Cooling-down Causes Rapid Consumption of Blood Lactate and No Increase of Urinary Bicarbonate

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(Received 23 October, 2012)

Abstract

Present study was aimed to examine the effects of cooling-down exercise on the levels of blood lactate and urinary bicarbonate after continuous ball-game exercise, in order to clarify the practical significance of erythrocytic Bohr effects. Twelve healthy young students participated in 50 minutes basketball game and 120 minutes resting without cooling-down exercise or 90 minutes resting with 30 minutes cooling-down exercise. When these subjects took 120 minutes rest alone, blood lactate decreased gradually, but urinary bicarbonate and pH began to increase extensively during resting period. When cooling down exercise was introduced immediately after ball-game exercise, blood lactate decreased rapidly to normal levels within 30 minutes, and no increase of urinary bicarbonate and urine pH was observed for resting period. Such drastic changes could be explained by the facts that cooling-down exercise enhanced both the oxidative metabolism of lactate and the respiratory rate, resulting in quick discharge of CO_2 into expired air, and finally causing the reduced discharge of bicarbonate into the urine. Without cooling-down exercise, intracellular pH of the circulating erythrocytes was postulated to be acidified, possibly causing cyanosis of peripheral tissues during resting period, which may be caused by the increase Bohr effects in erythrocytes.

Key words: Cooling-down exercise, Urinary bicarbonate, Urine pH, Bohr-effects

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In 1904, Bohr, Hasselbalch and Krogh[1)] discovered that oxygen affinity of erythrocytic hemoglobin was reduced by increasing amounts of carbon dioxide. This phenomenon was explained by the increased hydrogen ion, which is produced by the reactions: $CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^-$ catalyzed by carbonic anhydrase in erythrocytes and remains in the cells, while HCO_3^- is discharged from the cells to plasma. The remaining HCO_3^- in erythrocytes can be converted to CO_2 by the reverse reaction, and is expelled to the expired air from the lung. Therefore, under the conditions where CO_2 is overproduced in the peripheral tissues and the expelling of CO_2 from the lung is limited, hydrogen ion and HCO_3^- will be extensively produced in erythrocytes, resulting in the acidification of intracellular pH and the overflow of HCO_3^- from the cells to the plasma, and finally into the urine over the threshold of HCO_3^- in the blood[6)]. We postulated that such phenomenon would occur after the termination of heavy muscular exercise, when blood lactate, as an oxidative fuel, is extensively accumulated and the discharge of CO_2 from the lung is limited due to sedation of respiration.

Our previous study showed that there were marked changes in blood lactate, and respiratory and cardiac parameters during heavy muscular exercise with 10%, 30% and 80% one repetition maximum knee extension[4)] or with submaximal cycling exercise[5)]. In particular, blood lactate accumulated extensively during these heavy exercises and decreased gradually during resting period. Inversely, urinary bicarbonate and pH began to increase to a large quantity, 1 hour after beginning of the rest, in subjects loaded with heavy exercises[4) 5)]. The following three metabolic processes were given for this; 1) Lactate accumulated during heavy muscular exercises was aerobically metabolized, resulting in the production of CO_2[2) 11)]. 2) Since expelling of CO_2 into the expired air from the blood to the lung was limited due to relatively rapid sedation of the expiration rate after the termination of exercise, and CO_2 in the blood was then inevitably changed to bicarbonate by erythrocytic carbonic anhydrase. 3) Excessive bicarbonate would be discharged from erythrocyte to plasma, and further into the urine over the threshold of bicarbonate in the blood (about 24-28mM) as indicated by Pitts[6)]. Therefore, there would be leftover hydrogen ion in the erythrocyte, which would be buffered mostly by erythrocytic hemoglobin. Increased production of hydrogen ion in the erythrocytes may increase the Bohr effect on hemoglobin[1)], reducing the oxygen-carrying capacity of the erythrocytes and resulting in cyanosis, and finally may be relevant to physical fatigue after the end of heavy exercise.

