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Original Article

Multivariate analysis by exploratory machine learning model indicates orienteering race as an immunometabolically safe stimulus, but with differences between age groups

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Abstract:
Orienteering running, a sport that over time has been associated with bone and joint injuries but which today is known to present no more significant problems than other running sports, fully develops the individual, as it presents stimuli that are not only physical but also other sports, but cognitive and mental. A sportomic and multi-analysis study aimed to investigate the immunometabolic impact generated, comparing its results in two different age groups. Twelve male individuals participated, three adolescents and nine adults, all practitioners of the method. Venous blood was collected before and immediately after testing in a real competition. In relation to the hydration status, mild dehydration was indicated in the adult group and mild hydration in the adolescent group. With regard to renal function, the impact was higher in the adult group, with a statistical difference in this biomarker. Energy metabolism showed a greater demand for triglycerides in the adult group, similar alterations in glycemia, a greater formation of urate in the adult group, a greater decrease in insulin concentrations also in the adult group, with higher values in the group of adults. adolescents only for lactate. A possible greater myolysis was observed, due to the acute increase in LDH in the adult group, with an increase in cortisol for the same group, different from the decrease shown by adolescents. Total leukocytes, phagocytes there was a greater acute impact in the group of adolescents, which was also observed for lymphocytes and eosinophils, but with an antagonistic behavior for the platelets.
Key-Words: Data mining, Machine Learning, Sports Medicine, Physical Education.

Introduction

Orienteering, historically associated with bone and joint injuries (Fong et al., 2007), does not show higher injury rates than other sports involving running. Recently, it has been pointed out as an excellent model of exercises with combined cognitive and physical stimulation, the complete sport in all age groups (Östlund-Lagerström et al., 2020). Nowadays, this popular modality of physical education has become an emerging sport that integrates knowledge, competition, fun, and collaborative attributes already applied to students, from basic to higher education around the world, with several benefits acquired over time term (Li, 2021; Bao et al., 2022; Turkmen & Bicer, 2022). The objective of the present study was, based on sportomics, cross-sectional, descriptive, observational, and multi-analysis study, to identify the acute immunometabolic impact of an orienteering running session in male individuals divided into two groups, adolescents and adults.

Material & methods

This is a cross-sectional, descriptive, and observational study based on a sportomics strategy with young and adult practitioners of orienteering running.
Subjects The convenience sample consisted of 12 male individuals divided into two groups, adults (n = 09) and adolescents (n = 03).
Experimental Design Blood samples were collected at two times:
Pre - immediately before the test; Post – immediately after the end of the test.

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Venous blood was collected, obtained from perforation with a needle and device for vacuum collection in the median cephalic vein, collected in three different types of tubes, the first containing EDTA, which is for hematology, and the second in a tube without anticoagulant and without clot-forming gel for biochemical analyzes using serum and the third with sodium fluoride for blood glucose and lactate, by qualified and trained professionals, under the supervision of those responsible for the study.

Complete blood count was performed by automation, triglycerides by enzymatic method-Trinder, cortisol, myoglobin, and insulin by ELISA, urea, AST, and ALT by UV kinetic method, creatinine, total proteins, glucose, LDH, lactate, and urate by UV colorimetric method.

Statistical Analysis

Initially, descriptive statistics were performed on the data, with measurements of position (mean, median, mode, and percentiles) and dispersion (amplitude, variance, standard deviation, and standard error). Afterward, the univariate analysis of these data was performed using the Shapiro-Wilk normality test (because the sample was smaller than 30 individuals). The equal variance test would be applied if the Shapiro-Wilk test presented a result indicating normal distribution ($P>0.05$). For results with $P>0.05$, the paired T-Student test would follow; if $P\leq0.05$, the paired T-Student test would follow the non-parametric Mann-Witney test. If the Shapiro-Wilk test presented a result indicating non-normal distribution ($P\leq0.05$), the non-parametric Mann-Witney test would be applied directly.

Still, in the phase of the univariate analysis, the analysis of repeated measures ANOVA One Way dependent was performed because they were the same individuals in different conditions and moments. At the end of the univariate analysis phase, it became clear that individuals (male and female) could not be evaluated together to investigate the placebo effect (Figure 1). So, for a better interpretation of the data, the individuals were divided into two groups according to their sex.

