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Oral chronic sulforaphane effects on heavy resistance exercise: implications on inflammatory and muscle damage parameters in young practitioners

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Running head: *Sulforaphane intake and muscle damage*

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Abbreviations:

1-RM: one-repetition maximum, CK: creatine kinase, IL-6: interleukin-6, ROS: reactive oxygen species

Abstract

Objective: Sulforaphane is a phytochemical that is commonly found in broccoli and broccoli sprouts. However, whether chronic sulforaphane ingestion suppresses heavy resistance exercise-induced muscle damage parameters in humans remains unknown. Therefore, this study investigated the effects of oral chronic sulforaphane ingestion on heavy resistance exercise-induced muscle damage parameters.

Research Methods & Procedures: The study had a randomized, double-blind, placebo-controlled, crossover design. Ten healthy young males (age: 22.0 ± 0.3 years; body weight: 62.6 ± 2.4 kg; height: 171.0 ± 0.1 cm) were administered placebo or sulforaphane (30 mg/day) for 4 weeks at first trial, then after 4 weeks wash out period, at second trial, participants changed opposite treatment for 4 weeks. The individuals were subjected to heavy resistance exercise (bench press, 85% of one-repetition maximum for three times with eight repetitions) after each administration, and blood samples were collected before and 30 min and 24 h after exercise each session.

Results: Four weeks of sulforaphane intake decreased plasma levels of creatine kinase (CK), especially the extent of change in CK levels from 30 min to 24 h and baseline to 24 h. Moreover, the extent of change in the levels of interleukin-6 (IL-6) significantly decreased from baseline to 30 min upon prolonged intake of sulforaphane.

Conclusions: Taken together, these findings suggest that the oral chronic intake of sulforaphane suppressed heavy resistance exercise-induced increase in muscle damage parameter and expression of inflammatory cytokines. The chronic use of sulforaphane may be a novel therapeutic candidate for the prevention of muscle damage in athletes training daily with high intensity exercise.

Keywords: Creatine kinase, supplementation, exercise, cytokine, tissue damage

Introduction

The ingestion of nutritional supplements before and after exercise prevent exercise-induced muscle damage not only athletes, but also in regular physical activity partitioners. Such supplements enhance fat oxidation, ensure rapid recovery from fatigue, and improve dietary nutritional imbalance, thereby providing the most efficient and effective exercise-induced benefits [1].

Exercise training, especially eccentric muscle contraction, increases the incidence of muscle damage. the combination of high force and reduced recruitment of fiber number during eccentric contractions causes a high mechanical stress on the involved structures that may lead to focal microlesions of the muscle fibers [2]. Moreover, vigorous exercise cause myofibrillar damage, extracellular matrix disruption as well as membranes damage, concomitantly induce loss of calcium homeostasis, increases the synthesis of reactive oxygen species (ROS) as well as the synthesis and release of proinflammatory cytokines (IL-6 and CK) what resulted in muscle damage or delayed-onset muscle soreness [3]. The exercise-induced inflammation responses are mediated by various growth factors and actions of exercise responsive cytokines such as IL-6, proinflammatory cytokines such as tumor necrosis factor-α and interleukin-1β [4]. Aerobic exercise that involves downhill running for 60 min induces an increase in CK and IL-6 levels and delays the onset of muscle soreness [5]. Sastre et al. revealed that a single bout of high intensity treadmill running increases ROS production and oxidative stress markers, thereby resulting in

muscle damage [6]. Moreover, individuals who experience periods of intense exercise, such as marathon runners, may experience a chronic necrotic state of muscle fiber, leading to an increase in CK so that it is often used as a marker of exercise-induced skeletal muscle damage [7].

