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Research Paper

# Effect of chronic supplementation with methylsulfonylmethane on oxidative stress following acute exercise in untrained healthy men

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## Abstract

**Objective** This study was conducted to assess the effects of chronic daily methylsulfonylmethane (MSM) supplementation on known markers of oxidative stress following acute bouts of exercise in untrained healthy young men.

**Methods** Eighteen untrained men volunteered for this study. Participants were randomized in a double-blind placebo-controlled fashion into two groups: MSM ($n = 9$) and placebo ($n = 9$). The participants took supplementation or placebo daily for 10 days before running. Participants ran 14 km. The MSM supplementation was prepared in water at 50 mg/kg body weight. The placebo group received water. Serum malondialdehyde (MDA), protein carbonyl (PC) and plasma oxidized glutathione (GSSG) were measured as markers of oxidative stress. The plasma-reduced glutathione (GSH) level and the GSH/GSSG ratio were determined as markers of plasma antioxidant capacity.

**Key Findings** Acute exercise led to elevated levels of serum MDA, PC and plasma GSSG. MSM supplementation maintained PC, MDA and GSSG at lower levels after exercise than the placebo. The plasma level of GSH and the ratio of GSH/GSSG were significantly higher in the MSM supplemented group.

**Conclusions** These results suggest that chronic daily oral supplementation of MSM has alleviating effects on known markers of oxidative stress following acute bouts of exercise in healthy young men.

**Keywords** antioxidant; GSH; malondialdehyde; methylsulfonylmethane; protein carbonylation

## Introduction

Physical exercise may increase the accumulation of free radicals and induce oxidative stress.[1] Oxidative stress is a condition in which the existing balance between free radical production and their subsequent reduction via the antioxidant defence system becomes skewed in favour of free radical expression.[2] Free radicals or, more generally, reactive oxygen/nitrogen species (RONS) are well known for playing both deleterious and beneficial roles since they can be either harmful or beneficial to living systems.[3] Evidence for increased RONS production during and following exercise is provided by numerous investigations that have noted an increase in various oxidative stress biomarkers following both acute aerobic and anaerobic exercise.[2] During exhaustive exercise, an insufficiency of endogenous antioxidants may cause antioxidant defence systems to be temporarily overwhelmed. Supplementation of these systems with antioxidants may therefore reduce oxidative stress.[4] Measurement of various antioxidant or oxidant parameters can be used to determine the risk of oxidative stress or the effectiveness of antioxidant supplementation.[5]

Methylsulfonylmethane (MSM) is a natural chemical that is a new candidate antioxidant. It is a sulfur-containing compound found in a wide range of human foods, including fruit, vegetables, grains and beverages.[6] Recently, MSM has received wide attention as a dietary supplement in the treatment of osteoarthritis.[7–9] It has been shown that MSM is effective in seasonal allergic rhinitis,[10] interstitial cystitis,[11] autoimmune disease[12] and cancer chemoprevention.[13,14] The sulfur of MSM may be incorporated into methionine and cysteine, sulfur-containing amino acids, and act as a source of sulfur.[15] It has also been found that

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MSM has anti-inflammatory[16] and antioxidant activities, which may lead to lower production of free oxygen radicals.[17] Recently, the anti-inflammatory effect of MSM on a rat model of colitis has also been demonstrated.[18]

It is believed that MSM is non-toxic.[12] A 30-day study that used a 2600 mg/day dosage revealed no side effects.[10] However, no study has examined long-term supplementation with MSM. One study of MSM in rats showed that oral administration at a dose of 1.5 g/kg/day for 90 days did not cause any adverse side effects or increased mortality.[19,20] Kim *et al*. also pointed out that MSM introduced no more side effects than placebo in patients with osteoarthritis pain of the knee.[7]

The effect of MSM in racehorses following a jumping exercise was reported by Maranon *et al*.,[21] who noted that MSM was able to protect the animals from oxidative and inflammatory injury. Nevertheless, based on a literature survey, the alleviating or augmenting action of MSM on oxidative stress following acute exercise in humans has not been reported. The present study was therefore undertaken to evaluate the possible effects of chronic daily administration of MSM (50 mg/day) for 10 days on markers of oxidative stress following acute exercise in untrained healthy young men.

