann-Whitney U Test). Effect size was calculated using Cohen's d. An explanatory model (Multiple Logistic Regression) was created. Statistical significance was set at p<.05. The Wilcoxon test for marathon runners observed significant differences (p≤.001) in the white series. In ultratrail runners, the results were similar, with significant differences (p≤.001) in the platelet series. The Mann-Whitney U test showed the same *pre-race* hematological conditions for both groups, except for hematocrit and corpuscular values (p≤.001). Significant differences (p≤.001) were also found in the *post-race* leukocyte and neutrophil values. Significant differences in hematological parameters have been observed both between and within the groups. The differences with greater significance corresponded to the leukocyte series.

Key words: running, red blood cells, white blood cells, hematological changes, effort

Introduction

In recent decades, a positive relationship has been recognized between an optimal state of health and the regular practice of physical exercise (Sánchez-González, Rivera-Cisneros, & Tovar, 2003), in addition to a decrease in mortality in athletes compared to sedentary individuals (Müler, et al., 2017; Schwarz, et al., 2018). All this has led to a notable boom in sports, especially in those activities related to medium- and long-distance races. Both, road and trail marathons have exponentially increased their number of participants in recent years worldwide (Areces, et al., 2014; Nikolaidis & Knechtle, 2018; Rama, Minuzzi, Carvalho, Costa, & Teixeira, 2016; Wu, et al., 2004). This popularity is evidenced in the increase of sporting events, as can be seen in the information offered by the International Association of Athletics Federa-

tions (IAAF), which reports more than ten thousand competitions registered per year worldwide.

Medium- and long-distance races, especially marathons, characterized by exceeding the distance of 42 km, and ultratrails, which involve the completion of 115 km, are usually carried out under extreme environmental conditions (Hoffman & Fogard, 2011; Knechtle, 2012; Rama, et al., 2016). Physical capabilities and technical skills required for mountain races and marathons are quite different from each other. The orography of terrain, altitude, air temperature and the presence or lack of inclines, among other variables, make mountain races and marathons differ in their characteristics and in requirements runners need to meet to face them (Clemente, 2011). Moreover, runners' performance will be influenced by environmental factors and internal factors, among which we can include age, sex, anthropometry, running technique, as well as

training habits (Areces, et al. 2014). Therefore, the effort required will vary between two types of races (Clemente, 2011).

Marathon races' speed and duration of ultratrails subject the athlete's organism to strenuous conditions. Such exposure to stress causes a decrease in strength and loss of muscle mass along with the appearance of muscle damage. This can be evidenced by high levels of myoglobin, creatine kinase (CK), alanine aminotransferase (ALT) and hydroxybutyrate dehydrogenase, together with a notable elevation of lactic dehydrogenase (LDH) and troponins (Bernat-Adell, et al., 2019; Clemente, 2011; Hoffman, Ingwerson, Rogers, Hew-Butler, & Stuempfle, 2012; Nie, et al., 2011) in the blood. Such modifications remain altered for days in adults. At the end of any long-distance race, decreased triglyceride values are usually present since lipids are considered the main source of energy, while glucose can be altered in relation to carbohydrate consumption throughout the race (Clemente, 2011).

It is important to highlight risks linked to this type of sport practices that can compromise health and even life of runners. Among them are extreme fatigue, thermal and dehydration disorders, hypoglycemia or acute coronary syndrome that can lead to acute myocardial infarction (Sánchez-González, et al., 2003) although literature only indicates a prevalence of one sudden death for every fifty-five thousand runners in the first decade of this century (Roberts & Maron, 2005).

Regarding alterations at the hematological level, changes and alterations in the blood count have been observed, which influence both the red and white series with high levels of hemolysis being determined after strenuous exercise (Kratz, et al., 2002; Peters, Robson, Kleinveldt, Naicker, & Jogessar, 2004; Robach, et al., 2014; Ruiz Vicente, et al., 2013).

The consequence of exercise-induced hemolysis or "sports anemia" is primarily related to hemodilution, which is defined as an increase in plasma volume that exceeds the increase in the total mass of red blood cells and causes a reduction in their recount as well as a reduction in hemoglobin (Hb) or hematocrit (Htc). The cause of sports anemia is multifactorial and may be motivated by oxidative stress induced by exercise, iron deficiency, gastrointestinal bleeding, hematuria and hemolysis being a result of continuous foot strokes and is not always related to the massive destruction of red blood cells (Chiu, et al., 2015; Robach, et al., 2014). Most studies on hematological parameters have been carried out with professional runners, whose characteristics may differ from those that define amateur runners (Ruiz-Vicente, et al., 2013), so it is interesting to know more precisely the variations of hematological parameters in amateur marathon and ultratrail runners. Knowing hematological deviations in non-professional runners may be helpful for runners themselves to improve their performance as well as for coaches and medical staff.

