CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

ANALYSIS OF ERYTHROCYTE FUNCTION CHANGE DURING MOTION FROM NUTRITIONAL INTERVENTION PERSPECTIVE

Zheng Cheng* and Wei Huang

Department of Physical Education of Jiangsu University, Zhenjiang Xuefu 301 China 2012013
Corresponding author: hw431@yeah.net*

Article history:
Received:
25 November 2015
Accepted:
15 August 2016

Keywords:
Erythrocyte function change;
Nutritional intervention perspective;
Sports anemia model.

ABSTRACT
In this paper, first set up sports anemia model through animal experiment; make research on the oxidative stress state and energy metabolism function of erythrocyte based on sports anemia model and anti-sports anemia agent; make qualitative and quantitative research on the aging erythrocyte with adoption of advanced flow cytometry and laser co focal technique; at the same time, observe the change of protein of erythrocyte membrane with membrane one-dimensional and two-dimensional electrophoresis technology; adopts advanced image analysis system to make quantitative analysis of protein of erythrocyte membrane. Make a series of erythrocyte index test for 12 sports anemia athletes and 12 normal athletes through human experiment and make anti-sports anemia agent treatment for them by one month to explore how exercise causes erythrocyte damage, the mechanism of causing sports anemia and also how to present.

1. Introduction

Higher incidence of anemia exists among athletes. Anemia has serious influence on exercise ability, training effect, recovery and immunity after exercise and other functional status; it becomes the cause of excessive training sometimes. The relationship between anemia and physical load as well as nutritional status has aroused extensive attention of the medical community (Lukaski, 2005). The purpose of this research is to establish animal model of sports anemia and it studies the erythrocyte membrane changes of rats which make long-term training during different periods to understand the influence of exercise training on erythrocyte, especially the changing regularity of erythrocyte membrane when sports anemia or potential sports anemia happens to the rats, which can provide sensitive monitoring index for reflecting potential anemia accurately and preventing the happening and development of sports anemia(Pan, 2007). At the same time, combine hemoglobin, parameters of iron metabolism and other indexes to evaluate sports anemia and increase the diagnosis accuracy for sports anemia and provide basis for preventing the happening and development of sports anemia. Make research on the erythrocyte membrane change of rats which has made eight weeks of training and further discuss the mechanism of sports anemia.

2. Materials and methods

The simplest index of anemia evaluation is hemoglobin. Usually, the hemoglobin of male is higher than that of female. The standards of diagnosing sports anemia at home and abroad are different (Neuberger et al., 2007). The hemoglobin standard for diagnosing anemia in European countries and America is female (120g/l,) male<140g. The domestic adult standard is female<105g, male<105g, for children below 14 years old, both are (120g/l. Hematocrit and blood viscosity are in close relationship with hemoglobin. Physiology

believes that the best hematocrit is at the high value part within the normal range, which is about 45%. When the hematocrit is at 45%, the value of hemoglobin equals to about 16g%. Normally, hematocrit and blood viscosity present a rising curve, while the situation changes at high hematocrit (such as globalism). So it can't be simply believed that the higher hemoglobin, the better (Lewis et al., 1988).

In 1959, the Japanese scholar Yoshimura firstly put forward "sports anemia", which did not draw the attention of sports medicine. In recent twenty years, the development of research on people with sports blood deepens people's understanding. For most of athletes, sports anemia is a kind of relative anemia. The premise of proposing sports anemia is considered from the ideal value of hemoglobin when the athletes engaged in endurance make aerobic exercise (Mao et al. 2011). Because the function of hemoglobin is to transfer oxygen, which is the main factor deciding the max oxygen uptake of athletes. Therefore, the quantity of hemoglobin affects the motion ability obviously and hemoglobin is also used to judge the functional status of athletes (Konstam et al., 1982).

