＜原著論文＞

一過性の自転車エルゴメーター運動は赤血球合成を促進する

A single bout of exercise on a cycle ergometor enhances the biosynthesis of red blood cells.

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Abstract

The aim of this study is to investigate the effect of a single bout of cycling exercise on degradation using the osmotic fragility of red blood cells (RBC) and haptoglobin (hp) concentrations, and on biosynthesis using the activity of δ-aminolevulinate dehydratase (ALAD), erythropoietin (EPO) concentration and reticulocytes. These parameters were measured before, 0, 1, 3, and 6 hours after exercise. A single bout of the cycling exercise did not affect the levels of serum iron, ferritin, % saturation of total iron binding capacity (Tf), osmotic fragility and hp. However, δ-ALAD activity increased at 3 hours ($p<0.05$) and 6 hours ($p<0.01$) after exercise, respectively, compared to that of 0 hours after exercise. Reticulocytes were the highest at 6 hours after exercise. We conclude that RBC biosynthesis measured by δ-ALAD activity, EPO and reticulocytes, was triggered by a single bout of exercise stimulation, and was independent of exercise induced hemolysis and iron depletion.

keywords : *single bout of exercise, osmotic fragility, δ-ALAD activity, erythropoietin, reticulocytes*

Introduction :

The average life span of a red blood cell (RBC) is approximately 120 days under normal conditions. It has been also reported that the rate of RBC aging might be increased and the life span of RBC might be shortened during some forms of intensive training (28, 31). Although there were many reports on the mechanism of the life span of RBC induced by training or exercise, most of them were on exercise-induced hemolysis or iron depletion (RBC depletion). Direct comparative measurement of key factors concerned with the degradation and biosynthesis of RBC during some forms of training are totally insufficient at present.

Low concentrations of hemoglobin (Hb), iron and ferritin have been studied in various groups of the population engaged in physical activities (3, 4, 5, 9); soldiers during severe prolonged exercise for 4 or 5 days (17), triathletes (19), untrained men undergoing a 3-week training program (22) and in swimmers (25). These lowed iron stores in athletes are explained by the increased blood loss into feces and urine, changes in the RBC membrane from oxidative damage, foot strike hemolysis, hemolyzing factors released from the spleen, or iron losses through sweat (17, 19, 28).

It has been also reported that increased physical activity might augment iron demand. Although the general process of RBC synthesis with physical activity is well described; elevated numbers of RBC levels with lower mean corpuscular hemoglobin (MCH) values (24), the direct effect of physical activity both on RBC degradation and biosynthesis has not been well documented in the literature.

δ-aminolevulinate dehydratase (ALAD) is a second rate limiting enzyme in biosynthesis of RBC. This enzyme is a sensitive indicator of lead exposure and is well documented in the literature. Lead

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poisoning is occasionally associated with iron deficient anemia (1, 16, 20, 30). Significantly lowered activity of δ-ALAD in blood was also reported in lead treated animals (14). Davis and Avram (6) confirmed a linear correlation between reticulocytes proportion and the activity of RBC δ-ALAD in rats with anemia at birth growing to adulthood. Therefore, changes in δ-ALAD activity might be a good biomarker of RBC biosynthesis.

This study was undertaken to establish the mechanism of RBC aging during exercise. We therefore compared a single bout of exercise of cycle ergometer-induced changes both in osmotic fragility and serum haptoglobin (hp) concentrations as an index of degradative factors, and in δ-ALAD activity, erythropoietin concentrations and reticulocytes as biosynthetic indexes of RBC.

