ORIGINAL ARTICLE

Effects of allicin supplementation on plasma markers of exercise-induced muscle damage, IL-6 and antioxidant capacity

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Abstract To investigate the effects of allicin supplementation on exercise-induced muscle damage (EIMD), interleukin-6 (IL-6), and antioxidative capacity, a double-blinded, placebo-controlled study was conducted in well-trained athletes. Subjects were randomly assigned to an allicin supplementation group (AS group) and a control group, and received either allicin or placebo for 14 days before and 2 days after a downhill treadmill run. Plasma creatine kinase (CK), muscle-specific creatine kinase (CK-MM), lactate dehydrogenase (LDH), IL-6, superoxide dismutase (SOD), total antioxidative capacity (TAC), and perceived muscle soreness were measured pre and post exercise. AS group had significantly lower plasma levels of CK, CK-MM and IL-6, and reduced perceived muscle soreness after exercise, when compared with the control group. AS group also demonstrated a trend toward reducing plasma concentration of LDH after exercise ($P = 0.08$), although not statistically significant. Allicin supplementation induced a higher value of TAC at rest, and this higher value was maintained 48 h after exercise, however, there was no difference in SOD values after exercise between the two groups. The results suggested that allicin might be a potential agent to reduce EIMD. Further studies concerning anti-inflammatory and anti-oxidative effects of allicin on EIMD are needed.

Keywords Allicin · Eccentric exercise · Creatine kinase (CK) · Interleukin-6 (IL-6) · Antioxidative capacity

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Introduction

It has been widely reported that exercise-induced muscle damage (EIMD) frequently occurs after strenuous unaccustomed exercise, particularly if the exercise contains high frequencies of eccentric contractions (Proske and Morgan 2001; Clarkson and Hubal 2002; Kendall and Eston 2002). Several direct and indirect indicators of muscle damage have been observed, including ultrastructural disruption, delayed onset of muscle soreness, sustained loss of muscle function, and changes in many serum indicators, such as creatine kinase (CK), lactate dehydrogenase (LDH), interleukin-6 (IL-6), and superoxide dismutase (SOD) (Proske and Morgan 2001; Clarkson and Hubal 2002; Kendall and Eston 2002).

Although the exact mechanisms to explain exercise-induced muscle damage have not been delineated, the clinical symptoms include swelling, muscle soreness, and increase in inflammatory markers such as cytokines suggest that an inflammatory response subsequent to the mechanical disruption of the fiber occurs in damaged muscle and whole-body (Nieman et al. 2005; Peake et al. 2005a). Studies dealing with mechanism of EIMD have found that oxidative stress was also associated with skeletal muscle

damage after intensive exercise. Li et al. (1999) studied the effects of exhausting exercise and exercise training on skeletal muscle mitochondrial membrane fluidity and lipid peroxidation in rats, and found the exhausting exercise decreased membrane fluidity and increased lipid peroxidation. Uchiyama et al. (2006) examined the relationship between oxidative stress in muscle tissue and weight-lifting-induced muscle damage, and found an increase in reactive oxygen species (ROS) production in the muscle was observed after weight-lifting exercise, as confirmed indirectly by the significant increase in the activities of the antioxidant enzymes SOD, glutathione peroxidase, and catalase. Their results suggested that ROS-induced muscle fiber damage occurred as a consequence of weight-lifting exercise. Studies by other researches also provided evidence that the EIMD was associated with oxidative stress (Judge and Dodd 2003; Aoi et al. 2004).

The majority of studies that have been conducted in an attempt to reduce EIMD have focused on taking medicines, using massage, antioxidants supplementation and using other potential agents (Peake et al. 2005b; Hilbert et al. 2003; Lu et al. 2006; Davis et al. 2007; Savage and Clarkson 2002). However, the results of these studies varied greatly, and more studies of new treatments for reducing EIMD are needed.

