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The cytoprotective role of taurine in exercise-induced muscle injury

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Summary. Intense exercise is thought to increase oxidative stress and damage muscle tissue. Taurine is present in high concentration in skeletal muscle and may play a role in cellular defenses against free radical-mediated damage. The aim of this study was to determine if manipulating muscle levels of taurine would alter markers of free radical damage after exercise-induced injury. Adult male Sprague-Dawley rats were supplemented via the drinking water with either 3% (w/v) taurine (n = 10) or the competitive taurine transport inhibitor, β-alanine (n = 10), for one month. Controls (n = 20) drank tap water containing 0.02% taurine and all rats were placed on a taurine free diet. All the rats except one group of sedentary controls (n = 10) were subjected to 90 minutes of downhill treadmill running. Markers of cellular injury and free radical damage were determined along with tissue amino acid content. The 3% taurine treatment raised plasma levels about 2-fold and 3% β-alanine reduced plasma taurine levels about 50%. Taurine supplementation (TS) significantly increased plasma glutamate levels in exercised rats. Exercise reduced plasma methionine levels and taurine prevented its decline. Taurine supplementation increased muscle taurine content significantly in all muscles except the soleus. β-alanine decreased muscle taurine content about 50% in all the muscles examined. Lipid peroxidation (TBARS) was significantly increased by exercise in the extensor digitorium longus (EDL) and gastrocnemius (GAST) muscles. Both taurine and β-alanine completely blocked the increase in TBARs in the EDL, but had no effect in the GAST. Muscle content of the cytosolic enzyme, lactate dehydrogenase (LDH) was significantly decreased by exercise in the GAST muscle and this effect was attenuated by both taurine and β-alanine. Muscle myeloperoxidase (MPO) activity was significantly elevated in the gastrocnemius muscle, but diet had no effect. MPO activity was significantly increased by exercise in the liver and both taurine and β-alanine blocked this effect. There was no effect of either exercise or the diets on MPO activity in the lung or spleen. Running performance as assessed by a subjective rating scale was improved by taurine supplementation and there was a significant loss in body weight in the

β-alanine-treated rats 24 hours after exercise. In summary, taurine supplementation or taurine depletion had measurable cytoprotective actions to attenuate exercise-induced injury.

Keywords: Taurine – β-Alanine – Exercise – Oxidative stress – Muscle injury

Introduction

There is evidence that intense bouts of exercise induce oxidative stress and consequently muscle tissue is a target for damage by free radical mechanisms (Ji, 1999; Clanton et al., 1999). Taurine (2-aminoethanesulfonic acid) is present in high concentrations (mM) in skeletal muscle and it is known to regulate osmotic balance, ion channel function, calcium levels and contractility (Huxtable, 1992). Numerous reports have demonstrated the cytoprotective actions of taurine against ischemia, hypoxia and reperfusion injury in various tissues including the heart (Milei et al., 1992; Timbrell et al., 1995; Raschke et al., 1995). Muscle tissue is protected by various enzymatic and nonenzymatic antioxidant defense systems. Taurine may complement the cellular armamentarium against oxidative damage by various mechanisms. Unfortunately, few studies have directly assessed the potential of taurine to protect skeletal muscle against oxidative stress, despite taurine being present in many commercially available dietary products touted to enhance muscle development or provide protection against muscle injury. Therefore, the present study was designed to test the hypothesis that taurine supplementation would attenuate indices of oxidative damage in a model of exercise-induced muscle injury.

Material and methods

Animal model and dietary treatments

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) 180–200 grams body weight were used in these studies. Groups of 10 rats were maintained on one of 3 experimental diets. All rats consumed a soy-based taurine free diet (Purina 5729C-M) and controls were supplemented with 0.02% (w/v) taurine in the drinking which is equivalent to the taurine content of standard laboratory rat chow (Dawson et al., 1999). Two groups of controls were maintained, one group was not subjected to the exercise protocol and served as sedentary controls (CON group). The second group of controls was subjected to the exercise protocol (EC group). The second dietary group received 3% (w/v) β-alanine in the drinking water (ENT group) to competitively inhibit taurine transport and deplete tissue stores of taurine (Schaffer and Kocsis, 1981). The final group was given 3% taurine in the drinking water (ET group). All the rats (except the CON group) were maintained on these diets for one month and then subjected to exercise training. Food and water intake was measured every 48–72 hours as were body weights. At the end of the exercise session the rats were allowed 24 hours to recover and then were sacrificed by decapitation. The rats were processed for tissue collection in squads of 4 (2 squads per day) so that two rats from each experimental condition were processed daily and the order of sacrifice was randomized. Blood and tissue samples were collected and stored at $-80°C$ until assay. Tissue samples were rapidly removed and rinsed in ice cold phosphate-buffered saline (PBS), blotted dry and immediately snap frozen on dry ice. Body weight, food and water

intake after exercise and general behavioral observations were recorded for all experimental groups. The exercise protocol consisted of 90 minutes of intermittent running (18 bouts of exercise, 5 minutes duration, each separated by 2 minutes rest) at a speed of 16 m/min down a −16° grade on a treadmill (Schwane and Armstrong, 1983; Armstrong et al., 1983). All running sessions were performed at approximately the same time of day (10 AM–3 PM). Behavioral observations were recorded for each rat to index the ease and vigor of the running responses as well as the amount of aversive stimulation (electrical shocks) required to motivate and sustain running. The rats were rated on a scale of 1–3, with 3 being an excellent runner not requiring shocks or prompting by the observer and 1 being a slow runner, receiving multiple shocks and prompting by the observer.