(1) Relative anemia caused by plasma dilution

There is evidence proves that the endurance athletes have bigger plasma volume, while the endurance training 11 increases with the plasma volume. There are also researches indicate that the athletes are with bigger total hemoglobin, while the training increases with total hemoglobin. The decrease of hemoglobin concentration is caused by the disproportionate increase between plasma volume and erythrocyte or total hemoglobin. Brotherhood has compared the material of endurance runners and the control group and observed the similar results. The total hemoglobin of these athletes is higher than control group by 20%, but the hemoglobin concentration is relatively low. It can be seen that for some athletes, the increase of plasma volume caused by training is higher than the increase of total hemoglobin. The increase of plasma volume is an adaptive response of body and its result is to increase the stroke volume and max output of heart, which is helpful to transfer the oxygen to the surrounding tissues during strenuous exercise, where a situation of one reaction compensating the other reaction appears. Because the strenuous exercise can increase the maximum oxygen uptake, the positive adaptive response of cardiovascular function compensates the negative adaptive response causing relative anemia to a great extent. At the moment, the problem is that the training effect will be improved greatly if the dilution of hemoglobin can be avoided.

(2) Exercise worsens the destruction of erythrocyte in the blood. Exercise training of high intensity causes permeable and oxidative damage to erythrocyte membrane, decreases the deformation of erythrocyte and causes hemolytic; during anaerobic fermentation training of high intensity, the blood PH decreases due to lactic acid accumulation, which has influence on erythrocyte membrane with -3 protein; exercise affects the activity of $Na^{+}K$ eleven ATP enzyme on erythrocyte, and then changes the osmotic pressure of erythrocyte and decreases the deformability. The adverse effect of exercise training on the structure of erythrocyte membrane and deformability worsens the erythrocyte damage (Shin et al., 2015).

(3) Sports iron deficiency and iron-deficiency anemia, the iron deficiency of athletes may be possibly caused by three reasons: 1. Demand of iron increases during exercise, for example, the demand of iron of female increases due to the menstruation; athletes who need to reduce the fat usually eat low calorie diet, causing the inadequate intake of iron. 2. The decrease of iron absorption, under normal situation, the body will increase the iron absorption when the storage of iron is not enough, but the athletes with iron deficiency are different, who are usually with absorption barrier (Mooren et al. 2003). Ehn and others have observed that the iron absorption rate of long distance runners with iron deficiency is only 16.4%, while the control group with inadequate iron storage is 30%. The observation result of

Clement and others for female long distance runners is similar and the reason needs to be elaborated. 3. The increase of iron loss is caused by the great sweating of athletes (iron lass increases by 100%), stool excretion, appearance of hemoglobinuria, myohemoglobinuria, hematuria as well as the menstruation of female athletes etc. (Weder and Egan, 1988).

(4) Exercise causes the change of hormone. Exercise stress causes the increase of adrenaline, adrenaline causes spleen contraction and releases hemolytic factor, which increases the damages to erythrocyte.

3. Results and discussions

3.1. Experimental methods

This animal experiment is made based on sports anemia model of incremental load treadmill exercise which has been established successfully but not published yet. There are 65 male Westar rats, weighted 200g and provided by Institute of Medical Laboratory Animals of Chinese Academy of Medical Sciences, animal license number SCXKn-00-0006 and with animal grade of grade II. Select and divide into three groups randomly, which include control group (21 rats) and incremental load treadmill exercise group (short as sports group, 44 rats). The animal feed is nutrient pellet feed at full price, which is provided by Beijing Keao Xieli Feed Co., Ltd; environmental temperature of animal feeding is 23 ℃ with humidity 40-60%; raised in separate case and each with five rats, free diet, natural lighting. The nutrient components include ginseng, herb distances, yinyanglei, radix astragal, meddler, home iron, lycoypene and compound vitamin etc; the supplement time and method is gavages after training until the accomplishment of the experiment. The grouping of experimental animals is as shown in Table 1.

The rats of training group make incremental treadmill training (BCPT-96 model) by five weeks with treadmill degree at zero degree, speed at 30m/m, training for 6 days per week and for the first two weeks, training one time per day and in the following weeks, training one time in the morning and in the evening respectively and rest on Sunday (Sharif et al., 2010); The training arrangement is as following: the training time for the first time is one minute, followed by increase of 2 minutes/time and the last training time is 97 minutes (Hassel et al., 2013). If serious exhaustion symptom happens to rats during training, such as continuous mechanical stimulation make the rats can't run any more or their abdomens touch the ground seriously after get off the treadmill presenting "turtle type", then they are allowed to rest for 2 to 5 minutes. Select 22 rats randomly after five weeks to make nutritional intervention with anti-sports anemia complex and kill them in the 8th week, seen in Figure 1.