Then, the calculation of percentage variation was applied:

$$\Delta\% = \frac{(Final\ value - Initial\ value)}{Initial\ value}\ x\ 100$$

Cohen's equations (Cohen, 1992) were used to calculate the effect size for all variables to obtain Cohen d and r values:

$$d = \frac{M1 - M2}{\sqrt{\frac{SD1^2 + SD2^2}{2}}}$$

$$r = \frac{d}{\sqrt{((D^2)+4)}}$$

Where, M represent the means of observations and SD their respective standard deviations.

Table 1. Effect size values and their interpretation.

Effect	Small	Medium	Large
R	**0.10**	**0.30**	**0.50**
D	**0.20**	**0.50**	**0.80**

Adapted from Cohen (1992).

Next, multivariate data analysis was performed using data mining and machine learning techniques. In this phase, in order to seek a bivariate measure between the data, because the observations contain quantitative values, the Pearson and Spearman correlation tests were applied, with the Spearman correlation being used for a visual analysis using the heat map strategy and the Pearson test as an initial measure for the following machine learning analyses. As exploratory models of machine learning: CLUSTER - Classical Clustering (Agglomerative Hierarchical Method) and Nearest neighbor (single linkage); ORDINATION – Principal component Analysis (PCA) and Correspondence Analysis (CA).

The Z score was previously applied because the observations contained non similar measurement units. SigmaPlot 14.5 (Academic Perpetual License - Single User – ESD Systat® USA) and, Past 4.03 (Free version for Windows) were used to carry out the different statistical tests and produce the graphs.

Ethical Approval

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and the experiment met the requirements of research using human subjects (National Health Council, 2012). This study was approved by the Ethics and Research Committee (number 2,230,073) of the Federal University of Mato Grosso (UFMT) and was registered at clinicaltrials.gov (NCT 03522883). The individuals were informed that they could withdraw from the study at any time. Written informed consent was obtained from each subject, who was instructed on the nature of the research and the procedures involved.

Results

Table 2 revealed the percentage variations between the pre and post exercise times, as well as Cohen's r and d value for the comparison between the same times. In this, it was possible to observe that in relation to the hydration status, predicted by the behavior of erythrocytes, hemoglobin and hematocrit, mild dehydration was indicated in the adult group and mild hydration in the adolescent group, but both without statistically significant difference.

Table 2. Percent change, Cohen's d and r (effect size).

Variable	**Practicing Adults**			**Practicing Teenagers**		
	Δ %	**d Cohen**	**r Cohen**	**Δ %**	**d Cohen**	**r Cohen**
Erythrocytes	0.85	0.26	0.13	-0.89	0.61	0.29
Hemoglobin	0.21	0.06	0.03	-0.86	0.54	0.26
Hematocrit	0.24	0.06	0.03	-1.29	0.97	0.44
VCM	-0.61	0.21	0.10	-0.42	0.58	0.28
HCM	-0.57	0.21	0.10	0	0	0
CHCM	-0.03	0.02	0.01	0.20	0.12	0.06
RDW	-0.85	0.17	0.08	-1.57	0.87	0.40
Leukocytes	15.27	0.98	0.44	44.30	1.32	0.55
Rods	21.09	0.43	0.21	65.17	1.34	0.56
Segmented	23.13	0.81	0.37	68.83	1.63	0.63
Neutrophils	23.06	0.80	0.37	68.70	1.63	0.63
Eosinophils	-16.51	0.20	0.10	-25.15	0.39	0.19
Monocytes	2.76	0.12	0.06	43.00	0.99	0.44
Lymphocytes	7.08	0.22	0.11	18.80	0.36	0.18
Platelets	16.81	1.68	0.64	7.55	0.45	0.22
Triglycerides	-29.57	0.40	0.19	9.00	0.23	0.11
Glycemia	-0.71	0.03	0.02	-1.74	0.23	0.11
AST	6.33	0.22	0.11	4.17	0.28	0.14
ALT	-1.25	0.02	0.01	0	0	0
Total PTNs	5.73	0.86	0.39	0.82	0.52	0.25
Urea	7.24	0.28	0.14	7.27	0.15	0.08
Creatinine	25.40	1.81	0.67	9.23	0.77	0.36
Urate	11.53	0.76	0.35	0.67	0.38	0.19
LDH	21.50	1.22	0.52	8.75	0.60	0.29
Lactate	369.59	5.18	0.93	500.00	3.47	0.87
Cortisol	34.68	0.72	0.34	-14.41	0.30	0.15
Insulin	-58.27	0.89	0.41	-34.00	0.43	0.21

With regard to renal function, the impact was similar on urea, which can be explained by the fact that this biomarker requires a longer time to change, but in relation to creatinine, they were higher in the adult group, with a statistical difference in this biomarker.