Amino acid analysis

Plasma and tissue amino acids were measured by high performance liquid chromatography (HPLC). Tissue content of taurine and other amino acids were determined in perchloric acid extracts using HPLC with electrochemical detection of the ophtaldehyde derivatives as previously described (Dawson and Wallace, 1992). Plasma samples were deproteinized with methanol, diluted with mobile phase and derivatized as described previously (Dawson et al., 1999).

Markers of cellular injury

Muscle damage was indirectly assessed by measuring the muscle content of the cytosolic enzymes lactate dehydrogenase (LDH) and creatine kinase (CK). If the muscle cell membranes had been damaged and were "leaky", we predicted a drop in muscle content of these cytosolic enzymes. Muscles were homogenized in 20 volumes (w/v) of PBS and LDH and CK enzyme activity was measured using commercially available assay kits (Sigma, St Louis, MO). Enzyme activity was expressed as units of activity per gram of tissue wet weight.

Markers of free radical damage

Lipid peroxidation was estimated using the thiobarbituric acid test (TBARS). Samples were homogenized in 0.1 M perchloric acid containing 100 mg/L Na_2EDTA. TBARS were assayed by a modification (Dawson et al., 2000) of the method described by Wills (1987). Malonaldehyde (TBAR) formation was quantitated using 1,1,3,3-tetraethoxypropane as a standard. The absorbance of TBARs was read at 530 nm in a Beckman spectrophotometer. Acid soluble and protein thiol levels were measured in tissue samples homogenized in 0.2 M PCA containing 0.1 mM Na_2EDTA. The samples were centrifuged at 15,000 × g at 4°C for 15 minutes. The supernatant was assayed for acid soluble thiols (GSH, cys, cys-cys) by a modification (Eppler and Dawson, 2001) of the method of Ellman (Boyne and Ellman, 1972). Protein thiols were determined by a modification of the Ellman procedure (Boyne and Ellman, 1972). Tissue proteins were precipitated with 0.2 M PCA and the pellet was treated with 1% SDS in 5 mM phosphate buffer and assayed by a 30 min reaction with 5,5′-dithiobis-(2-nitrobenzoic acid) (Boyne and Ellman, 1972). This method indexes oxidative damage to protein sulfhydryl groups. SDS solublized proteins prepared as described above were also measured using a fluorometer. Tryptophan fluorescence was determined using an excitation wavelength of 280 nm and emission wavelength of 345 nm (Davies et al., 1987). The data are expressed as nmoles of tryptophan per gram tissue wet weight. Damage to proteins can result in a loss of tryptophan fluorescence due to oxidative modification of tryptophan residues (Davies et

al., 1987). Cytosolic antioxidants (ascorbic acid and uric acid) were measured in tissue perchloric acid extracts by HPLC with electrochemical detection. A Varian (Walnut Creek, CA) C18 HPLC column (4.6 × 100 mm) was used. The mobile phase consisted of 50 mM phosphate buffer (pH = 3.0) containing 200 mg/L Na_2EDTA. A flowrate of 0.5 ml/min was used and the electrochemical detector was set at a 0.65 volt oxidation potential.

Myeloperoxidase (MPO) activity

Due to differences in the levels of MPO activity in specific tissues, two different tissue preparation and assay methods were employed. The liver, spleen and lung tissues were prepared according to the method outlined by Marcinkiewicz et al. (1998). These samples were assayed using o-dianisidine dihydrochloride (Marcinkiewicz et al., 1998). Muscle samples were prepared according to the method outlined by Kuebler et al. (1996). These samples were assayed using tetramethylbenzidine (Kuebler et al., 1996).

Statistical analysis

In general the data were subjected to analysis of variance (ANOVA) followed by Neuman-Keuls multiple comparison test to compare treatment means. The rating scale data were analyzed by the Kruskal-Wallis nonparametric ANOVA followed by Dunn's multiple comparison test. The effects of exercise on body weights were assessed by a paired t-test to compare body weights for individual rats before and after exercise. All statistical analyses were performed using GraphPad Prism software (San Diego, CA).

