

January 7, 2013

Dear Editors,

Please find enclosed the edited manuscript in Word format (file name: ESPS Manuscript NO: 1432)).

Title: Effects of exercise on leukocytosis and blood hemostasis in 800 healthy young females and males.

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Name of Journal: World Journal of Experimental Medicine

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The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Reviewer # 1

1) *The variability in findings may be attributable other than to the intensity and duration of the exercise protocols used, age and gender, also if not mainly, to the initial fitness levels of the people studied. The authors should give this information and analyze based on this evidence.*

All subjects were asked to evaluate their own fitness level ranking from 1-4, where 1 indicates lack of training and 4 was athletic (training sports on national level). Only 4 subjects in total reported fitness level 1, and that group was not further analyzed. Analyzing data based on fitness level showed no significant results on leukocytosis either after running or after cycling. The only significant differences found was a lower number of WBC's circulating in resting situation between members of group 4 as compared to those designated in group 2 (mean  $5.8 \times 10^9/L$  for group 4 and  $6.6 \times 10^9/L$  for group 2) and also a larger change after-before for group 4. This was true for both for subjects whether running or cycling. For those who cycled there was significantly higher change in heart rate after exercise for those in groups 3 and 4 as compared to those in group 2. No significant differences were found when APTT or D-dimer was analyzed based on fitness levels.

We have added this comment to the results section. Also, a new table 2 was added to showing these findings.

2) *Comparison was done among healthy individuals performing two different kinds of exercise. However, It is not clear why blood samples were not collected also soon after exercise in the first group, and after resting in the second group.*

Previous studies have investigated leukocytosis and found that in the two exercise setups the findings are more pronounced after resting for the first group and soon after exercise for the second group (Ref. 1-6). Our aim was to examine the hematological and hemostatic characteristics at these selected moments in a large group of healthy subjects.

3) *It is not clear whether all laboratory exams (Hematological tests, Coagulation tests, and Sedimentation Rate) were executed in all cases. In this respect the authors indicated 800 subjects studied in the introduction section, however in the results section a total number of 795 females and 506 males were examined for SR and, finally, in the research frontiers section the authors stated that their large study involved more than 1200 healthy females and males; 800 of them were included in the evaluation. Please clarify in a table the exact number of cases entered this study, giving the exact information on sex, age and blood exams done. Sedimentation rate was tested on all individuals involved in the project, including those that were not evaluated further. Hence, not all individuals were tested for hematological and coagulation tests. Age was only collected for the complete class together with SR values. Table 1 with an overview of individuals involved in the study has now been added to the manuscript.*

4) *The median age was 23 years. Results should be given also on the basis of age and comparative figures or tables should be done, i.e. cases <23y versus cases > 23y. Are there any difference?*

We did not collect age specifically for the subjects who exercised. This was partly done to ensure anonymity.

5) *It could be of interest to show data as fold increase (post exercise/basal) for a clearer wbc count modification of each single case. Also, how many cases exceeded normal values? In the group running for 1 h and resting for 3 h, 83 subjects had a total amount of WBC higher than normal value ( $> 11 \times 10^9/L$ ). In the other group, 5 minutes after cycling, we observed that 65 subjects had a total amount of WBC higher than normal value.*

A new figure 4 shows this increase.

6) *Is there any relationship between heart rate and blood tests?*

There was a significant ( $P < 0.0001$ ) relationship among changes in number of WBC's and heart rate (New Fig. 5). Linear regression heart rate and PCV for the subjects cycling for 5 minutes, shows a significance of  $P=0.0048$ .

1- *The authors must to explain, from a rational point of view (based on scientific literature), what are the expected differences among the gender, concerning the proposed study.*

A sentence regarding this point has been added to the introduction.

2- *The inclusion and/or exclusion criteria must be included. How was the lifestyle of the included subjects? The diet? The use of any medication? Please, address this. The only inclusion criteria were that the students volunteered to perform the exercise protocol as instructed, and SR within normal values.*

We have added to the manuscript a new Table 1 to show the numbers of individuals and the sort of exercise they performed to clarify this point. The fitness level was the only lifestyle information given and this has been added as Figure 1. As indicated all subjects were asked to evaluate their own fitness level ranking from 1-4, where 1 indicates lack of training and 4 was athletic (training sports on national level). Only 4 subjects in total reported fitness level 1, and that group was not further analyzed.

3- *How many exercise training section each subject performed? It is not “clear” on the manuscript.*

Each individual performed only 1 training bout for the leukocytosis. A new table 1 shows the numbers of individuals involved in this study and the type of exercise they performed.

4- *The authors must better explain the differences in the protocol design. In the first group the blood collection was done 3h after exercise whereas in the second group the blood collection was done immediately after exercise. The difference in the time of blood collection (after exercise) could contribute to the presented results?*

We have added new figures and tables to address the referee's concerns.

5- *Additionally, it is not clear when heart rate measurement was performed (immediately after exercise or after a resting period). Please, address this point.*

*We thank the referee for the observation.*

We have added to the materials and methods section the following sentence “heart rate was measured before and after exercise at the same time blood was collected for the two different groups”.

6- *The coagulation test was performed only to second group (intense exercise)? What are the reasons?*

We added to the material and methods this sentence: “in these tests, the coagulation was performed on an independent set of individuals performing only intense exercise

7- *Are the data distributed according to normal distribution (Gauss distribution)? If not, a non parametric test (Mann-Whitney, I think) must be more suitable for the analysis. Please, address this point.*

A quick test done shows normal distribution of all data collected.

## RESULTS

8- *Concerning the PCV data: The increase in this parameter after intense exercise (both in male and female) could be due to dehydration or not? In positive case, the resting in the first group (3h) could be responsible for differences presented here?*

The differences in PCV after the 2 different bouts of exercise can be explained by the time blood was taken, but again this was a parameter that was measured at these points since the phenomenon is well documented.

9- *The authors must explain the number (n) of individuals included in analyzes. Indeed, in general 120-136 female and 121-140 male were included (according to Figures data). Numbers can be found in the new Table 1.*

10- *Concerning Leukocytosis examination after 5 min intense exercise (page : Please, include neutrophils when female data are presented.*

The results are included in Figure 3.

11- **Concerning Hemostasis shifts into a higher equilibrium after 5 min intense exercise: Why this test was done exclusively in the group under intense exercise (second group)? Please, address this from a rational point of view. Additionally, the n of individuals included in this analysis must also be explained.**

It was only tested on intense exercise but it should be emphasized that this group was independent of the leukocytosis groups.

## DISCUSSION

12- *The increases in the WBC are, in general, associated with an inflammatory process. In this case (under the protocol used) is possible to assert that the exercise induces an inflammatory process (even transient)? What are the physiological implications of this? Please, address this.*

We have briefly commented on this point in the Discussion part

13- *The authors do not present any evidence of increase in the levels of catecholamine, cortisol, and others under their experimental protocol. This purely speculative discussion must be avoided. Please, discuss it from reported results.*

We have changed the text to reflect the concern raised by the referee.

14- *The difference in the APTT data (among male and female) must be discussed.*

We have added a sentence in the Discussion regarding this point.

15- *Are the differences, reported here, expected to occur in athletes or not?*

Some of our test subjects are/were athletes competing at international level in skiing, soccer, kickboxing and 5000 meter running a.o.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the World Journal of Experimental Medicine.

Sincerely yours,

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