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Effects of *Allium* Vegetables on Energy Stores and Utilization in Exercising Rats

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Abstract

This study investigated the effect of *allium* vegetable intake on the storage and utilization of energy substrates before, during, and after exercise in tissues of rats. Ninety rats were fed either a control diet or a diet with added *Allium sativum* (AS, garlic), *Allium cepa* (AC, onion), *Allium fistulosum* (AF, spring onion), or *Allium tuberosum* (AT, Chinese chives) for 4 weeks and were then subdivided into 3 groups: before-exercise (BE); during-exercise (DE); after-exercise (AE). The DE group was exercised on treadmill for 1 hour just before being sacrificed at the end of 4th week of the dietary treatment rats in the AE group were allowed to recuperate for 2 hours after being exercised like the DE group. The levels of glycogen (GLY), triglyceride (TG) and protein (PRO) were compared in liver and skeletal muscle. In the AS diet animals, the level of liver GLY was significantly higher than those of control animals in the BE, DE and AE groups. The level of muscle TG also tended to be higher in BE, but lower in AE than in control animals. In AC animals, the level of muscle GLY was significantly lower than those of control animals in BE, DE and AE. The level of muscle TG also tended to be higher than those of control animals in BE and DE but tended to be lower in AE. In AF animals, the level of muscle GLY was significantly lower than those of control animals in BE, DE and AE. The level of muscle TG was also significantly lower than those of control animals in BE, DE and AE groups. In AT animals, the level of muscle GLY was significantly lower than those of control animals in BE, DE and AE. These results suggest that *Allium sativum* diets enhance the capacity to store fuel before as well as during exercise and increases the potential to utilize the stored fuel during exercise.

Key words: *Allium sativum*, *Allium cepa*, *Allium fistulosum*, *Allium tuberosum*, exercise, stored fuel

INTRODUCTION

When the body is involved in physical exertion, certain metabolic processes occur to assure that adequate energy is provided to the exercising muscles. Carbohydrate and fat are the primary energy substrates used for exercise; the availability of carbohydrate to working muscle becomes a limitation to the ability to perform prolonged high intensity exercise. Because carbohydrate becomes increasingly important as a fuel for muscular exercise as the intensity of the exercise increases, and because the amount of carbohydrate stored in the body is limited, muscle and liver glycogen depletion can become limiting factors during prolonged exercise. Accordingly, nutritional ergogenic aids and dietary manipulations intended to promote increased fuel storage and utilization of stored fuel have been studied for increasing exercise performance (1-6).

It has been reported indirectly that *allium* vegetables may modulate energy metabolism. Organosulfur compounds in *allium* vegetables have cholesterol and lipid lowering effects (7-10). Furthermore, flavonoids found in *allium* vegetable have been reported to play a role in preventing oxidative damage induced by active oxygen radicals and non-enzymatic lipid oxidation (11-15). Evidence is accumulating that strenuous exercise induces an imbalance between free radical production and the body's antioxidant defense systems, especially in untrained people. Given that high intensity exercise can increase free radical production, antioxidant supplements may offer benefits during prolonged aerobic activity and reduce fatigue (16-21). However, there is no direct evidence that *allium* vegetables affect energy metabolism during exercise, nor have effects on exercise capacity of muscles been reported.

Therefore, the aim of this study was to investigate the effect of *allium* vegetable intake on the storage and utilization of the stored fuels before, during, and after exercise in tissues of rats *in vivo*.

