

# Effects of Fructose-Electrolyte-Antioxidant Vitamin Commercial Sports Drink Ingestion on Muscle Damage and Energy Metabolic Response during Exhaustive Exercise

## 攝取含果糖-電解質-抗氧化維生素之市售運動飲料 對力竭運動中肌肉損傷及能量代謝的反應

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### Abstract

The purpose of this study was to investigate the effects of drinking a fructose -electrolyte- antioxidant vitamin commercial sports drink on muscle damage and energy metabolic response. A randomized double-blind controlled cross-over study was designed. Eight untrained healthy males completed twice cycling exercise of 70%  $\dot{V}O_{2\max}$  until exhaustion. During exercise subjects took of 170 mL commercial sports drink or placebo every 15 min. Concentrations or activities of CK, CK-MB, glucose, lactate, SOD, GSH and MDA+4-HNE in blood were measured pre-exercise (Pre-Ex), at 30 min during exercise (Ex-30), and exhaustion (Post-Ex). There were no significant differences in the antioxidant status parameters between the two treatment groups. However, the CK and CK-MB activities at Post-Ex in the supplement group were significantly lower than the values in the placebo group. The plasma lactate concentration at Ex-30 in the supplement group was significantly lower than that in the placebo group ( $5.6\pm 3.4$  vs  $7.1\pm 3.4$  mmol/L,  $p<0.05$ ). The blood glucose concentration at Post-Ex in the supplement group was significantly higher than the value in the placebo group ( $6.2\pm 1.4$  vs  $4.7\pm 0.5$  mmol/L,  $p<0.05$ ). In conclusion, ingesting the commercial sports drink used in this study alters energy metabolic response during exercise and may attenuate the degree of muscle cell damage.

Keywords: antioxidant, creatine kinase, glucose, lactate

### 摘要

本研究之目的在探討攝取含果糖、電解質及抗氧化維生素的市售飲料對肌肉損傷及能量代謝反應的影響。研究以8位未受過訓練的健康男性為對象，採雙盲、交叉設計。受試者以 70% $\dot{V}O_{2\max}$  的強度進行腳踏車運動至力竭，運動期間每15分鐘補充170毫升的市售飲料或安慰劑，並於運動前、運動中30分鐘及運動採集血液測量其中之肌酸激酶、葡萄糖、乳酸、超氧化物歧化酶、

穀胱甘肽及丙二醛。結果顯示二組間之抗氧化能力並無差異，但補充劑組在運動後的CK及CK-MB活性與運動中30分鐘的血乳酸值均顯著低於安慰劑組 ( $p<0.05$ )；且運動後的血糖值亦顯著高於安慰劑組 ( $6.2\pm 1.4$  vs  $4.7\pm 0.5$  mmol/L,  $p<0.05$ )。此結果證實補充市售之運動飲料可改變運動中的能量代謝反應及減少肌肉損傷。

關鍵詞：抗氧化劑、肌酸激酶、葡萄糖、乳酸

## Introduction

Prolonged submaximal aerobic exercise has been demonstrated to induce an oxidative stress (Cavas & Tarhan, 2004; Marzatico et al., 1997). The increase in free radical production is primarily due to a dramatic increase in oxygen uptake. Malondialdehyde (MDA) is one of the final lipid peroxidation products and is always be used as an indicator of lipid peroxidation. Dekkers et al. (1996) reviewed some rodent and human studies and concluded that MDA significantly increased after exercise to exhaustion. MDA can be changed by plasma antioxidant levels and antioxidant enzyme. Kanter et al. (1988) have demonstrated that plasma activities of creatine-kinase (CK) and MB isoenzyme of creatine kinase (CK-MB) elevations after exercise may relate to an exercise-induced lipid peroxidation. Muscle damage resulting from exercise can indirectly be assessed by plasma CK activity (Saunders et al., 2004). Ingesting various antioxidants can detoxify the peroxides produced during exercise (Horton & Fairhurst, 1987). Research conducted by Viguie et al. (1993) reports that supplementation with  $\beta$ -carotene, vitamin E, and vitamin C daily for 2 months enhances the antioxidant capacity of the human blood glutathione system and diminishes the extent of exercise-induced muscle damage.

