Nutritional Sciences 6(3), August, 2003, pp. 155-159

The Effect of *Allium* Vegetable Intake on the Utilization and Recuperation of Plasma Fuel in Acute-Exercising Rats

Youn-Ok Cho and Eun-Young Kong

Department of Food and Nutrition, Duksung Women's University, Seoul 132-714, Korea

Ninety rats were fed either a control diet or one of several *allium* vegetable diets (*allium sativum* (AS), *allium cepa* (AC), *allium fistulosum* (AF) or *allium tuberosum* (AT)) for 4 weeks and were separated into 3 groups : non-exercise (NE), exercise (EX), and exercise and recuperation (ER). The EX group was exercised on a treadmill for 1 hour just before sacrifice at the end of 4th week of diet intake and the ER group was recuperated for 2 hours after exercise. The levels of glucose (GLU), (PRO), triglyceride (TG), free fatty acid (FFA) and hemoglobin (Hb) were compared in plasma. In the NE group, GLU levels of AS and AC tended to be higher than those of the control group. There were no differences in GLU levels between the control group and the *allium* vegetable groups in EX, whereas GLU levels of AS, AF and AT tended to be lower than that of control group in ER. There were no differences in PRO among the groups NE, EX and ER. TG and FFA levels of AS, AC, AF and AT tended to be lower than those of the control group in NE, EX and ER. Hb levels of AS, AC, AF and AT were lower than that of the control group in NE and ER and tended to be lower than that of EX. These results suggest that *allium* vegetable diets have the potential to enhance the capacity to oxidize fatty acid and to recover triglyceride after recuperation, although there is compensation among stored fuel utilization during exercise

Key words : *allium sativum, allium cepa, allium fistulosum, allium tuberosum*, exercise, fuel sources

INTRODUCTION

The metabolic events that occur during exercise provide the energy to the exercising muscle of the body and the energy used for exercise in animals is derived predominantly from carbohydrate and fat. During exercise, skeletal muscle can rely on both fat and carbohydrate oxidation to cover the need for chemical energy. Under resting conditions, fatty acid oxidation contributes considerably to total energy provision. With increasing exercise intensity, carbohydrate utilization becomes important in maintaining muscle power output[1,2] but the amount of carbohydrate stored in the body is relatively small, so the availability of carbohydrate to the working muscle becomes a limitation on the ability to perform prolonged high intensity exercise.

Attempts have been made to induce a greater oxidation of fat during exercise, which should reduce the utilization of limited carbohydrate resources of the body and thereby improve endurance capacity.[3-5] However, intravenous infusion of nutrients during exercise is practically impossible and chronic feeding induces metabolic adaptation. Thus, in order to increase the blood glucose level or to induce a greater fat oxidation during exercise, athletes are usually advised to consume special diets or ergogenic aids to stimulate energy yielding nutrient catabolism.

It has been reported indirectly that *allium* vegetable may be involved in this fuel metabolism. Recent studies have shown that *allium* vegetable contains active compounds regulating many metabolic diseases. Organosulfur compounds in *allium* vegetable have cholesterol and lipid lowering effects.[6-10] Also, flavonoids found in *allium* vegetable have been reported to play a role in preventing oxidative damage induced by active oxygen radicals in living systems and non enzymatic lipid oxidation.[11-17] Accumulating evidence has shown that untrained and strenuous exercise induces an imbalance between free radical production and the body's antioxidant defense systems. Given that high intensity exercise can increase free radical production, antioxidant supplements may offer benefits during prolonged aerobic activity and reduce fatigue.[18-22] However, direct evidence of *allium* vegetable's effect on body fuel metabolism during exercise and its effect on the endurance capacity of muscle has not been reported. Therefore, the aim of this study was to investigate the effect of an *allium* vegetable diet on the utilization and recuperation of body

* This study was supported by the research fund of Korea Science and Engineering Foundation(R06-2002-004- 01005-0)
Accepted : August 15, 2003
§ To whom correspondence should be addressed.

fuel during exercise in vivo.