Liver function showed no difference in both groups.

Energy metabolism, on the other hand, showed a greater demand for triglycerides in the adult group, similar alterations in glycemia, a greater formation of urate in the adult group, a greater decrease in insulin concentrations also in the adult group, with higher values in the group of adults. adolescents only for lactate, perhaps due to a slower speed in its removal by the Cori cycle in this group still in formation. With regard to stress, a possible greater myolysis was observed, due to the acute increase in LDH in the adult group, with an increase in cortisol for the same group, different from the decrease shown by adolescents. Finally, regarding the immune system, total leukocytes, phagocytes (neutrophils, segmented, rods and monocytes) there was a greater acute impact in the group of adolescents, which was also observed for lymphocytes and eosinophils, but with an antagonistic behavior for the platelets. Considering only the biomarkers with the statistical difference found by the univariate tests, in both groups, there was an increase in formation and lactate, with an increase only in the group of adults for platelet creatinine and LDH (Figure 1).

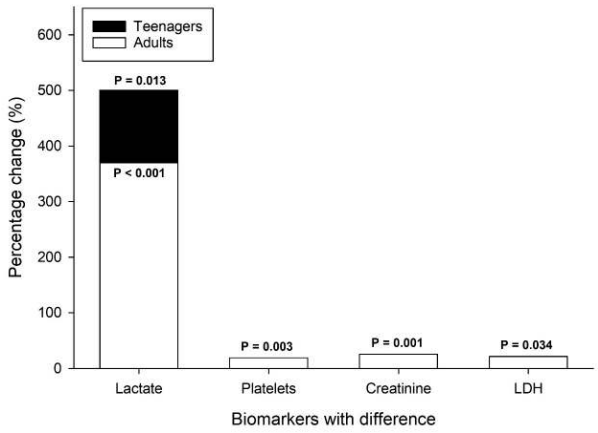

Figure 1. Exercise caused similar energy stress between groups (lactate) but higher on platelet de-margination, renal function (creatinine), and myolysis (LDH) in adults.

As the study variables had different units of measurement and magnitudes, the calculation of the Z Score was applied before carrying out the multivariate analyses, aiming at standardizing the observations.

To provide a holistic and integrated evaluation of the data, avoiding the dogmas and paradigms presented by the univariate analysis, Pearson's correlation coefficient was initially calculated to correlate the behavior of the variables so that the results were presented with variation between the highest negative correlation (-1) in shades of red and the highest positive (1) in shades of blue, these being represented by a heat map with the intensity (Correlation coefficient) being represented by the size of the circles and placing within of a square the correlations with the value of P<0.050 (Figure 2).

A **B**

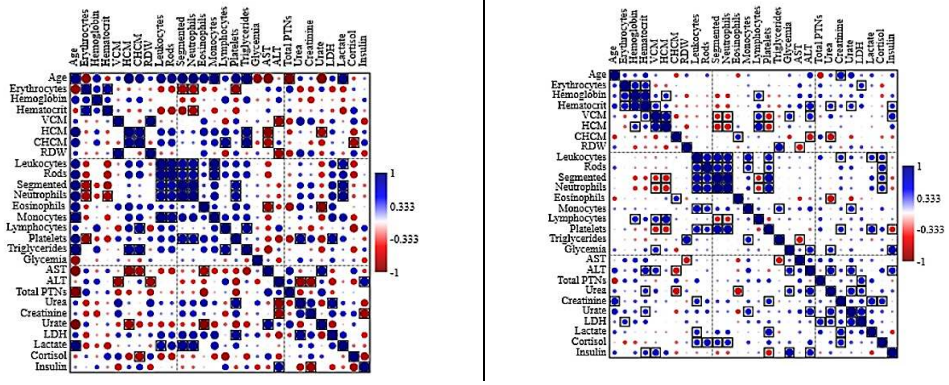

Figure 2. Pearson Correlation (In the box P<0.050). {A - teenagers; B - Adults}.