The use of garlic as a food and as a medicinal agent has ancient origins in Asia (Rivlin 2001). In ancient Chinese medicine, garlic was prescribed to aid respiration and digestion, most importantly diarrhea and worm infestation (Woodward 1996). Recently, garlic and garlic constituents prepared by various means have been shown to have antioxidant, anti-inflammatory and various other biological actions (Amagase et al. 2001; Amagase 2006; Makris et al. 2005).

Allicin is produced during the crushing of garlic cloves resulting in a chemical interaction between the non-protein amino acid allicin and the enzyme allicinase (Lawson and Gardner 2005). Since allicin is the main component responsible for the biological activity of garlic, it has been the most extensively studied of garlic compounds. Although allicin has not been considered a biologically active component of garlic (Amagase et al. 2001), recently, its bioavailability has been identified by a well-designed study conducted under both simulated gastrointestinal condition and in vivo (Lawson and Gardner 2005).

Son et al. (2006) conducted a study in vitro and found that allicin was capable of inhibiting gamma-irradiation induced expression of intercellular adhesion molecule-1 which plays an important role in the regulation of cellular inflammatory responses. Based on these findings, they argued that allicin might be a potential therapeutic agent for treating various inflammatory disorders. Allicin is also considered an effective antioxidant, since it prevents lipid peroxidation (Xiao and Parkin 2002), and has the ability to scavenge hydroxyl radicals (Prasad et al. 1995).

We hypothesize that allicin may inhibit the inflammatory response and oxidative damage after eccentric exercise, and eventually reduce EIMD. Therefore, the present study was designed to investigate primarily the effect of allicin, a major component of garlic, on established markers of eccentric exercise-induced muscle damage, IL-6, and anti-oxidative capacity in trained athletes.

Methods

Subjects

Eight male and eight female athletes, aged 18–20, volunteered to take part in this study. Subjects were randomly assigned in to an allicin supplementation group (AS group) and a control group by sex and sports participation. A medical exam, including measurement of height, weight, blood pressure and electrocardiogram, was performed for each subject before the study. Subjects were advised to maintain their normal dietary habits and avoid taking antioxidant supplements, such as vitamin C and vitamin E during studying period. Brief interviews were also conducted for all subjects at baseline and endpoint. Subjects were asked about their health condition and life style (including dietary and sleeping) and training intensity. No subject had infectious diseases or was currently taking anti-inflammatory and anti-fatigue medicine during the study period. The purpose and procedures of this study, and all of the measurements were fully explained, and written informed consent was obtained from all subjects before the study. This study has been approved by the Ethics Committee of Chengdu Physical Education Institute, and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Supplementation

The supplementation consisted of capsules containing 80 mg of allicin (for AS group), whereas the placebo consisted of similar capsules containing 80 mg of maltodextrin (for control group, Petersen et al. 2001). Both were provided by Xinbei Pharmacy Co., Ltd., Xinjiang, China. Capsules were administered orally and daily for 2 weeks before the exercise protocol was performed and continued 2 days after.

Exercise protocol

All the subjects completed a downhill running protocol on a motorized treadmill after 2 weeks of supplementation. A

5-min period warm-up was given with the treadmill speed set at 6 km h^{-1} and grade at -10%. After a 3-min rest, exercise started with a 3-min stage of downhill running at the same speed and grade performed during the warm-up period, and the speed was increased 2 km h^{-1} every stage until 16 km h^{-1} or the subjects reached a heart rate of 170 beats min^{-1}, after which the speed remained constant. Subjects were encouraged to run to exhaustion and ratings of perceived exertion (RPE) were assessed using the Borg Scale at 15 s before the end of each stage (Borg 1998). During the last stage, each subject reached a heart rate of 180 beats min^{-1} or more and RPE ≥ 19. The average exercise time was 44.6 ± 3.3 min.