Objectives

The objectives were to determine the hematological values in amateur runners *pre* and *post* road marathon and *pre* and *post* ultratrail; to compare the within- and between-group hematological parameters obtained at two time points, and to determine the relationship between the hematological variables and age, sex, body mass index (BMI) and running time.

Methods

A descriptive and comparative observational study was performed. The hematological values were compared in two stages: *pre-* and *post-race* within each group and then between the groups.

Population of the road marathon group

The population of marathon runners were the participants in the *Trinidad Alfonso EDP Marathon*, held in the city of Valencia on November 20th, 2016 (N=18,800). They previously received a communication via email describing the characteristics of the study with the request for their participation. A total of 456 runners responded, all of them non-professional athletes. The sample was formed by the runners who voluntarily gave their explicit consent to participate in the study (n_1=103 runners) and who met the following inclusion criteria: being between 30 and 44 years of age, having a body mass index (BMI) between 16 and 24.99 kg/m^2, having previously completed a marathon with an accredited time: between 3 hours and 4 hours for men and between 3:30 hours and 4:30 hours for women, and having passed the medical tests prior to the study (electrocardiogram, blood pressure, echocardiography, and stress test). The following exclusion criteria were established: presenting any documented renal pathology, presenting any documented cardiological pathology or history of ischemic heart disease in first-degree relatives, and presenting any process that involved taking medication continuously.

Population of the ultratrail mountain group

The population of ultratail runners were participants in the *Penyagolosa Trails in the CSP* modality, of 107.4 km, with an uphill level of 5604 and downhill level of 4356 m respectively, which was held on May 9th, 2015 (N=600). They previously received a communication via email describing the characteristics of the study with the request for their participation. The sample was formed by all those who voluntarily gave their explicit consent to participate in the study (n_2=50 runners) and who fulfilled

the following inclusion criteria: having previously completed a race with a distance greater than 60 km and having passed the medical tests prior to the study (electrocardiogram and blood pressure). The following exclusion criteria were established: being under 18 or over 65 years of age, having been hospitalized during the previous year, presenting a history of cardiovascular or renal pathology, suffering from any type of neoplasia, inflammatory or infectious disease at the time of the study, having presented a hemorrhagic event or having received a blood transfusion in the previous four months or presenting a process that involved taking medication continuously.

The final sample size corresponded to a value of n=153 runners, resulting from the sum of the samples of both groups (n_1=103 marathon runners and n_2=50 ultratrail runners).

Procedure

The data were obtained in the *Trinidad Alfonso EDP Marathon*, which had 42 km and 195 metres of route on asphalt and an incline of between one and 27 metres above sea level, and in the *Penyagolosa Trails in the CSP* modality, which had a total distance of 115 km, with 5,439 metres of positive slope and 4,227 metres of negative slope. For the sample participants in both races, a survey was first conducted to know the training habits of each participant and their medical history (Hernando, et al., 2018). The BMI was determined with the data of height and body mass obtained by measuring with a SECA-123 stadiometer and a SECA-770 digital floor scale. Body mass of the participants was measured *pre-* and *post*-race.

For each participant, a total of two blood determinations were performed. Blood samples were extracted from an antecubital vein, first of the one and then of the other arm. The blood sample was collected in Vacutainer® tubes specific for this type of analyses and the Roche / Hitachi cobas c analyzer was used. The first extraction was made 24 hours before the race, at the Runner's Fair, and the next extraction was performed at the finish line. The blood samples were transported in cold medium, within two hours after the extraction. Samples were processed upon arrival at the *Hospital 9 d'Octubre de Valencia* laboratory, in the case of the marathon. The mountain race analyses were processed in the laboratory of the *Jaume I Hospital in Castellón*.

Variables

The independent variables were sociodemographic (age and biological sex) variables, biological (BMI, in kg/m^2) variables *pre-* and *post-test*, and the race time variable (which indicated hours invested in the race: from start to the finish line).

