Influence of exercise on plasma ammonia and urea after ingestion beverages of carbohydrate electrolyte

Gusbakti Rusip1*, Sri Mukti Suhartini2, Ang Boon Suen3
1Department of Physiology, Faculty of Medicine, Muhammadiyah University of Sumatera Utara
2Programme doctor, Faculty of Medicine, University of Indonesia
3Departement of Exercise Physiology, Faculty of Medicine, University of Sains Malaysia

* Email: gusbakti@nusa.net.id

Abstract. Ingestion of beverages with carbohydrate electrolyte during exercise can delay fatigue. Fatigue caused by the decreasing of glycogen deposit source and indefensible reproduced ATP result in the improvement of IMP and ammonia during fatigue. The aim of this research was to observe the alteration of plasma ammonia and urea before, during and after exercise, after ingestion beverages of carbohydrate - electrolyte. Ten male subjects (age 18-30 years) were subjected to there cycle ergometer at 60% of VO_{max} with a pedal speed of 60 rpm until there is fatigued. The subject was given a drink of carbohydrate-electrolyte at a concentration of 6%, 12% and a flavored water placebo (P) to consume the volume of 3 ml/kg BW every 20 minutes. Blood samples were taken at rest and every 20 minutes until fatigue for analyzing plasma ammonia and urea. Mean exercise until fatigue show that no difference for three beverages. However, plasma ammonia and urea were significantly increase compared before and after exercise (p<0.001). Results of plasma ammonia before exercise for beverages CHO 12% (HC) (31.86±1.93µml/l vs 86.50±5.13µml/l), for CHO 6% (MC) (33.08±1.43µml/l vs 90.68±3.41µml/l), for no carbohydrate (P) (33.64±1.93µml/l vs 93.12 ± 2.91µml/l). Whereas plasma urea before exercise for beverages CHO 12% (4.75±0.12mmol/l vs 5.44±0.10mmol/l), for CHO 6% (4.88±0.20mmol/l vs 5.22±0.10mmol/l), for Placebo (4.88±0.20mmol/l vs 5.54±0.24mmol/l). Conclusions that increase of plasma ammonia of during fatigue, can become the criteria for determining intensity exercise until fatigue results are better than plasma lactate.

Keywords: exercise cycling ergometer, plasma ammonia, urea

1. Introduction
Ingestion beverages carbohydrate-electrolyte during exercise can improve performance. Recently it has been reported that carbohydrate ingestion during prolonged strenuous exercise (i.e., 74% of max O2 uptake) can delay the development of fatigue [1,2]. Carbohydrate administration may result in increased utilization of blood glucose with a proportional slowing of muscle glycogen depletion [3]. Their fatigue during exercise is related to elevated levels of ammonia, which is one of the products of metabolism
protein [4,5]. Fatigue usually occurs due to decreased levels of muscle glycogen to exercise at moderate intensity [2,6]. In the prolonged exercise, can a reduction in muscle glycogen resources and failure re-phosphorylation ADP. Source reduction of glycogen during fatigue cannot maintain re-synthesis ATP and causes increased inosine monophosphate (IMP) and ammonia. Several studies have manipulated the supply of carbohydrate to contracting the skeletal muscle in an attempt to alter ammonia production during prolonged exercises at moderate intensities [5,6,7,8]. The effect of low glycogen content on metabolism ammonia is currently unclear because one study reported that muscle ammonia production was enhanced in this circumstance, whereas another found no change. Some studied trained cyclists the influence of carbohydrate ingestion combined with altered muscle glycogen content during 2 hours of cycling exercise [3,8,9]. However, during the first 20 minute of exercise when exercise intensities were identical, plasma ammonia concentration was elevated in the subjects depleted of carbohydrates, and higher plasma ammonia concentration was attributed an increased muscle ammonia production derived from amino acid catabolism.

Ammonia production intense exercise has often associated with the development of fatigue. Depletion of glycogen stores limits ATP re-synthesis, which impairs exercise capacity due to resulting increases in fatigue [10]. The main sources of exercise ammonia are adenosine monophosphate (AMP) deaminase and catabolism of branched chain amino acids. During high intensity exercise, ammonia mainly from the deamination of AMP. The energy for muscular contraction is provided by the dephosphorylation of ATP to ADP. When ATP use during exercise cannot be matched by re-synthesis, ATP depletion occurs due to irreversible deamination to form ammonia and inosine monophosphate (IMP) [12,13]. This is associated with a rising level in plasma ammonia, this terms “metabolic stress” because the impact on muscle contraction and accompanied by fatigue in healthy subjects [14,15]. The accumulation of plasma ammonia can be used as an indicator of ATP loss and the development of metabolic stress in exercising skeletal muscle [16,17]. Skeletal muscle depletion and plasma ammonia accumulation have been demonstrated during cycling ergometer in this study.