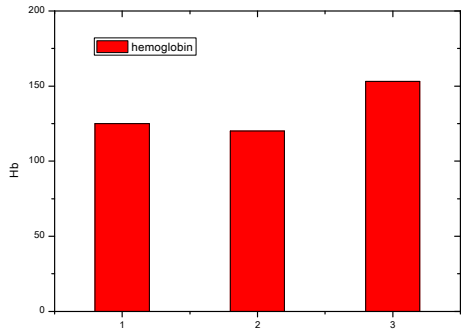

Figure 1. Running, swimming movement of hemoglobin

Select four rats randomly from control group, sports group and sports + nutrition group to make scanning electron microscope observation (Da et al., 2014). Erythrocyte classification refers to RBC form classification method reported by Keji Lias well as classification method introduced in clinical hematology written by Jading Deng; observe abnormal RBC form and each observed sample should not be less than 1000 RBC and calculate abnormal rate of erythrocyte (Kanda et al., 2015). The specific methods for treatment of erythrocyte by scanning electron microscope are in Table 2.

(1) Put 10Oul (about one drop) whole blood into pre-mixed SOOul normal saline at room temperature and EP tube of SOul biotin,

supplement to 1.5ml with normal saline at 37℃ complex; the time is 20 minutes(Mcmorrow et al. 2012);

(2) Low speed centrifugation (less than 150OrPm), add 0.25% glutaraldehyde solution to fix after removing supernatant, room temperature, coincidence minutes, as is shown in Table 3;

(3) Make centrifugal separation for erythrocyte and use 30k glutaraldehyde for the 2nd fixation, room temperature, and coincidence minutes;

(4) Centrifugation, water bath;

(5) Overlay;

(6) 50%, 70%, 90%, 100% gradient ethanol dehydration;

(7) Critical point drying;

(8) Spraying;

(9) Observe erythrocyte form under JEOLJSM-5600Lv scanning electron microscope.

Table 1. Experimental animal group

group	N	weight
The control group	20	204.3
Animal groups	15	205.9

Table 2. Treadmill and swimming exercise influence on rat blood index and weight

group	N	hemoglobin	Red blood cell count	Red blood cells deposited	weight
The control group	7	126.3	9.01	50.23	362.59
The treadmill group	8	112.5	6.32	44.26	345.25
The swimming team	9	153.6	9.25	44.85	353.68

Table 3. Exercise and nutrition intervention effects on erythrocyte antioxidant enzyme activity

group	SOD	GSH-PX	CAT	Ery-SOD	Ery-GSH-PX	Ery-CAT
1	985.32	123.65	32.65	906.23	7.06	0.52
2	858.23	69.85	21.58	785.21	4.58	0.26
3	904.68	85.45	33.69	779.36	5.62	0.48

3.2. Data statistical method

Make data analysis with SPSS10.0 statistical software. Each index is expressed with mean and standard deviation. Make variance analysis and correlation analysis of each index. P<0.05 with significance.

It can be seen from Figure 2 that the sports change the antioxidant enzyme activity of plasma and erythrocyte. (1) Compared with control group, the plasma SOD and erythrocyte SOD decrease obviously, decreasing from 951.48±20.33 and 903.97±234.42 to 898.45 ± 80.98 and 728.54 ± 129.95, decreased by 5.62% and 19.44% respectively, with obvious significance (P<0.05); the plasma SOD and erythrocyte SOD of sports + nutritional intervention group increase, increased by 0.83% and 6.96% respectively with obvious trend, but there is no significant difference; however, the plasma SOD and erythrocyte SOD of sports + nutritional intervention group decrease when compared with that of control group, but there is no significant difference (P(0.05). (as shown in Figure 2 and 3)