The dependent variables were the hematological parameters studied, including the red blood cell count, hematocrit value (Htc), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, platelets and platelet volume.

Data analyses

For the univariate analysis purposes, the quantitative and continuous variables were presented as means and standard deviations. Qualitative variables were expressed in frequencies and percentages. The Kolmogorov-Smirnov test showed no normal distribution of the variables, therefore bivariate analyses were performed using the Wilcoxon Signed-Rank Test to observe the intragroup differences between the values obtained at two time points for related samples; to compare the hematological parameters between the groups across both time points, the Mann-Whitney's U test was used for independent samples.

In both statistics, the strength of association was measured through the Cohen's d; being non-parametric statistics, the expression of Cohen's d for the Wilcoxon test was expressed in r_{bis} and for the Mann-Whitney's U test in d_{pppc2}.

The influence of the independent variables on the dependent variables in both groups was analyzed using multiple logistic regression. The analyzes were performed using IBM SPSS® version 25. A value of p<.05 was accepted as statistically significant.

Ethical considerations

The present study was approved by the Deontological Commission of the Jaume I University. The study has respected the Declaration of Helsinki (WMA, 2013) guidelines for studies with human beings. Random numbers were created from the bib number of each runner to anonymize personal data. The data is kept in archives protected by the principal investigator of the project at the Jaume I University.

The volunteers were informed of the objectives, risks, and benefits of the study, and signed an informed consent authorizing their participation. In the case of the marathon test, the study protocol was approved in May 2016. The project is registered in the ClinicalTrials.gov database with code NCT 03155633.

Results

A total of 119 runners took part in the study. The total sample consisted of 103 men (86.6%) and 16 women (13.4%), out of whom 84 (men 85.7% and women 14.3%) were marathon runners and 35 (men 88.6% and women 11.4%) ultratrail

runners. The mean age was 38.55±3.62 years for the marathon runners and 39.2±7.34 years for the ultratrail runners. The BMI at Runner's Fair was 23.03±1.68 kg/m² in the road marathon group, which at the finish line decreased to 22.6±1.52 kg/m². In the case of the ultratrail group, the mean BMI value was 24.09±2.38 kg/m², which decreased to 23.42±2.31 kg/m² after the race. The mean race time used to complete the 42 km of the marathon was 3hours:34minutes:54seconds (±20minutes:45seconds) versus the mean time of 22hours:25minutes:47seconds (±3hours:44minutes: 41seconds) that was required to complete the ultratrail. Table 1 shows the descriptive results of both races.

After the descriptive analysis, an intragroup comparison was performed using the Wilcoxon test. The results of analyzing the differences between the *pre-* and *post-race* hematological parameters for both groups are shown in Table 2.

Table 1. Descriptive results of blood tests for the road marathon and ultratrail groups

	Pre-marathon (n=84)	Pre-ultratrail (n=35)	Post-marathon (n=84)	Post-ultratrail (n=35)
	M±SD	M±SD	M±SD	M±SD
Red blood cells (10^12/L)	4.94±.34	4.79±.32	4.98±.34	4.79±.38
Hemoglobin (g/dL)	14.7±.93	14.36±1.08	14.84±1	14.43±1.25
Hematocrit (%)	45.25±2.57	41.93±2.62	45.07±2.74	41.33±3.09
MCV (fL)	91.75±3.73	87.78±30.04	90.6±3.72	86.42±4.28
MCH (pg)	29.77±1.4	30.04±1.58	29.82±1.39	30.14±1.61
MCHC (g/dL)	32.45±.75	34.22±.79	36.59±33.73	34.88±.98
RDW	13.16±.67	13.28±.74	12.95±.58	13.2±.8
Leukocytes (10^9/L)	9.04±11.29	6.56±1.76	17.36±3.94	13.42±2.69
Neutrophils (10^9/L)	4.12±1.33	3.69±1.37	14.48±3.66	10.14±2.54
Lymphocytes (10^9/L)	2.25±.67	2.05±.52	1.58±.60	1.88±.53
Monocytes (10^9/L)	.54±.15	0.58±.18	1.22±.38	1.30±.44
Eosinophils (10^9/L)	.19±.13	0.17±.14	.03±.04	0.04±.06
Basophils (10^9/L)	.03±.01	0.03±.01	.03±.01	0,02±.01
Platelets (10^9/L)	220.25±39.97	219.88±44.32	256.36±50.87	249.25±44.33
Platelet Vol. (fL)	11.4±.74	4.79±.32	11.59±.82	4.79±0.38

Note. Mean value expressed as (M). Value of the standard deviation expressed with (SD). MCV: mean corpuscular volume. MCH: mean corpuscular hemoglobin. RDW: red blood cell distribution width.

