

Effects of black garlic supplementation on exercise-induced physiological responses

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Abstract Antioxidants, including garlic, are beneficial to suppress exercise-induced oxidative stress (EIOS), and black garlic (BG) is a recently-developed whole food with strong antioxidant properties. This study investigated the effects of BG supplementation on physiological responses, especially on EIOS and recovery of muscle function. Nineteen untrained males were assigned to either a BG group (n=11, GG) or placebo group (n=8, PG), with a similar age and body mass index, during a 14-day-study. Before and after eccentric exercise of elbow flexors, we measured muscle function, blood and urinary biochemistries concerning muscle injury proteins, inflammatory cells, cytokines, reactive oxygen metabolites (d-ROMs), and antioxidative potential (BAP). Maximal voluntary contraction strength decreased by 35% immediately post-exercise in both groups. Recovery of circumference of biceps brachii in GG was significantly faster than in PG during 3-7 days post-exercise. d-ROMs level was lower in GG than in PG during 1-3 days post-exercise, but no significant difference in BAP was observed between groups. Exercise induced leukocytosis, and monocytes, lymphocytes, and neutrophils all exhibited significant time effects. A significantly greater creatine kinase level was found on day 3 post-exercise in PG than in GG. Lipid peroxide concentration was lower during 3-7 days post-exercise in GG than in PG, and the 8-iso-prostaglandin F_{2a} level was significantly greater in PG than in GG at every post-exercise point. These results suggest that BG supplementation had certain effects on suppression of physiological responses, including EIOS, and might promote the recovery of edema in injured tissue.

Keywords : black garlic, exercise-induced oxidative response, muscle function, inflammation

Introduction

Intense exercise initiates an immune response, manifested as increased blood concentrations of leucocytes, inflammatory cytokines, and acute-phase proteins¹. Intense eccentric exercise has been reported to injure skeletal muscle at various levels of ultrastructure, the extracellular matrix, and possibly the capillaries². Following mechanical injury of muscle fibers, immediately after intense exercise, infiltration of tissue liquid and plasma proteins, as well as an increase in inflammatory cells may occur.

Previous studies have reported that eccentric exercise can increase xanthine oxidase in local endothelial cells³, blood serum creatine kinase, iso-prostaglandin F_{2a} and malondialdehyde levels, as well as decrease oxygen radical absorbance capacity (ORAC)⁴. Even a single bout of exercise can result in activation of several distinct

systems of radical generation that may be separated into primary as well as secondary sources, suggesting that muscular contraction is associated with reactive oxygen species (ROS) production⁵. The production of ROS has been thought to play an important role in both the initiation and progression of muscle fiber injury after the initial mechanical insult. Increased release of ROS from injured tissues and activation of both phospholipase and protease stimulates an increase in inflammatory cells, which clear away the residue of destroyed tissue and promote repair or regeneration of injured tissues. However, excessive inflammation may again damage the injured tissues¹. A secondary increase in inflammatory cells and bimodal strength reduction are associated with increased ROS release from injured tissues, and the increase in oxidative stress is considered the principle explanation for this².

Garlic is among the oldest of all cultivated plants and has been used as a medicinal agent for thousands of years. In ancient Greece, Hippocrates, the father of medicine,

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used garlic as an essential component of his therapeutic armamentarium⁶. There is evidence that garlic was ingested at the original Olympic games as a “performance-enhancing agent”⁷. Studies have confirmed several pharmacological effects of garlic, including antibacterial, antifungal, hypolipidemic, hypoglycemic, antithrombotic, antihypertensive, and anticancer effects⁸⁻¹², and it has been shown in many cases that the protective effects of garlic are associated with its antioxidant properties¹³. The antioxidant properties of garlic against ROS have been widely recognized, including a superoxide dismutase (SOD)-like effect against superoxide radicals ($O_2^{\cdot-}$)^{14,15}, a catalase-like effect against hydrogen peroxide (H_2O_2), and scavenging of the most reactive and toxic ROS, the hydroxyl radical (OH^{\cdot})¹⁶. The compound(s) involved in the scavenging by garlic extracts are essentially heat-stable, and their scavenging ability is not related to alliinase activity¹⁷.

Studies regarding the effects of raw garlic supplementation on EIOS have also been reported. Gun-Ae Y¹⁸ indicated that, a compulsory exercise (swimming, 40 min/day, 5 days/week) model induced an obvious oxidative stress response in rats, and 2% garlic supplementation lowered either the plasma TRARS level or activities of hepatic catalase, and increased hepatic superoxide dismutase (SOD) activity. Gun-Ae Y demonstrated that anti-oxidative defense against oxidative stress could be enhanced by garlic supplementation through the induction of antioxidant enzymes. Another recent study¹⁹ in humans showed that, 30 min aerobic exercise (75% $\dot{V}O_2$ max) induced a significant increase of MDA and total peripheral leukocytes counts, and a significant decrease of TAC, while short-term garlic extract (700mg/day) supplementation significantly affected these variables, suggesting that garlic short-term supplementation is able to decrease exercise-induced oxidative damages (lipid peroxidation) and inflammatory indices (leukocytosis) in non-athletic men.