Corroborating and giving robustness to the previous findings, it was possible to observe in Figure 2 a similar behavior between the groups for total leukocytes and phagocytes, with differences in red cells, platelets, lymphocytes, insulin, cortisol, lactate, LDH, and other markers between the study groups.

In order to seek similarity between the behaviors of the variables, the Euclidean similarity index was initially calculated, with its findings represented in three different ways, through classical clustering (Figure 3), with the neighbor-joining clustering strategy (Figure 4), and with the network plot and Fruchterman-Reingold algorithm (Figure 5).

A **B**

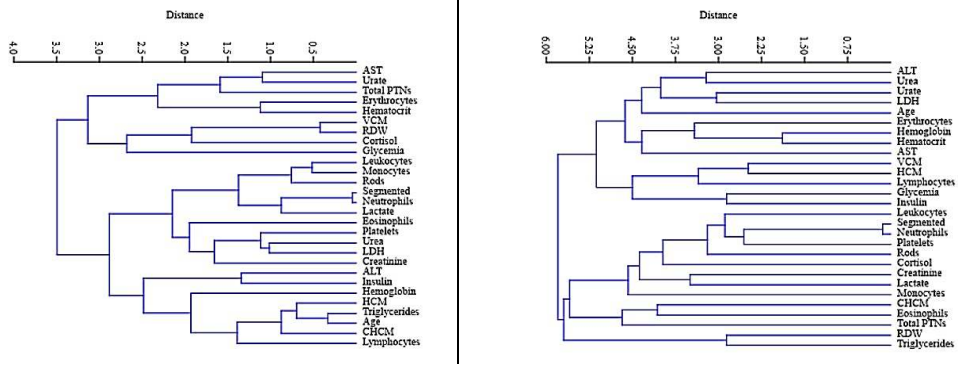

Figure 3. Classical Clustering dendrogram with Euclidian similarity index {A - teenagers; B - Adults}.

A **B**

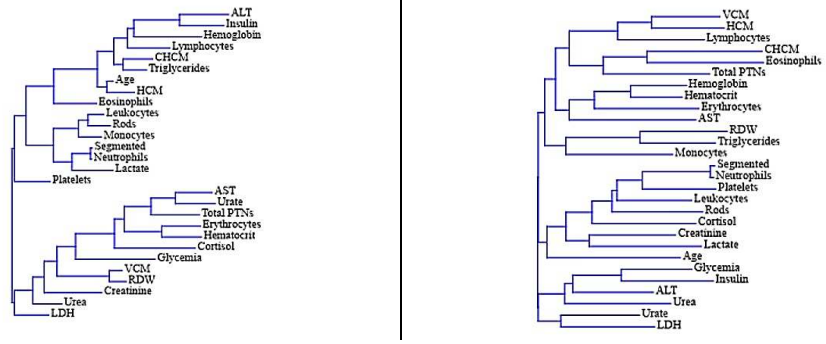

Figure 4. Neighbor joining clustering dendrogram with Euclidian similarity index {A - teenagers; B - Adults}.