## Methods

### Participants

Eighteen untrained healthy young men volunteered for this study. Each participant completed a pre-exercise health status questionnaire. Individuals were not eligible to enroll to the study if they: a) had a history of medical or surgical procedures that might significantly affect the study outcome, including cardiovascular disease or metabolic, renal, hepatic, or musculoskeletal disorders; b) were smokers or used medication that might have significantly affected the study outcome; c) used any nutritional supplements (i.e. creatine, protein drinks, amino acids, vitamins) in the 8 weeks before the beginning of the study; d) had participated in another trial or ingested another experimental product in the 30 days before screening and enrollment.

All participants were verbally informed of the aim of the study and written informed consents were obtained. The protocol of the study was approved by the university ethics committee in accordance with the Helsinki Declaration.

### Experimental design

All procedures were undertaken at the laboratory of the Ardabil Sports Medicine Committee. Two weeks prior to the main test, participants underwent a Bruce test on a treadmill to determine their $VO_{2max}$ (maximal oxygen consumption). Participants were randomized in a double-blind placebo-controlled fashion into two groups: MSM ($n = 9$) and placebo ($n = 9$). They arrived at the laboratory after overnight fasting. A baseline blood sample was taken. Afterwards, subjects consumed either placebo (200 ml water) or MSM supplement (50 mg/kg MSM in 200 ml water [the dose was adapted from the work of Kim *et al*.[7]]) daily for 10 days. On day of the test, participants attended the athletics arena after overnight fasting. After a second blood sample was taken, participants had a 10-min warm-up involving running at 50% $VO_{2max}$ for 10 min and stretching for 5 min. Then participants ran 14 km. They were allowed to consume water *ad libitum* throughout the exercise. Blood samples were taken immediately and at 30 min, 2, 24 and 48 h after exercise.

### Blood sampling and analysis

Approximately 10 ml of blood was withdrawn at each time point. Three millilitres of blood was placed in heparinized tubes and centrifuged at 3000 rpm for 10 min at 4°C. Plasma was transferred to microtubes and stored at −80°C for subsequent analysis. The rest of the blood was allowed to clot and centrifuged at 5000 rpm for 10 min. Serum was removed and aliquoted in 0.2 ml volumes and stored at −80°C until analysis.

Serum malondealdehyde (MDA) was determined by the method of Mateos *et al*.[22] In brief, an aliquot of 100 µl of serum was placed in a 1.5 ml microtube and 20 µl of 6 M NaOH was added. Alkaline hydrolysis of protein-bound MDA was achieved by incubating this mixture in a 60°C water bath for 30 min. Protein was precipitated with 50 µl of 35% (v/v) perchloric acid, and the mixture was centrifuged at 2800*g* for 10 min. A 100 µl volume of supernatant was transferred to a microtube and mixed with 100 µl of 2,4 nitrophenylhydrazine (DNPH) prepared as a 5-mM solution in 2 M hydrochloric acid. Finally, this reaction mixture was incubated for 30 min at room temperature protected from light. An aliquot of 20 µl of the reaction mixture was injected onto an HPLC system equipped with a C18 column (4.6 × 250 mm, 5 µ).

Protein carbonyls (PC) were measured using DNPH reagent according to a method described elsewhere,[23] with slight modifications as reported by Baltacioglu *et al*.[24] The carbonyl content was calculated from the peak absorption (360 nm) using an absorption coefficient (e) of 22 000 $M^{-1}cm^{-1}$. Each sample was read against the control sample (treated with 2.5 M HCl). The protein carbonyl content was expressed as concentration (µmol/l) in serum.

Plasma reduced glutathione (GSH) and oxidized glutathione (GSSG) determinations were performed using ion exchange chromatography with an NH2 column (4.6 × 150 mm), as described by Giustarini *et al*.[25] but with slight modification. Briefly, 250 µl of plasma sample was reacted with 50 µl of 310 mM N-ethylmaleimide and incubated for 5 s. The mixture was diluted with an equal volume of trichloroacetic acid (5% w/v final concentration) and centrifuged at 15 000*g* for 2 min. After supernatant alkalization, the sample was reacted with an equal volume of 2,4-dinitrofluorobenzene solution (1.5% v/v in ethanol) for 3 h at room temperature in the dark. After acidification with 10 µl HCl (37% v/v initial concentration), 20 µl of the sample was loaded onto the HPLC.