Despite the finding that heating before or after garlic cutting was unable to eliminate the capacity of raw garlic or its extracts to scavenge $O_2^{\cdot-}$, H_2O_2 , and OH^{\cdot} , various studies have suggested that the method of garlic preparation may influence its medicinal properties. Cooking, roasting or broiling may make the garlic effects much milder, while raw garlic may have some severe side effects, for example, leading to gastrointestinal disturbance, anemia, bleeding, and even severe allergy, etc.^{8,10,13-15,20,21}.

A black garlic (BG) manufacturing process has been developed in recent years using a one-month maturing process at a certain temperature and humidity, which removes the stimulating smell of raw garlic and produces new constituents, such as S-Allyl-cysteine and large quantities of maillard compounds. Compared to raw garlic, BG contains 3 times the amount of polyphenols and 1.5 times the amount of free amino acids, and has 10-fold stronger antioxidant properties²². These strong antioxidant properties and large quantity of free amino acids might be expected to affect exercise-induced inflammatory response, de-

layed occurrence of muscle soreness, and the recovery of muscle fatigue or muscle injury. In fact, BG has been recommended in some sports clubs before and after physical training, and lots of subjects experienced better fitness, feeling more energetic or less fatigue, and faster recovery from intense physical activities. Moreover, the one-month maturing process removed the unpleasant garlic odor and pulled out the natural sweetness and flavor, making it much easier to be eaten by all ages.

Hence, concerning its tasteful flavor and strong anti-oxidative effects, we investigated the effects of BG supplementation on some physiological responses, including exercise-induced oxidative stress, biological anti-oxidative potential and the process of recovery from muscle injury, especially in regard to anti-inflammatory properties.

Materials and Methods

Study population. Twenty-three healthy, nonsmoking, untrained males were recruited from Osaka City University 1 month before the study. During the screening process, an extensive interview to clarify the extent and nature of each individual’s exercise habits was conducted, specifically to ascertain whether or not a regular weight-training program was a component thereof. Those subjects with a regular weight-training program and prior history of injury to the biceps brachii or elbow region were excluded. Additionally, subjects were instructed to maintain current exercise levels and diet habits, and to abstain from anti-inflammatory or analgesic drugs throughout the study. Further, to encourage adherence to study guidelines and supplementation schedule, the following steps were taken: 1) at the beginning of the study, subjects were strongly urged to take the supplements 3 times a day at mealtime; 2) the supplementation ball packages were to be returned at the next time of measurement, to confirm that each individual was taking the BG or placebo completely as instructed; 3) subjects were contacted on a daily basis via e-mails and telephone calls; 4) the contents of diet were photographed to confirm that no drastic changes in diet occurred. In addition, subjects were required not to take vitamin/mineral supplements for 1 month before the study and throughout the experimental period. Other criteria for exclusion included recent bacterial infection (<2 wk), acute febrile illness in the prior 2 months, and hypertension (systolic blood pressure >130 mmHg or diastolic blood pressure >90 mmHg). The study was conducted under a protocol approved by the Ethics Committee of Osaka City University. Each subject signed an informed consent form before participation.

Study design. This was a 2-wk, double-blind, parallel-design study involving comparison of a dietary supplement (BG group: GG) and a placebo (Placebo group: PG). The subjects were randomly assigned based on matched-paired design of age and body mass index (BMI) to

Table 1. Physical characteristics of subjects.

	Black Garlic Group (n=12)	Placebo Group (n=8)
Age (yr)	23.2 ± 6.4	22.4 ± 5.6
Body mass index (kg/m²)	22.4 ± 3.9	23.0 ± 3.9
Body fat (%)	18.3 ± 9.4	18.9 ± 5.3
Heart rate (bpm)	68 ± 8	73 ± 10
Systolic blood pressure (mmHg)	116 ± 12	112 ± 9
Dystolic blood pressure (mmHg)	67 ± 7	66 ± 6
Maximal arm eccentric contraction strength (kg)	28.2 ± 10.0	25.0 ± 3.2

All values represent the mean ± SD.

receive either BG balls (n=12) or placebo balls (n=11) for 7 d (days) before and then 7 d after an acute bout of eccentric exercise. After the start of the study, 2 smoking subjects and 1 vitamin-taking subject, all in PG were reported, and these 3 subjects were removed from the study, thus leaving 8 subjects in PG (Table 1). Because blood samples from 1 subject in GG could not be collected at all experimental time points, the blood-sampled subjects in GG numbered 11.

Subjects reported to the exercise laboratory room, and blood and urinary samples were collected. Changes in maximal voluntary contraction strength (MVC strength) on arm flexion were measured at every post-exercise point during the study period. The subjects also completed a subjective evaluation of muscle soreness and had range of motion (ROM) and circumference of the upper arm assessed for the involved arm.