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MATERIALS AND METHODS

Experimental animals and diets

Ninety male Sprague-Dawley rats (Daehanbiolink Co., Korea) weighing 95~105 g were fed either the control diet or one of the *allium* supplemented diets with either *Allium sativum* (AS, garlic), *Allium cepa* (AC, onion), *Allium fistulosum* (AF, spring onion), or *Allium tuberosum* (AT, Chinese chives). The control diet was a vitamin-free casein based semisynthetic diet which met AIN-93 recommendation (22). The composition of *allium* vegetable diet was the same as that of control diet except for the amount of cellulose. The diets were adjusted to be isocaloric by adding 10% cellulose to the control diet and 10% dried *allium* vegetable powder to the *allium* vegetable diets (Table 1). Thus, all experimental diet contained 14% protein, 4% fat, 67% carbohydrates, 10% fiber and 3600 kcal/kg by weight. Dried *allium* vegetable powders were purchased at a local market (Bumi Food Co., Korea). Animals received 10% *allium* vegetable diets or control diet for 4 weeks.

Exercise and sample collection

At the end of week 4, animals in each dietary group were subdivided into 3 exercise groups: before-exercise (BE); during-exercise (DE); after-exercise (AE). BE groups were sacrificed without exercise at the end of week 4. Exercised groups were exercised on a treadmill (100° incline, 0.5~0.8 km/h) for 1 hour; animals in the AE groups were allowed to recuperate for 2 hours after exercise. At the respective time points, animals were sacrificed by decapitation under light ether anesthesia. Immediately following decapitation, liver and skeletal muscle (gastrocnemius) were rapidly removed and stored at -40°C until analyzed.

Biochemical analysis

Glycogen was measured by a colorimetric procedure (23). After tissue samples were homogenized in cold sodium phosphate buffer (0.02 M, pH 7.0), aliquots of the tissue homogenate were analyzed for protein and triglyceride. Total protein was determined using a commercial kit based on the Biuret reaction (Asan Pharmaceutical Co., Korea). Triglyceride was analyzed with a commercial kit utilizing the glycerol phosphate oxidase-quinoneimine coloring method (Asan Pharmaceutical Co., Korea).

Statistical analysis

All data were subjected 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

There were no differences in body and liver weights between the control group and *allium* vegetable diet groups except for body weight in the AS group and lower liver weight in the AT group (Table 2). Because differences in body weight and liver weight were relatively small, the amount of fuel storage was assumed to have not been influenced by the differences of body weight or liver weight.

Table 3 shows the effect of *Allium sativum* (AS) diet on fuel sources in liver and skeletal muscle. In AS, the level of liver glycogen was significantly higher than those of control animals in BE, DE and AE. The level of liver triglyceride also tended to be higher than those of control animals in BE, DE and AE although these

Table 1. Composition of diet

Ingredient (g/kg)	CONT[1)]	AS	AC	AF	AT
Casein	140	140	140	140	140
Sucrose	100	100	100	100	100
Soybean oil	40	40	40	40	40
t-Butylhydroquinone	0.008	0.008	0.008	0.008	0.008
Cornstarch	465.692	465.692	465.692	465.692	465.692
Dyetrose	155	155	155	155	155
Cellulose	150	50	50	50	50
Salt mix #210050	35	35	35	35	35
Vitamin mix #310025	10	10	10	10	10
L-Cystine	1.8	1.8	1.8	1.8	1.8
Choline bitartrate	2.5	2.5	2.5	2.5	2.5
AS		100			
AC			100		
AF				100	
AT					100
Total	>1100	>1100	>1100	>1100	>1100

[1)]CONT, Control; AS, *Allium sativum*; AC, *Allium cepa*; AF, *Allium fistulosum*; AT, *Allium tuberosum*.

Table 2. The effect of *allium* vegetables on body and liver weights[1)]

	CONT[2)]	AS	AC	AF	AT
Body weight (g)	248.13±16.11[a3)]	220.83±15.61[c]	240.56±9.12[ab]	249.72±13.98[a]	234.39±13.20[b]
Liver weight (g)	10.54±1.27[a]	10.82±1.06[a]	10.62±1.17[a]	10.91±1.18[a]	9.42±1.26[b]

[1)]Values are mean±SD, n=18.
[2)]CONT, Control; AS, *Allium sativum*; AC, *Allium cepa*; AF, *Allium fistulosum*; AT, *Alium tuberosum*.
[3)]Values in the same row with different superscripts are significantly different at $p<0.05$.