A **B**

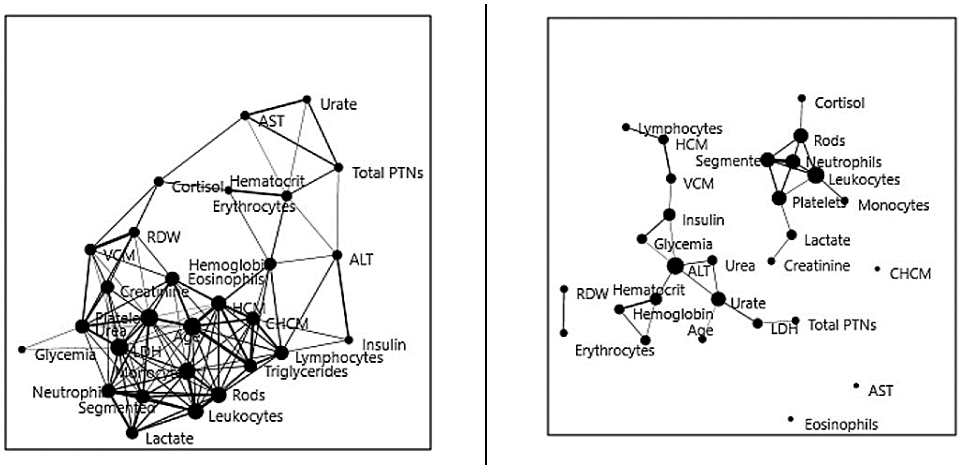

Figure 5. Network plot (Fruchterman-Reingold Algorithm) and Euclidian similarity index {A - teenagers; B - Adults}.

The correlations and similarities presented in the figures will be discussed in the sequence of the work.

Discussion

The present sportomics, holistic and integrated study follows the model previously presented (Gonçalves et al., 2012; Gonçalves et al., 2022) and improved for the problem in question. Cluster analysis was applied to group the observations into groups that are homogeneous among themselves and heterogeneous among groups through an agglomerative hierarchical method, where the number of clusters was defined throughout the analysis, and the scheme used was the nearest neighbor with a single linkage.

Regarding the unsupervised and exploratory model of machine learning, a principal component analysis (PCA) was used. Initially, Pearson's correlation coefficient for PCA feasibility is calculated, correspondence analysis (CA) is applied, a valuable data science visualization technique to discover and display the relationship between the categories. The differences between the groups concerning the immune system can be explained by a better efficiency of this system in adults, which is impaired by the arrival of old age, but improved by physical activity and eating habits (Weyh et al., 2020). Higher metabolic efficiency in adults could also explain the higher lactate concentration in adolescents. And also, the differences in blood glucose and insulinemia.

Glycemic control is essential for homeostasis (Beylot, 1996). Increased blood glucose signals the pancreas to increase insulin secretion, which promotes the opening of glucose transporters, with glucose uptake by cells (Thorens & Mueckler, 2010). When metabolized, pyruvate is formed, which can be used as a substrate for the pyruvate dehydrogenase complex, creating acetyl-coenzyme A, being oxidized following the Krebs cycle, or following other paths such as lipogenesis (Fuller & Kim, 2021), or it will be used as a substrate for the enzyme lactate dehydrogenase (LDH), receiving two protons from the environment and forming lactate for cell Ph homeostasis (Market, 1984). During exercise, there is an increase in energy demand with the degradation of glucose to form energy. This demand promotes an accumulation of protons in the intracellular environment, requiring a greater formation of dose-dependent lactate (intensity or duration-dependent) that will be released in the blood (Brooks, 2018).

This lactate formed and available in the blood will be again converted into glucose in the liver and will return to the blood, this conversion being through a metabolic pathway known as the Cori cycle (Cori & Cori, 1946; Schwartz, 1976; Hoffer, 1990).

In this way, the exercise may have been more intense for adolescents, or the Cori cycle was more efficient in the adult group. Since the concentrations of cortisol and LDH may point to greater stress in the adult group. And added to the fact that adults have shown greater de-margination of platelets and greater accumulation of plasma creatinine.

Conclusions

The present sportomics and multi-analysis study presents, in addition to reference values for different biomarkers in male individuals who practice orienteering, it also presents the impact caused by this modality in adolescents and adults, in addition to pointing out the immunometabolic safety for the insertion of methods in schoolchildren, as it is an excellent cognitive and physical stimulus, in addition to presenting sensorimotor challenges for practitioners with future adaptations, not only physical but cognitive and social.

Limitations

Because a convenience sample was chosen, and after applying the inclusion and exclusion criteria, there was a reduction in the sample size. However, the results are highly relevant with the advent of selected statistical methods.

Practical applications

The orienteering run can be applied and developed in adolescents and schoolchildren, with metabolic impacts even lower than those generated in adults, attributing safety to the method.

Competing interests

The authors declare that they have no competing interests.

Financial competing interests

The authors declare that they have no financial competing interests.

Authors' contributions

MKMG, MVAV, RLN, AMMN and LCOG: essential contributions to the conception and design of the study protocol; acquisition, analysis and interpretation of data; and involvement in drafting of the manuscript. MKMG, MVAV, LSM, ASG, NCSF, RLN, AMMN and LCOG: critical revisions for important intellectual content. All authors read and approved the final manuscript.