### Statistical analysis

Results are expressed as mean ± standard error of mean (SEM), and $P < 0.05$ was considered statistically significant. Data were analysed for time and group intervariability using two-way repeated measures of analysis of variance. Group intervariability was reported as treatment effect (impact of placebo or MSM administration on examined markers). If the data showed significance, a Fisher LSD test was performed to determining time points of significance.

**Table 1** Subjects' characteristics in MSM and placebo groups

| Groups | Age (year) | Height (cm) | Body mass (kg) | $VO_{2max}$ (ml/kg/min) |
|---|---|---|---|---|
| Placebo | 23.9 ± 2.9 | 178.0 ± 9.7 | 78.7 ± 9.9 | 42.9 ± 2.2 |
| MSM | 22.4 ± 3.2 | 177.1 ± 5.1 | 77.9 ± 7.8 | 44.7 ± 3.6 |

Values are mean ± SEM ($n = 9$); $VO_{2max}$ maximal oxygen consumption.

## Results

### Participants' characteristics

The characteristics of participants, including age, height, BMI and preliminary $VO_{2max}$, are summarized in Table 1.

### GSH level

The results of two-factor ANOVA revealed a significant treatment effect ($P = 0.027$) for plasma GSH level. A significant difference in plasma GSH levels at the post-exercise time point ($P < 0.001$) was observed when comparing MSM and placebo. As depicted in Figure 1a, exercise tends to lower plasma GSH levels, while supplementation with MSM maintains and even increases plasma GSH levels.

### GSSG level

There was a significant treatment and time effect on plasma levels of GSSG ($P = 0.31$ and $P < 0.001$, respectively). A significant increase in plasma GSSG levels was detected at the post-exercise time point for placebo and MSM groups ($P < 0.001$ and $P = 0.024$, respectively). However, the increase in plasma GSSG level in the placebo group was higher than for the MSM group. Again, significant differences between placebo and MSM groups were observed at the post-exercise point ($P = 0.014$), and at 30 min ($P = 0.026$) and 2 h ($P = 0.003$) after exercise (Figure 1b).

### GSH/GSSG ratio

The main effects of treatment and time were detected for GSH/GSSG ratio ($P = 0.003$ and $P = 0.029$, respectively). When compared with pre-exercise values, a significant decrease in the GSH/GSSG ratio at the post-exercise time point was observed in placebo group ($P = 0.035$). Significant differences were also found for the GSH/GSSG ratio at the pre-exercise ($P = 0.045$), post-exercise ($P = 0.005$), 30-min ($P = 0.027$) and 2-h ($P = 0.021$) points after exercise when comparing the placebo and MSM groups (Figure 1c).

### MDA level

A significant effect of treatment was observed for the serum MDA level ($P = 0.047$). There was a significant elevation of MDA levels after exercise only in the placebo group ($P = 0.012$). MDA levels were significantly higher in the placebo group than in the MSM group at the post-exercise ($P = 0.015$) and the 2-h and 24-h time points ($P = 0.029$, $P = 0.027$, respectively) (Figure 2).

### PC content

The PC content of serum was significantly higher immediately after exercise compared to pre-exercise values ($P = 0.033$) in the placebo group. There was a significant