(2) The changing trend of GSH-PX is in line with SOD. Compared with control group, the

plasma GSH-PX and erythrocyte GSH-PX of sports group decrease obviously, decreasing from 123.04±71.80 and 7.09±3.51 to 63.87±56.87 and 4.82±2.03 respectively, decreased by 48.9% and 31.98% respectively, the difference is with obvious significance (P<0.05); the plasma GSH-PX and erythrocyte GSH-PX of sports + nutritional intervention group increase, increased by 33.28% and 16.23%, the trend is obvious but without obvious significance. However, the plasma GSH-PX and erythrocyte GSH-PX of sports + nutritional intervention group present decreasing trend compared with control group (as shown in Figure 4 and 5).

(3) Compared with control group, the plasma CAT and erythrocyte CAT of sports group decrease obviously, decreasing from 36.26±13.13 and 0.52±0.14 to 21.52±10.98 and 0.29±0.045, decreased by 40.73% and 44.16% respectively, the difference is with high degree of significance (P<0.01); the plasma CAT and erythrocyte CAT of sports + nutritional intervention group increase obviously then sports group, increasing from 21.52±10.98 and 0.29±0.045 to 33.13±13.24 and 0.41±0.17, increased by 34.97% and 41.44% respectively, the difference is with significance ((P<0.05)). However, the plasma CAT and erythrocyte CAT of sports + nutritional intervention group decrease compared with control group, but with no obvious significance(as shown in Figures 6 and 7).