Blood sampling

CK, muscle-specific creatine kinase (CK-MM) and LDH were used as markers of exercise-induced muscle damage. The CK is a dimeric protein found as three principle iso-enzymes: muscle (MM), heart (MB), and brain (BB). Within skeletal muscle, CK occurs mostly in the MM isoform (approximately 90% of TCK) and only minutely in the MB and BB forms (Hyatt and Clarkson 1998). Total antioxidative capacity (TAC), SOD were used to estimate plasma antioxidative capacity, and IL-6 was examined to detect whether allicin can inhibit the inflammatory response in the damaged muscle.

Blood sampling was scheduled as follows: before allicin supplementation for AS group (baseline), pre-exercise, immediately, 24 and 48 h post-exercise for both groups.

Biochemical analyses

Plasma CK and LDH were determined spectrophotometrically by an OLYMPUS AU-5400 analyzer (Olympus Co., Tokyo, Japan) and a HITACHI 7170A analyzer (Hitachi, Ltd., Tokyo, Japan), using test kits (Biosino Biotechnology Company Ltd, Beijing, China).

CK-MM was separated and quantified by agarose gel electrophoresis in the Rapid Electrophoresis system (Helena Laboratories, Beaumont, Texas, USA).

The antioxidant defense system consists of enzymatic and non-enzymatic antioxidants, which are able to reduce Fe^{3+} to Fe^{2+}. TAC was measured by reaction of phenanthroline and Fe^{2+} using a spectrophotometer at 520 nm. At 37°C, a TAC unit is defined as the amount of antioxidants required to make absorbance increase 0.01 in 1 ml serum (Feng et al. 2001). The TAC detecting kit was obtained from Nanjing Jiancheng Bioengineering Institute, Nanjing, China.

The concentration of serum SOD was measured by the xanthine oxidase method using standard test kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Serum IL-6 concentration was determined by enzyme-liked immunosorbent assay (ELISA) using a test kit (Jingmei Biotech Co., Ltd. Shenzhen, China).

Muscle soreness assessment

The perceived leg muscle soreness of subjects was assessed using the Borg CR-10 scale (Borg 1998). The Borg's scale ranges from 0 (no pain) to 10 (maximal pain). At the beginning of the exercise test, subjects were given a typewritten set of standardized instructions for the use of the CR-10, and were instructed to give CR-10 values at pre-exercise, immediately, 24 and 48 h post-exercise.

Statistical analyses

Bivariate analyses were conducted to compare characteristics of subjects and blood markers at baseline and demographic variables between the AS and the control groups. Proportional differences were tested for significance using the χ^2 test, differences between means were calculated and significance levels were obtained using the unpaired t tests.

The differences in blood markers between pre-allicin supplementation and pre-exercise were compared using paired t tests for AS group.

A one way ANOVA with repeated measure were used to compare within-group changes in all variables from pre-exercise to each time point after exercise.

A two-way repeated measure analysis of covariance (ANCOVA) was used to detect statistically significant between- and within-subject effects. To adjust for differences between AS and control groups at pre-exercise, TAC ($P < 0.001$) was used as a covariate in the corresponding statistical model.

An unpaired t test was used to analyze difference between the two groups with respect to subject characteristics (i.e., age, height, weight). The Statistical Package for the Social Sciences (SPSS) version 12.0 was used in all statistical analyses, and $P < 0.05$ was considered statistically significant.

Results

Characteristics of subjects

As shown in Table 1, there were no significant differences in the characteristics of subjects between the AS and the control groups.

Allicin supplementation and changes of blood markers

When comparing plasma concentrations of all blood markers between pre-allicin supplementation and

Table 1 Comparison of characteristics between AS group and control group

Variables	AS group (n = 8)	Control group (n = 8)	P values
Age (years, mean ± SD)	18.9 ± 0.8	19.0 ± 0.9	0.781
Females (%)	50.0	50.0	1.000
Height (cm, mean ± SD)	169.4 ± 13.9	170.4 ± 11.1	0.876
Weight (kg, mean ± SD)	59.5 ± 13.6	61.3 ± 6.0	0.730
Track and field athletes (%)	62.5	62.5	1.000
Weight lifter (%)	37.5	37.5	1.000

pre-exercise (after 2 weeks of supplementation) in AS group we found TAC and SOD were significantly increased at pre-exercise ($P = 0.001$ and 0.002). No significant differences between pre-supplementation and pre-exercise in CK, CK-MM, LDH and IL-6 were found.