With continuous exercise, the body water will further decrease as a result of fluid loss by sweating and insensible water loss from the lung. Electrolytes are easily lost in sweat during physical activity. Besides water and electrolytes, carbohydrate supply is also important for exercise. Carbohydrate can contribute to the maintenance of a normal blood glucose level and will lead to sparing of the endogenous carbohydrate reserves (Brouns, 2002). Carbohydrate-electrolyte drink is commonly used in sports to provide enough fluid and energy. Studies show that the ingestion of carbohydrate-electrolyte solution during exercise will alter the energy metabolism, such as blood lactate (Khanna & Manna, 2005), blood glucose and plasma free fatty acid (Morris et al., 2003). The fructose, differ to glucose, is passively and thus slowly absorbed. It can maintain a

normal blood glucose level while not influencing insulin secretion. However, this may lead to intestinal side effects due to fructose accumulation in the intestine whenever the fructose supply exceeds the rate at which it is absorbed (Brouns, 2002).

The importance of antioxidant vitamins for exercise is the same as carbohydrate-electrolyte drink. Of the many human supplementation studies in beverage, the majority regards the ingesting sports drink (Desbrow et al., 2004; Jeukendrup et al., 1997; Morris et al., 2003) or antioxidant (Meydani et al., 1993; Sen et al., 1994) alone. During exercise, supplement combination antioxidant vitamins with drink is the most efficiency way for supply. A commercial sports drink containing antioxidant vitamins, fructose and electrolytes might have more benefits for exercise-induced injury. Therefore, in the present study, we investigated the influences of a commercial sports drink on muscle damage and energy metabolic responses during exercise.

## Materials and Methods

### Subjects

Eight untrained healthy males, with a mean age ( $\pm$  SD) of  $21.4\pm 2.3$  yrs, weight  $69.5\pm 9.9$  kg, height of  $171.6\pm 6.3$  cm, and maximal oxygen consumption ( $VO_{2\max}$ ) of  $41.1\pm 4.9$  mL/ kg-min, participated in the experiment.

### Experimental design

A randomized double-blind controlled cross-over study was designed. Eight subjects were randomly allocated into two treatment groups, placebo and commercial sports drink (supplement). The supplement contained 5.5 g fructose, 49 mg  $Na^+$ , 20 mg  $K^+$ , 2 mg  $Ca^{2+}$ , 0.6 mg  $Mg^{2+}$ , 60 mg  $Cl^-$ , citric acid 0.2 g, lactic acid: 0.2 g, vitamin E, 100 mg vitamin C, and 0.6 mg  $\beta$ -carotene per 100 mL (Energen, Otsuka Pharmaceutical Co., Japan). The placebo was aspartame and orange flavored water, with the same taste and color with the supplement.

### Exhaustive exercise test

An exhaustive test was performed 1 week after the  $\dot{V}O_{2\max}$  test. Subjects had a light breakfast 2 hours before the test. They began with 60 rpm cycling at 70% load of the maximal exercise intensity reached in the  $\dot{V}O_{2\max}$  test until exhaustion. During exercise, 170 mL (half can) of supplement or placebo was supplied to subjects every 15 min. Following a 1 week washout period, the subjects from the supplement group were switched into the placebo group, and vice versa. The exhaustive exercise test was then repeated.

### Blood collection and analysis

Four milliliters of antecubital venous blood was collected from each subject prior to exercise (Pre-Ex), at 30 min during exercise (Ex-30), and immediately after exercise (Post-Ex). Blood samples were immediately transferred to chilled heparinized glass tubes and centrifuged (2,500 rpm, 4 °C, 15 min). The plasma fraction was stored at -30 °C until analyses. The blood cell fraction were resuspended to the original blood volume and washed with a cold isotonic saline solution to get the erythrocyte fraction.