On the other hand, the cooling-down exercise have been introduced on an empirical basis after the termination of heavy muscular exercise or sports, and are known to beneficial to cardiovascular and respiratory system after maximal exercise[3)] and to the recovery of post-exercise heart rate[8)]. However, the physiological roles of cooling-down exercises have not been sufficiently clarified, in particular, in terms of changes in blood and urinary metabolites. It may be possible that if cooling-down exercises were introduced after the termination of heavy muscular exercise, the increased lactate would be consumed more rapidly during a short period, and a large amount of CO_2 produced by aerobic metabolism of lactate would be discharged with expired air, and urinary bicarbonate that would otherwise have accumulated during the rest period after the termination of heavy exercise would be maintained at the normal levels. The present study investigated whether this working hypothesis would be possible or not, examining the changes in blood lactate, urinary bicarbonate and urine pH in the subjects playing the programmed basketball game with 20 minutes exercise, 10 minutes rest and 20 minutes exercise, accompanied with or without subsequent cool-down exercises.

Methods

Subjects

Subjects were 12 healthy male student volunteers (age: 21-24 years old, height: 170.0±5.0 cm, weight: 67.5±4.2 kg, means ± SE). The study was performed in the air-conditioned gymnasium of Tokyo Medical University approximately at 10℃ , on scheduled days in December. The present study was carried out according to the policy of the Declaration of Helsinki. After explanation of the study protocol and requirements, written informed consent was obtained before the testing began. There was no history of any endocrine disorders, glucose intolerance, or chronic diseases that might have influenced their responsiveness to strenuous exercise. Subjects were instructed to maintain their normal diet and to avoid physical activity for 24 hours before the experimental trials.

Exercise and resting without introducing cooling-down exercise

On the first day, they arrived at the Department of Biochemistry, Tokyo Medical University, at 11:00. From 11:00 to 12:00, they ate lunch and rested. These subjects were involved in the 50 minutes programmed basketball game (20 minutes game, 10 minutes rest and 20 minutes game), held in the air-conditioned gymnasium, from 13:00 to 13:50 (Fig. 1A & B). After the exercise was completed, they rested in chairs for 120 minutes, without any cooling-down exercises (Fig. 1A).

Urine and blood samples were collected at scheduled intervals as indicated by the arrows in Fig. 1A: just before starting the basketball game (-50 minutes, 13:00), just after the end of the game (0 minutes, 13:50), 30 minutes after the game (14:20), 60 minutes after the game (14:50), 90 minutes after the game (15:20) and 120 minutes after the game (15:50).

Exercise followed by cooling-down exercise and resting

On the second day, the basketball game program was performed according to the same schedule mentioned above, and then 30 minutes cooling-down exercise was introduced (Fig. 1B). The details of 30 minutes active cooling-down exercise are as follows: 15 minutes of jogging (heart rate: 90-120 beats/minutes) immediately after the basketball game, and 15 minutes of muscle static stretching. In order to perform cooling-down exercise, 12 subjects jogged slowly until the heart rate became 20 to 30 beats above resting rate for 15 minutes, then they stretched slowly into the desired position, as far as possible without pain, and hold the stretch for 30 seconds. These subjects repeated to stretch each of the major muscle groups involved in the exercise, using the static stretching techniques for 15 minutes (The muscle groups included shoulders, quadriceps, hamstrings, calves and lower back). After the end of basketball game and cooling-down exercise, the subjects rested in chairs for 90 minutes.

Urine and blood samples were collected at scheduled intervals as indicated by the arrows in Fig. 1B: just before starting the basketball game (-50 minutes, 13:00), just after the end of the game (0 minutes, 13:50), just after the cooling-down exercise for 30 minutes (14:20), 60 minutes after the game (14:50), 90 minutes after the game (15:20) and 120 minutes after the game (15:50).

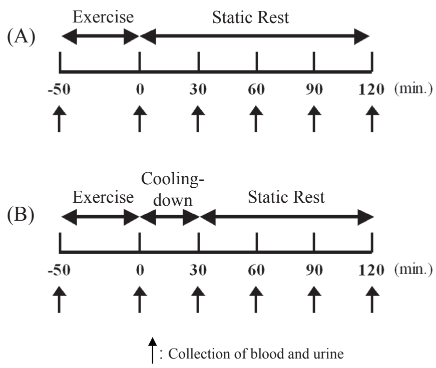

Fig. 1 Protocol of continuous muscular exercise including 50 minutes basketball game, and resting with or without 30 minutes cooling-down exercise. The blood and urine samples were collected at the points indicated by the arrows in the figure. (A): Group of exercise without cooling-down, (B): Group of exercise with 30 minutes cooling-down.