Methods

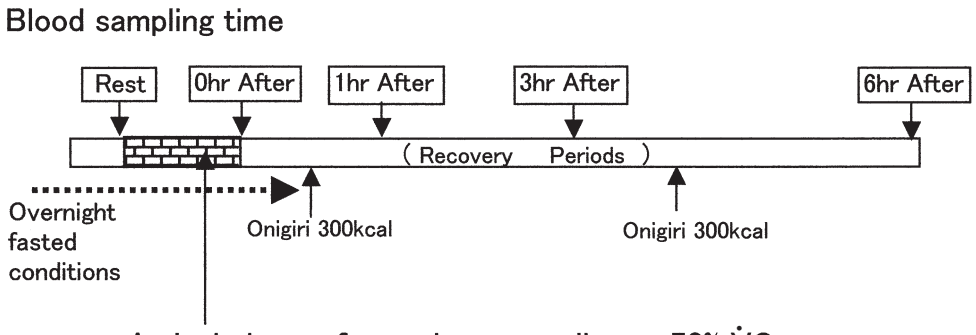
Subjects

Five female sedentary collegiate students who had not participated in a regular physical activity for the previous 6 months were voluntary recruited. No anemia diagnosed by a decrease in Hb less than 12g/dl was observed prior to this experiment. All subjects were non-smokers with normal blood pressure.

To create appropriate testing conditions, all participants were instructed to refrain from strenuous activity for the preceding 24 hours, and were asked to fast overnight. Prior to this study, each subject gave their written informed consent in accordance with the procedures approved by the Ethics Committees of the Japan Women's College of Physical Education.

Determination of Peak Oxygen Consumption

Values for O_2 consumption ($\dot{V}O_2$) of each individual were determined using a continuous incremental loading test to volitional exhaustion on an electrically braked cycle ergometer (Colibal 400, Lode Co. Ltd., Holland). After a standard familiarization and warm up procedure, each subject began cycling at 0W. The workload increased stepwise every three minutes by 25W until exhaustion. Expired air was collected continuously with an automated gas analysis system (Metabolic Measurement Cart 2900: Sensor Medics Co. Ltd., USA). R was monitored throughout the test. The gas analyzer was calibrated to a known volume of air, known concentrations of O_2 and CO_2, and to an atmospheric pressure before each exercise session. $\dot{V}O_2$ and $\dot{V}CO_2$ were obtained at 1-minute intervals. The $\dot{V}O_2$ peak was determined as the highest $\dot{V}O_2$ value attained when a subject could no longer cycle at the specified



Female sedentary students perticipated this experimental protocol.
This experimental procedure was accordance with the Ethical Committee on Human Experimentation at the Japan Women's College of Physical Education.

Fig. 1 Experimental Design

50rpm. The work rate for exercise corresponding to 70% $\dot{V}O_2$ peak was calculated from this test (93±6W).

Experimental Protocol

Subjects exercised on a cycle ergometer at 70% $\dot{V}O_2$ peak for 35.3±4.8 (28-43) minutes and consumed a mean energy of 248.8±3.1kcal. Environmental conditions during the experiment were maintained at 23-25°C and 48.5-51.3% relative humidity.

Venous blood samples were obtained by venepuncture from an antecubital vein before (baseline or pre), immediately after (post or 0 hours), 1, 3 and 6 hours after the exercise (Fig 1). Subjects were required to fast until the first blood sample was obtained after exercise. At 30 minutes and 210 minutes after exercise, a standardized meal was provided (two pieces of ONIGIRI, 300kcal each, which is a cooked rice ball, and a cup of green tea, giving a total energy intake of 600kcal during recovery).

Dietary Analysis

Food intake for the consecutive three days prior to this experiment were determined by a well-trained dietitian using the 24-hour recall method. The intake of individual nutrients were computer calculated based on the Standard Tables of Food Composition for Japanese (4^{th}) (29).

Blood Analysis

Blood samples were collected in EDTA-2K tubes. Haematological parameters, including RBC counts, Hb concentration and hematocrit (Ht) levels were measured. Percentage change in plasma volume was estimated from Ht and Hb pre- and post-exercise values using the procedure of Dill and Costill (8).

The following biochemical analysis was run in duplicate. The assay for osmotic fragility of RBC was followed by the method of Beutler (2). δ-ALAD activity was assayed in the heparinized RBC fraction as described previously (13, 14). 2ml of heparinized blood was centrifuged at 3,000rpm for 10 minutes at 5°C and the resultant supernatant was discarded. The precipitated RBC were washed three times with 2ml of cold phosphate buffered saline (pH 7.4), filled up with the same buffer and stored at −70°C until assay. Resultant RBC was incubated at 37°C for 30 minutes with δ-aminolevulinic acid. The reaction was stopped with a TCA-$HgCl_2$ mixture and the color was developed using modified Ehrlich reagent. Enzyme activity was calculated using the molar absorption coefficient (6.1×10^4) of the final Ehrlich color salt at 553nm, and the results were expressed as nmol porphobilinogen/ml RBC/hr.