Table 2. Wilcoxon test

	Road marathon			Ultratrail		
Variable	Z	p	r_{bis}	Z	p	r_{bis}
Red blood cells	-1.110	.267	**.918**	-.303	.762	**.966**
Hemoglobin	-1.953	.051	**.816**	-.534	.594	**.878**
Hematocrit	-1.403	.160	**.893**	-1.696	.090	**.968**
MCV	-6.805	**<.0001*****	**.952**	-4.284	**<.0001*****	**.998**
MCH	-1.995	**.046***	**.892**	-1.356	.175	**.989**
MCHC	-6.522	**<.0001*****	**.812**	-4.154	**<.0001*****	.743
RDW	-5.647	**<.0001*****	.660	-2.353	**.019***	**.975**
Leukocytes	-6.859	**<.0001*****	**.993**	**-5.160**	**<.0001*****	**.833**
Neutrophils	-7.961	**<.0001*****	.692	-5.086	**<.0001*****	**1**
Lymphocytes	-6.505	**<.0001*****	.762	-2.154	**.031***	**.912**
Monocytes	-7.909	**<.0001*****	**.887**	-5.160	**<.0001*****	**.548**
Eosinophils	-7.945	**<.0001*****	.266	-5.072	**<.0001*****	.770
Basophils	-1.459	.145	.334	-.667	.505	.578
Platelets	-7.343	**<.0001*****	**.929**	-4.540	**<.0001*****	**.988**
Platelet Vol.	-3.033	**.002***	.608	-4.633	**<.0001*****	**.876**

Note. p value: p<.05*, p<.01**, p <.001*** / There is no difference if the value is between ±1.96.
Association power – Cohen's d values (r_{bis}): .2-.3 low / .5-.7 medium / >.8 strong
MCV: mean corpuscular volume. MCH: mean corpuscular hemoglobin. MCHC: mean corpuscular hemoglobin concentration. RDW: red blood cell distribution width.

In addition, the differences in both *pre-* and *post-race* hematological parameters between the ultratrail and marathon runners were studied using a Mann-Whitney's U test, and the results are represented in Table 3. The power of association of these differences was measured using Cohen's d in both intra- and inter-group comparisons.

Finally, a prediction map was generated using multiple linear regressions with the intro method to explain the differences found in the pre- and post-race hematolological parameters for runners of each type of race. A level of confidence was accepted (CI=95%). The integrated variables were age, sex, BMI, and running time. The last variable only took into account *post-race* values. The results are shown in Table 4.

Table 3. Mann-Whitney's U test

	Pre	Post	d_{ppc2}
Red blood cells	**.019***	**.021***	.119
Hemoglobin	.095	.157	.072
Hematocrit	**<.0001*****	**<.0001*****	.162
MCV	**<.0001*****	**<.0001*****	.054
MCH	.266	.284	-.034
MCHC	**<.0001*****	**<.0001*****	**4.558**
RDW	.392	.136	-.188
Leukocytes	**.036***	**<.0001*****	.152
Neutrophils	.055	**<.0001*****	**2.908**
Lymphocytes	.222	**.003***	-.792
Monocytes	**.949**	**.675**	.439
Eosinophils	.308	.605	-.225
Basophils	.647	.057	**.998**
Platelets	.984	.111	**1.09**
Platelet Vol.	**.001***	.241	.256

Note. p value: p<.05*, p<.01**, p<.001***. Association power – Cohen's d values (d_{ppc2}): .2-.3 low /. 5-.7 medium / >.8 strong. MCV: mean corpuscular volume. MCH: mean corpuscular hemoglobin. RDW: red blood cell distribution width. MCHC: mean corpuscular hemoglobin concentration.