Hemoglobin was measured by the standard cyanmethaemoglobin method (Metertech Model 710, Metertech Inc, Taiwan). To evaluate blood antioxidant status, erythrocyte SOD (superoxidase dismutase) activity and GSH (glutathione) content were measured using commercial kits (Randox Lab Ltd., Crumlin, Antrum, UK and Calbiochem-Novabiochem Co., USA, respectively), and were expressed as units/g protein and mmol/L. As an index of plasma lipid peroxidation, plasma MDA+4-HNE concentrations were determined by a commercial kit (Calbiochem-Novabiochem Co., USA), and then analyzed by a Shimadzu UV-1201 spectrophotometer (Shimadzu Corporation, Analytical Instruments Division, Kyoto, Japan). Other blood biochemical parameters were determined using a Johnson & Johnson DT60 II analyzer (Orthoclinical Diagnostics, Rochester, NY, USA).

### Statistical analysis

Data were expressed as the mean  $\pm$  standard deviation (SD). Time and differences between supplementation were tested by analysis of variance for repeated measurements (ANOVA). When F-values were significant, further analysis was carried out using the Tukey post hoc test. Statistical significance was considered at  $p < 0.05$ .

### Results

The average time to complete the 70%  $\dot{V}O_{2\max}$  of cycling exercise test in group of supplement and placebo were  $69.3 \pm 27.1$  min and  $62.1 \pm 15.9$  min. There was no significant difference between the two groups. Plasma MDA+4-HNE, erythrocyte SOD activity, and GSH concentration are given in Table 1. These parameters at Pre-Ex and Post-Ex did not differ statistically between two groups. Further, no significant treatment effects on MDA+4-HNE, SOD, and GSH levels were observed.

The changes in CK and CK-MB activities during different periods are shown in Fig. 1. During the exhaustive exercise, CK activity increased significantly at Ex-30 and Post-Ex in placebo group as compared with that at Pre-Ex (from  $297.9 \pm 216.7$  to  $409.9 \pm 312.9$  and  $385.6 \pm 254.7$  U/L,  $p < 0.05$ ). However, CK activity still maintained the same level during different periods in group of supplement. Furthermore, the CK activity at Post-Ex in the supplement group was significantly lower than the value in the placebo group ( $233.9 \pm 120.5$  vs  $385.6 \pm 254.7$  U/L,  $p < 0.05$ ). The CK-MB activity increased significantly at Post-Ex in placebo group as compared with that at Pre-Ex (from  $16.6 \pm 7.6$  to  $23.1 \pm 7.0$  U/L,  $p < 0.05$ ). The group of supplement, in contrast, did not show any significant differences in CK-MB activity at different periods. Moreover, the CK-MB activity at Post-Ex in the supplement group was significantly lower than the value in the placebo group ( $13.3 \pm 5.9$  vs  $23.1 \pm 7.0$  U/L,  $p < 0.05$ ). Thus, supplement beverage ingested during exercise had a significant effect on these changes.

The plasma lactate concentrations in the two groups are presented in Fig. 2 (A). At Ex-30, the plasma lactate significantly increased in both supplement and placebo groups ( $p < 0.05$ ). Plasma lactate at Post-Ex in the placebo group was significantly higher than that at Pre-Ex. However, plasma lactate in the supplement group had returned to the baseline level. Moreover, the plasma