The concentrations of serum iron, ferritin, total iron binding capacity (TIBC), erythropoietin (EPO) and hp were measured by Medical Labo, Co. Ltd. (Tokyo, Japan).

Reticulocytes stained with new methylene blue dye were counted under light microscopy (Alpha-photo-2 YS2-H, Nikon Co. Ltd. JAPAN).

Data Analysis

All values in the Tables and Figures are expressed as means±standard deviation in each group. Statistical significance was calculated by one-way repeated measures ANOVA. Specific statistical difference between means was then determined by the paired Wilcoxon test. The level of significance was taken as $p<0.05$.

Results

Subject Characteristics

Eight female sedentary students volunteered for this study. The medical history and use of medicine were asked of each individual. Three subjects were excluded because of abnormal RBC and Hb concentrations at the baseline hematological test. The resultants were five female students; mean age 21.0±0.2yr, height 157.0±4.0cm, body mass 49.0±1.7kg, and $\dot{V}O_2$ peak 38.0±5.4ml/kg/min. Daily mean intakes of energy, protein, carbohydrate, iron, vitamin A and vitamin C were 1867±553kcal/day, 56.9±21.5g/day, 261±66g/day, 14.8±19.1mg/day,

Table 1 Hematological response of female sedentary students

(n=5)

	Rest	0hr After	1hr After	3hr After	6hr After
RBC ($\times 10^4$ / μl)	427 ±16	440 ±29	440 ±29	442 ±32	442 ±33
Hb (g/dl)	13.0 ±0.3	13.3 ±0.8	13.4 ±0.7	13.6 ±0.7	13.6 ±0.9
MCV (μ^3)	90.8 ±1.2	90.0 ±1.7	90.0 ±1.3	90.0 ±1.3	90.0 ±1.7
MCH ($\gamma\gamma$)	30.6 ±0.5	30.4 ±0.5	30.6 ±0.5	30.8 ±1.2	31.0 ±0.6
MCHC (%)	33.8 ±0.4	33.6 ±0.5	34.0 ±0.0	34.2 ±0.7	34.4 ±0.5

All values are means±SD. No significant differences($p>0.05$) present among the various time points.

Abbreviations: RBC; red blood cells, Hb; hemoglobin, MCV; mean corpuscular volume, MCH; mean corpuscular hemoglobin, MCHC; mean corpuscular hemoglobin concentration.

2542±2806IU/day, and 41.9±10.3mg/day, respectively.

Haematological Changes

A significant change was observed in Ht and Hb concentrations ($p<0.01$) during the recovery period, reflecting the gradual increase in plasma volume from the end of exercise (0 hours or post) to 1 hour, 3 hours, and 6 hours. No significant changes in MCH concentration were observed during this experimental session (Table 1).

Therefore, the following estimation was continued to take PV change into consideration. Using formulae of Dill and Costil (8), RBC counts and Hb levels did not appear to change during these experimental conditions (Table 1). Serum iron levels were 91.2±12.3μg/dl at the time point of pre exercise, and 104.8±25.1μg/dl at 6 hours after exercise. Transferrin saturation (Tf) was 25.4±11.9% and 28.1±6.2% at pre and 6 hours after exercise, respectively. Concentrations of serum ferritin were 27.0±9.0ng/ml and 27.5±7.3ng/ml, at pre and 6 hours after exercise, respectively. No significant differences in levels of serum iron, Tf and ferritin were observed during this study.

Osmotic fragility

Osmotic fragility was expressed as NaCL concentrations of 50% hemolysis. NaCl concentrations as an index of 50% hemolysis did not change during this experiment (Table 2). Hp concentrations did not change either under our experimental conditions.

Table 2 Changes in 50% hemolysis and haptoglobin concentrations.

(n=5)

	50% hemolysis (NaCl %)	Haptoglobin (mg / dl)
Rest	0.478 ± 0.013	53.0 ± 21.8
0hr After	0.469 ± 0.007	56.4 ± 24.4
1hr After	0.473 ± 0.010	52.0 ± 19.4
3hr After	0.467 ± 0.004	52.8 ± 19.3
6hr After	0.463 ± 0.006	54.3 ± 20.6

Both levels of osmotic fragility and haptoglobin were unchanged throughout this experiment ($p>0.05$).