MATERIALS AND METHODS

1. Animals and diets

A total of 90 male Sprague-Dawley rats (Daehan-biolink Co., Korea) of 95-105g were divided into 5 groups of 18 rats with similar body weights : Control, *allium sativum* (AS), *allium cepa* (AC), *allium fistulosum* (AF) and *allium tuberosum* (AT). Dried *allium* vegetable powder was purchased at a local market (Bumi Food Co., Korea). Animals received 10% *allium* vegetable diets or a control diet for 4 weeks. The control diet was a vitamin-free casein-based semisynthetic diet that met the AIN-93 recommendation.[23] To get the same amount of calories as that provided by the control diet, 10% cellulose for the control diet and 10% dried *allium* vegetable powder for the *allium* vegetable diets were added. Thus, all experimental diets contained 14% protein, 4% fat, 67% carbohydrates, 10% fiber and 3600 Kcal/kg by weight. Prior to initiating the respective vegetable diets, the rats were given ad libitum access to the control diet for 1 week to allow them to adapt to the diet and feeding schedule and to bring all the rats to a similar metabolic status.

2. Exercise and sample collection

At the end of week 4, the EX group was exercised on a treadmill (10°, 0.5-0.8 km/h) for 1 hour and the ER group was recuperated for 2 hours with the corresponding diet after exercise. At the respective times, (non-exercise, after one hour of exercise, 2 hours recuperation after exercise), the animals were sacrificed by decapitation under light ether anesthesia. Immediately following decapitation, blood was collected in heparinized tubes and centrifuged to separate the plasma. The plasma was stored at -40℃ until analyzed.

3. Biochemical analysis

Plasma glucose was analyzed with a commercial kit based on enzymatic method (Asan Pharmaceutical Co., Korea). Total protein was determined using a commercial kit based on the Biuret reaction (Asan Pharmaceutical Co., Korea). Triglyceride (TG) was analyzed with a commercial kit utilizing a glycerol phosphate oxidase-Quinoneimine coloring method (Asan Pharmaceutical Co., Korea). Free fatty acid (FFA) was analyzed with a commercial kit utilizing acyl CoA synthetase-Acyl CoA oxidase (NEFAZYME-S, Eiken Chemical Co., Japan). Hemoglobin concentration was determined using a commercial kit based on the cyan-methemoglobin method (Asan Pharmaceutical Co., Korea).

4. Statistical analysis

All data were subject to an analysis of variance and tested for significant differences by Duncan's multiple range test (SAS Institute, Cary, NC). A p value < 0.05 was considered to be significant.

RESULTS

As shown in Table 1, at week 4, there were no differences between the control group and *allium* vegetable diet groups, except the AS group, in which there were differences in body weight and feed efficiency ratio (FER). Compared to the control group, the AS group showed a lower body weight and higher FER. Table 2 shows the effect of the *allium sativum* (AS) diet on fuel sources in plasma. GLU levels of AS tended to be higher in the NE, but lower in the ER than those of the control group, although these differences were not statistically significant. There were no differences between the AS and control in PRO levels, regardless of exercise or recuperation. The FFA level of AS was higher than that of the control group in EX. TG and FFA levels of AS tended to be lower in the ERthose of the control group, although these differences were not statistically significant due to the large standard deviation. Table 3 shows the effect of the *allium cepa* (AC) diet on fuel sources in plasma. There were no differences between AC and control in GLU and PRO levels regardless of exercise or recuperation. TG and FFA levels of AC tended to be lower in the NE and ER than those of the control group, although these differences were not statistically significant due to the large standard deviation. There were no differences between the AC and the control in EX. Table 4 shows the effect of the *allium fistulosum* (AF) diet on fuel sources in plasma. GLU and PRO levels of AF tended to be lower in the NE and ER but higher in the EX than those of the control group, although these differences were not statistically significant. TG and FFA levels of AF tended to be lower than those of the control

