Amino Acids (2004) 26: 203–207
DOI 10.1007/s00726-003-0002-3

Amino Acids
Printed in Austria

Role of taurine supplementation to prevent exercise-induced oxidative stress in healthy young men

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Received December 2, 2002
Accepted January 3, 2003
Published online May 9, 2003;

Summary. To evaluate the protective effects of taurine supplementation on exercise-induced oxidative stress and exercise performance, eleven men aged 18–20 years were selected to participate in two identical bicycle ergometer exercises until exhaustion. Single cell gel assay (SCG assay) was used to study DNA damage in white blood cells (WBC). Pre-supplementation of taurine, a significant negative correlation was found between plasma taurine concentration before exercise and plasma thiobaribituric-acid reactive substance (TBARS) 6 hr after exercise ($r = -0.642, p < 0.05$). WBC showed a significant increase in DNA strand breakage 6 hr and 24 hr after exercise. Seven-day taurine supplementation reduced serum TBARS before exercise ($p < 0.05$) and resulted in a significantly reduced DNA migration 24 hr after exercise ($p < 0.01$). Significant increases were also found in VO_2max, exercise time to exhaustion and maximal workload in test with taurine supplementation ($p < 0.05$). After supplementation, the change in taurine concentration showed positive correlations with the changes in exercise time to exhaustion and maximal workload. The results suggest that taurine may attenuate exercise-induced DNA damage and enhance the capacity of exercise due to its cellular protective properties.

Keywords: Taurine – DNA damage – Exhaustive exercise – Lipid peroxidation – Antioxidant

Introduction

Exhaustive exercise is known to increase free radical production which can induce a chain of events referred to as lipid peroxidation (Alessio, 1993). The highly reactive free radicals are known to modify several critical cellular components leading to tissue damage. It has also been shown that damage to DNA may occur as a result of oxidative stress following exercise (Hartmann et al., 1994; Niess et al., 1999). The potential of dietary antioxidants to detoxify the peroxides produced during exercise has received increasing attention in recent years.

Taurine, 2-aminoethanesulfonic acid, is one of the most abundant free amino acids in mammalian cells. Taurine has cytoprotective and enantiostatic properties through its actions on antioxidation, detoxification, osmoregulation, membrane-stabilization and intracellular calcium flux regulation due to its molecular structure (Huxtable, 1992). It has been reported that taurine can protect against oxidative damage under many conditions (Redmond et al., 1998; Wright et al., 1986; Qi et al., 1995), and attenuate cell necrosis and apoptosis (Wang et al., 1995 and 1996). Therefore, it is reasonable to hypothesize that taurine may exert a beneficial effect on the prevention of DNA damage in white blood cells (WBC) due to exhaustive exercise induced oxidative damage. The effect of taurine supplementation on exercise performance variables was also investigated.

Material and methods

Subjects and test protocol

Eleven male college students aged 18–20 years volunteered to enroll in the study. All were sedentary (performed no regular exercise), nonsmokers, in good health and with normal weight-for-height. They had not taken any medication for at least one month prior to the experiment. All subjects gave informed consent to participate in this study. For at least one week preceding the test, subjects were asked to refrain from strenuous physical exercise. First, all subjects performed on a bicycle ergometer at room temperature at a rate of 60 rpm with an increased work load of 20 W/min. The subjects had a 4 min warming-up exercise of 0 W/min to reach the required speed. The test was stopped when the test persons declared themselves to be exhausted. During the exercise, blood pressure, heart rate and electrocardiogram (ECG) were monitored every two minutes. The primary criterion for attainment of maximal oxygen consumption (VO_2max) was a plateau of VO_2 when workload was increased. If a plateau did not occur, a respiratory exchange ratio $\geqq 1.1$ or a maximal heart rate within 10 beats/min of the age-predicted maximum (220-age) was considered to satisfy the requirements for VO_2max. In addition to

VO_2max, maximal heart rate, exercise time to exhaustion and maximal workload values were determined after the test. After 7-day of taurine supplementation, an identical exhaustive test procedure was repeated at the same time of day.

Taurine supplementation protocol

After the first exercise test, the subjects received supplements of a daily dose of 6 grams (2 grams three times a day) taurine powder (Taisho, Tokyo, Japan) for 7 days prior to the second exercise test. No subject registered any complaint during the period of taurine supplementation.