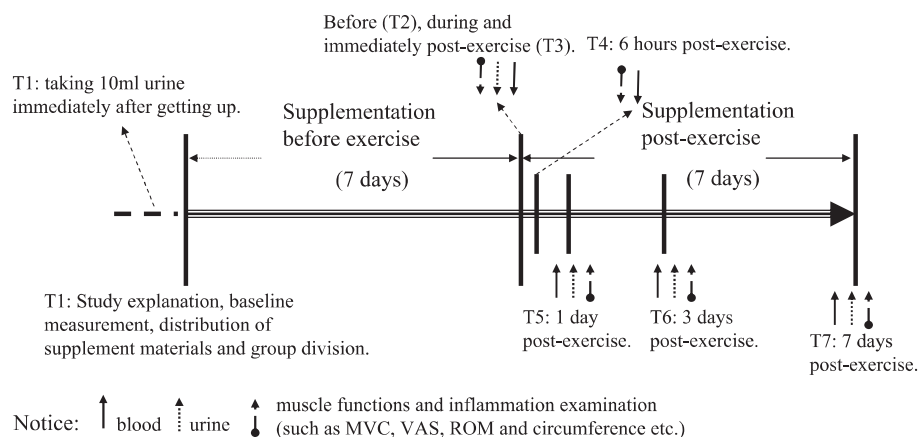
Supplementation. Supplementation, which was initiated on the first day of the 14-d period, consisted of daily doses of 9 g (due to the content of BG freeze-dried powder in the ball, the real weight of BG was equivalent to 11.2 g BG per day) BG balls (Umeken Co., Ltd. Osaka, Japan), of which 50% was BG paste, 25% BG freeze-dried powder, and 25% excipient. The balls were manufactured

with a weight of 200 mg each, and 15 garlic balls (3 g) were taken orally at each meal. The formulation of placebo balls consisted of starch from rice, corn, and sweet potato completely identical in weight and appearance to the BG balls. Each dose of 15 balls was packaged to make supplementation more convenient.

Exercise protocol. An arm curl machine (TG-071, Evernew Co., Japan) was used for the eccentric exercise protocol. Subjects performed three sets of ten repetitions at 70% of their eccentric one-repetition maximum using only the non-dominant arm. The maximal eccentric contraction strength was determined when the elbow flexors could not keep the 90-degree elbow flexion anymore as the weight from the curl machine increased. Each repetition was performed over a 6-s (second) duration. Two-minute rest periods were given between sets and repetitions were continued until completion. This type of exercise causes pain and edema for several days post-injury²³.

Blood and urine collection. Blood was taken from the antecubital vein of the uninvolved arm at 7 d pre-exercise (T1), immediately pre-exercise (T2), immediately post-exercise (T3), 6 h post-exercise (T4), 1 d post-exercise (T5), 3 d post-exercise (T6), and 7 d post-exercise (T7)

Experimental protocol



between 11:30~12:30, except for T4 at 17:00-17:30, and collected into two tubes with VenoJect II for serum acquisition, and EDTA-2K for whole-blood. The blood samples were then centrifuged at 3000 rpm for 10 min at 4°C. Serum was allocated to storage tubes, and then stored immediately in a freezer in multiple aliquots. Five early-morning urine samples were collected immediately after arising on every measuring day.

Biochemical analysis of blood and urine. Both creatine kinase and lactate dehydrogenase (CK and LDH) activities were measured, by methods of ultraviolet spectrophotometry standardized by the Japanese Society of Clinical Chemistry, using the N-assay CPK-L (Nitobo, Inc.) and L-type LDH J (Wako, Inc.) kits, respectively. Differential counts including neutrophils, monocytes and lymphocytes were performed three times with a KX-21 multiple automatic blood cell counting apparatus (Sysmex, Co. LTD.), and the average values were calculated. C-reactive protein (CRP) was measured using a latex agglutination test with a N-assay LA CRP-s kit (Nitobo, Inc.). Granulocyte colony-stimulating factor (G-CSF) was immunoassayed with a cyclizer-kit (KAINOS, Laboratories, Inc.). Lipid peroxide (LP) was measured using the hemoglobin-methylene blue method with a detamina-LPO (Kyowa Medics, Inc.). Oxidative stress and antioxidant potential were evaluated by determination of reactive oxygen metabolites (d-ROMs, with the unit of UCarr) and biological antioxidant potential (BAP) tests (Diacron, Grosseto, Italy), respectively, which were carried out using a FRAS4 analytical system (Free Radical Analytical System, Iram srl, Parma, Italy). Urinary 8-isoprostanglandin $F_{2\alpha}$ (8-iso-PGF $_{2\alpha}$) was immunoassayed with an 8-isoprostane/EIA-kit (Cayman Chemical Company, USA).

MVC strength, muscle soreness, ROM, and circumference. MVC strength was measured pre-exercise, and immediately, 6 h, 1 d, 3 d and 7 d post-exercise, using a strain gauge attached to a force transducer machine GT-310 (OG GIKEN, Inc. Japan). Subjects lay on a bed with both feet pushing firmly on the side metal railing, and the upper arm on the bed, with the elbow flexed at 90 degrees during contraction. Three trials were performed with appropriate rest of about 10 s between trials, and each trial was 3 s in duration. Subjective measures of muscle soreness and ROM were also measured before and post-exercise. Muscle soreness was assessed using a visual analog scale (0-10 cm, with 0 = no pain and 10 = extreme pain)²⁴. Soreness measures were subjectively evaluated through palpation for edema and subject reports of severity of soreness. ROM (degrees) was assessed as active arm flexion from full extension using standard goniometry²⁵. Circumference (cm) of the upper arm was assessed using a soft measure around the middle point of the biceps brachii, which was determined by finding out the highest point when bending the working elbow to 90 degrees. A

semi-permanent skin marker was used to mark the middle point of the biceps brachii to ensure reproducibility in day-to-day measurement. The means of three trials for MVC strength, ROM, and circumference were used to obtain mean measurements. All of the above examinations of muscle function were performed by the same person to assure standardization and reliability.