Table 3 Changes in δ-ALAD activity, erythropoietin concentration and reticulocyte counts.

(n=5)

	Rest	0hr After	1hr After	3hr After	6hr After
ALAD (nmoles/mlRBC/h)	562 ±67	546 ±90	634 ±54	670 ±68[a]	695 ±84[b,c]
Erythropoietin (mU/ml)	14.4 ±5.4	15.2 ±5.7	19.1 ±3.7	19.0 ±6.2	20.7 ±6.0
Reticulocytes (‰)	19.6 ±8.2	20.6 ±4.8	21.1 ±5.8	20.4 ±3.5	31.9 ±8.2[d]

Abbreviation: ALAD; δ-aminolevulinate dehydratase activity. All values are means±SD.

a: significantly different from the 0hr after the exercise level($p<0.05$).

b: significantly different from the rest level($p<0.05$).

c: significantly different from the 0hr after the exercise level($p<0.01$).

d: significantly different from the other time points ($p<0.05$).

Synthetic capacity of RBC

δ-ALAD activity was significantly higher at 3 hours ($p<0.05$) and 6 hours ($p<0.01$) after exercise compared to immediately (0 hours) after exercise (Table 3).

EPO concentrations gradually increased from the baseline to 6 hours after exercise, but this was not significant. Reticulocytes were the highest at 6 hours after exercise compared to all other time points ($p<0.01$).

Discussion

In this current study, a single bout of exercise on a cycle ergometer at 70% $\dot{V}O_2$ peak for about 30 minutes, did not change plasma volume-corrected levels of RBC, Hb, hp concentrations and osmotic fragility, but δ-ALAD activity significantly increased at 3 hours and 6 hours after exercise, EPO concentrations tended to increase during recovery periods, and reticulocytes were highest at 6 hours

after exercise. These results suggest that the RBC degradation is independent of this cycling exercise bout, but RBC biosynthesis is enhanced.

In our present study, we had chosen 250kcal as an additional energy expenditure level, which was the same as The Recommended Daily Exercise for Japanese (18). It is well known that many Japanese people get less energy expenditure by recent automobilization. Japanese minister of Health, Labour and Welfare saied in their report that people need to expend more energy by an additional exercise loading (18).

Rotstein et. al. (21) have reported the effect of supramaximal, short duration intermittent exercise and different recovery conditions on plasma volume (PV). They found that PV significantly decreased immediately after exercise, increased significantly during recovery and returned to its pre-exercise values within 40 minutes of recovery. Gastmann et. al. (12) examined blood chemical changes in nine ultra-triathletes, and reported that PV increased by 15.4-15.9% during prolonged heavy exercise. They suggested that this might be caused by an osmotic gradient due to multiple carbohydrate intakes of approximately 50-80g per hour, followed by water movement from intracellular to extracellular space (10).

In our experimental conditions, the calculated average of PV change using "hematological formulae" (8) was 4.2%, 7.6% and 8.7% at 1, 3 and 6 hours after exercise, respectively, consistent with previous report (27). Each subject underwent a single bout of cycle-exercise at 70% $\dot{V}O_2$ peak, not extremely hard and about 250kcal of energy expenditure, and consumed 2 pieces of ONIGIRI (boiled rice : 31.7g carbohydrate×2 pieces each) at 30 and 210 minutes after exercise. The carbohydrate eating might explain the reason for the elevated PV level during recovery in our study (10). This suggests that dietary conditions should be considered in hematological estimations.

It is still unclear whether a single bout of cycle-ergometer exercise can affect osmotic fragility or biosynthesis of RBC. Exercise-induced hemolysis has been described after marathons (15), ultramarathons (8, 24), and triathlons (19). Many explanations have been proposed on hemolysis in athletes ; foot-strike hemolysis, renal ischemia ; hypoxic damage to the kidney, and the release of a hemolyzing factor. No significant change in the levels of RBC, Hb, 50% hemolysis and hp were observed under our experimental conditions, indicating that exercise-induced hemolysis did not develop. This suggests cycle-ergometer exercise under our experimental conditions doesn't have any direct connection with hemolytic factors such as foot-strike stress (15) and lysolecithin (26), and that the exercise did not develop RBC degradation.