Blood sampling and biochemical analysis

Pre- and post-supplementation of taurine, venous blood samples were taken for the detection of plasma taurine and serum thiobaribituric acid reactive substance (TBARS) before exercise and serum TBARS 6 hr after the exercise tests. Ear lobe blood samples taken before, 2 hr, 6 hr and 24 hr after exercise tests were used for the single cell gel (SCG) assay.

Blood samples were centrifuged immediately and serum was kept at $-80°C$ until analysis. Plasma was separated from heparinized whole blood for the detection of taurine. Serum TBARS was determined according to the procedure of Yagi (1976). Lipid peroxide content was estimated by measuring the TBARS produced in terms of malondialdehyde (MDA).

Plasma taurine concentration was determined by capillary electrophoresis with Waters capillary ion analyzer (Millipore Co. Milford, MA, USA) and Waters AccQ-Fluor Reagent Kit (Millipore). First, 10 μl of plasma was delivered to a 0.5 ml micro reaction tube. Then 70 μl of AccQ-Fluor borate buffer was added and the mixture was vortexed. Finally, 20 μl of AccQ-Fluor reagent was added and vortexed at once for 10 sec. After that, the sample was heated for 10 min at 55°C. A fused silica capillary with a length of 75 μm I.D. $\times$ 70 cm was used to separate the derivatized samples. The UV absorbance signal was measured at 254 nm. The applied voltage was 20 kV and the hydrostatic injection time was 60 sec. The eluent was borate buffer (120 mM sodium tetraborate and 0.5 M SDS). Electrophoretic data were collected and analyzed by a 805 Data Station software (Millipore).

SCG assay

The procedure described for the SCG assay by Hartmann et al. (1994) was followed with minor modifications. Agarose (50 μl of 0.75%) diluted in Ca- and Mg-free buffer was placed on microscope slides, and immediately smeared with another slide. When the agarose smear was dried in a refrigerator, 300 μl of the same agarose solution was placed on the slides, covered with a coverslip (24 $\times$ 32 mm) and kept at 4°C for 5 min. After solidification of the agarose, the coverslips were removed, and then 4 μl of whole blood mixed with 96 μl of 0.5% low melting agarose was placed on the slides. The slides were covered again with coverslips and kept in the refrigerator for 5 min to solidify the agarose. Then the cover slips were removed, 100 μl of 0.75% agarose was added and the slides were again kept cold for 5 min. After removal of the cover slips, the slides were immersed in a jar containing cold lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% sodium lauroylasrcosine, pH 10; 1% Triton X-100 and 10% dimethyl sulfoxide were added freshly). The slides were kept at 4°C for 1.5 hr. After lysis, the slides were placed in a horizontal electrophoresis box. The unit was filled with freshly made alkaline buffer (300 mM NaOH and 1 mM EDTA, pH 13) to a level 2.5 mm above the slides. The cells were exposed to alkali for 20 min to allow unwinding of the DNA. Electrophoresis was applied for 20 min at 20 V in a 4°C cold room. All of these steps were conducted under dim light to prevent additional DNA damage. After electrophoresis, the slides were washed with Tris buffer (0.4 M Tris, pH 7.5) to neutralize the excess alkali for 5 min. This last step was repeated three times. Finally, 50 μl of 20 μg/ml ethidium bromide was added to each slide. The slides were covered with cover slips, kept in a humidified box and observed by fluorescence microscopy. Photomicrographs of the cells under the fluorescence microscope were taken at 100 $\times$ magnification using RPH 135 transparency film, ASA 400. Images of 35 randomly selected cells were analyzed from each sample. The length of DNA migration was determined on an epidiascope at 8 $\times$ magnification by measuring the length (mm) of nuclear DNA and the migrating DNA.

Statistical analysis

Results are given as mean $\pm$ standard deviation (SD). Comparisons were performed by analysis of variance (ANOVA) with Tukey HSD's post hoc tests. Associations between variables were analyzed by calculating the Pearson rank correlation coefficients. A value of $p < 0.05$ was regarded as significant.