Table 3. The effect of *Allium sativum* diet on fuel sources in rats[1)] (mg/g)

		BE		DE		AE	
		CONT[2)]	AS	CONT	AS	CONT	AS
Liver	Glycogen	25.93±7.11[b3)]	33.43±8.56[a]	16.38±9.71[c]	29.74±6.17[ab]	17.66±7.26[c]	25.10±6.26[b]
	Triglyceride	3.83±0.75[b]	4.83±0.91[ab]	4.68±1.24[ab]	5.31±0.84[a]	4.59±0.71[ab]	5.04±0.57[a]
	Protein	270.82±24.30[ab]	259.50±18.37[b]	285.00±17.70[a]	265.50±7.53[ab]	279.00±29.02[ab]	271.50±8.85[ab]
Muscle	Glycogen	1.09±0.56[a]	1.10±0.71[a]	1.06±0.57[a]	1.05±0.51[a]	1.36±0.46[a]	1.38±0.66[a]
	Triglyceride	4.76±0.89[ab]	5.63±1.26[a]	4.01±0.84[bc]	4.07±2.18[abc]	4.73±1.16[ab]	3.06±0.80[c]
	Protein	162.57±18.30[a]	177.45±15.04[a]	173.00±16.55[a]	163.50±44.63[a]	172.20±10.00[a]	162.00±37.53[a]

[1)]Values are mean±SD, n=6.
[2)]CONT, Control; AS, *Allium sativum*; BE, before exercise; DE, during exercise; AE, after exercise.
[3)]Values in the same row with different superscripts are significantly different at $p<0.05$.

differences were not statistically significant. There were no differences between AS and control in liver protein, muscle glycogen and protein levels regardless of exercise. Compared to control animals, muscle triglyceride level of AS tended to be higher before exercise but was significantly lower after exercise.

Table 4 shows the effect of *Allium cepa* (AC) diet on fuel sources in liver and skeletal muscle. There were no differences between AC and control in liver glycogen, triglyceride and protein and muscle protein levels regardless of exercise. The level of muscle glycogen of AC animals was significantly lower than those of control animals in AE. Compared to control animals, muscle triglyceride level of AC tended to be higher before and during exercise but tended to be lower after exercise.

Table 5 shows the effect of the *Allium fistulosum* (AF) diet on fuel sources in liver and skeletal muscle. There were no differences between AF and control in liver glycogen, triglyceride and protein and muscle protein levels regardless of exercise. Muscle glycogen and triglyceride of AF animal was significantly lower than those of control animals in BE, DE and AE.

Table 6 shows the effect of the *Allium tuberosum* (AT) diet on fuel sources in liver and skeletal muscle. There were no differences between AT and control in liver and muscle protein levels regardless of exercise. Compared to control animals, liver triglyceride concentrations in AT were higher than in BE and AE. In AT animals, the level of liver glycogen was significantly lower than those of control animals in BE and muscle glycogen was significantly lower than those of control animals in BE, DE and AE. Compared to control animals, muscle triglyceride level of AT tended to be higher before exercise but tended to be lower after exercise.

DISCUSSION

A number of studies have shown that physical ex-

Table 4. The effect of *Allium cepa* diet on fuel sources in rats[1)] (mg/g)

		BE		DE		AE	
		CONT[2)]	AC	CONT	AC	CONT	AC
Liver	Glycogen	25.93±7.11[a3)]	24.51±5.82[a]	16.38±9.71[b]	13.50±5.19[b]	17.66±7.26[b]	13.91±7.69[b]
	Triglyceride	3.83±0.75[ab]	2.94±0.37[b]	4.68±1.24[a]	4.88±1.44[a]	4.59±0.71[a]	4.13±0.56[a]
	Protein	270.82±24.30[a]	273.00±9.30[a]	285.00±17.70[a]	286.50±20.01[a]	279.00±29.02[a]	289.50±13.25[a]
Muscle	Glycogen	1.09±0.56[ab]	0.70±0.71[b]	1.06±0.57[ab]	0.76±0.58[b]	1.36±0.46[a]	0.79±0.52[b]
	Triglyceride	4.76±0.89[ab]	6.21±1.91[a]	4.01±0.84[b]	5.49±1.52[ab]	4.73±1.16[ab]	3.93±1.82[b]
	Protein	162.57±18.30[a]	184.20±22.33[a]	173.00±16.55[a]	166.86±13.67[a]	172.20±10.00[a]	186.60±16.00[a]