Statistics. Means \pm SDs were calculated. Physical characteristics of subjects, including age and BMI, were matched between groups for comparability at randomization into the double-blind phase. Two-way ANOVA (supplement \times time) followed by post-hoc multiple comparison tests were applied by using the analysis software JMP 10, developed by SAS. Correlations between d-ROMs and BAP, (change in) leucocytes and d-ROMs or change in circumference were examined by determination of Pearson's correlation coefficients. The $P < 0.05$ criterion was used to establish statistical significance.

Results

Muscle functions. Changes in MVC strength, muscle soreness, circumferences, and ROM before to post-exercise are shown in Fig 1. MVC strength (a) exhibited an approximate 35% decrease in both groups immediately post-exercise (T3), the decrease continued to day 10 (T6), and a significant time effect ($p < 0.05$) was found. However, we could not find a significant difference between groups. By day 14 (T7), MVC strength had nearly returned to baseline (T1) in both groups. Muscle soreness (b) and ROM (d) exhibited significant changes post-exercise (time effects: $P < 0.05$, respectively), but finally recovered to the baseline level (T7), with no differences between groups. Circumference of biceps brachii (c) around working arm flexors was significantly lower on day 10 (T6) and day 14 (T7) in GG than in PG ($P < 0.05$, both); it finally returned to the baseline level (T7) in GG, but still tended to be greater in PG at T7.

d-ROMs and BAP. d-ROMs (Fig. 2a) indicated significant supplement and time effects ($p < 0.05$, both) during the study, and post-hoc multiple comparison test showed significantly lower values at T5 and T6 in GG than in PG ($P < 0.05$, both). However, there existed no differences in BAP values (Fig. 2b) at the same points between groups, even though the time effect was significant ($P < 0.05$). A positive correlation ($r = 0.46$, $p < 0.05$) between d-ROMs and BAP 1 d post-exercise existed (Fig. 3), but no significant correlation at other points was found. Furthermore, a visual downward change in d-ROMs value coincided with a visual upward change in BAP 6 h post-exercise.

Leucocytes and blood biochemistry tests (Table 2). Exercise induced significant leukocytosis post-exercise, with significant time effects in leukocytes, lymphocytes

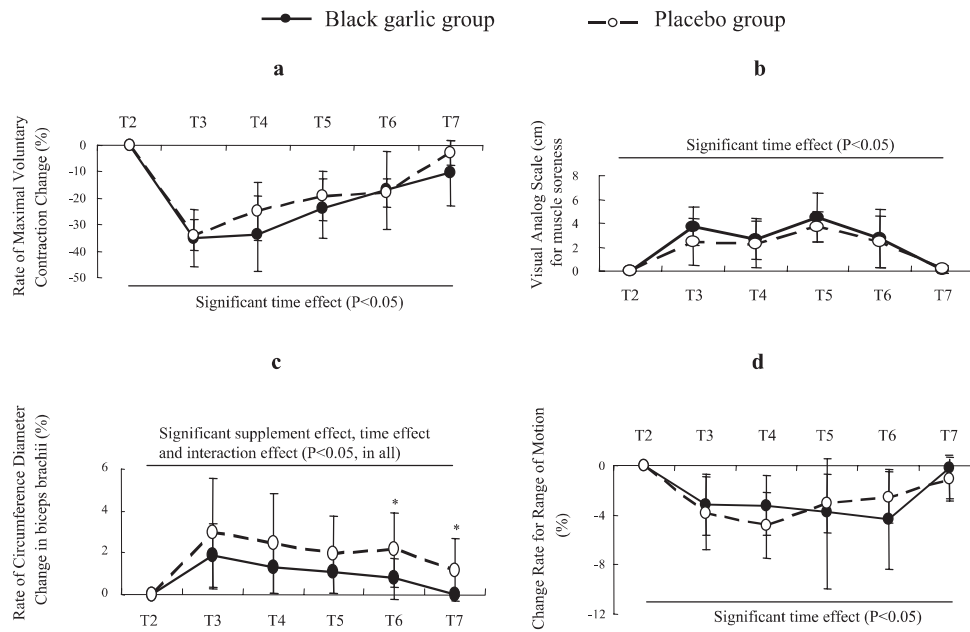


Fig. 1 (a) Maximal voluntary contraction strength, (b) pain, (c) circumference/diameter of biceps brachii and (d) range of motion before and after an eccentric arm exercise in subjects receiving a placebo (PG, $n=8$) and black garlic supplement (GG, $n=12$). Both groups indicated significant time effects in each investigated muscle function. * $P < 0.05$ between groups in the same time points. T2 (immediately pre-exercise), T3 (immediately post-exercise), T4 (6 h post-exercise), T5 (1 day post-exercise), T6 (3 day post-exercise), and T7 (7 day post-exercise).