lactate at Ex-30 in the supplement group was significantly lower than in the placebo group ( $5.6 \pm 3.4$  vs  $7.1 \pm 3.4$  mmol/L,  $p < 0.05$ ). Fig. 2 (B) shows the plasma glucose concentrations for the two groups during a cycling exercise. It can be observed that glucose concentration does not display any significant differences during different periods in the placebo group. Nevertheless, the glucose concentration at Ex-30 in the supplement group was significantly higher than that at Pre-Ex ( $6.1 \pm 0.6$  vs  $5.4 \pm 0.8$  mmol/L,  $p < 0.05$ ). Furthermore, the plasma glucose concentration at Post-Ex in the supplement group was significantly higher than the value in the placebo group ( $6.2 \pm 1.4$  vs  $4.7 \pm 0.7$  mmol/L,  $p < 0.05$ ). Actually, the percent changes in plasma glucose concentration decreased 3.71% in the placebo group but increased 17.20% in the supplement group. The results indicated that the commercial sports drink supplementation might lessen lactate accumulation and maintain blood glucose.

## Discussion

Our results showed that CK and CK-MB activities in the placebo group significantly increased after exercise but the values in the supplement group did not. Muscle injury from eccentric exercise is also associated with the appearance of intra muscular enzymes (such as CK) in the plasma (Aguilo et al., 2005; Kondo & Itokawa, 1994). It has been demonstrated that plasma activities of CK and CK-MB elevations after exercise may be related to an exercise-induced lipid peroxidation (Kanter et al., 1988). Although there were no significant differences in plasma MDA+4-HNE, erythrocyte SOD activity and GSH concentration between Pre-Ex and Post-Ex in the placebo group in this study, we speculate that the exercise protocol of this study still induced slight lipid peroxidation. The result of our study was similar to the study of Morillas-Ruiz et al. (2005). Morillas-Ruiz et al. showed that exercise at 70%  $\dot{V}O_{2\max}$  did not increase oxidative stress significantly, but the protein carbonyls (as the marker of protein oxidation) and 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-OHdG) (as the indicator of DNA oxidative) significantly increased. Further investigation is necessary to explain whether the intensity of 70%  $\dot{V}O_{2\max}$  exhaustive cycling exercise can apparent induce lipid peroxidation and muscle damage.

Moreover, the decrease of CK and CK-MB activities in the supplement group may have resulted from the protection of electrolytes in the beverage. Electrolytes of sodium, magnesium, calcium and potassium help the cells to function normally and provide the key to muscle function, mental focus and body cooling. Magnesium deficiency impaired intracellular calcium homeostasis by altering the integrity of the sarcoplasmic reticulum membrane (Konig et al., 1998). The study of Laires et al. (1993) showed significant correlations between plasma magnesium and CK. The reduction of plasma CK and CK-MB may be due to the magnesium protection effect on cell membrane. Further research is necessary, with a larger number of subjects and variables, to obtain a better understanding of these interactions.

Elevated lactate concentrations due to exercise in our study agree with results reported by other studies (Carnevale & Gaesser, 1991; Gohil et al., 1988). Lactate is a by-product of energy metabolism. The more intense the exercise, the more lactate is produced and taken up by working muscles. The accumulation of lactate can cause muscle fatigue, pain, and soreness. With a high intensity and duration of exercise, lactate accumulation can reduce performance. In the present work, lactate concentrations of post-exercise in the supplement group were significantly lower than in the placebo group. Some investigations also showed that carbohydrate-electrolyte drink supplementation during exercise can keep blood lactate at lower levels and removes it faster (Khanna & Manna, 2005). The mechanism may be due to the combining effect of carbohydrate and electrolyte in the drink.