Comparison of changes in blood markers between the AS and control groups at pre- and post-exercise

There were no significant differences in pre-exercise level of CK, CK-MM, LDH, SOD and IL-6 between the AS group and control group. However, the AS group had higher plasma concentration in TAC ($P < 0.001$) compared with the controls at pre-exercise.

Both the AS and control groups showed a similar pattern of changes in plasma concentration of CK and CK-MM: Both increased immediately post-exercise, and reached their maximum values at 24 h post-exercise then decreased (Fig. 1). Two-way ANCOVA showed a significant main effect for group (AS group vs control group) on CK and CK-MM responses after exercise ($P < 0.001$), and the AS group demonstrated significantly lower CK and CK-MM response compared with the control group at 24 and 48 h post-exercise (Fig. 1).

The peak concentration of plasma LDH occurred immediately post-exercise in both the groups, then returned to the pre-exercise level. The AS group showed a faster return, and had lower values of LDH at 24 and 48 h post-exercise compared with the controls (Fig. 1), although there was only a trend towards main effects of group ($P = 0.08$).

The plasma concentration of IL-6 increased in response to exercise in both groups. Peak values were reached immediately post-exercise for both groups, with values increasing 1.9- and 8.9-fold for AS group and control group, respectively. When compared to the control group, the AS group showed significantly lower IL-6 values at all time points after exercise, and retuned to pre-exercise level at 24 h after exercise (Fig. 2).
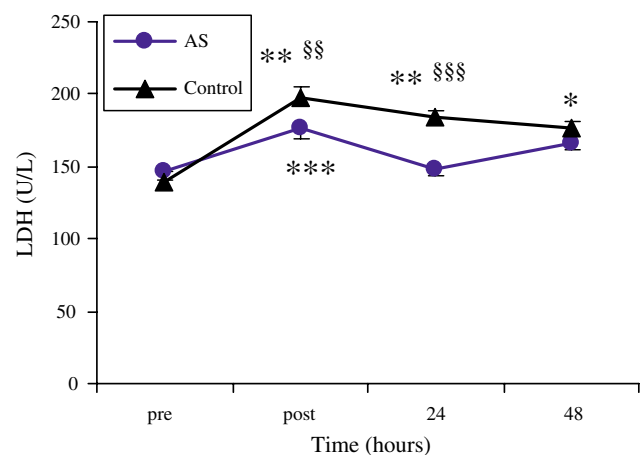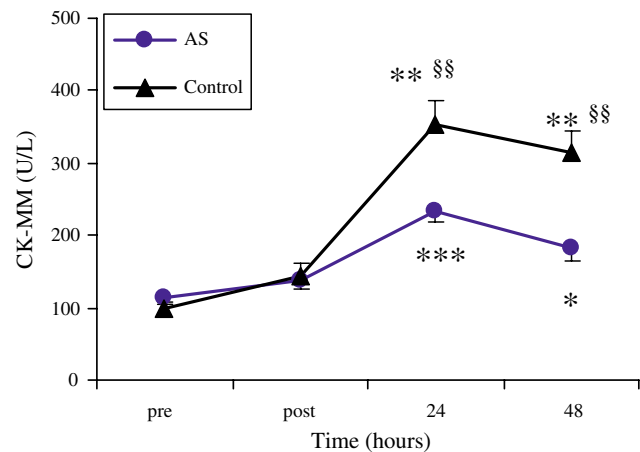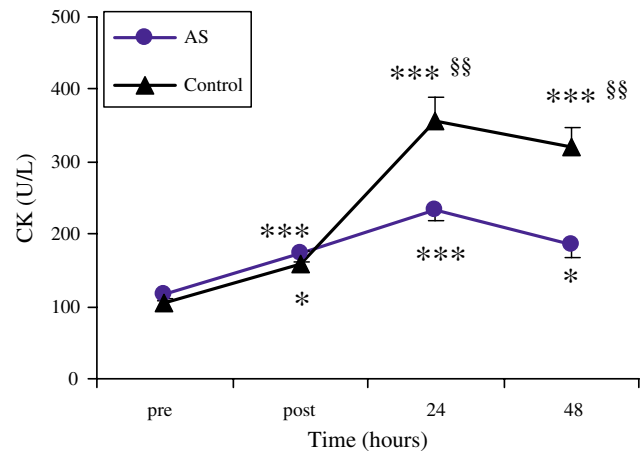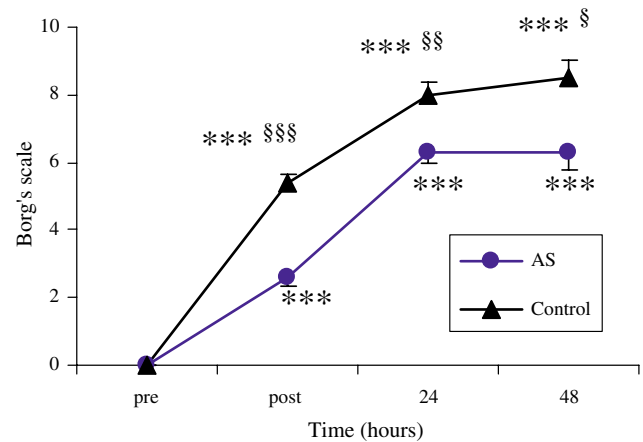