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Data availability All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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Supp. Table 1. Descriptive statistics (position and dispersion measures) of minors.

Variable	Pre exercise							Post exercise						
	X	Me	Mo	S^2	S	E	CV	X	Me	Mo	S^2	S	E	CV
Erythrocytes	5,3	5,2	A	0,0037	0,06	0,03	1,16	5,2	5,2	A	0,0081	0,09	0,05	1,73
Hemoglobin	15,5	15,5	15,5	0,0033	0,06	0,03	0,37	15,4	15,2	15,2	0,1200	0,35	0,20	2,25
Hematocrit	46,2	46,4	A	0,4433	0,66	0,38	1,44	45,6	45,8	A	0,3233	0,57	0,33	1,25
VCM	87,9	87,8	A	0,5700	0,75	0,44	0,86	87,5	87,7	A	0,2233	0,47	0,27	0,54
HCM	29,5	29,7	A	0,1433	0,38	0,22	1,28	29,5	29,7	A	0,1433	0,38	0,22	1,28
CHCM	33,7	33,9	A	0,2800	0,53	0,30	1,57	33,8	33,8	A	0,3033	0,55	0,32	1,63
RDW	12,7	12,7	A	0,0633	0,25	0,14	1,98	12,5	12,6	A	0,0433	0,21	0,12	1,66
Leucocytes	5089	5620	A	1.306	1143	659	22,46	7343	7950	A	4.515.625	2125	1227	28,95
Rods	89,0	112,0	A	1.957	44,24	25,54	49,70	147,0	159,0	A	1789	42,30	24,42	28,77
Segmented	2521	2700	A	211.600	460	266	18,27	4256	3436	A	2.062.096	1436	829	33,74
Neutrophils	2610	2817	A	253.009	503	290	19,30	4403	3578	A	2.149.156	1466	846	33,30
Eosinophils	214,6	176,0	A	26.399	162,48	93,81	75,69	161,0	159,0	A	12.434	111,51	64,38	69,40
Monocytes	387,7	411,0	A	5884	76,70	44,29	19,79	554,3	636,0	A	51.012	225,8	130,4	40,74
Lymphocytes	1873	1798	A	311.140	557,8	322,1	29,78	2225	2002	A	1.575.025	1255	725	56,44
Platelets	256,0	250,0	A	1.183	34,39	19,86	13,43	275,3	254,0	A	2.457	49,57	28,62	18,00
Triglycerides	103,7	114,0	A	1.340	36,61	21,14	35,32	113,0	108,0	A	1.999	44,71	25,81	39,57
Glycemia	96,0	93,0	A	49	7,00	4,04	7,29	94,3	91,0	A	57	7,57	4,37	8,03
AST	24,0	22,0	A	19	4,36	2,52	18,16	25,0	24,0	A	7	2,65	1,53	10,58
ALT	27,0	26,0	A	73	8,54	4,93	31,64	27,0	26,0	A	91	9,53	5,50	35,33
Total PTNs	8,1	8,1	A	0,02	0,15	0,09	1,88	8,2	8,2	A	0,01	0,10	0,06	1,22
Urea	18,3	17,0	A	65	8,08	4,67	44,09	19,67	16,00	A	82	9,07	5,24	46,14
Creatinine	0,87	0,87	A	0,01	0,10	0,06	12,12	0,95	0,99	A	0,01	0,10	0,06	10,79
Urate	5,2	5,2	A	0,81	0,90	0,52	17,31	5,3	5,6	A	1,24	1,11	0,64	19,95
LDH	243,7	236,0	A	856	29,26	16,89	12,01	265,0	255,0	A	1.675	40,93	23,63	15,44
Lactate	1,87	1,90	A	0,02	0,15	0,09	8,18	11,20	9,80	A	14,4	3,80	2,19	33,92
Cortisol	11,3	14,0	A	42,5	6,52	3,77	57,55	9,70	8,10	A	17,9	4,23	2,44	43,64
Insulin	19,90	9,20	A	400	20,02	11,56	100,6	13,13	9,70	A	88,9	9,43	5,44	71,81

X = average; Me = median; Mo = mode; S2 = variance; S = standard deviation; E = standard error; CV = Coefficient of variation; A = a modal.