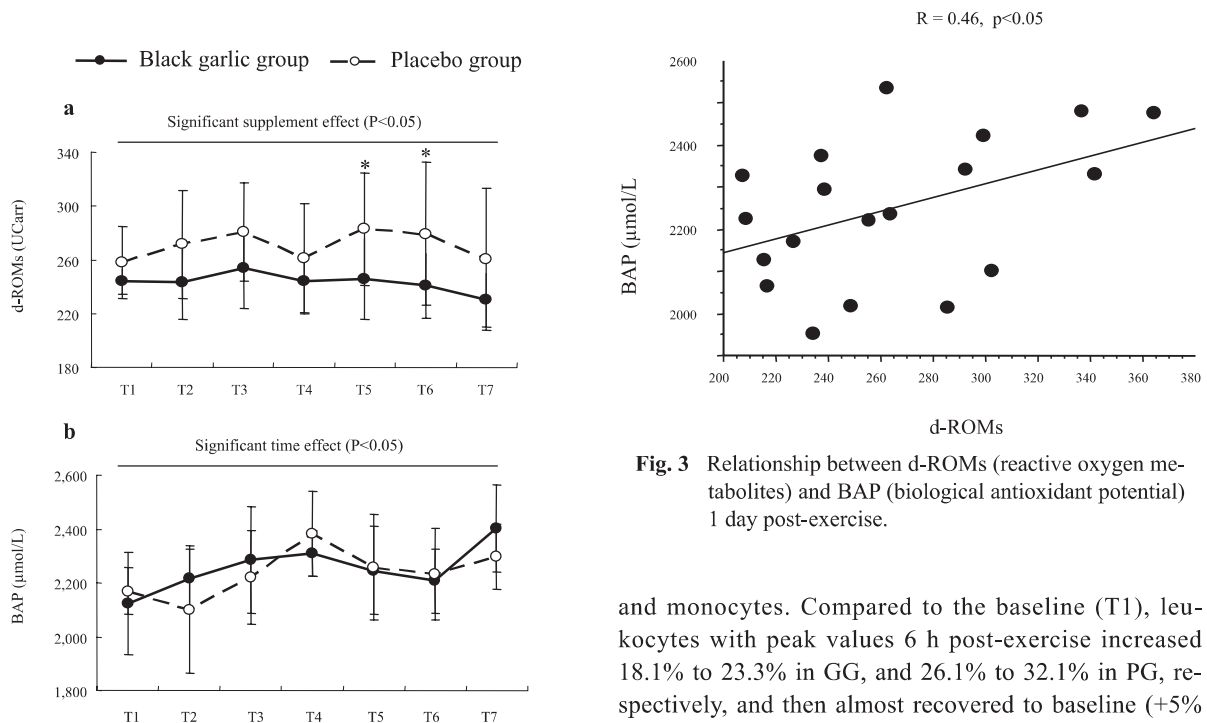


Fig. 2 Change in reactive oxygen metabolites (a, d-ROMs) and biological antioxidant potential (b, BAP) during the experimental period. * $P < 0.05$ between groups in the same time points. T1 (7 day pre-exercise), T2 (immediately pre-exercise), T3 (immediately post-exercise), T4 (6 h post-exercise), T5 (1 day post-exercise), T6 (3 day post-exercise), and T7 (7 day post-exercise).

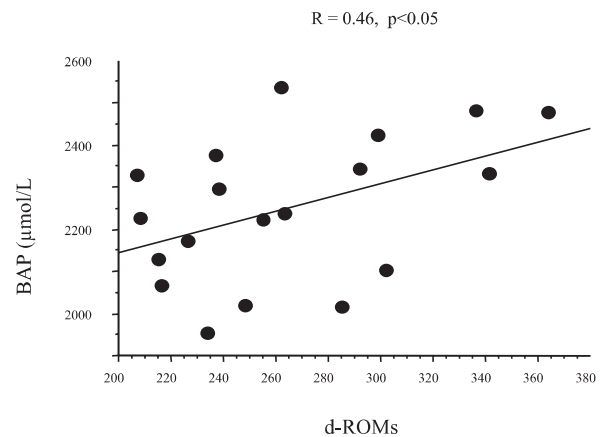


Fig. 3 Relationship between d-ROMs (reactive oxygen metabolites) and BAP (biological antioxidant potential) 1 day post-exercise.

and monocytes. Compared to the baseline (T1), leucocytes with peak values 6 h post-exercise increased 18.1% to 23.3% in GG, and 26.1% to 32.1% in PG, respectively, and then almost recovered to baseline (+5% approximately) in GG, while still greater (approximately +12.6%~18.4%) in PG 1-3 d post-exercise. A significant CK supplement effect was found, and it was greater in PG than in GG ($p < 0.05$) after 3 d post-exercise (T6). A significant increase after exercise (time effect, $p < 0.05$) in LDH was seen, and both CK and LDH peaked at T6. CRP and G-CSF exhibited no significant changes in either group during the study period (Table 2).

Table 2. Leukocytes and its subdivisions, and blood biomarkers examination in healthy human subjects receiving a placebo (n=8) or black garlic (n=11) supplementation.