Fig. 1 Changes in CK, CK-MM and LDH before and after eccentric exercise. Values are means and SE. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; comparison of the changes from pre-exercise level for each group; §§ $P < 0.01$, §§§ $P < 0.001$, comparison of the changes from pre-exercise level between the AS and the control groups

As shown in Fig. 3, TAC values were significantly increased only at 48 h post-exercise in control group. AS group had a significantly higher TAC value at pre-exercise when compared with control group ($15.0 ± 2.0$ vs.
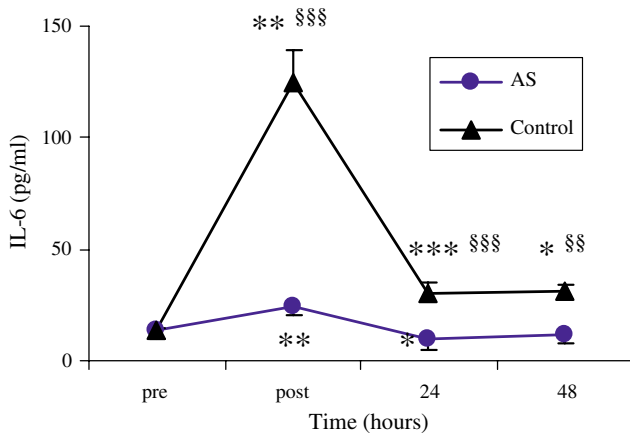
Fig. 2 Changes in IL-6 before and after eccentric exercise. Values are means and SE. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; comparison of the changes from pre-exercise level for each group; §§ $P < 0.001$ §§§ $P < 0.001$, comparison of the changes from pre-exercise level between the AS and the control groups