Measurement of urinary bicarbonate and pH

Immediately after the excretion of urine into a 200 ml paper cup, urine was transferred via syringe into a 10 ml rubber-capped vacuum glass tube (Veno-ject; Terumo Co., Ltd., Tokyo)[9) 10)]. Urinary pH and bicarbonate were examined within 2 hours after the termination of the present exercise program on both the first day and the second day. Urine pH and Urinary HCO_3^- were analyzed by a Hitachi 8800 automatic blood gas analyzer (Hitachi Co. Ltd., Tokyo). During the training period, all subjects received the same meals at the same time.

Measurement of blood lactate

Blood lactate was measured with a Lactate Pro (Arkray Inc., Kyoto) using an enzymatic method as described in a previous report[7].

Statistics

Statistical analysis was performed by two-way factorial ANOVA and multiple comparison test (Bonferroni) for the data in Fig 2, Figs 3(A) and 3(B). p value less than 0.05 was considered to indicate a statistically significant. All values were given as means ± SE.

Result

In the present study, 12 healthy young adults were involved in the continuous muscular exercise including 50 minutes basketball game (20 minutes exercise, 10 minutes rest and 20 minutes exercise) and 120 minutes resting without cooling-down exercise or 90 minutes resting with 30 minutes cooling-down exercise according to the protocols in Fig. 1. Since these subjects were instructed to maintain their normal diet and to avoid physical activity for 24 hours before the experimental trials, and they played the basketball strenuously 20 minutes, twice, the values of each physiological indexes obtained did not deviate from the average values. Expiration and cardiac rate returned normal within 15 minutes and 10 minutes, respectively, after the end of 50 minutes basketball game. In the present study, we had special attentions to the changes in blood lactate, urinary bicarbonate and urine pH, which are considered to be important to evaluate the physiological conditions after the termination of exercise as described previously in the heavy muscular exercise with submaximum knee extension[4] or with submaximal cycling exercise[5].

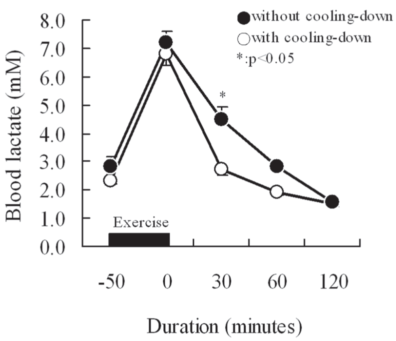

Fig. 2 Changes in blood lactate before and after continuous muscular exercise, with or without 30 minutes cooling-down exercise. (●):without cooling-down exercise; (○): with 30 minutes cooling-down exercise. *: $p<0.05$ vs cooling down exercise.

As shown in Fig. 2, lactate increased from 2.3 mM to 6.8 mM during 50 minutes basketball game in the blood of each of all subjects who played basketball game and took 120 minutes rest (solid dots). Lactate accumulating in the blood decreased gradually to the baseline level, 60 to 120 minutes after the exercise (solid dots in Fig. 2). In the subjects who played 50 minutes basketball game followed by 30 minutes cooling-down exercise and 90 minutes rest, blood lactate increased from 2.9±1.0 mM to 7.2±0.7 mM during 50 minutes basketball game. However, when 30 minutes cooling-down exercise including 15 minutes jogging and 15 minutes static stretching were introduced after the basketball game (open circles in Fig. 2), blood lactate decreased rapidly to the normal levels within 30 minutes. The difference in the levels of lactate in the blood between the subjects without cooling-down exercise and those with cooling-down exercise was 2 mM, 30 minutes after the end of the basketball game. Figure 3A and 3B show the changes in urinary bicarbonate and pH, respectively, before and after the 50 minutes basketball game followed or not by the 30 minutes cooling-down exercise. As shown by solid dots in Fig. 3A, urinary bicarbonate increased from 3.3±1.1 mM to 11.8±3.7 mM, during 120 minutes resting period when 30 minutes cooling-down exercise was not introduced. Urine pH also increased from 6.05 to 6.70, 120 minutes after the end of the exercise (solid dots in Fig. 3B). When 30 minutes cooling-down including 15 minutes jogging and 15 minutes static stretching were introduced after the exercise, urinary bicarbonate and pH rather decreased as shown by the open circles in Fig. 3A and 3B. Urinary bicarbonate decreased from 6.5±3.4 mM to 5.2±2.1 mM (open circles in Fig.3A), and urine pH from 6.20 to 6.15 during cooling-down and resting period (open circles in Fig.3B).