	T1		T2		T3		T4		T5		T6		T7	
	GG	PG	GG	PG	GG	PG	GG	PG	GG	PG	GG	PG	GG	PG
LEUK	4718 (1082)	4750 (561)	4991 (1096)	5013 (671)	5573 (1084)	5988 (1181)	5818 (995)	6275 (1087)	4973 (1271)	5625 (1572)	4927 (940)	5350 (1228)	4827 (1190)	5088 (491)
LYM	1582 (649)	1500 (342)	1791 (534)	1913 (368)	1918 (471)	2088 (606)	1882 (546)	2263 (515)	1636 (518)	1725 (602)	1673 (465)	1763 (526)	1618 (464)	1725 (477)
MXD	436 (180)	338 (130)	527 (110)	538 (160)	509 (158)	613 (230)	582 (199)	600 (207)	473 (142)	575 (231)	509 (164)	563 (272)	427 (110)	525 (175)
NEUT	2627 (916)	2663 (910)	2945 (932)	3250 (859)	3145 (1049)	3288 (1002)	3355 (808)	3488 (743)	2836 (1188)	3175 (1359)	3200 (1448)	3025 (904)	2991 (1126)	3088 (610)
CK (U/L)	—	—	141 (32)	163 (149)	146 (34)	167 (153)	158 (38)	174 (138)	373 (420)	405 (469)	2138 * (3887)	2696 * (4515)	778 (1147)	777 (1154)
LDH (U/L)	—	—	155 (32)	158 (38)	161 (33)	170 (39)	162 (29)	162 (31)	160 (32)	180 (31)	201 (75)	231 (59)	154 (31)	167 (25)
CRP (mg/dL)	—	—	0.05 (0.01)	0.05 (0.01)	0.06 (0.02)	0.06 (0.02)	0.06 (0.03)	0.06 (0.02)	0.06 (0.02)	0.08 (0.07)	0.05 (0.02)	0.16 (0.28)	0.06 (0.01)	0.07 (0.04)
G-CSF (pg/mL)	—	—	22 (16)	22 (7)	—	—	27 (27)	22 (8)	21 (15)	27 (17)	—	—	—	—

All values represent the mean \pm SD (below in parentheses). Measurements were conducted at 7 d pre-exercise (T1), immediately pre-exercise (T2), immediately post-exercise (T3), 6 h post-exercise (T4), 1 d post-exercise (T5), 3 d post-exercise (T6) and 7 d post-exercise (T7). * $P < 0.05$ between groups in the same time points. LEUK (leukocytes) LYM (lymphocytes), MXD (monocytes), NEUT (neutrophils), CK (creatinine kinase), LDH (lactate dehydrogenase), CRP (C-reative protein), and G-CSF (granulocyte colony-stimulating factor).

The increase in lymphocytes positively correlated with circumference change at 6 h post-exercise ($r = 0.52$, $P < 0.05$) (Fig. 4a), as did neutrophil count with blood d-ROMs immediately post-exercise ($r = 0.47$, $P < 0.05$) (Fig. 4b).

Although no time effect and supplement \times time effect were observed in blood LP, a significant supplement effect ($P < 0.05$) was found, and GG had significantly lower values at T6 and T7 than PG ($P < 0.05$, both) (Fig. 5a). 8-iso-PGF_{2 α} level (Fig. 5b) indicated significant supplement effect, time effect and supplement \times time effect ($P < 0.05$, all), GG had lower values than PG post-exercise at T4, T5, T6, and T7 ($P < 0.05$, all).

Discussion

The major findings of this study indicate that supplementation of BG can significantly attenuate blood levels of CK, d-ROMs, LP and urinary 8-iso-PGF_{2 α} concentrations, which are induced by a brief, acute, intense eccentric exercise; and BG might promote the recovery of edema in injured muscle. This is the first study we are aware of that assessed physiological responses, especially of oxidative stress following eccentric exercise with supplementation of BG, a whole food popular in Japan with its strong antioxidant properties.

The significant post-exercise decreases of d-ROMs in GG (BG supplementation group) conformed to our assumption that this recently developed tasteful natural an-

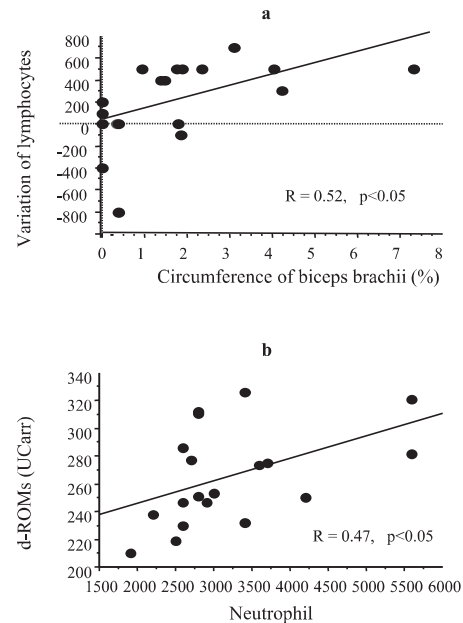


Fig. 4 Relationship between the increase of lymphocytes and the circumference change rate at 6 h post-exercise (a), between blood reactive oxygen metabolites (d-ROMs) and neutrophil immediately post-exercise (b).

tiioxidant food may be beneficial to inhibit EIOS. Despite the fact that no significant differences of BAP values between the two groups were found, the significant time effect on BAP denotes that this exercise protocol had