Fig. 4 Changes in score of Borg's scale before and after eccentric exercise. Values are means and SE. *** $P < 0.001$; comparison of the changes from pre-exercise level for each group; § $P < 0.05$, §§ $P < 0.01$, §§§ $P < 0.001$, comparison of the changes from pre-exercise level between the AS and the control groups
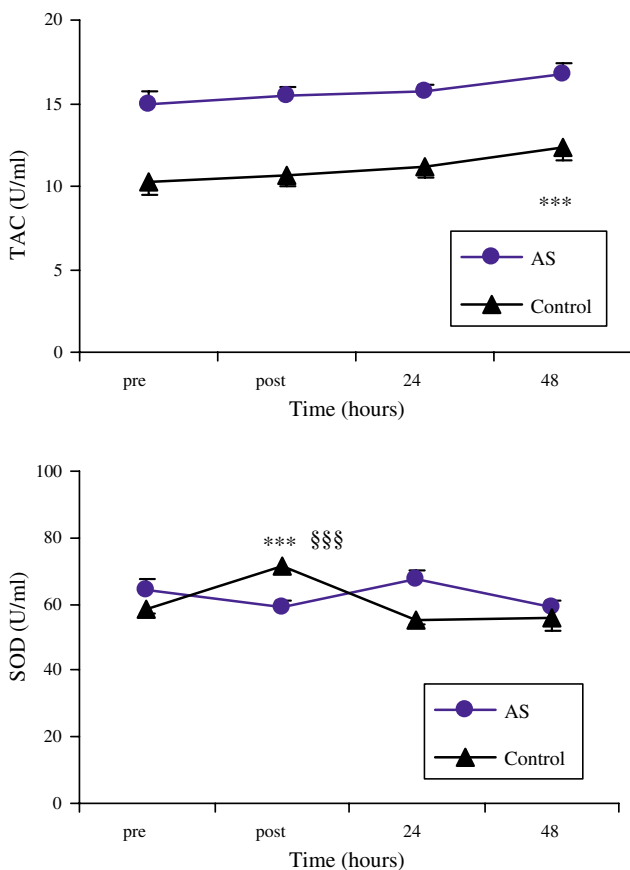
Fig. 3 Changes in TAC and SOD before and after eccentric exercise. Values are means and SE. *** $P < 0.001$; comparison of the changes from pre-exercise level for each group; §§§ $P < 0.001$, comparison of the changes from pre-exercise level between the AS and the control groups

10.2 ± 0.9, $p < 0.0001$), and maintained higher values at all time points (group effect, $P = 0.0001$). However, the two groups showed similar patterns of change in TAC after exercise (time × group effect was not significant).

AS group and control group showed different patterns of change in SOD after exercise. In the AS group, SOD decreased immediately post-exercise, then returned to the pre-exercise level at 24 h post-exercise. In contrast, the control group demonstrated an increase immediately post-exercise, and returned to the pre-exercise level at 24 h post-exercise. Although two-way ANCOVA revealed a significant interaction between allicin supplementation and time on plasma concentration of SOD, no significant group effects were found (Fig. 3). Thus the effect of allicin supplementation on plasma concentration of SOD was not identified in this study.

Comparison of changes in muscle soreness after exercise between AS group and control group

Leg muscle soreness increased significantly after exercise in both groups (Fig. 4, time effect, $P = 0.004$). AS group had significantly lower scores at all time points except at pre-exercise (group effect, $P = 0.0001$), and the effect of allicin supplementation on reducing leg muscle soreness was identified (time × group effect, $P = 0.0001$).

Discussion

The main finding of this study was that allicin supplementation blunted eccentric exercise-induced increases in plasma CK (including CK-MM), LDH and IL-6, and

alleviated muscle soreness. A higher pre-exercise TAC level, that may be induced by allicin supplementation, appears to also contribute to reducing the exercise induced muscle damage.

When muscle is damaged, there is a disruption to the cell membrane allowing muscle proteins, such as CK and LDH, to move from the cell into the interstitium then enter the blood stream (Rodenburg et al. 1994). Therefore, CK is commonly used as a blood marker of muscle damage (Clarkson and Hubal 2002; Volfinger et al. 1994). In line with previous studies (Clarkson and Hubal 2002; Brown et al. 1997), we also found the plasma concentration of CK increased immediately post-exercise, and reached peak values at 24 h post-exercise. However, the AS group showed a lower peak value and an earlier recovery compared with the controls. Allicin supplementation also demonstrated a trend toward reducing plasma concentration of LDH after exercise, although not statistically significant. In addition to the evidence that AS group had lower perceived muscle soreness after exercise, it is suggested that allicin might reduce, in some degree, the magnitude of EIMD.