Sulforaphane is a phytochemical that is mostly found in broccoli and broccoli sprouts. Additionally, sulforaphane is widely known that the most potent inducer of phase 2 antioxidant enzymes. These enzymes mainly contributed to the detoxication and enhancement of antioxidant potential [8], and might reduce the risk of exercise-induced cellular damage attributed to oxidative stress or inflammation. Bauman et al reported that the short-term intake of sulforaphane activates Keich-like ECH-associated protein 1 (Keap1) - nuclear erythroid 2 p45-related factor 2 (Nrf2) signaling, the major regulator of cytoprotective responses to oxidative and electrophilic stress, upregulates phase II enzymes, and reduces the number of tumor cells in rats with carcinogen-induced cancer [9]. Moreover, sulforaphane intake-induced upregulation of phase II enzymes was accompanied by a significant increase in the expression of Nrf2, which correlates with an increase in total antioxidant capacity and decrease in plasma lactate dehydrogenase (LDH) and CK activities in vastus lateralis muscle of rats that underwent exhaustive exercise [10]. Therefore, sulforaphane acts as an antioxidant in the skeletal muscle and is critical in the modulation of the muscle redox status, thereby leading to the prevention of exhaustive exercise-induced muscle damage. However, the effect of chronic consumption of sulforaphane on acute heavy resistance exercise-induced increase in CK and proinflammatory cytokine, IL-6 production remains to be understood in humans.

Here, we hypothesized that the oral chronic (4 weeks) sulforaphane intake suppressed a single bout of vigorous intensity resistance exercise-induced increase in the levels of CK, a biomarker for muscle damage, and IL-6, a proinflammatory cytokine. Thus, we determined the plasma levels of CK and IL-6 before, 30 min, and 24 h after vigorous resistance exercise.

Materials and Methods

Participants

This study was approved by the Human Research and Ethics Committee at the Graduate School of Human Development and Environment, Kobe University. Participants provided written informed consent before commencement of the study, which conformed to the principles embodied by the *Declaration of Helsinki*. Ten healthy young men (aged 22.2± 0.3 years) comprised the study cohort, and participants with a history of smoking, medication, and dietary nutritional supplementation were excluded from this study. The participants were examined by a physician to confirm that no one had medical problems that might preclude participation or affect the study findings. Room temperature was maintained at 22–24°C throughout the experiment. The study was designed in a double-blind, randomized, and crossover manner (n=10). Participants were administered sulforaphane (30 mg/day) or placebo for four weeks at first trial, and after 4-week washout periods sulforaphane or placebo groups changed opposite treatment at second trial according to previous study [11].

Exercise Protocol

We performed the one-repetition maximum (1-RM) strength test for bench press before the experiments according to a previous study [12]. To avoid a possible learning effect, the 1-RM test was performed twice at least 3 days after the first 1-RM test. The same investigator measured the 1-RM strengths using the same levels of vocal encouragement. The weight used the resistance exercise portion of this study was 85% of each subject's predetermined 1-RM for 3 sets of 8 repetitions. The rest period between sets was 3 min. The weight was increased for each subject when his rating of perceived exertion (RPE) was <16 for the 8th repetition of the 3rd set for bench press.

Blood Sampling

Fasting blood samples were obtained to avoid the effect of food as described previously [12,13]. On arrival at the laboratory, blood samples were collected from the fingertips and stored in MBS Capillary (MBS, Tokyo, Japan) that contains EDTA-2Na according to previous study [14]. Blood samples were also collected immediately and 24 h after exercise. Plasma samples were immediately centrifuged at 1,500 ×*g* for 15 min at 4°C. The supernatant was immediately transferred to polypropylene tubes and stored at -30°C until further analysis.

Sandwich Enzyme Immunoassay

The plasma levels of interleukin-6 (IL-6; R&D Systems, Inc., Minneapolis, MN, USA) and CK (Abcam, Cambridge, U.K.) were determined using a sandwich enzyme immunoassay kit according to previous studies [15, 16]. Immobilized polyclonal antibodies were used to detect IL-6 and CK. The secondary horseradish peroxidase-conjugated

antibodies were monoclonal. Optical density at 420 and 450 nm was determined using a microplate reader (Thermo Fisher Scientific, Multiskan FC, Yokohama, Japan). All samples were assayed in duplicates.

Statistical Analysis

Data have been represented as mean±standard error of the mean. Differences were analyzed using two-way analysis of variance (group × time). Post-hoc tests were used to correct for multiple comparisons (Bonferroni test) for data with significant differences. $P<0.05$ was considered statistically significant.