The purpose of this study was to investigate the changes in plasma ammonia and urea before and after exercise after ingestion beverages of carbohydrate electrolyte.

2. Methods
2.1 Subjects
Ten healthy recreational athletes were subjected to exercise on a cycle ergometer (Lode). Subjects were tested for maximal oxygen uptake (VO$_2$max) before any of the experimental trials initiated. Percentage age of body fat was estimated form skin fold. The solutions to be tested were two carbohydrate-electrolyte drinks and an artificially flavored water placebo. Each subject was tested in a random order under each of three conditions using a double-blind design has within two weeks the each trial. All subjects had given their consent before participation.

2.2 Materials
- Bicycle ergometer (Lode NVL-77)
- spectrophotometer
- Infusion catheter (butterfly needle)
2.3 Experimental design

Subjects were instructed to maintain a constant diet and training schedule throughout the experimental period, especially 2 days prior to each exercise bout. For all trials, subjects had reported to the laboratory in the morning after a period of 10–12 hours fasting. An 18-gauge catheter was inserted into the superficial forearm vessel. Electrodes were secured to the skin for monitoring heart rate (HR) respectively. In the morning, subjects were allowed to consume water but no solid food. Five minutes prior to start cycling, subjects had consumed 3 ml/kg/body weight of MC, HC and water placebo and kept consuming the same schedule in during every 20 minutes exercise.

| Ingestion Beverage 3 ml/kg BW |
|-------------------------------|
| Rest                         |
| VO2max 50%                   |
| VO2max 60%                   |
| Warm up                      |
| Time (minute)                |
| -5                            |
| 0                             |
| 20                            |
| 40                            |
| 60                            |
| 80                            |
| End                           |

Blood sample 5 ml

2.4 Biochemical analysis
Blood samples (5 ml) were obtained at rest and during exercise in every 20 minute until fatigued, centrifuged at 4°C, and plasma samples deposit temperature -70°C were used to analysis ammonia for measuring ammonia (Boehringer M.G.BH, ammonia) and Analysis urea (Boehringer M.G.BH, test combination urea S).

2.5 Statistical Analysis
All data were analyzed using student’s t-test, was used for determining the level of significance (P<0.05). All data reported as mean ± SE.

3. Results
The characteristic subjects age 24.6 ± 0.3 years, body weight 60.7 ± 2.3 kg, body height 166.3 ± 0.5 cm, capacity vital 4.0 ± 0.0 l, VO2max 44.6 ± 0.5 ml.kg⁻¹.min⁻¹, heart rate max 173.6 ± 1.1 beat.min⁻¹

3.1 Changes in plasma levels of ammonia
During exercises, plasma ammonia levels increase significantly on the three drinks were given, when compared before exercise, during exercise, and the end of the exercise showed a significant difference (p <0.001). Where the plasma levels of ammonia for drinks CHO 12% (HC) before 31.86 ± 1.93 μm l/l and the end of exercise 86.50 ± 5.13 μm l/l and CHO 6% (MC) before 33.08 ± 1.43 μm l/l and the end of exercise 90.68 ± 3.41 μm l/l, whereas the placebo drink without carbohydrate (P) before 33.64 ± 1.93 μm l/l.
and end of exercise 93.12 ± 2.91 μmol/l. The three types of beverages no significant difference, but when compared to before exercise and the end of exercise seen a significant difference (p <0.001).

Figure 1. Changes plasma ammonia during exercise of cycling at VO₂max 60%, Rest, 0: warm up, end exercise, HC = high carbohydrate 12 %, MC: moderate carbohydrate 6%, P: no carbohydrate

3.2 Changes in plasma levels of urea
During exercises, plasma urea levels increase significantly on the three types of drinks given before exercise when compared the end of the exercise (p <0.001). Urea levels before exercise for drinks CHO 12% (HC) 4.75 ± 0.12 mmol/l and the end of exercise 5.44 ± 0.10 mmol/l, and for drinks CHO 6% (MC) before 4.88 ± 0.20 mmol/l and the end of exercise 5.22 ± 0.10 mmol/l, whereas for placebo (P) before 4.88±0.20 mmol/l and the end of exercise 5.54 ± 0.24 mmol/l. From the following description does not a significant difference for all three types of drinks and significant of difference compare for before and the end of exercise (Figure 2).
Figure 2. Changes plasma urea during exercise of cycling at \( \text{VO}_{2\text{max}} \) 60%, Rest, 0: warm up, end exercise, HC: high carbohydrate 12 %, MC: moderate carbohydrate 6%, P: no carbohydrate