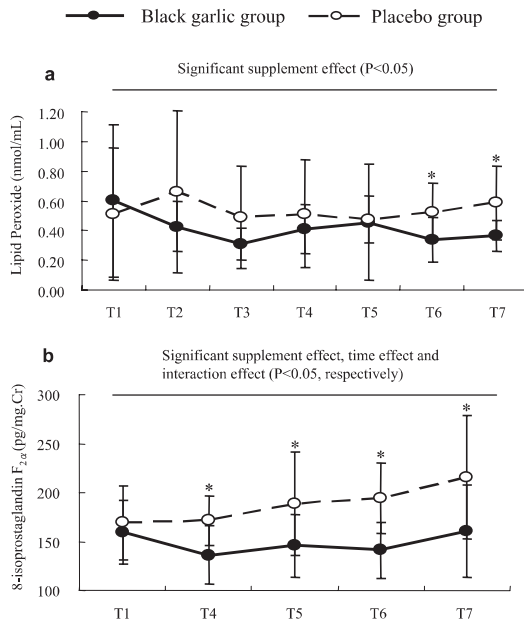


Fig. 5 Changes of blood lipid peroxide (a) and urinary 8-isoprostaglandin $F_{2\alpha}$ (b) in the experimental period. * $P < 0.05$, between groups in the same time points. T1 (baseline), T2 (immediately pre-exercise), T3 (immediately post-exercise), T4 (6 h post-exercise), T5 (1 day post-exercise), T6 (3 day post-exercise), and T7 (7 day post-exercise).

brought on increases in both d-ROMs and BAP, which is in agreement with the results caused by various ergometry exercises at maximal or sub-maximal intensities²⁶.

The decline in d-ROMs coincided with an increase in BAP, 6 h post-exercise in the placebo group, is very interesting. We believe that the biological adaptation stimulated by exercise provoked recruitment of biological antioxidants several hours later and, consequently, attenuated oxidative stress. Increased oxidative stress, caused by intense exercise, leads the visceral organs (especially liver and kidney) to release stored antioxidants into the blood, which counteract increased oxidative stress. This suggests that a biological response system may automatically protect tissues from attack, when necessary, from ROS and free radicals. However, the nature of the correlation between d-ROMs and BAP is still unclear. Positive correlation²⁶ and negative correlation²⁷ were concurrently reported. d-ROMs increased significantly after intense bicycle racing and marathons, and antioxidant supplementation significantly decreased d-ROMs²⁸, but intense intermittent exercise increased both d-ROMs and BAP²⁶. The present study found only a positive correlation between d-ROMs and BAP 1 d post-exercise, located in the chronic inflammatory response of secondary injury. The correlation between d-ROMs and BAP may be influenced by the extent of oxidative stress and the quantity of antioxidants stored in blood or tissues. Homeostasis between d-ROMs and BAP might be lost due to an excessive increase in

oxidative stress, or recovered by supplementation of antioxidants, resulting in a decrease in oxidative stress.

8-iso-PGF_{2α} has been identified as the most promising biomarker for use in human nutrition intervention studies establishing the role of antioxidants and their optimal intakes in decreasing the risk of chronic disease²⁹. Consistent with other investigations on the effects of garlic, BG supplementation significantly decreased urinary 8-iso-PGF_{2α} at every post-exercise point, and blood LP concentrations 3-7 d post-exercise. One in vitro study found that garlic extract prevented human LDL oxidation through an O₂⁻ scavenging effect and chelation of Cu²⁺, and also curbed lipid oxidation³⁰. In an animal study, garlic administration decreased LP and prevented a decline in GSH peroxidase activity in erythrocytes²¹. Furthermore, a human study reported that garlic extract, ingestion for 14 days, reduced plasma and urinary concentrations of 8-iso-PGF_{2α} by 29% and 37% in nonsmokers and by 35% and 48% in smokers; and that 14-day cessation of ingestion resulted in a return to values not differing from those before ingestion¹⁰. Our findings imply that 8-iso-PGF_{2α} is sensitive to the supplementation of BG. Even though urinary 8-iso-PGF_{2α} concentration was significantly greater post-exercise in PG (placebo group) than in GG (BG group), and the values were all above the normal range (<150pg/mL), we could not discover any correlation between the increase in 8-iso-PGF_{2α} and changes in muscle strength, muscle soreness or circumference; and thus could not clarify the clinical significance of the increased concentrations over the normal range. Our results are in agreement with another study, in which no direct causal relationship was found between the values of 8-iso-PGF_{2α} and exercise performance or chronic disease³¹. The effects of ROS on exercise performance may be exerted in a more complicated system.

The exercise protocol used in this study is reported to induce elbow flexion muscle (biceps brachii) injury with lower cardio-respiratory activity and short duration²³. MVC strength, measured immediately post-exercise, decreased about 35%, and the decrease continued to 3 d post-exercise. In addition, the responses of other variables including muscle soreness, ROM, circumference (edema), leucocytes, and blood muscle injury proteins suggest that this protocol caused both acute and chronic inflammation responses following muscle injury. Despite the obvious evidence of muscle injury after eccentric exercise in this study, the changes of CRP and G-CSF were quite minor, suggesting that eccentric exercise-induced muscle injury is not associated with the significant release of cytokines into systemic circulation, which agrees with a previous study that performed 6 sets of 5 repetitions of eccentric exercise on elbow flexors³².