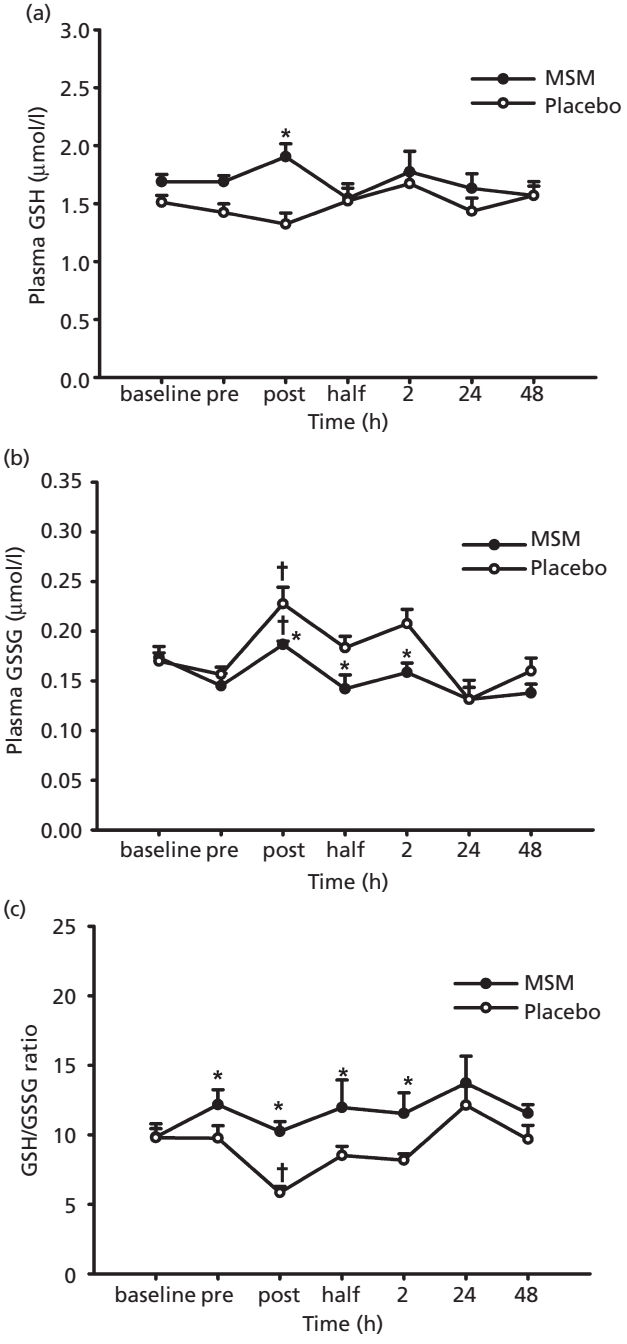


**Figure 1** Effect of MSM on plasma levels of a) GSH, b) GSSG and c) the ratio of GSH/GSSG in healthy young men after an acute bout of exercise. Values represent mean ± SEM ($n = 8$). $^{\dagger}P < 0.05$ vs pre-exercise values; $^{*}P < 0.05$ vs placebo. Pre, pre exercise; post, post exercise.

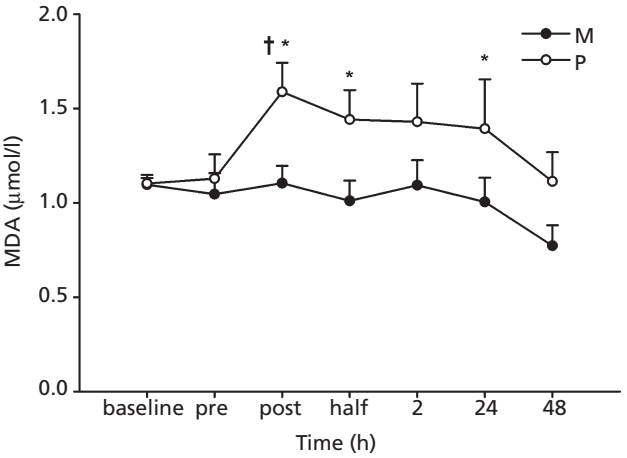


**Figure 2** Effect of MSM on serum MDA levels in healthy young men after an acute bout of exercise. Values represent mean ± SEM ($n = 8$). $^{\dagger}P < 0.05$ vs pre-exercise values; $^{*}P < 0.05$ vs placebo; Pre, pre exercise; post, post exercise.

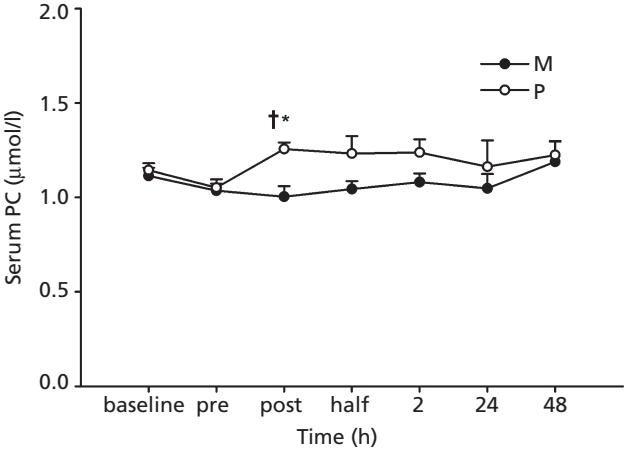


**Figure 3** Effect of MSM on serum PC content levels in healthy young men after an acute bout of exercise. Values represent means ± SEM ($n = 8$). $^{\dagger}P < 0.05$ vs pre-exercise values; $^{*}P < 0.05$ vs placebo; Pre, pre exercise; post, post exercise.