Results

The subjects averaged 170.1 ± 8.0 cm in height, 61.5 ± 10.2 kg in body weight, and 20.8 ± 2.2 kg/m^2 in body mass index (BMI). Room temperatures at pre- and post-supplementation test periods were $23.4 \pm 1.9°C$ and $23.7 \pm 1.6°C$; humidity was $48.5 \pm 3.5\%$ and $47.8 \pm 2.6\%$, respectively. These values showed no significant differences. The mean plasma concentration of taurine significantly increased from 51.7 ± 10.2 nmol/ml to 88.3 ± 39.8 nmol/ml after 7-day supplementation period ($p < 0.05$).

The changes in serum TBARS before and 6 hr after exercise in both pre- and post-supplementation trials are shown in Fig. 1. Pre-supplementation of taurine, serum TBARS was slightly increased by 3.9% (no significant difference) 6 hr after exercise. Taurine supplementation did not influence TBARS level 6 hr after exercise (3.1 ± 0.5 nmol/ml) when compared with that before supplementation (3.2 ± 0.3 nmol/ml). However, taurine supplementation reduced pre-exercise serum TBARS concentration from 3.1 ± 0.3 nmol/ml to 2.7 ± 0.4 nmol/ml ($p < 0.05$).

In the pre-supplementation test, migration of DNA from WBC increased significantly 6 hr (9.8 ± 1.4 mm, $p < 0.01$)

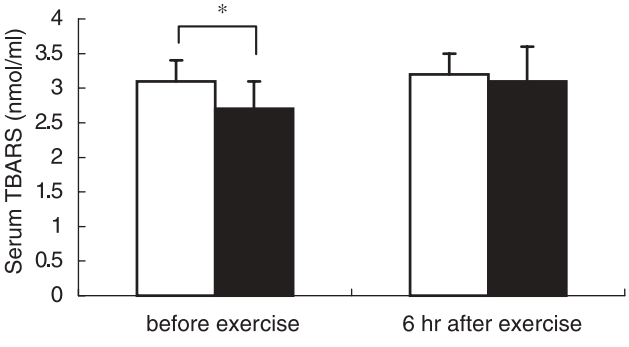

Fig. 1. Serum concentration of TBARS (nmol/ml) before and 6 hr after exhaustive exercise in tests of pre-supplementation (open bars) and post-supplementation (solid bars) of taurine (means $\pm$ SD, $n = 11$). $^*p < 0.05$

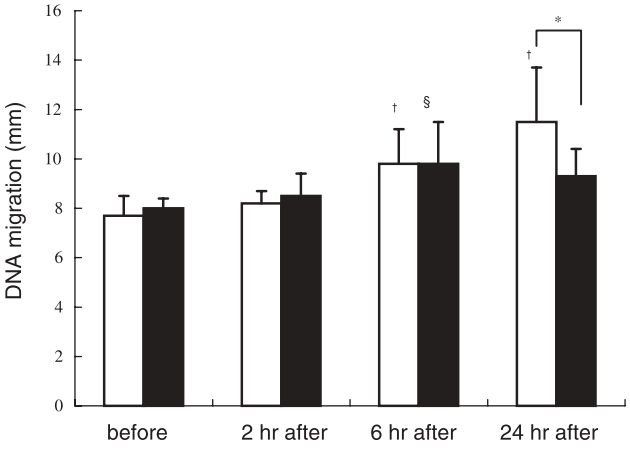

Fig. 2. DNA migration before, 2 hr, 6 hr and 24 hr after exercise in tests of pre-supplementation (open bars) and post-supplementation (solid bars) of taurine (means ± SD, $n = 11$). † Compared with before exercise pre-supplementation of taurine ($p < 0.01$). § Compared with before exercise post-supplementation of taurine ($p < 0.05$). * ($p < 0.01$)