Table 4. Results obtained by applying a prediction map

		Pre			Post	
	Test	p	R	Test	p	R
Hematocrit	M	**<.0001*****	**31.8%**	M	**<.0001*****	**31%**
	U	.076	19.6%	U	.414	11.9%
Red blood cells	M	.554	1.7%	M	.280	5.4%
	U	**.020***	**24.3%**	U	.387	10.4%
MCV	M	.344	4%	M	.563	3.6%
	U	.472	7.7%	U	.367	13%
MCHC	M	**.007***	**14.1%**	M	.404	4.9%
	U	.151	15.5%	U	.395	12.3%
Leukocytes	M	.853	1%	M	.215	7%
	U	**.021***	**26.6%**	U	.107	**21.8%**
Neutrophils	M	.481	3%	M	.358	5.3%
	U	**.028***	**25.2%**	U	.149	19.6%
Lymphocytes	M	.511	2.8%	M	.645	3.1%
	U	.121	16.8%	U	**.028***	**29.7%**
Platelet Vol.	M	.736	1.6%	M	.589	3.5%
	U	.635	5.3%	U	.635	5.3%

Note. p value: p<.05*, p<.01**, p<.001***. M = marathon. U = ultratrail.
MCV: mean corpuscular volume. MCHC: mean corpuscular hemoglobin concentration.

Discussion and conclusions

There are few studies that analyze hematological changes in medium- and long-distance runners in detail (Wu, et al., 2004). The results of this study allow us to describe the characteristics of hematological values in amateur marathon and ultratrail runners as well as to show which hematological values change between baseline (*pre-race*) and the finish line in both groups. The changes observed were similar in both the road marathon group and the ultratrail mountain group, although the conditions of both tests were different.

Regarding the red blood cells, no significant differences were evidenced. In the case of marathon runners, there was a slight increase, while in ultratrail mountain runners the values remained almost the same. Similar results were obtained by Minuzzi, Teixeira, Carvalho, da Costa, and Rama (2018), who also did not find significant differences in the values of Hb and Htc. The differences in hemoglobin and hematocrit were not significant, with a slight increase in hemoglobin values, while the hematocrit remained constant. These results were similar to those obtained by Clemente (2011) and Robach et al. (2014). Previous studies did determine significant changes between baseline and finish line conditions in the case of marathon runners; Duca et al. (2006) showed a significant increase in the post-marathon red blood cell count without presenting changes in hemoglobin values; Nuviala, Lapieza, Anson, Castillo, and Giner (1995) found significant increases in red blood cell count, hemoglobin and hematocrit. However, Sierra et al. (2019), in a sample of similar characteristics, found a significant reduction in the hematocrit value in marathon runners, and this reduction remained significant until 15 days after the race. Kratz, Wood, Siegel, Hiers, and Van Cott (2006) and Sánchez-González et al. (2003) found an increase in hemoglobin, which they associated with hemoconcentration, and they described an increase in all the variables studied. Rama et al. (2016) and Wu et al. (2004) also showed a decrease in erythrocyte count, hematocrit and hemoglobin at the end of the race in the mountain context.

As for the mean corpuscular parameters, significant differences were found between both times under the study and between both race modalities, with the results showing a strong association power. The MCV was reduced after the effort, as in previous studies (Kratz, et al., 2002, 2006; Ruiz-Vicente, et al., 2013), and this difference could be the product of oxidative stress associated with great efforts that generated alteration of cell homeostasis secondary to dehydration, motivating a decrease in cell size (Ruiz Vicente, et al., 2013; Wu et al., 2004). However, previous studies showed that the MCV was increased after a marathon race (Kratz, et al., 2002; Remacha, 1992; Sánchez-González, et al.,

2003). It should be noted that previous studies with mountain runners did not show significant changes in any of the corpuscular values according to Wu et al. (2004), or showed that the values of MCV and MCH remained constant (Rama, et al., 2016; Robach, et al., 2014).

Regarding MCH, significant alterations were only found in the marathon group, remaining unchanged in the ultratrail runners. Coinciding with Wu et al. (2004), the MCHC increased significantly in the *post-race* determination in both groups. Rama et al. (2016) associated this increase with hemoconcentration due to a loss of intracellular fluid in erythrocytes. The fact that both the size and the corpuscular volume were reduced, in the case of the red series cells, coincides with a decreased RDW value, unlike in the study by Robach et al. (2014). Interestingly, literature supports that a normal or decreased value of RDW is associated with a lower risk of inflammatory disease (Horta-Baas & Romero-Figueroa, 2019). However, and despite the differences found, athletes adapt to such changes without detriment (Rama, et al., 2016).