Results

Body weight, height, percent fat, and body mass index were not significantly different throughout the experiments (Table 1; $P < 0.05$). The levels of CK increased after heavy resistance exercise in the placebo and sulforaphane-administered groups, and suppressed the exercise-induced increase in CK levels 24 h after exercise upon the administration of sulforaphane (Fig. 1A). Moreover, although IL-6 levels increased 30 min after exercise in both groups, sulforaphane intake suppressed the exercise-induced increase in IL-6 as compared to that in the placebo group (Fig. 2A; $P < 0.05$).

Table 1: Physical characteristics of the patients

	Trial 1	Trial 2
Age (years)	22 ± 0.3	22 ± 0.3
Height (cm)	171.42 ± 3.12	171.44 ± 3.24
Body Weight (kg)	62.62 ± 2.43	63.34 ± 2.55
Body Fat (%)	11.37 ± 2.36	13.35 ± 1.82
Body Mass Index	21.27 ± 2.34	21.53 ± 2.78

Values represent mean ± standard error.

Alterations in CK levels

There were no changes in CK levels across the pre, 30 min, and 24-h time points after exercise in the sulforaphane and placebo groups. However, the extent of change in CK levels from baseline to 24 h after exercise suppressed (Fig. 1B; $P < 0.05$) the exercise-induced increase of CK levels upon the administration of sulforaphane.

Extent of change in IL-6 levels

Although the extent of changes in IL-6 levels in the placebo group was not altered between baseline, 30 min, and 24 h after exercise, the changes in IL-6 level reduced from baseline to 30 min after exercise (Fig. 2B; $P < 0.05$) in the sulforaphane group than that in the placebo group.

Discussion

Oral chronic intake of sulforaphane induced a decrease in CK levels 24 h after vigorous resistance exercise. Moreover, exercise-induced increase in IL-6 levels were suppressed upon the ingestion of sulforaphane 30 min after exercise when compared with the levels in the placebo group. Furthermore, the extent of change in CK levels from baseline to 24 h after exercise decreased along with the extent of change in IL-6 levels from baseline to 30 min after exercise, upon the administration of sulforaphane. Therefore, vigorous resistance exercise-induced increase in biomarker of muscle damage may be ameliorated by oral chronic intake of sulforaphane.

Sulforaphane has gained considerable attention over the recent years for its chemo-preventive function, as it is known to be a strong inducer of endogenous antioxidants [8]. Previous studies have demonstrated the effect of sulforaphane on muscle or tissue damage in rodents after aerobic exercise; however, the induction of the defense mechanisms underlying human muscle damage upon resistance exercise remain to be elucidated. Although a previous study reported that a single bout of resistance exercise increased CK and IL-6 levels after 30 min of exercise [17], the present study showed that oral chronic sulforaphane intake suppressed resistance exercise-induced increase in CK and IL-6 levels. The reasons that sulforaphane intake suppressed exercise-induced increase in CK and IL-6 would be reported previous study. The acute ingestion of sulforaphane in rodents (25 mg/kg) increased the synthesis of NAD(P)H quinone dehydrogenase 1 (NQO1), which is one of the two highly inducible quinone reductases in mammalian systems and plays multiple roles in the adaptation to cellular stress [10]. Moreover, sulforaphane intake also upregulates heme oxygenase, an essential enzyme in heme catabolism that cleaves heme

to form biliverdin (subsequently converted to bilirubin by biliverdin reductase) and carbon monoxide, a putative neurotransmitter. Sulforaphane induces the overexpression of glutathione S-transferase and glutathione reductase that in turn suppresses the synthesis of ROS [10]. Therefore, chronic sulforaphane intake upregulates antioxidant enzymes such as NQO1 and may suppress the exercise-induced increase in CK and IL-6 levels observed in the present study. To elucidate the effect of sulforaphane intake on muscle damage in humans, further studies would be needed to investigate the relationship among other antioxidant enzymes, proinflammatory cytokines, muscle soreness, and stiffness, which is measured by magnetic resonance images (MRI), visual analog scale, or a questionnaire.