The mechanism of reducing EIMD by allicin supplementation might be associated with its anti-oxidative effects. It is well established that intense bouts of exercise induce oxidative stress and consequently muscle tissue is a target for damage by free radical-mediated mechanisms (Kendall and Eston 2002). Sjödin et al. (1990) indicated that high intensity exercise may result in a markedly enhanced rate of free radicals production from semiquinone and xanthine oxidase, and an attack of free radicals on the cell membranes may lead to a loss of cell viability and to cell necrosis and could initiate the skeletal muscle damage. Recently, Okada et al. (2006) conducted a detailed study to investigate the anti-oxidative mechanism of allicin. Based on experimental results, they argued that the antioxidant property of allicin is due to scavenging the chain-carrying peroxyl radicals of the substrates by transfer of this allylic hydroperoxides, and speculated the allylic hydrogen of allicin contributes to its antioxidant activity. In the present study, we found 2 weeks of allicin supplementation increased the plasma values of TAC and SOD at rest, and the elevated TAC was maintained after exercise. The higher values of TAC pre and post exercise might have a positive impact on reducing EIMD.

The evidence that allicin supplementation lowered the increase of plasma IL-6 indicated the possibility that allicin attenuated the inflammatory response after eccentric exercise. IL-6 is a pleiotropic cytokine that has important roles in the regulation of the immune response, inflammation, and hematopoiesis (Nishimoto and Kishimoto 2006). It has drawn a lot of attention from researchers in recent years, since it is markedly increased after exercise,

and this increase was considered to be associated with an inflammatory response in working muscles and whole body (Pedersen and Fischer 2007). Inflammation after muscle injury occurs to clear debris from the injured area in preparation for generation (Clarkson and Hubal 2002). This inflammatory response is characterized by infiltration of fluid and plasma proteins into the injured tissue and increases in inflammatory cell populations (MacIntyre et al. 1995). Thus, it is naturally considered that anti-inflammatory agents may reduce the muscle damage induced by exercise. Previous studies have reported that the use of anti-inflammatory drugs could reduce plasma level of CK and muscle soreness after eccentric exercise (Pizza et al. 1999; Tokmakidis et al. 2003). In recent years, researches have studied anti-inflammatory mechanism of Allicin from different points of view. Sela et al. (2004) examined the in vitro effects of allicin on the functioning of T cells related to their entry to inflamed extravascular sites. They found that allicin inhibits the SDF-1α (CXCL12)-induced T cell migration through fibronectin, and that this inhibition is mediated by the down-regulation of the reorganization of cortical actin and the subsequent T cell polarization, and T cell adhesion to fibronectin. It is suggested that allicin may be used therapeutically to down-regulate inflammatory reactions, and used clinically to inhibit T cell-mediated diseases. To elucidate the anti-inflammatory mechanism of allicin, a research group (Mo et al. 2003; Son et al. 2006) in Korea conducted studies focusing on the regulation of intercellular adhesion molecule-1 (ICAM-1), which plays an important role in the T-cell mediated host defense system and in migration of (activated) leukocytes to sites of inflammation. They found allicin inhibited the TNF-α induced expression of ICAM-1 on human umbilical endothelial cells in a dose-dependent manner, and allicin also inhibited the production of NO and H_2O_2 induced by TNF-α, which suggests that the inhibition of ICAM-1 expression by allicin may be due to the modulated production of the reactive oxygen/nitrogen components (Mo et al. 2003). As mentioned above, in another study, their results demonstrated that allicin downregulated gamma-irradiation induced ICAM-1 expression via inhibition of both AP-1 activation and the c-Jun NH_2-terminal kinase pathway (Son et al. 2006). Furthermore, allicin also showed an anti-inflammatory effect on autocrine cytokine TNF-α in *Mycobacterium tuberculosis* infected monocytes (Hasan et al. 2006, 2007). Based on the evidence that garlic resulted in a dose-dependent reduction of IL-6 in endotoxin-stimulated human placental explants (Makris et al. 2005), we speculate that allicin supplementation reduced the increase in plasma IL-6 after eccentric exercise and that this change was due to its anti-inflammatory property, and this property may also contribute to a reduction in EIMD.