With regard to platelets, these significantly increased at the end of both races as well as their volume, with the difference associated with ultratrail athletes being greater and with a strong size effect. Similar results were evidenced in previous literature, with an increase in absolute platelet values, both in road and mountain runners, regardless of the distance (Kratz, et al., 2002; Lippi, et al. 2010; Minuzzi, et al., 2018; Rama, et al., 2016; Remacha, 1992; Ruiz-Vicente, et al., 2013; Sánchez-González, et al., 2003; Smith, Garbutt, Lopes, & Pedoe, 2004; Wu, et al., 2004). In a sample of 99 runners, Schwarz el al. (2018) obtained similar results to this study; in the *post-race* evaluation, leukocytosis, the increase in platelet count together with a reduction in lymphocytes were significant. These results could be associated with an acute response to tissue injury secondary to strenuous exercise and direct mechanical stress caused by the continuous impacts against the ground throughout the test (Clemente, 2011; Kratz, el al., 2006; Rama, et al., 2016; Ruiz-Vicente, et al., 2013).

Tissue damage was manifested by leukocytosis at the expense of the increase in absolute neutrophil values in both the road and mountain runners. The results were in accordance with previous studies, as both the marathon and the ultratrail are conditioned by an inflammatory process at a systemic level, without the need for the presence of a pathological factor (Coso, et al., 2012; Duca, et al., 2006; Kratz, et al., 2006; Lippi, et al., 2010; Neubauer, Reichhold, Nersesyan, Konig, & Wagner, 2008; Rama, et al., 2016;; Remacha, et al., 1992; Ruiz-Vicente, et al., 2013; Smith, et al., 2004; Wu, et al., 2004). The decrease in eosinophils presented in both race modalities and the decrease in monocytes in the

ultratrail runners could be related to muscle damage and an increase in the secretion of adrenal hormones such as cortisol (Henson, et al., 2019; Rama, et al., 2016; Sánchez-González, et al., 2003). A significant increase in the absolute values of lymphocytes and monocytes has been determined, while basophils have not changed after exertion.

In general, the changes observed in both groups are similar at the hematological level, despite the differences in the characteristics and conditions of the races. When comparing the baseline conditions of both groups of athletes, significant differences were found in the value of hematocrit, red blood cells, MCV, MCHC, total leukocyte value and platelet volume, with a weak association power except for MCHC. At the end of the tests, the same prior differences were maintained to which changes in neutrophils were added, responsible for the early and rapid response to situations of muscle injury and, less significantly, in lymphocytes. The runners, after the completion of 42 km on road or 115 km on mountain, finished with the same values in terms of platelet count and volume. It should be noted that the power of association was mild, despite having a reliable significance.

The regression model explained that age, sex, and BMI determine some of the differences found in the *pre-race* evaluation in the case of marathon runners, affecting baseline hematocrit and MCHC. In the *post-race* evaluation, by adding the running time, the model explained only the changes in the hematocrit value. In the case of ultratrail runners, before starting the test, the variability was given by the same factors for the red blood cells, the MCHC and in the absolute values of leukocytes and neutrophils. At the end of the 115 km, lymphocytes were determined by sex, BMI, and age in approximately 30% of cases. It is necessary to continue researching in this field in order to obtain more conclusive results.

In conclusion, the existence of significant differences between the baseline and *post-race* conditions of the runners has been evidenced, in both the road marathon and ultratrail mountain runners. They were similarly affected, with special interest drawn to the changes in corpuscular values and in the white series. Furthermore, the comparison between the groups, at the *pre-race* time, started with baseline differences in red blood cells, hematocrit, MCV, MCHC, platelet volume and leukocyte count in favor of marathon runners. At the end of both tests, the athletes maintained the previous differences and added significant differences in the absolute values of neutrophils and lymphocytes.

Finally, it has been determined that the biological sex, age, BMI, and running time only explain the differences in hematocrit (*pre-* and *post-race*) and MCHC (at baseline) of marathon runners, while in mountain athletes, they explain the changes in red blood cells, leukocytes and neutrophils in the baseline conditions and the variation in lymphocytes at the end of the test.

Limitations of the present study were related to the differences between the two races and the physical and training differences of the respective runners. Regarding the aspects to be improved for future studies, the fact of quantifying dehydration and hemoconcentration should be included as well as monitoring the presence of hemolysis.

It should be noted that these data could be of help at a practical level for the interpretation of analytics in endurance athletes after prolonged efforts, more adjusted to reality than the reference values indicated for the general population. Knowing these, hematological changes may help the runner training and prevent risks associated with sports of a high physical demand.

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