Although regular physical exercise is important in preventing chronic diseases, such as obesity, type 2 diabetes, hypertension, and other metabolic syndromes, exercise or untrained exercise increases the synthesis of ROS, depending on the intensity/load and duration of exercise. Moderate habitual exercise improves health and skeletal muscle trophism, and stimulates cellular antioxidant defense [18, 19]. In contrast, acute and vigorous exercise is a risk factor for muscle injuries or soreness owing to the increase in oxidative stress in muscles, thus limiting exercise performance [20]. Exposure to high level of ROS induces exhausting of the enzymatic and non-enzymatic antioxidant system and results in impaired cellular function, macromolecule damage, apoptosis, and necrosis [21, 22, 23], although not invariably [24, 25], these contradictory results possibly ascribe to nutritional status of antioxidants and training level in participants. In fact, ROS and proinflammatory cytokines were essential in cellular development and optimal function, especially in untrained individuals. Although all participants have experienced resistance training in the present study, they did not habitually train or exercise. According to previous

studies, sedentary subjects could exhibit exercise-induced oxidative stress as well as trained subjects [26]. However, the response of ROS synthesis and proinflammatory cytokines by vigorous intensity exercise should be compared between trained and untrained subjects in future studies.

Nrf2 is a transcription factor that enhances the production of phase Ⅱ enzymes [10]. However, Nrf2 is degraded by Keap1 under non-oxidative conditions to attenuate its activity as a transcription factor. Keap1-mediated degradation of Nrf2 is inhibited in the presence of electrophiles, such as carcinogens and ROS in the body; this allows the nuclear translocation of Nrf2 to bind DNA and increase the production of phase Ⅱ enzymes [27]. Sulforaphane also has electrophilic properties which has slight adverse effects on the human body, as its ingestion promotes Keap1-Nrf2 signaling and increases the production of phase Ⅱ enzymes [28]. Notably, the short-term administration of sulforaphane induces the overexpression of phase Ⅱ enzymes via the activation of Keap1-Nrf2 signaling, thereby decreasing tumor cell numbers [9]. A similar study reported that a low dose of sulforaphane (30 mg/day) improves hepatic function by decreasing oxidative stress [29]. A four-week administration of sulforaphane decreases ROS levels and improves inflammation in the colon [30]. Similarly, three-day administration of sulforaphane (25 mg/kg body weight) reduces muscle damage in rodents via the activation of phase Ⅱ enzyme production and decrease in ROS levels [10]. Although the activation of phase Ⅱ enzymes was not measured in this study, the duration and dose of sulforaphane administration was adequate to suppress vigorous resistance exercise-induced increase in CK levels, presumably via the upregulation of Keap1-Nrf2 signaling and phase Ⅱ enzymes.

In conclusion, the oral intake of sulforaphane for four weeks suppressed the vigorous resistance exercise-induced increase in IL-6 and CK levels. Thus, sulforaphane may suppress vigorous exercise-induced muscle damage augmented by increasing levels of ROS or inflammatory cytokines. Although further studies would be needed to elucidate the mechanism(s) underlying the protective effect of sulforaphane against exercise-induced muscle damage in humans, sulforaphane may have promising clinical applications.

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Figure Legends

Figure 1: **The effect of vigorous intensity resistance exercise on CK level (A) before exercise (Pre), after 30 min and 24h exercise, and (B) the extent of change for CK which was calculated by 30 min after exercise minus pre(30min-pre), 24 h after exercise minus 30 min (24h-30min), and 24 h after exercise minus pre (24h-pre).** Data expressed mean ± SE. * $P < 0.05$ vs Placebo group, † $P < 0.05$ vs Pre.

Figure 2: **The effect of vigorous intensity resistance exercise on IL-6 level (A) before exercise (Pre), after 30 min and 24h exercise, and (B) the extent of change for IL-6 which was calculated by 30 min after exercise minus pre(30min-pre), 24 h after exercise minus 30 min (24h-30min), and 24 h after exercise minus pre (24h-pre).** Data expressed mean ± SE. * $P < 0.05$ vs Placebo group, † $P < 0.05$ vs Pre.