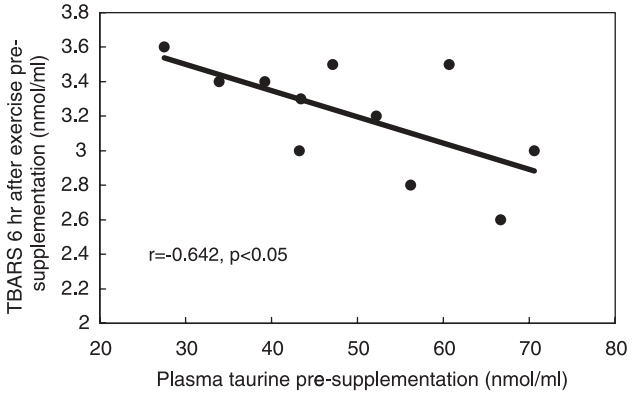

Fig. 3. Correlation of plasma taurine levels before exercise with serum TBARS levels 6 hr after exercise pre-supplementation

and 24 hr (11.5 ± 2.2 mm, $p < 0.01$) after exercise when compared with that of before exercise (7.7 ± 0.8 mm). Seven-day of taurine supplementation did not affect the migration values before exercise (8.0 ± 0.4 mm), 2 hr (8.5 ± 0.9 mm) and 6 hr (9.8 ± 1.7 mm) after exercise, but resulted in a significantly decreased DNA migration 24 hr after exercise (9.3 ± 1.1 mm, $p < 0.01$), when compared with that at the same time in pre-supplementation test (Fig. 2).

Pre-supplementation of taurine, a significant negative correlation was found between plasma taurine concentration before exercise and serum TBARS 6 hr after exercise ($r = -0.642$, $p < 0.05$, Fig. 3). The serum TBARS 6 hr after exercise showed no significant correlation with either DNA migration 6 hr or 24 hr after exercise.

Table 1 shows the mean values for VO_2max, maximal heart rate, exercise time to exhaustion and maximal workload in the tests of pre- and post-supplementation. Significant increases were evident in VO_2max, exercise time to exhaustion and maximal workload after one week of taurine supplementation ($p < 0.05$). The change in plasma taurine concentration post-supplementation correlated with the change in exercise time to exhaustion ($r = 0.616$, $p < 0.05$) and with the change in maximal workload

Table 1. Exhaustive exercise performance variables in tests of pre- and post-supplementation of taurine (means ± SD, $n = 11$)

	VO_2max (ml/kg · min)	HR_{max} (beat/min)	Exercise time (min)	$Workload_{max}$ (Watts)
pre-supplementation	43.7 ± 4.7	183 ± 14	18.8 ± 3.2	234 ± 65
post-supplementation	46.7 ± 5.2 *	185 ± 13	19.3 ± 3.4 *	243 ± 67 *
change [1]	2.4 ± 3.6	1.2 ± 5.6	0.4 ± 0.5	8.6 ± 9.8

VO_2max, maximal O_2 consumption; *HR_{max}*, maximal heart rate; *$Workload_{max}$*, maximal workload;
* significantly different from the pre-supplementation test, $p < 0.05$

Table 2. Correlations between the change in plasma taurine concentrations and the changes in exercise performance variables post-supplementation ($n = 11$)

	VO_2max	HR_{max}	Exercise time	$Workload_{max}$
coefficient (r)	0.397	0.412	0.616	0.620
p value	0.226	0.208	0.043	0.042

VO_2max, maximal O_2 consumption; *HR_{max}*, maximal heart rate; *$Workload_{max}$*, maximal workload

($r = 0.620$, $p < 0.05$) post-supplementation as shown in Table 2.

Discussion

Increased serum TBARS with a peak level at 6 hr after a downhill run in man has been reported (Maughan et al., 1989). However, in our study, we did not find a significant increase in mean serum TBARS concentration 6 hr after exercise on a cycle ergometer. Changes in plasma and serum TBARS due to exercise are not reported consistently. A 77% increase in plasma TBARS concentration from athletes following an exhaustive running exercise was found in one study (Kanter et al., 1988). However, in another study, runners completing a half marathon had no change in plasma TBARS (Viinikka et al., 1984). As serum TBARS levels and the peak value subsequent to exercise may vary according to exercise intensity, we cannot exclude the possibility that we missed the maximal serum values of TBARS.