Table 1. The effect of allium vegetable diet on body weight and feed efficiency ratio[1)]

	CONT[2)]	AS	AC	AF	AT
BW	248.13[a3)] ±16.11	220.83[c] ±15.61	240.56[ab] ±9.12	249.72[a] ±13.98	234.39[b] ±13.20
FER	0.34[b] ±0.05	0.38[a] ±0.04	0.35[b] ±0.04	0.35[ab] ±0.04	0.33[b] ±0.03

1) Values are mean±SEM, n=6
2) CONT : Control; AS : *allium sativum*; AC : *allium cepa*; AF : *allium fistulosum*; AT : *allium tuberosum*; BW : body weight; FER : feed efficiency ratio
3) Within a given raw, those values with different superscripts are significantly different at p<0.05

Table 2. The effect of allium sativum diet on fuel sources in plasma[1)]

	NE		EX		ER	
	CONT[2)]	AS	CONT	AS	CONT	AS
GLU	165.13$^{b3)}$ ±10.15	173.05^{ab} ±17.40	189.85^{a} ±20.46	182.12^{ab} ±21.48	190.03^{a} ±28.10	177.33^{ab} ±10.02
PRO	5.68^{ns} ±0.38	5.51 ±0.29	5.63 ±0.71	5.64 ±0.41	5.74 ±0.41	5.40 ±0.25
TG	125.36^{ns} ±47.08	91.17 ±29.40	124.50 ±24.89	112.83 ±30.52	108.33 ±63.60	81.67 ±37.94
FFA	1158.55^{ab} ±531.15	1124.17^{ab} ±558.87	1238.67^{ab} ±508.17	1725.67^{a} ±768.09	1056.83^{b} ±487.46	575.67^{b} ±119.62

1) Values are mean±SEM, n=6
2) CONT : Control; AS : *allium sativum*; NE : non-exercise; EX : exercise; ER : recuperation after exercise; GLU : plasma glucose; PRO : plasma protein; TG : plasma triglyceride; FFA : plasma free fatty acid
3) Within a given raw, those values with different superscripts are significantly different at $p<0.05$; ns : not significantly different at $p<0.05$

Table 3. The effect of *allium cepa* diet on fuel sources in plasma[1)]

	NE		EX		ER	
	CONT[2)]	AS	CONT	AS	CONT	AS
GLU	165.13$^{ns3)}$ ±10.15	181.00 ±19.95	189.85 ±20.46	185.18 ±33.24	190.03 ±28.10	189.93 ±18.48
PRO	5.68^{ns} ±0.38	6.04 ±0.53	5.63 ±0.71	5.51 ±0.58	5.74 ±0.41	5.68 ±0.44
TG	125.36^{ns} ±47.08	92.17 ±19.80	124.50 ±24.89	111.67 ±42.81	108.33 ±63.60	87.50 ±39.02
FFA	1158.55^{ns} ±531.15	959.17 ±606.37	1238.67 ±508.17	1258.00 ±331.17	1056.83 ±487.46	746.00 ±491.11

1) Values are mean±SEM, n=6
2) CONT : Control; AC : *allium cepa*; NE : non-exercise; EX : exercise; ER : recuperation after exercise; GLU : plasma glucose; PRO : plasma protein; TG : plasma triglyceride; FFA : plasma free fatty acid
3) Within a given raw, those values with ns superscripts are not significantly different at $p<0.05$

Table 4. The effect of *allium fistulosum* diet on fuel sources in plasma[1)]

	NE		EX		ER	
	CONT[2)]	AS	CONT	AS	CONT	AS
GLU	165.13$^{bc3)}$ ±10.15	157.29^{c} ±15.13	189.85^{ab} ±20.46	202.01^{a} ±34.97	190.03^{ab} ±28.10	176.61^{bc} ±11.24
PRO	5.68^{ab} ±0.38	5.43^{b} ±0.28	5.63^{ab} ±0.71	6.10^{a} ±0.68	5.74^{ab} ±0.41	5.53^{ab} ±0.35
TG	125.36^{ns} ±47.08	109.17 ±40.83	124.50 ±24.89	93.00 ±33.61	108.33 ±63.60	75.33 ±25.87
FFA	1158.55^{ab} ±531.15	1646.33^{a} ±535.59	1238.67^{ab} ±508.17	1041.17^{b} ±197.21	1056.83^{ab} ±487.46	768.50^{b} ±501.42