Figure 1
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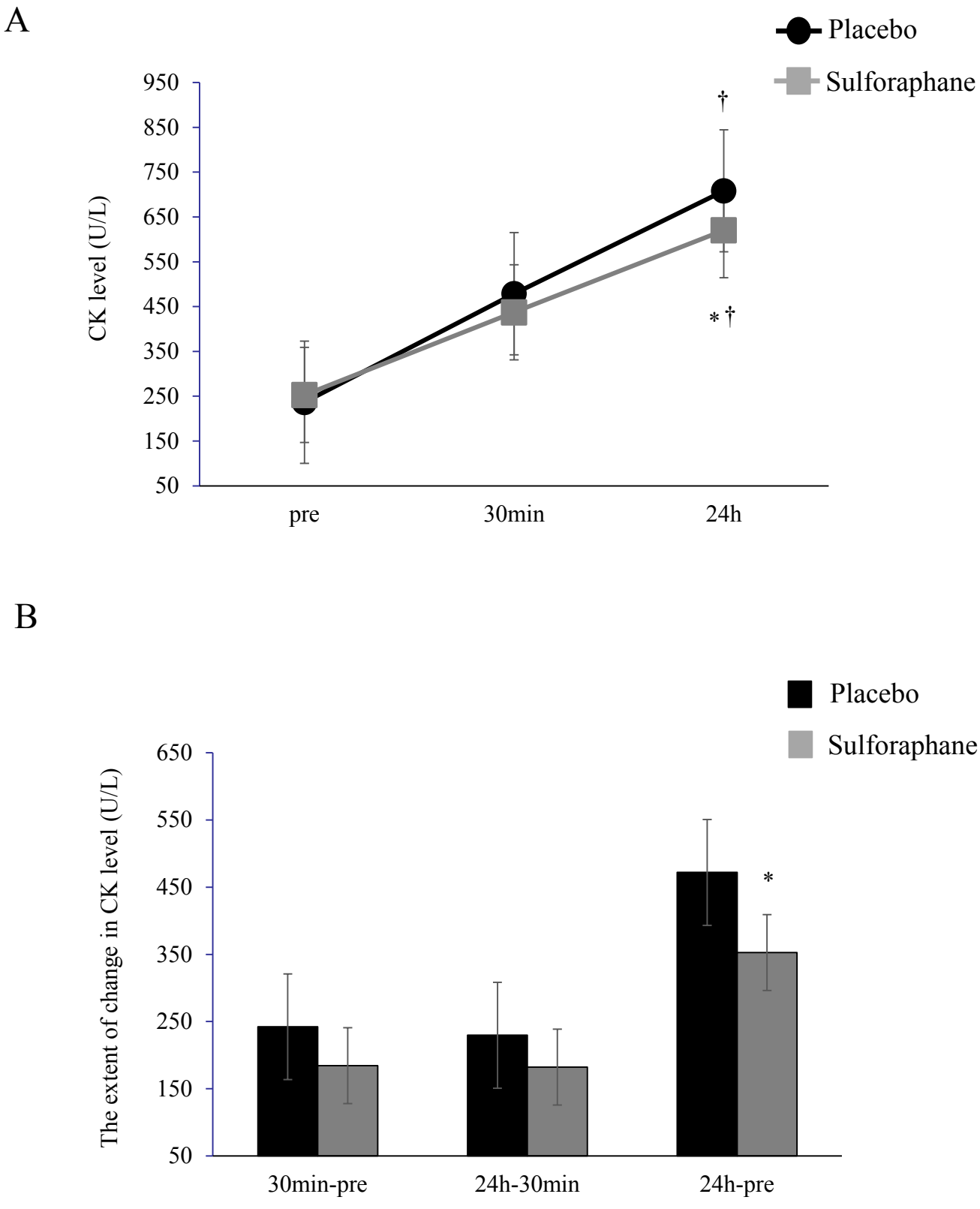

Figure 2
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