δ-ALAD activity is a second rate limiting enzyme for porphobilinogen proven in vivo. Davis and Avram (6) confirmed a linear correlation between reticulocyte proportion and the activity of RBC δ-ALAD in rats with anemia at birth growing to adulthood, suggesting that δ-ALAD activity might be a good parameter of RBC biosynthesis. On the other hand, Schmidt et. al. (23) examined the effect of different exercise regimens on serum immunoreactive EPO concentration, and indicated that submaximal exercise on a cycle ergometer for 60 minutes at 60% of $\dot{V}O_2$ peak, had no immediate effect on serum EPO, whereas under hypoxia the higher EPO level was observed at one and two days after exercise. This could result from haemodilution (8, 10). Furthermore, they reported that the number of reticulocytes increased after all hypoxic experiments. These observations were similar to our findings.

In our experimental condition, δ-ALAD activity was significantly higher at both 3 hours and 6 hours after exercise compared with that of the post level. This increase in δ-ALAD activity might be caused by accelerated neural functions during exercise. It is well known that physical activity stimulates a functional activity of the central nervous system and a release of hormones such as adrenocorticotrophic hormone (ACTH), norepinephrine and cortisol. It seems likely that δ-ALAD activity might be influenced by some neural activity or hormones (11).

Serum EPO concentrations were gradually increased from 0 hours to 6 hours after exercise, but not significantly. Reticulocyte counts were highest at 6 hours after exercise. The increase in EPO levels might be related to a release of reticulocytes from bone marrow into peripheral blood (23), suggesting the possibility that erythropoiesis is enhanced by this submaximal exercise.

In summary, with this level of exercise, hemolysis did not occur. Consequent iron deficiency was not observed either, but increased EPO and reticulocytes were observed. The activity of δ-ALAD was enhanced by this level of exercise, and was not triggered by hemolysis or iron deficiency, indicated that RBC biosynthesis was stimulated by δ-ALAD, activated by some kind of exercise induced hormonal or neuronal stimulation. An enhancement in the new RBC biosynthesis may increase the need for RBC synthetic materials such as iron, protein and vitamin B groups etc.. Therefore, people who have a habit of exercising should have much more nutrients properly, corresponding with their physical activity, as compared to sedentary people.

Further examinations are needed to clarify this possibility.

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Reference :