It has been suggested that damage to DNA may occur as a result of oxidative stress, highly intensive anaerobic exercise induces up to a 40 fold increase in oxygen consumption which may enhance oxygen free radical release from the electron transfer chain in mitochondria (Benzi, 1993). The DNA migration in peripheral white blood cells significantly increased 6 hr after exercise and the maximal increase was found at 24 hr after exercise, which confirms previous findings (Hartmann et al., 1994; Niess et al., 1996 and 1998). Even though there is rapid removal of free radicals, their creation and removal within the cell could still result in single DNA strand breaks and base shifts that are likely to alter normal cell function (Alessio, 1993). However, peripheral white blood cell DNA migration did not reach its maximum directly after exercise in our study and in other investigations (Hartmann et al., 1994; Niess et al., 1996 and 1998). Moreover, no significant correlations were found between serum TBARS levels 6 hr after exercise and DNA migration 6 hr and 24 hr after exercise. DNA genotoxic products of lipid peroxidation such as malondialdehyde-deoxyguanosine adduct are potential mediators of DNA damage (Chaudhary et al., 1994). On the other hand, acute dynamic exercise increases levels of the end product of nitric oxide in circulating plasma in normal subjects (Node et al., 1997). As NO induces DNA damage by causing DNA strand breaks and inhibiting DNA repair enzymes, it may also be involved in the onset of DNA damage (Gross et al., 1995). Therefore, in addition to oxidative damage directly induced by free radicals, the delay in onset of the DNA damage might have been due to secondary mediators.

Taurine is an effective free radical scavenger and normally is concentrated in cells and tissues that possess a considerable potential for producing oxidants (Huxtable, 1992; Redmond et al., 1998). Taurine has been reported to reduce oxidative damage to DNA caused by free radicals (Messina et al., 2000). Furthermore, Dawson et al. (2002) found a cytoprotective role of taurine in exercise-induced muscle injury by blocking the increase in TBARS in the fast twitch fiber type muscle. In our study, plasma taurine concentration pre-supplementation showed a significant negative correlation with plasma TBARS 6 hr after exercise, which suggests that the lower taurine level may result in the higher degree of oxidative stress induced by exercise. TBARS levels were decreased before exercise by 7-day of taurine supplementation; nevertheless exercise reversed this taurine effect. A significant reduction in DNA migration at 24 hr after exercise in post-supplementation test was observed. As the protective effect of taurine on the onset of DNA damage occurs only 24 hr after exercise and no effect of taurine in reducing TBARS after exercise, it may imply that an indirect antioxidative action of taurine possibly plays a more important role in protecting DNA damage in our study. Taurine has been reported to attenuate NO and reactive oxygen intermediate-dependent hepatocyte apoptosis and necrosis (Redmond et al., 1996). By regulating the production of NO, taurine might be playing a role in preventing exercise-induced DNA damage. As NO was not measured in this study, this hypothesis will require further study. In addition, oxidative stress is thought to contribute to membrane damage through increasing permeability (Pasantes-Morales et al., 1984). Taurine may also mediate cellular protection through its membrane stabilizing property. However, the mechanisms leading to DNA damage and the protective effect of taurine are complex and still have to be elucidated.

Exercise after taurine supplementation showed significantly increased VO_2max, exercise endurance time and maximal workload. Although the ''training'' effect of the first exercise test might affect the performance of the second one, the change in plasma taurine concentration after supplementation was positively correlated with changes in exercise time to exhaustion and maximal workload in our study, which suggests that exercise performance is related with taurine levels. Dawson et al., (2002) found 3% taurine in drinking water could improve running performance in adult male rats. However, mechanisms of taurine in augmenting exercise performance are poor understood. It has been established that taurine is positively inotropic for heart tissue exposed to

sub-physiological concentrations of Ca^{2++} (Franconi et al., 1982a and b). In addition, taurine supplementation improves the electrical and contractile properties of skeletal muscle membrane from aged rats (Pierno et al., 1996). We hypothesize that taurine may improve exercise performance perhaps by regulating Ca^{2++} homeostasis and then enhancing myocardial and skeletal muscle contraction under exhaustive conditions.

In conclusion, our results demonstrate that taurine supplementation attenuates the DNA damage in WBC from untrained subjects induced by exhaustive exercise. This action of taurine may be due to not only the direct scavenging of free radicals, but also indirect antioxidant and other detoxifying properties. Also, taurine supplementation is associated with a higher exercise performance.

Acknowledgements

This study was supported by a grant from Taisho Pharmaceutical Co., Ltd., Japan. We are grateful to the volunteers who participated in this study. We also thank Dr. M. Sekine and Dr. H.B. Wang for helpful discussions.

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