1) Values are mean±SEM, n=6
2) CONT : Control; AF : *allium fistulosum*; NE : non-exercise; EX : exercise; ER : recuperation after exercise; GLU : plasma glucose; PRO : plasma protein; TG : plasma triglyceride; FFA : plasma free fatty acid
3) Within a given raw, those values with different superscripts are significantly different at $p<0.05$; ns : not significantly different at $p<0.05$

group in EX and ER, although these differences were not statistically significant due to the large standard

Table 5. The effect of *allium tuberosum* diet on fuel sources in plasma[1)]

	NE		EX		ER	
	CONT[2)]	AS	CONT	AS	CONT	AS
GLU	165.13$^{b3)}$ ±10.15	162.40^{b} ±12.67	189.39^{a} ±20.46	180.39^{ab} ±17.41	190.03^{a} ±28.10	180.64^{ab} ±21.64
PRO	5.68^{ns} ±0.38	5.38 ±0.42	5.63 ±0.71	5.27 ±0.23	5.74 ±0.41	5.56 ±0.53
TG	125.36^{a} ±47.08	80.50^{ab} ±26.79	124.50^{a} ±24.89	77.33^{ab} ±38.29	108.33^{ab} ±63.60	59.83^{b} ±22.45
FFA	1158.55^{ns} ±531.15	942.33 ±493.56	1238.67 ±508.17	1096.67 ±258.57	1056.83 ±487.46	863.83 ±554.25

1) Values are mean±SEM, n=6
2) CONT : Control; AT : *allium tuberosum*; NE : non-exercise; EX : exercise; ER : recuperation after exercise; GLU : plasma glucose; PRO : plasma protein; TG : plasma triglyceride; FFA : plasma free fatty acid
3) Within a given raw, those values with different superscripts are significantly different at $p<0.05$; ns : not significantly different at $p<0.05$

deviation. Table 5 shows the effect of the *allium tuberosum* (AT) diet on fuel sources in plasma. There were no differences between AT and control in glucose and PRO levels in the NE, EX and ER. TG and FFA levels of AT tended to be lower than those of the control group although these differences were not statistically significant due to the large standard deviation and regardless of exercise or recuperation. Fig 1 shows the effect of the *allium* vegetable diet on blood hemoglobin levels. Hb levels of AS, AC, AF and AT were lower than those of the control in the NE and ER, and tended to be lower in the EX.

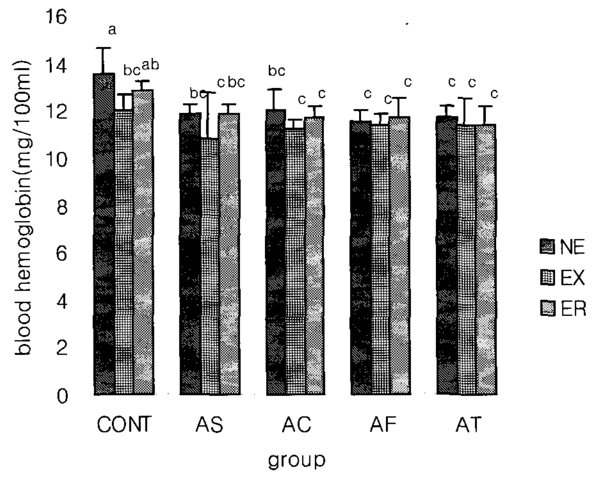

Fig 1. The effect of allium vegetable diet on blood hemoglobin level
1) Values are mean±SEM, n=6
2) CONT : Control; AS : *allium sativum*; AC : *allium cepa*; AF : *allium fistulosum*; AT : *allium tuberosum*; NE : non-exercise; EX : exercise; ER : recuperation after exercise

DISCUSSION

There were no differences between the control group and *allium* vegetable diet group, except the AS group, in which there were differences in body weight and feed efficiency ratio (FER). Even the difference in body weight and FER between the control group and the AS was less than 10%. Thus, the biochemical indices might not be influenced by the difference inn body weight and FER due to the difference in diet taste and intake.