4. Discussion
The predominant source of ammonia production during exercise, seems primary due to the deamination of AMP muscle by adenylate deaminase. Production of ammonia from other tissue has been observed experiment no specifically concerted with exercise e.q. chemical poisoning, oxygen toxicity, and also contribute to an elevated blood ammonia during exercise[18]. In this later study, blood samples were taken as long as 4 minute after the onset of exercise. The present study has investigated the kind course of blood ammonia concentration in man, in samples taken serially to a 20’s interval during continuous ramp test to exhaustion on a bicycle ergometer [14,15,17].

In the study, it was clear that an increase in plasma ammonia was found during submaximal exercise intensity prolonged. One source of ammonia in the blood plasma is the activity of AMP deamination in muscle cells. Broberg and Sahlin, 1989 suggested that adenine nucleotide fragments may be caused low glycogen in muscle [5]. Brouns et al. (1990), also that the levels high of ammonia in the muscle is associated with muscle fatigue during prolonged exercise [15]. Although high levels of ammonia were secondary muscle glycogen levels decrease, but it is not clear whether the increased ammonia or low carbohydrate reserves is accelerating fatigue. In this study, an increase in plasma levels of ammonia on the three types of drinks are almost the same, although a little lower on the provision of carbohydrate drinks 12% (Figure 1). From the results of this study found an increase in plasma levels of ammonia caused by deamination of AMP may be more dependent on oxygen supply. Under normal circumstances 47-65 \( \mu \text{mol/l} \), during exercise can increase up to 20 folds. In a study undertaken by Babij et al. (1983) showed that in the resting state ammonia levels of arterial blood 50% taken up by the skeletal muscles, in addition Babij also estimates that only 28% of the total ammonia produced by deamination of adenine nucleotides that due to accumulate of ammonia in the blood and in the muscles of ammonia as a buffer to the synthesized of alanine, glutamic and glutamine [13,18]. Activities glucose-alanine cycle, in a derivative of glucose to form pyruvate alanine also found to increase during exercise. Alpha ketoglutarate and pyruvate in skeletal muscle is a precursor which showed an increase during exercise and tend to make ammonia as a buffer. Increased blood ammonia simultaneously with the addition of
exercise intensity, may be a reciprocal relationship to changes in the synthesis of ammonia through glutamate, glutamine and alanine. The production of glutamine and alanine in skeletal muscle is affected by pH in the muscle during its formation. However, the expenditure of alanine and glutamine by muscle is greater than the expenditure of lactic acid, which contributes amino acids are controlled by pH. The time of recovery intensity of the exercise will result in a rapid increase, following a decline in blood ammonia and lactic acid blood. This situation has a significant connection between the workloads with increased blood ammonia and lactic acid. This increase is a reciprocal relationship between the production of substrates in the muscle, diffusion into the blood and changes the substrate through several metabolic pathways.

In this study, plasma levels of urea rise to the three types of drinks, increase significantly evident when compared before and end the exercise, the same result is also find in previous studies conducted by Fallowfied & William (1993). There is also found an increase in plasma urea during exercise and increased plasma urea concentration is a sign of metabolic protein [16]. Thus, increased plasma urea can estimate fatigue during exercise. Carbohydrate supplementation avoids the no use of amino acids. It shows that the role of the protein helps the final energy metabolism, and therefore known that changes in plasma urea have nothing correlation of carbohydrates during exercise.

5. Conclusion

The production of ammonia during exercise is an indicator of exercise intensity. The state exercise can increase the capacity of the muscles, changes in ammonia levels are associated with increased synthesis of glutamine. Ammonia and amino acid metabolism in the peripheral and central nervous system, an integration of physical activity, important that this increase may be affected by metabolic pathways during exercise. In this study found meaningless changes in plasma ammonia and urea after ingestion beverages of the three types, but significant changes were an apparent increase during exercise when compared before and the end exercise. Level blood lactic acid did not reach the summit due to the intensity of exercise until final few minutes of exercise and vary with blood ammonia levels that reach a maximum at the end of exercise and decreased slowly when exercise is stop. Therefore, increased levels of blood ammonia are equivalent to endpoint is fatigue. It appears that the criterion for the metabolism of proteins with the intensity of exercise until exhaustion occurs better than lactic acid.

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