1. Aly MH, Kim HC Renner SW, Boyarsky A, Kosmin M, and Pagli DE (1993) Hemolytic anemia associated with lead poisoning from shotgun pellets and the response to Succimer treatment. Am J Hemtol 44 : 280–3.
2. Beutler E, Kuhl W, West C (1982) The osmotic fragility of erythrocytes after prolonged liquid storage and after reinfusion. Blood 59 : 1141–7.
3. Clarkson P, Haymes EM (1995). Exercise and mineral status of athletes : calcium, magnesium, phosphorus, and iron. Med Sci Sports Med 27 : 831–843.
4. Clement DB, Asmundson RC, Medhurst CW (1977) Haemoglobin values : comparative survey of the 1976 Canadian Olympic team. Can Med Assoc J 117 : 614–6.
5. Clement DB, Asmundson RC (1982) Nutritional intake and hematologyical parameters in endurance runners. Physician Sports Med 10, 37–43.
6. Davis JR, Avram MJ (1978) Developmental changes in delta-aminolevulinic acid dehydratase (ALAD) activity and blood reticulocyte percent in the developing rat. A brief note. Mech Ageing Dev 7 : 123–9.
7. Dickson DN, Wilkinson RL, Noakes TD (1983) Effects of ultramarathon training and racing on hematological parameters and serum ferritin levels in well-trained athletes. Int J Sports Med 3 : 111–7.
8. Dill DB, Costill DL (1974) Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. J Appl Physiol 37 : 247–8.
9. Elliot DL, Goldberg L and Eichner ER (1991) Hematuria in young recreational runner. Med Sci Sports Exerc 23 : 892–4.
10. Fellmann N (1992) Hormonal and plasma volume alterations following endurance exercise. A brief review. Sports Med 13 : 37–49.
11. Flora GJ, Khanna VK, Seth PK (1999) Changes in neurotransmitter receptors and neurobehavioral variables in rats co-exposed to lead and ethanol. Toxicol Lett 109 : 43–9.
12. Gastmann U, Dimeo F, Huonker M, Bocker J, Steinacker JM, Petersen KG, Wieland H, Keul J, Lehmann M (1998) Ultra-triathlon related blood chemical and endocrinological responses in nine athletes. J Sports Med Phys Fitness 38 : 18–23.
13. Granick JL, Sassa S, Levere RD and Kappas A (1973) orrelation between the ratio of activated to inactiveated δ-ALAD of whole blood and the blood lead levels. Biochem Med 8 : 149–159.
14. Gupta V and Gill KD (2000) Influence of ethanol on lead distribution and biochemical changes in rats exposed to lead. Alcohol 20 : 9–17.
15. Jordan J, Kiernan W, Merker HJ, Wenzel M, Beneke R (1998) Red cell membrane skeletal changes in marathon runners. Int J Sports Med 19 : 16–9.
16. Kaul B, Rasmuson JO, Olsen RL, Chanda CR, Slazhneva TI, Granovsky EI, Korchevsky AA (2000) Blood lead and erythrocyte protoporphyrin levels in Kazakhstan. Indian J Pediatr 67 : 87–91.
17. Lindemann R., Ekanger R., Opstad P.K., Nummestad M., Ljosland R (1978) Hematological changes in normal men during prolonged severe exercise. Am Corr Ther J 32 : 107–110.
18. Ministry of Health Labour and Welfare (1999) 6th Recommended Dietary Allowances. Dietary Reference Intakes, Daiichi Shuppan (Tokyo).
19. O'Toole ML, Hiller WDB, Roalstad MS, Douglas PS (1988) Hemolysis during triathlon races : its rela-

tion to race distance. Med Sci Sports Exerc 20 : 272–5.
20. Osterode W, Barnas U, Geissler k (1999) Dose dependent reduction of erythroid progenitor cells and inappropriate erythropoietin response in exposure to lead : new aspects of anaemia induced by lead. Occup Environ Med 56 : 106–9.
21. Rotstein A, Falk B, Einbinder M, Zigel L (1998) Changes in plasma volume and hot environmental conditions. J Sports Med Phys Fitness 38 : 24–9.
22. Schmidt W., Maassen N., Trost F., Boning B (1988) Training induced effects on blood volume, erythrocyte turnover and haemoglobin oxygen binding propertyes. Eur J Appl Physiol 57 : 490–8.
23. Schmidt W, Eckardt KU, Hilgendorf A, Strauch S, Bauer C (1991) Effects of maximal and submaximal exercise under normoxic and hypoxic conditions on serum erythropoietin level. Int J Sports Med 12 : 457–461.
24. Seiler D, Nagel D, Franz H, Helstern P, Leitzmann P, Jung K (1989) Effects of long-distance running on iron metabolism and hematological parameters. Int J Sports Med 10 : 357–362.
25. Selby GB, Eichner ER (1986) Endurance swimming, intravascular hemolysis, anemia, and iron depletion. Am J Med 81 : 791–4.
26. Shiraki K (1968) The effect of spleenectomy on sports anemia. J. Physiol. Soc. Jap. 30 : 96–108.
27. Shirreffs SM, Taylor AJ, Leiper JB, Maughan RJ (1996) Post-exercise rehydration in man : Effects of volume consumed and drink sodium content. Med Sci Sports Exerc 28 : 1260–71.
28. Smith JA (1995) Exercise, training and red blood cell turnover, Sports Med 19 : 9–31.
29. The Science and Technology (1998) 4th Standard Tables of Food Composition in Japan. Hitotubashi Shuppan, Tokyo.
30. Wright RO (1999) The role of iron therapy in childhood plumbism. Curr Opin Pediatr 11 : 255–8.
31. Yoshimura H, Inoue T, Yamada T, and Shiraki K (1980) Anemia during hard physical training (sports anemia) and its causal mechanism with special reference to protein nutrition. Wld Rev Diet 35 : 1–86.

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