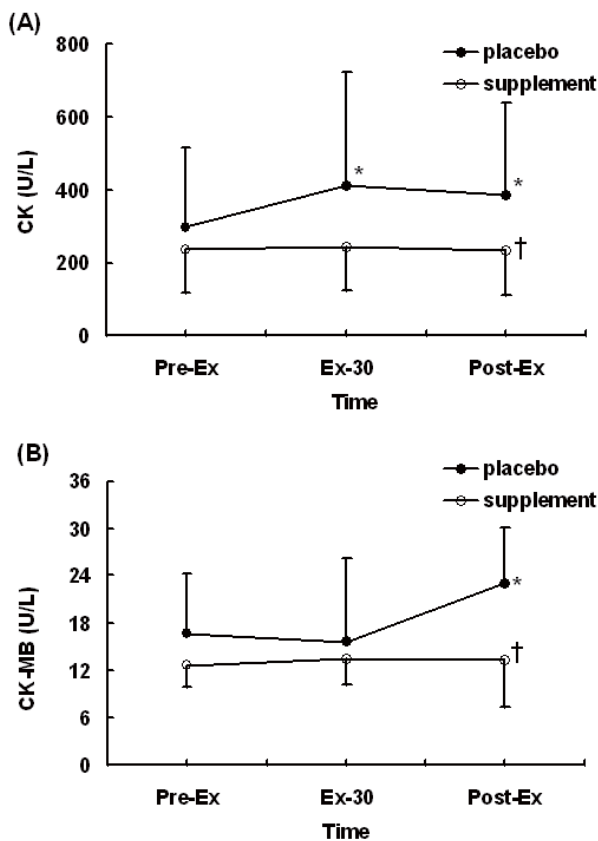
In conclusion, this study demonstrates that the commercial sports drink used in this study might decrease the degree of muscle damage and alter energy metabolic response during exercise to benefit endurance exercise.

**Table 1. Blood MDA+4-HNE, GSH, SOD levels during a cycling exercise with commercial sports drink (supplement) or placebo supplementation (mean ± SD).**

	Pre-Ex		Post-Ex	
MDA+4-HNE (mmol/L)				
supplement	37.3	± 30.0	40.5	± 27.8
placebo	37.6	± 23.4	34.8	± 28.2
GSH (µmol/L)				
supplement	255.4	± 65.2	285.6	± 155.1
placebo	286.3	± 122.0	245.3	± 151.0
SOD (U/g protein)				
supplement	2.3	± 1.1	2.8	± 0.8
placebo	3.2	± 1.0	2.8	± 0.9

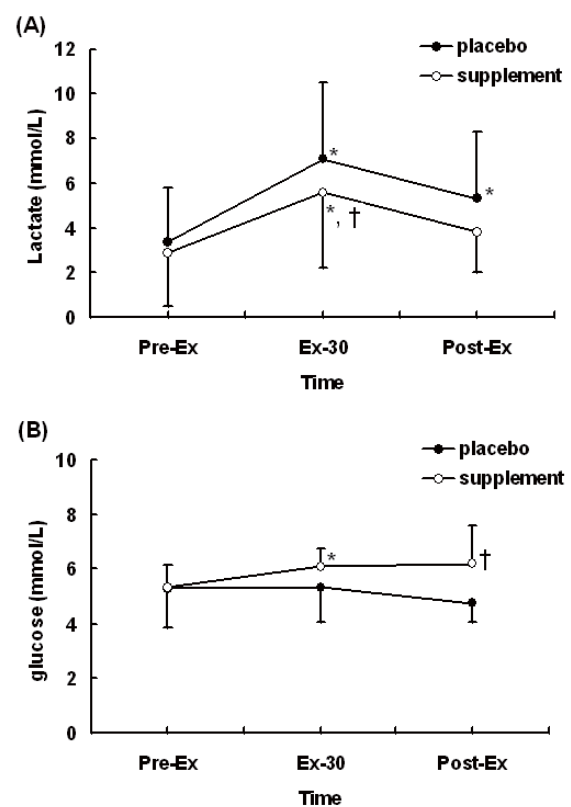
**Figure 1. Blood CK and CK-MB activities during a cycling exercise with commercial sports drink (supplement) or placebo supplementation (mean±SD).**

\* Significant difference (p<.05) from Pre-Ex.  
 † Significant difference (p<.05) from placebo group.



**Figure 2. Blood lactate and glucose concentrations during a cycling exercise with commercial sports drink (supplement) or placebo supplementation (mean±SD).**

\* Significant difference (p<.05) from Pre-Ex.  
 † Significant difference (p<.05) from placebo group.



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