difference in PC levels at the post-exercise time point ($P < 0.013$) when comparing the placebo and MSM groups (Figure 3).

## Discussion

The protective effect of MSM on exercise-induced oxidative damage in racehorses has been published previously.[21] To our best knowledge, the present study is first report of the effects of chronic administration of MSM on exercise-induced oxidative stress in men.

The findings from this study suggest that MSM supplementation alleviates known markers of oxidative stress following acute exercise in healthy young men and is in agreement with the work of Maranon *et al*.[21]

In our preliminary study on the anti-inflammatory and antioxidant effects of MSM on carrageenan-induced rat paw edema (unpublished data), the linear portion of the dose-response curve is between 200 and 800 mg/kg, which translates to a human dosage range of 16–128 mg/kg using a simple equation.[26] Clinical trials of MSM on knee osteoarthritis also suggest a dosage range of 1.5–6 g/day (25–100 mg/kg).[7] The present study was therefore carried out with 50 mg/kg of MSM, which is the average of this range.

A decrease in GSH, an increase in GSSG and a decrease in GSH/GSSG ratio following acute exercise has been reported in many studies.[2,27,28] Glutathione status typically returns to basal level within 60 min of recovery.[28] In accordance with these findings, our study showed a significant increase in plasma GSSG level in both groups and a decrease in GSH/GSSG ratio only in the placebo group. Chronic supplementation with MSM attenuated the increase in plasma GSSG levels and maintained the GSH/GSSG ratio at higher levels. MSM's ability to prevent GSH depletion following exercise has previously been reported.[21] In accordance with these findings, the current study showed that plasma GSH level are significantly higher in the MSM group at the post-exercise time point. This is expected since MSM metabolism provides one of the precursors needed for GSH synthesis, therefore counteracting GSH depletion.[15]

The current study uses the MDA level of serum as a marker of lipid peroxidation. The serum MDA level was increased significantly above pre-exercise values immediately after exercise only in the placebo group. In accordance with our observations, an increase in lipid peroxidation following a single bout of exercise has been noted by many authors.[27,29,30] Our study shows that chronic daily administration of MSM is able to reduce the increase in serum MDA levels following acute exercise. This finding is in accordance with a previous study of MSM's effect on plasma lipid hydroperoxides in racehorses.[21] The exercise-induced increase in serum MDA levels is reduced by MSM supplementation, pointing to an improvement in the redox state of cells, which is in agreement with higher plasma levels of GSH. Furthermore, MSM has direct radical-scavenging activity,[12] which may play a role in lowering serum levels of MDA.

In the present study, serum PC content, as a marker of protein oxidation, showed significant elevation above pre-exercise values only in the placebo group. The enhancement of PC following training or a single bout of exercise has been observed by many investigators.[31–33] Our study demonstrated that MSM administration is able to suppress the increase in PC levels after exercise. This finding is in parallel with higher levels of GSH and GSH/GSSG ratio in the MSM-supplemented group.

In this study, the pro-oxidative condition induced by acute exercise, which is confirmed by the decreased ratio of GSH/GSSG, was accomplished by significant accumulation of lipid and protein oxidation by-products in plasma/serum and is tapered off by chronic daily administration of MSM.

## Conclusion

The present study demonstrates that an acute bout of exercise can induce oxidative stress in healthy young men. Chronic daily oral supplementation with MSM for 10 days has some alleviating effects on lipid peroxidation and protein

carbonylation and may alter the plasma GSH/GSSG redox state in favour of increased antioxidant capacity. Nevertheless, the exact mechanism of MSM in attenuating the markers of oxidative stress is not well established and further exploration is needed.

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### Conflict of interest

The authors declare that they have no competing interests to disclose.

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