[1)]Values are mean±SD, n=6.
[2)]CONT, Control; AC, *Allium cepa*; BE, before exercise; DE, during exercise; AE, after exercise.
[3)]Values in the same row with different superscripts are significantly different at $p<0.05$.

Table 5. The effect of *Allium fistulosum* diet on fuel sources in rats[1] (mg/g)

		BE		DE		AE	
		CONT[2]	AF	CONT	AF	CONT	AF
Liver	Glycogen	25.93±7.11[a3]	21.18±8.83[ab]	16.38±9.71[b]	17.11±4.80[b]	17.66±7.26[c]	20.87±7.29[ab]
	Triglyceride	3.83±0.75[ab]	3.21±0.33[b]	4.68±1.24[a]	4.73±1.61[a]	4.59±0.71[a]	4.23±0.75[ab]
	Protein	270.82±24.30[ab]	267.00±9.30[ab]	285.00±17.70[a]	286.50±3.67[a]	279.00±29.02[ab]	256.50±28.32[b]
Muscle	Glycogen	1.09±0.56[a]	0.51±0.25[b]	1.06±0.57[a]	0.32±0.09[b]	1.36±0.46[a]	0.42±0.23[b]
	Triglyceride	4.76±0.89[a]	2.59±2.19[bc]	4.01±0.84[ab]	1.67±2.22[c]	4.73±1.16[a]	1.31±1.06[c]
	Protein	162.57±18.30[b]	186.90±16.83[a]	173.00±16.55[ab]	182.40±30.54[ab]	172.20±10.00[ab]	170.40±13.60[ab]

[1]Values are mean±SD, n=6.
[2]CONT, Control; AF, *Allium fistulosum*; BE, before exercise; DE, during exercise; AE, after exercise.
[3]Values in the same row with different superscripts are significantly different at $p<0.05$.

Table 6. The effect of *Allium tuberosum* (AT) diet on fuel sources in rats[1] (mg/g)

		BE		DE		AE	
		CONT[2]	AT	CONT	AT	CONT	AT
Liver	Glycogen	25.93±7.11[a3]	14.20±7.46[b]	16.38±9.71[b]	13.58±4.59[b]	17.66±7.26[b]	13.64±6.27[b]
	Triglyceride	3.83±0.75[c]	6.12±1.42[ab]	4.68±1.24[bc]	5.16±0.33[bc]	4.59±0.71[c]	7.10±2.34[a]
	Protein	270.82±24.30[a]	297.00±18.00[a]	285.00±17.70[a]	288.50±18.88[a]	279.00±29.02[a]	279.00±18.88[a]
Muscle	Glycogen	1.09±0.56[a]	0.46±0.22[b]	1.06±0.57[a]	0.63±0.32[b]	1.36±0.46[a]	0.71±0.33[b]
	Triglyceride	4.76±0.89[ab]	5.99±2.02[a]	4.01±0.84[b]	3.44±0.25[b]	4.73±1.16[ab]	3.60±0.30[b]
	Protein	162.57±18.30[a]	177.45±15.48[ab]	173.00±16.55[ab]	189.15±38.17[a]	172.20±10.00[ab]	171.60±5.24[ab]

[1]Values are mean±SD, n=6.
[2]CONT, Control; AT, *Allium tuberosum*; BE, before exercise; DE, during exercise; AE, after exercise.
[3]Values in the same row with different superscripts are significantly different at $p<0.05$.