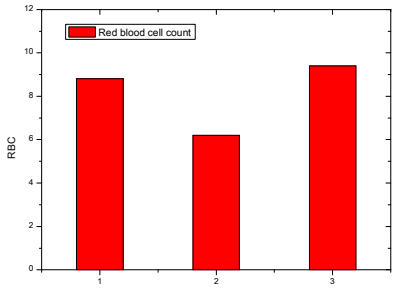

Figure 2. Running, swimming exercise effect on the number of red blood cells

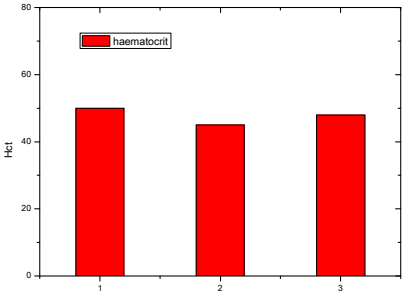

Figure 3. Run, the influence of the backlog of swimming exercise on blood cells

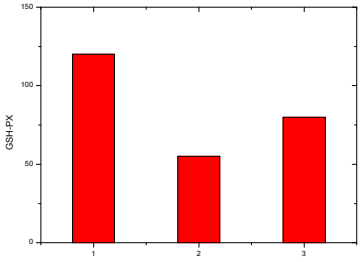

Figure 4 Sports and anemia agent affect plasma GSH – PX

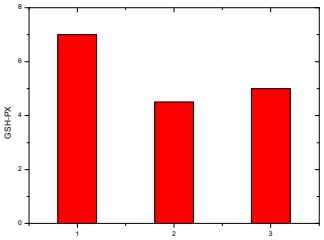

Figure 5. Sports and anemia agent effects on erythrocyte GSH – PX

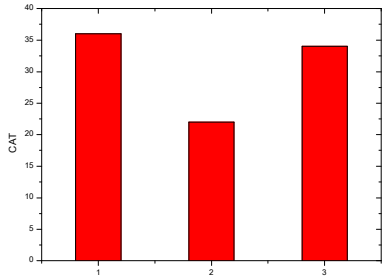

Figure 6. Sports and anemia agent affect plasma CAT

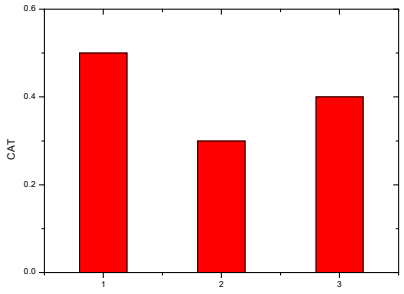

Figure 7. Sports and anemia agent effect on red blood cells to the CAT

4. Conclusions

In this experimental research, first set up sports anemia model through animal experiment; make research on the oxidative stress state and energy metabolism function of erythrocyte based on sports anemia model and anti-sports anemia agent; make qualitative and quantitative research on the aging erythrocyte with adoption of advanced flow cytometry and laser co focal technique; at the same time, observe the change of protein of erythrocyte membrane with membrane one-dimensional and two-dimensional electrophoresis technology; adopts advanced image analysis system to make quantitative analysis of protein of erythrocyte membrane. Make a series of erythrocyte index test for 12 sports anemia athletes and 12 normal athletes through human experiment and make anti-sports anemia agent treatment for them by one month to explore how exercise causes erythrocyte damage, the mechanism of causing sports anemia and also how to present. The following conclusions have been reached:

(1) This research results show that for the three standard indexes Hb for evaluating anemia in the 10 weeks' exhaustion of heavy load, treadmill sports group and control group presents very obvious statistical significance ($P<0.01$), for RBC and/or Hct in the 10 weeks' exhaustion of heavy load, treadmill sports group and control group do not have obvious statistical significance. Therefore, this sports anemia model is good. In addition, the author finds out that this model consumes too much time during experiment and the utilization of treadmill is no economical, therefore, it is suggested to adopt sports anemia model of incremental load treadmill.

(2) Based on sports anemia model caused by long time incremental exercise, the sports anemia athletes experiment proves that the erythrocyte abnormal rate of rats in sports group is higher than that of control group through observations with different times of electron microscope; moreover, each abnormality of sports group is higher than that of control group.

(3) Based on sports anemia model caused by long time incremental exercise, the sports anemia athletes experiment proves that erythrocyte free radical and lipid peroxide products increase, oxidation resistance ability decreases, presented as the decreased ability of antioxidant enzyme system and non-enzyme system, which explains the serious imbalance between erythrocyte oxidation and anti-oxidation.

(4) Based on sports anemia model caused by long time incremental exercise, the sports anemia athletes experiment proves that both erythrocyte Na^{+}-K-ATP enzyme activity and $Ca^{2+}Mg^{2+}$-ATP enzyme activity decrease, and ion imbalance appeared in erythrocyte, which has affect the permeability of erythrocyte membrane.

(5) Based on sports anemia model caused by long time incremental exercise, the sports anemia athletes experiment proves that both the erythrocyte glycolytic potential and phosphoric acid bypass metabolism of sports anemia athletes decrease, ATP and NADPH generations decrease, affecting body energy metabolism and GSH-PX activity; two energy metabolisms of erythrocyte glycolysis and pentose phosphate shunt decrease, causing the damage to erythrocyte.

(6) Based on sports anemia model caused by long time incremental exercise, the sports anemia athletes experiment proves that exercise has speeded up the aging process of erythrocyte; the SA of sports anemia group decreases obviously than that of control group; PS extrusion improves obviously than that of control group. The increase of erythrocyte aging after exercise is related to the increase of oxidative stress level of erythrocyte as well as the accumulation of calcium ion in the cell.

(7) Based on sports anemia model caused by long time incremental exercise, the sports anemia athletes experiment proves that when free radical generation increases and superoxide dismutase SOD, catalane CAT, glutathione peroxides GSH-Px etc. decrease, oxygen free radical damages many biomacromolecules, such as nucleon and protein membrane polyunsaturated acid, causes super oxidation reaction, decreases the content of actins and protein with -6 and brings about damages to skeleton structure and function of erythrocyte membrane. The observation result of erythrocyte membrane protein change with two-dimensional electrophoresis techniques reveals the change of erythrocyte membrane protein with -3. Statistics can't be made for lot of samples in this experiment, but the advantages can't be neglected.

5. References

Chou, S.L., Huang, Y.C., Fu, T.C., Hsu, C.C., Wang, J. S. (2016). Cycling exercise training alleviates hypoxia-impaired erythrocyte rheology. *Medicine & Science in Sports & Exercise*, págs. 48(1), 57-65.