Supp. Table 2. Descriptive statistics (position and dispersion measures) of adults.

Variable	Pre exercise							Post exercise						
	X	Me	Mo	S^2	S	E	CV	X	Me	Mo	S^2	S	E	CV
Erythrocytes	5,33	5,34	A	0,037	0,19	0,06	3,59	5,38	5,42	A	0,024	0,15	0,06	2,89
Hemoglobin	15,6	15,6	A	0,366	0,60	0,21	3,88	15,6	15,5	15,2	0,282	0,53	0,18	3,40
Hematocrit	46,2	45,9	A	3,525	1,88	0,62	4,07	46,3	45,7	45,7	2,44	1,56	0,52	3,38
VCM	86,6	86,9	A	6,315	2,51	0,84	2,90	86,1	85,9	A	6,92	2,63	0,87	3,05
HCM	29,3	29,1	29,1	0,575	0,76	0,25	2,59	29,1	29,3	29,9	0,68	0,83	0,27	2,84
CHCM	33,8	34,1	34,3	0,352	0,59	0,20	1,75	33,8	33,8	33,3	0,29	0,54	0,18	1,60
RDW	13,1	12,9	12,9	0,461	0,68	0,23	5,19	13,0	12,9	13,1	0,43	0,66	0,22	5,06
Leucocytes	7527	7380	A	1.056.784	1028	342	13,66	8677	8700	A	1.708.846	1307	435	15,06
Rods	138,0	141,0	A	4.485	66,98	22,32	48,53	167,1	174,0	174	4.807	69,34	23,11	41,49
Segmented	4280	4334	A	1.129.969	1063	354	24,85	5269	5309	A	1.864.162	1365	455	25,91
Neutrophils	4418	4413	A	1.236.544	1112	370	25,18	5437	5493	3520	2.013.103	1419	472	26,09
Eosinophils	225,4	177,0	A	34.848	186,7	62,2	82,80	188,2	121,0	87	36.743	191,7	63,9	101,8
Monocytes	612,1	602,0	A	21.089	145,2	48,4	23,72	629,0	612,0	A	18.550	136,2	45,4	21,65
Lymphocytes	2269	2140	A	257.475	507,4	169,1	22,36	2430	2349	A	782.064	884,3	294,8	36,39
Platelets	272,3	270,0	A	532	23,08	7,69	8,47	318,1	309,0	336	944	30,73	10,24	9,66
Triglycerides	233,0	181,0	A	54.419	233,3	77,7	100,1	164,1	147,0	A	5.056	71,10	23,70	43,33
Glycemia	109,5	100,0	100	789	28,10	9,37	25,65	108,8	106,0	98	259	16,10	5,37	14,80
AST	26,7	25,5	36	43	6,58	2,32	24,61	28,4	28,0	A	74	8,64	2,88	30,40
ALT	29,8	25,5	A	332	18,20	6,44	61,05	29,5	23,5	19,0	378	19,46	6,88	65,96
Total PTNs	7,75	7,80	A	0,19	0,43	0,14	5,58	8,20	8,10	A	0,34	0,59	01,9	7,16
Urea	32,2	31,0	A	77	8,82	2,94	27,40	34,5	34,0	34,0	59	7,68	2,56	22,23
Creatinine	0,98	1,01	0,96	0,01	0,11	0,04	11,82	1,22	1,15	A	0,02	0,16	0,05	12,66
Urate	6,23	6,30	5,7	0,72	0,85	0,28	13,61	6,95	6,80	A	1,07	1,04	0,35	14,92
LDH	192,2	184,0	A	764	27,65	9,22	14,38	233,5	216,0	200,0	1.543	39,28	13,09	16,82
Lactate	2,44	2,20	A	0,42	0,65	0,21	26,44	11,48	11,50	A	5,67	2,38	0,79	20,75
Cortisol	12,3	12,3	A	14	3,75	1,25	30,42	16,6	16,1	A	57	7,57	2,52	45,54
Insulin	20,6	15,5	7,3	337	18,38	6,11	88,97	8,6	7,6	A	24	4,95	1,65	57,51

X = average; Me = median; Mo = mode; S2 = variance; S = standard deviation; E = standard error; CV = Coefficient of variation; A = a modal.