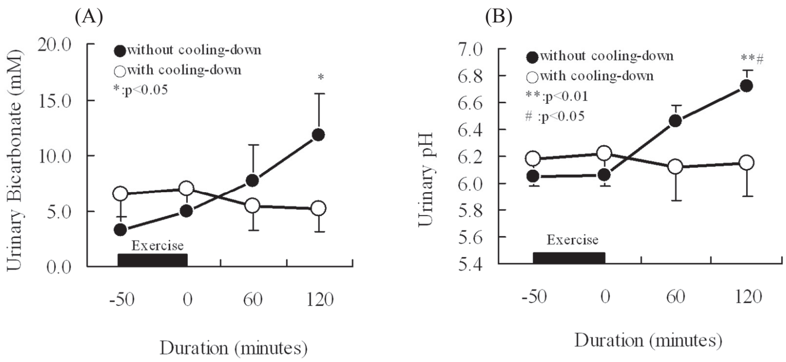

Fig. 3 Changes in urinary bicarbonate and pH before and after basketball game, with or without 30 minutes cooling-down exercise. (A): changes in urinary bicarbonate, (B): changes in urine pH. Solid dots (●) show the changes in urinary bicarbonate before and after the exercise without cooling-down exercise. Open circles (○) show those before and after exercise, with the accompanying cooling-down exercise. **: $p<0.01$, *$p<0.05$ vs pre-exercise. #: $p<0.05$ vs cooling-down exercise.

Discussion

In the present study, it was demonstrated that lactate accumulating in the blood during continuous heavy muscular exercise such as the presently programmed basketball game decreases gradually during 120 minutes resting period (solid dots in Fig. 2) and that according to the decrease in blood lactate, urinary bicarbonate (solid dots in Fig. 3A) and pH (dots in Fig. 3B) increased. Furthermore, we found that when the cooling-down exercise was introduced after the end of 50 minutes basketball game, blood lactate decreased rapidly to normal levels during 30 minutes cooling-down exercise (open circle in Fig. 2), and urinary bicarbonate (open circle in Fig. 3A) and pH (open circle in Fig. 3B) decreased to the levels below the baseline. These findings may be explained by the following views.

On the changes in blood lactate, urinary bicarbonate and pH during continuous muscular exercise and 120 minutes resting without cooling-down exercise

The decrease of blood lactate which initiated after the end of the basketball game may be explained by the facts that blood lactate was incorporated to muscles and liver (Fig. 4), and then will be metabolized aerobically by the tricarbonic acid cycle, producing a large quantity of CO_2. Then, carbon dioxide is released into the blood and penetrates into the erythrocytes, where carbonic anhydrase catalyzes the reaction: $CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^-$. Since a large amount of lactate was consumed during 120 minutes resting period, and approximately 60% of blood lactate is possibly metabolized oxidatively[2], yielding 3 mols CO_2 from 1 mol lactate, it is anticipated that 10.3 mM CO_2 may be produced by complete oxidation of lactate (decreased 5.7 mM lactate $\times 0.6 \times 3$ = 10.3 mM CO_2), and was converted to the equivalent amount of bicarbonate and hydrogen ion in the erythrocytes (Fig. 4). Excessive amounts of bicarbonate move from the erythrocytes to the plasma, and then will be discharged to the urine over the threshold of bicarbonate in the blood (about 24-28mM) as Pitts et al.[6] indicated. In this case, the discharge of CO_2 into the expired air from the blood to the lung would be limited due to sedation of the expiration rate after exercise (respiration rate and heart rate recovered to normal levels, within 15 minutes and 10 minutes, respectively, after the termination of the basketball game, data not shown), and bicarbonate released from the erythrocytes would inevitably be discharged into the urine. The data shown by solid dots in Fig. 3A that 8-11 mM bicarbonate was increased in the urine during 120 minutes resting period, are consistent with the value (10.3 mM) which was estimated from the changes in blood lactate (solid dots in Fig. 2).

