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Reply

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We would like to express our heartfelt thanks to Prof. L. Benov for his interest in and comments on our paper, ‘The Effect of Training Type on Oxidative DNA Damage and Antioxidant Capacity during Three-Dimensional Space Exercise’ [1]. First, he questioned the increase in erythrocyte superoxide dismutase (SOD) activity, which increased at least twice immediately after Orbotron exercise and dramatically decreased just 30 min after recovery. As a matter of fact, we never expected such a result, and thus do not clearly understand its mechanism. In any case, we frankly admit that our study was a limited preliminary one and that selection bias might have affected the outcome. Nevertheless, it was our view that, given the fact that all subjects who participated in Orbotron exercise showed a similar trend in the changes of SOD activity, it may not be advisable to dismiss the results as a chance effect, and hence it was thought that there must be a cause for the changes in SOD activity. As Prof. Benov mentioned, it is illogical to relate such changes to gene expression, inasmuch as erythrocytes’ nuclei are removed by denucleation after they have matured. What should not be overlooked is that the study pertained to subjects who were aviation cadets receiving special military training and three-dimensional space exercise, which is quite unlike the two-dimensional exercise that has been dealt with in previous studies [2]. Based on these facts, a hitherto unknown physiologic change may have occurred. Although we do not know of a clear mechanism, we assume that some form of allosteric changes might have occurred at the protein level, or SOD activity might have changed due to a post-translational modification that has so far been unknown [3]. To find the mechanism that changes SOD activity, a further study needs to be conducted on more subjects, in which the effect of chance may be minimized. In addition, the study should be performed at the molecular level in order to find out why SOD activity has changed. The plain fact is that well-trained aviation cadets can cope very well with oxidative stress caused by three-dimensional space exercise and become physiologically adapted to it.

Prof. Benov wondered how we measured erythrocyte SOD activity. Put simply, first, the whole blood collected from the peripheral veins of subjects was placed into a heparinized Vacutainer and the amount of hemoglobin was measured and recorded. Then, 1 ml of whole blood was mixed with 0.85% sodium chloride (NaCl), centrifuged at 3,000 rpm for 10 min, and the supernatant

was separated. This process was repeated 4 times. Next, cold distilled water was added to the mixture so that the total volume became 2.0 ml and this was then incubated at 4°C for 15 min. Finally, it was diluted 25 times using 0.1 mmol/l of the phosphate buffer, and analyzed using the autoanalyzer Cobas Mira (Roche Co. Ltd., Switzerland) [4].

Regarding the statement, ‘CuZnSOD displays modest peroxidase activity, but this does not mean it decomposes H₂O₂ to H₂O and O₂’ [5, 6], it is widely known that catalase is the enzyme that decomposes H₂O₂ into H₂O and O₂. We did not mean to suggest that SOD had catalase activity, but that SOD and catalase might be in the same region of the body. For example, it is known that both SOD and catalase are present in the liver [7].

Lastly, regarding in vivo experiments, Prof. Benov emphasized the importance of knowledge and understanding of the process at the molecular level. We are entirely in agreement with this observation, and this advice has made us review our study. However, our aim was to suggest that it might be advisable to perform a study of the physiological status, as its results could provide valuable insights that may lead to further studies. May we point out that there are many cases where physiological studies are more suitable than studies at the molecular level because of the expense and time involved, and thus we reckon that it would be appropriate to commence a study from the physiological perspective and later devise a study that would lead to an understanding of the molecular level. Of course, we do not mean to refute Prof. Benov’s opinion that in the case of in vivo experiments, previous knowledge and understanding of the process at the molecular level are very important. As mentioned above, we are entirely in agreement with our colleague’s advice, which is wise and merits consideration. We acknowledge that the remarks of Prof. Benov motivated us to review the limitations of our study and are grateful for his observations.

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