haustion is correlated with very low muscle glycogen levels. A depletion of liver glycogen may lead to hypoglycemia during exercise because gluconeogenesis normally cannot keep pace with glucose utilization by the muscles (24). It has been reported that as endurance athletes deplete the endogenous carbohydrate stores, the body catabolizes some of its protein for energy or eventual conversion to glucose. Protein catabolism has been shown to increase significantly even when muscle glycogen is depleted by only about 33~55 percent (25). The protein levels in animals fed the control and *allium* vegetable diets did not changed regardless of exercise or recuperation in this study, therefore, we can conclude that glucose production through gluconeogenesis was not increased and that liver glycogen was the major source of blood glucose in this study.

Carbohydrate stores in muscles and liver are important for sustained energy. Maintaining adequate stores to meet energy needs helps prevent fatigue during exercise, therefore, the observation that animals on the AS diet had significantly higher levels of liver glycogen than did control animals, regardless of exercise, suggests the possibility of *Allium sativum* has the potential for use as an effective nutritional ergogenic aid. When exercise is initiated energy turnover increases with rapid mobilization and oxidation of both carbohydrates and lipids stored within contracting muscle; increases in fat oxidation is most important in low intensity exercise (26, 27). A relative increase in the availability of free fatty acids during exercise has been shown to delay the onset of exhaustion (28). Free fatty acids may be released by adipose tissue triglycerides and travel through the blood to the muscle cells, and may also be derived from the muscle cell triglycerides. Especially, intramuscular triglyceride is an important form of fat for oxidation by muscle during exercise (29) and the increased content and use of muscle triglyceride may be the primary adaptive mechanism underlying the greater capacity of trained muscle to oxidize fatty acids during exercise (30). Compared to control animals, muscle and liver triglyceride levels of AS tended to be higher before exercise although this difference was not statistically significant, however, they were significantly lower after exercise. This utilization of muscle triglyceride during exercise could also be a factor that limits performance capacity in prolonged exercise. Thus, it is suggested that AS diets have a potential to increase glycogen stores and to oxidize muscle triglyceride more efficiently during exercise.

The AC diet had no significant effect on liver or muscle initial concentrations of glycogen, triglyceride, or protein, and therefore did not increase the fuel stores.

However, AC diet might have some effect on the utilization of muscle fuel since muscle triglyceride level of AC tended to be higher before and during exercise, but tended to be lower after exercise and muscle glycogen levels of AC were significantly lower than that of control after exercise. Intramuscular fat is an important energy substrate for endurance athletes (31) and protein appears to be a relatively minor source of energy, accounting for less than 5 percent of the total activity related energy utilization (32). Muscle glycogen becomes important as a fuel for muscular exercise as the intensity of exercise increases (27). Therefore, the AC diet might also help delay fatigue and improve exercise performance by facilitating the mobilization and oxidation fat and conserving limited carbohydrate stores.

The AF diet had undesirable effects on fuel stores; there were no differences between AF and control in liver glycogen, triglyceride and protein and muscle protein concentrations regardless of exercise, but muscle glycogen and triglyceride of the AF animals were 50% lower than those of control animals. The AT diet also had an undesirable effect on glycogen stores because liver and muscle glycogen stores in the AT animals were significantly lower than those of control animals before exercise, and there were no differences between AT and control in liver and muscle protein levels regardless of exercise.

This study demonstrates that dietary *Allium sativum* has the potential to increase glycogen stores and oxidization of muscle triglyceride during exercise. Dietary *Allium cepa* may also increase the utilization of muscle fuel during exercise although this diet has no effect on increasing fuel stores. *Allium fistulosum* and *Allium tuberosum* in the diet have undesirable effects on fuel stores. Therefore, *Allium sativum* and *Allium cepa* have the potential to be useful ergogenic agents where as *Allium fistulosum* and *Allium tuberosum* do not. Further research is justified to determine the effects of *Allium sativum* and *Allium cepa* on exercise performance.

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