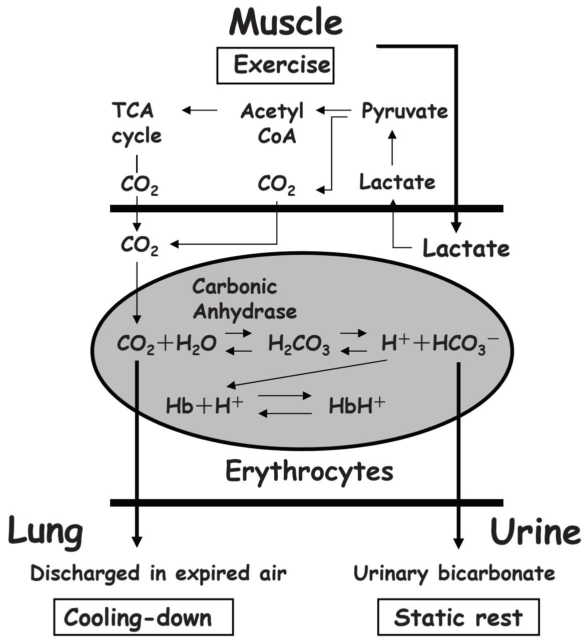

Fig. 4 Schematic process of production of CO_2 from blood lactate in the muscle, production of hydrogen ion and bicarbonate in the erythrocyte, the discharge of bicarbonate into the urine and discharge of CO_2 from the lung.

According to Bohr et al.[1], the oxygen affinity of hemoglobin in the erythrocytes was reduced by increasing amount of CO_2, which was demonstrated to be due primarily to a reduction in pH (named as Bohr effect). These processes may be explained by the conversion of CO_2 to bicarbonate and hydrogen ion by carbonic anhydrase in the erythrocytes as depicted in Fig. 4 ($CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^-$). Since intracellular hydrogen ion, which was produced by this reaction cannot move out of the cell membrane rapidly, and combines with hemoglobin, exerting the Bohr effect, our present results suggest that during the resting period after exercise, a large amount of hydrogen ion would be left in the erythrocytes, causing the reduction of intracellular pH and finally reducing oxygen affinity of intracellular hemoglobin and oxygen-carrying capacity of the erythrocytes. Thus, cyanosis in the tissues may occur. Such anaerobic conditions induce the production of lactate and acidification of the tissues, probably resulting in the physical fatigue.

Though we had previously postulated that the blood pH might be reduced by the leftover of hydrogen ion in the blood, after the discharge of a large amount of bicarbonate into the urine[4) 5)], this view should be changed to that the erythrocytic pH might be reduced.

Effect of cooling-down exercise on the changes in blood lactate, urinary bicarbonate and pH during resting period after continuous muscular exercise

When the cooling-down exercise was introduced after the end of 50 minutes basketball game, blood lactate, which was extensively accumulated during the game, decreased rapidly to normal levels during 30 minutes cooling-down exercise (open circles in Fig. 2). It may be possible that blood lactate was oxidatively metabolized in the muscle during the cooling-down exercise, producing a large amount of CO_2 (Fig. 4), which will be converted to hydrogen ion and HCO_3^- by carbonic anhydrase in the erythrocytes, then will be changed to CO_2 again, and will be expired to the lung. Therefore, accumulation of hydrogen ion and bicarbonate in the erythrocytes will not be caused. This view was supported by the result that urinary bicarbonate and pH were decreased to the levels below the baseline, when cooling-down exercise was introduced (Fig. 3A and B, open circles).

The present study showed that the increased urinary bicarbonate and pH during the resting period after continuous muscular exercise such as basketball game was abolished, and blood lactate accumulating during exercise period was rapidly diminished, by the introduction of 30 minutes cooling-down exercise. Our present results support the view that cooling-down exercises are beneficial to the physical recovery of human body after engaging in heavy exercises.

Acknowledgments

The present work was supported by a Grant-in Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (Grant no. 15500458, 2003-2006).

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