The researches focusing on the role of IL-6 in fuel metabolism introduced a new aspect of the IL-6 response to exercise. Stouthard et al. (1995) conducted a study to investigate the endocrinologic and metabolic effects of interleukin-6 in humans, and found that during infusion of recombinant human IL-6, there was a rapid and persistent increase in $\dot{V}O_2$, $\dot{V}CO_2$, and resting energy expenditure. Their results also showed that the interleukin-6 induced increases in plasma norepinephrine, cortisol, glucagon, plasma free fatty acids concentration, and rates of appearance of glucose. Steensberg et al. (2000) found that the active (but not the resting) upper leg of humans released significant amounts of IL-6 into the circulation during prolonged single-limb exercise, and IL-6 release is dependent on the pre-exercise glycogen content of the muscle, and not related to muscle damage or inflammation. Gleeson and Bishop (2000) also reported that the plasma IL-6 response to exercise is elevated when subjects begin exercise in a glycogen-depleted state. A study by Helge et al. (2003) provide evidence that the IL-6 release from working skeletal muscle is positively related to work intensity, glucose uptake and plasma adrenaline concentration. It is likely that IL-6 acts in a completely different context during exercise compared with during inflammation. Gleeson (2000) commented that the IL-6 response may be a signal indicating that muscle glycogen stores are reaching critically low concentration and that the active muscles' reliance on blood glucose as a source of energy is on the increase. This may signal the liver to increase its glucose output to prevent a drastic fall in the blood glucose concentration. In the present study, average time of downhill running was 45 min, thus the muscle glycogen stores might be decreased, and induced the IL-6 released into blood.

Many researches have paid attention to the possibility of glucose-lowering effects of garlic from 1970s (Mathew and Augusti 1973; Augusti and Mathew 1975). Study by Mathew and Augusti (1973) and Augusti and Mathew (1975) demonstrated that allicin lowered fasting blood sugar and improved oral glucose tolerance in rats with mild diabetes mellitus but had no effects on normal rats. However, several later studies confirmed the hypoglycemic effect of various garlic preparations in normal rats (Liu et al. 2007), and this effect was also identified in diabetes patients and normal males (Sobenin et al. 2007; Zhang el al. 2001). Liu et al. (2007) indicated that the mechanisms for garlic as a hypoglycemic agent may be related to its effects on improvement in insulin secretion and insulin sensitivity. In the presence of insulin, glucose utilized by skeletal muscle is oxidized and converted to glycogen and lactate. Hargreaves (2004) argued that the allicin may improve the insulin secretion, and the later increased the transport of glucose into muscle cells and converted to glycogen. In the present study, subjects in AS group had

used Allicin for 2 weeks before the exercise test, thus, the skeletal muscle of subjects in this group may contain glycogen more than the controls during downhill running. Since the IL-6 production is modulated by glycogen content (Pedersen et al. 2003), we suppose that the initiation time of IL-6 production may be delayed in the muscle containing more glycogen, and the amount of release of IL-6 will be decreased during exercise.

There was an important limitation in this study. To examine the effects of allicin supplementation on blood markers at rest, we only compared those variables at baseline and pre-exercise in AS group, without using a parallel control group. Thus, the evidence that allicin supplementation increased TAC and SOD at rest was relatively weak, although the interviews showed there were no differences in diet, taking medicines and antioxidants between AS group and control group.

In conclusion, the results of the present study suggested that allicin supplementation reduced the increases in markers of muscle damage such as CK, CK-MM and perceived muscle soreness, and inhibited an increase in plasma IL-6 in response to eccentric exercise (downhill running). A higher pre-exercise TAC level that may be induced by allicin supplementation, appears to also contribute to reducing EIMD.

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