Clarke-Jenssen, A.C., Fredriksen, P.M., Lilleby, V., Mengshoel, A.M. (2005). Effects of supervised aerobic exercise in patients with systemic lupus erythematosus: a pilot study. *Arthritis & Rheumatology*, 53(2), 308-12.

Da, B.M., Mastalurova, I., Brazaite, G., Mcgovern, N., Thompson, K., Gray, S.R. (2014). The effect of krill oil supplementation on exercise performance and markers of immune function. *Plos One*, 10(9).

Hassel, E., Berre, A. M., Skjulsvik, A. J., Steinshamn, S. (2013). Effects of exercise training on pulmonary vessel muscularization and right ventricular function in an animal model of copd. *Respiratory Research*, 15(1), 485-490.

Kanda, E., Muneyuki, T., Suwa, K., Nakajima, K. (2015). Alcohol and exercise affect declining kidney function in healthy males regardless of obesity: a prospective cohort study. *Plos One*, 10(8).

Konstam, M. A., Tu'Meh, S., Wynne, J., Beck, J. R., Kozlowski, J., Holman, B. L. (1982). Effect of exercise on erythrocyte count and blood activity concentration after technetium-99m in vivo red blood cell labeling. *Circulatio*n, 66(3), 638-42.

Lewis, R.D., Yates, C.Y., Driskell, J.A. (1988). Riboflavin and thiamin status and birth outcome as a function of maternal aerobic exercise. American *Journal of Clinical Nutrition*, 48(1), 110-6.

Lukaski, H.C. (2005). Low dietary zinc decreases erythrocyte carbonic anhydrase activities and impairs cardiorespiratory function in men during exercise. *American Journal of Clinical Nutrition*, 81(5), 1045-51.

Mao, T.Y., Fu, L.L., Wang, J.S. (2011). Hypoxic exercise training causes erythrocyte senescence and rheological dysfunction by depressed gardos channel activity. *Journal of Applied Physiology*, 111(2), 382-91.

Mcmorrow, R.C.N., Windsor, J.S., Hart, N.D., Richards, P., Rodway, G. W., Ahuja, V. Y., et al. (2012). Closed and open breathing circuit function in healthy volunteers during exercise at mount everest base camp (5300m). *Anaesthesia*, 67(8), 875–880.

Mooren, F.C., Golf, S.W., Völker, K. (2003). Effect of magnesium on granulocyte function and on the exercise induced inflammatory response. *Magnesium Research Official Organ of the International Society for the Development of Research on Magnesium*, 16(1), 49-58.

Neuberger, G.B., Aaronson, L.S., Gajewski, B., Embretson, S.E., Cagle, P.E., Loudon, J.K., et al. (2007). Predictors of exercise and effects of exercise on symptoms, function, aerobic fitness, and disease outcomes of rheumatoid arthritis. *Arthritis & Rheumatism*, 57(6), 943-52.

Pedralli, M.L., Waclawovsky, G., Camacho, A., Markoski, M.M., Castro, I., Lehnen, A.M. (2016). Study of endothelial function

response to exercise training in hypertensive individuals (sefret): study protocol for a randomized controlled trial. *Trials*, 17(1), 1-9.

Pan, X. (2007). Correlation between erythrocyte membrane functional changes of exercise-induced anemia rats and exercise modes. *Sport Science Research*.

Sharif, S., Thomas, J.M., Donley, D.A., Gilleland, D. L., Bonner, D. E., Mccrory, J. L., et al. (2010). Resistance exercise reduces skeletal muscle cachexia and improves muscle function in rheumatoid arthritis. *Case Reports in Medicine*, 2011(2), 205691-205691.

Shin, J.H., Lee, Y., Kim, S.G., Bo, Y.C., Lee, H.S., Bang, S.Y. (2015). The beneficial effects of tai chi exercise on endothelial function and arterial *stiffness in elderly women with rheumatoid arthritis. Arthritis Research & Therapy*, 17(1), 1-10.

Weder, A.B., Egan, B.M. (1988). Erythrocyte water, na+-k+ cotransport, and forearm vascular function in humans. *Hypertension*, 12(2), 199-203.