Since during exercise, glucose can be supplied from liver glycogen breakdown or increased glucose production through gluconeogenesis and an increased FFA utilization, this results in a slower rate ofutilization[24]. Because the protein level of the control and *allium* vegetable animals did not change, regardless of exercise or recuperation, glucose production through gluconeogenesis is not assumed to have increased and the higher level of plasma glucose in the EX and ER groups of control rats is thought to be derived from liver glycogen. The fact that a higher plasma glucose level in the ER group of AC, AF and AT rats was not observed might be attributed either to an increase in glucose release from the plasma to the other tissue or to a decrease in glucose uptake from the liver. It can be assumed that although glucose uptake from the liver increased, the epididymal fat pads in the *allium* vegetable animals were more permeable to glucose and the uptake of glucose into fats cell would increase, resulting in no change in plasma glucose level in the *allium* vegetable animals. The concentration of glycogen in the liver was not measured in the present study, but similar changes in the concentration of glucose in the blood in the control and AS groups suggest that carbohydrate metabolism during exercise was not influenced by the *allium* vegetable diet.

When exercise begins, energy turnover increases rapidly with rapid mobilization of carbohydrate and lipid stored within the contracting muscle. The rate of plasma fatty acid oxidation is apparently regulated by physiological concentrations of circulating fatty acid. At low intensities, the fat oxidation increased as the exercise time is increased.[25,26] In addition, there was a gradual increase in the uptake of glucose and FFA from the circulation supplying the contracting muscle. The relative increase in the availability of FFA during exercise was shown to delay the onset of exhaustion.[27] Compared to the control animals, the AS animals showed higher FFA levels during exercise. This result is consistent with the report that was studied in men.[28] Because FFA continue to utilize accompanied by a relative sparing of carbohydrate during the first few hours after intense exercise and glucose utilization proceeds at a rate usually associated with rest,[29] AS animals is thought to show the lower plasma FFA concentration after recuperation. Thus, it is suggested that AS diets have a potential to enhance the capacity to oxidize fatty acid and to improve performance during exercise. The FFA levels of the AC and AT animals were lower than those of the control animals, but they came to be similar to those of the control animals during exercise. Also, the FFA levels of AC and AT animals were lower than those of the control animals after recuperation. Thus, it can be suggested that the increased need for fat oxidation during exercise could have led to more FFA utilization in the AC and AT diet animals and caused them to reach the level of the control animals.

Another important form of fat for oxidation by muscle during exercise is intramuscular triglyceride.[30] Plasma triglyceride is a potential source of energy for muscle and is important for recovering intramuscular triglyceride during long periods between bouts of exercise.[31] For this reason, plasma TG level thought to have decreased after recuperation in both the control animals and the *allium* vegetable animals. Compared to the control animals, the *allium* vegetable animals showed a lower plasma TG concentration after recuperation. Thus, it is assumed that the plasma TG of the *allium* vegetable was taken up to recover intramuscular triglyceride after recuperation and resulted in lower levels of plasma TG.

Therefore, diets with *allium* vegetables, such as AS, AC, AT and AF, have the potential to enhance the capacity to oxidize fatty acid and to recover triglyceride after recuperation, although there is a compensation among stored fuel utilization during exercise. However, this study does not confirm the positive effect of *allium* vegetable diets on the health of athletes. In fact, the hemoglobin level of animals on *allium* vegetable diets tended to be lower than that of the control animals in all states.

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