Although strength, muscle soreness and ROM post-exercise did not differ significantly between the groups, the responses of edema did. The 2-phase increase in d-ROMs immediately (acute) and 1 d (chronic) post-exercise in the

placebo group suggests that this exercise protocol resulted in a 2-phase increase in oxidative stress, which might have indirectly decreased the fluidity of cell membranes (the passage of nutrients and waste through membranes) in local tissue, leading to local edema³³). Since BG supplementation markedly inhibited an increase of d-ROMs 1-3 d post-exercise, a decrease in cell membrane fluidity was suppressed, which might have promoted the recovery of edema in the treatment group 3-7 d post-exercise. Moreover, an *in vivo* study³⁴) indicated that garlic and its preparations could intensify phagocytosis by macrophages, an effect possibly related to the differences in recovery between the groups in this study. Our finding also indicated that an increase in lymphocytes positively correlated with a circumference change at 6 h post-exercise.

Blood leukocytosis was observed in both groups post-exercise, with significant time effects in leukocytes, lymphocytes and monocytes. Compared to the baseline, leukocytes 6 h post-exercise increased 23.3% in the treatment group, and 32.1% in placebo group, respectively. Leukocyte levels returned almost to the baseline level by 1-3 d post-exercise in the treatment group, but was still higher in the placebo group, indicating a suppressive effect of BG on the response of leucocytes, which can give rise to a diminished release of free radicals (e.g. O_2^- and nitric oxide radical) from neutrophils, monocytes, and macrophages. This effect may be beneficial in decreasing secondary injury of muscle, as well as affecting the repair of injured muscle. Absolute neutrophil count (ANC) or level immediately post-exercise correlated positively with blood d-ROMs, suggesting that increased oxidative stress might result from the increased leukocytosis immediately post-exercise. Muscle repair is dependent on the activity of inflammatory cells and mediators, though inflammation simultaneously facilitates and interferes with muscle repair³⁵). The effect of the inflammatory response is determined by their balance. It is therefore unclear whether it is necessary for the inflammatory response, observed in this study, to be curbed to improve recovery of muscle function, as reflected in muscle strength. The relationship between a decrease in strength and leucocyte number could not be determined.

Both blood CK and LDH concentrations obviously peaked 3 d post-exercise in both groups, but GG (BG group) showed a significantly lower blood CK level than PG (placebo group). This result implies that BG supplementation mitigated the extent of chronic muscle injury caused by exercise. Exercise-related blood CK response is characterized by large variability, and appears to be independent of sex, muscle mass and activity level of subjects²). A previous study found that serum CK activity was related to serum glutathione (GSH) activity, and that GSH served as a CK-preserving agent during the lifetime of the enzyme in circulation³⁶). The above findings suggest that BG supplementation might be the main reason for the significantly lower blood CK level 3 d post-exercise and

blood LP concentration 3-7 d post-exercise.

The 2-phase change in d-ROMs observed in this study resembles the bimodal strength reduction caused by an inflammatory response following eccentric exercise³⁷). The 2-phase change in d-ROMs may be related to the inflammatory response in blood, though we could not demonstrate this clearly, except for a positive correlation between d-ROMs and neutrophil count immediately post-exercise. Blood free radicals and ROS are generally believed to be released from inflammatory cells, injured muscle tissue, and vascular endothelial cells; and our findings suggest that the exercise protocol we used might have induced marked production of ROS and free radicals via systemic inflammatory response rather than local muscle tissue injury. In addition, there may be a time delay between blood d-ROMs production and the response of inflammatory cells.

Issues such as the mechanisms involved in exercise-induced oxidant production, oxidative stress, and strategies to minimize exercise-induced oxidative stress using antioxidant supplements have been addressed, though it is still difficult to determine which antioxidant supplement regimen is most effective. While antioxidants are mostly safe even when consumed at moderately high doses, it should be noted that the long-term effects of megadoses have not been determined in humans. Given that antioxidants act in inner networks, it would be more appropriate to speculate that taking a combination of various classes of antioxidants is prudent²⁰). BG contains large amounts of antioxidants, such as various organosulfonates, S-Allylcysteine (SAC), maillard compounds, polyphenols, saponin, and organo-selenium compounds, and is a tasteful natural whole food with strong antioxidant properties that can be considered as an ideal mixture of antioxidants. Although this study did not examine which preparations including raw garlic and other garlic preparations have stronger antioxidant properties, an *in vitro* investigation has shown that BG powder is the strongest antioxidant as evaluated by SOD activity among raw garlic, raw garlic powder, BG powder, and propolis²²).

In summary, BG supplementation at 11.2 g per day for 14 days: 1) promoted the recovery of local edema caused by eccentric exercise, but had no significant effect on the recovery of strength; 2) significantly curbed exercise-induced oxidative stress; 3) markedly decreased urinary 8-iso-PGF_{2 α} and blood LP concentrations; and 4) inhibited the increases in blood LDH and CK levels caused by exercise. In conclusion, BG supplementation can be expected to improve some physiological responses, such as chronic inflammatory responses (edema), and mitigate the extent of muscle injury due to eccentric exercise through decreasing oxidative stress.

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