Results

Physiological and behavioral measures

Overall, there was a significant ($p < 0.001$) effect for both dietary treatments and days of treatment on body weights (Fig. 1). Comparison of mean body weights for the dietary treatment groups on any given day were not statistically significant (Fig. 1). There was a nonsignificant trend for the EC group to have slightly higher body weights than the other groups. The average daily food and water intakes are presented in Table 1. There was no effect of the diets on food intake, but fluid consumption was significantly higher in taurine supplemented rats whereas β-alanine treated rats drank significantly less than the other groups (Table 1). All groups consumed less food in the 24-hour period after the 90-minute exercise session on the treadmill (Table 1). Water intake was less affected by exercise but was slightly reduced. The β-alanine treated rats maintained a lower level of fluid intake after exercise than the other 2 groups (Table 1). Body weights were recorded before and 24 hours after the exercise session and the β-alanine treated rats lost a significant amount of weight after exercise (Fig. 2). The observer rating of the rat's running performance is presented in Fig. 3 and ET rats were better runners than EC rats. EC rats did not differ from the ENT group in running performance (Fig. 3).

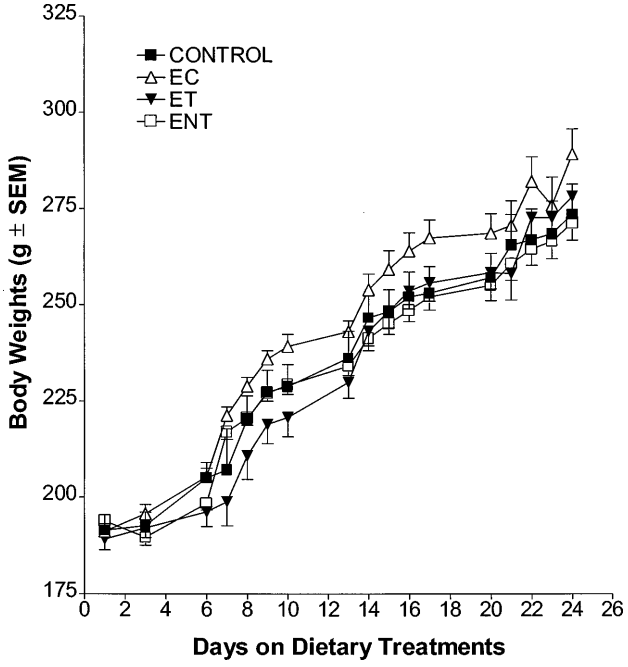

Fig. 1. Body weights for the experimental groups and the effects of dietary supplementation with 3% taurine (*ET*) or 3% β-alanine (*ENT*). Repeated measures ANOVA showed that body weights for all the groups significantly ($p < 0.001$) increased over the course of the study and there was an overall significant ($p < 0.001$) effect of the dietary treatments. Planned comparisons of the mean Body weights for the individual groups failed to detect a significant difference among the groups in body weights

Table 1. Average daily food and water intake and the effects of exercise

24 Hour Intake	CON	EC	ET	ENT
Food (g)	30.5 ± 0.6	31.2 ± 0.5	32.6 ± 0.8	32.5 ± 0.7
Food PE (g)	ND	17.5 ± 2.1	16.9 ± 2.8	16.9 ± 3.1
Water (g)	31.4 ± 0.8	32.4 ± 0.9	44.7 ± 1.0[a]	21.6 ± 0.5[b]
Water PE (g)	ND	27.7 ± 1.8	33.3 ± 5.1	16.3 ± 1.6[b]

Mean daily food and water intake for each group was averaged for the entire study. There was no effect of the diets on food intake, but ET rats drank significantly ([a]$p < 0.001$) more than the other groups and ENT rats drank significantly ([b]$p < 0.001$) less than the other groups. Food and water intake was measured for the 24-hour period post-exercise (*PE*) and the dietary treatments did not alter food intake, but the ENT rats continued to drink less than the other groups. *ND* not determined.

Amino acid alterations

Plasma amino acid data are presented in Table 2. As expected, dietary supplementation with 3% taurine significantly elevated plasma concentrations of taurine. β-Alanine produced an overall nonsignificant decline in plasma taurine levels, although there was about a 50% reduction in plasma

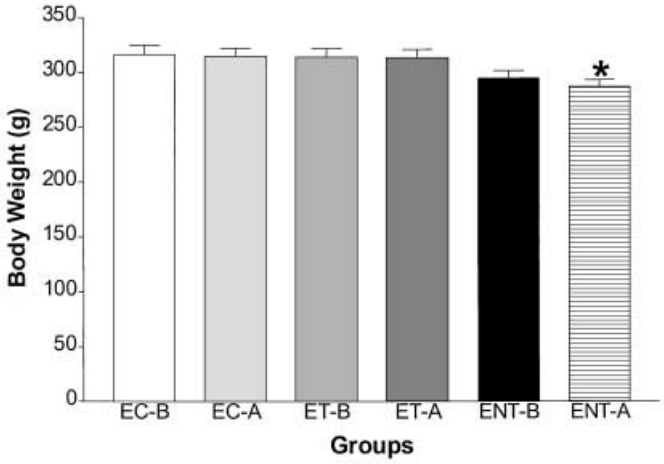

Fig. 2. The effects of 90 minutes of treadmill running on body weight 24 hours after exercise. Body weights for the groups were determined before (*B*) and 24 hours after (*A*) exercise and the difference in body weight for each rat was statistically analyzed. The β-alanine-treated (*ENT*) rats showed a significant (*$p < 0.05$) loss of body weight following exercise while the other groups were unaffected

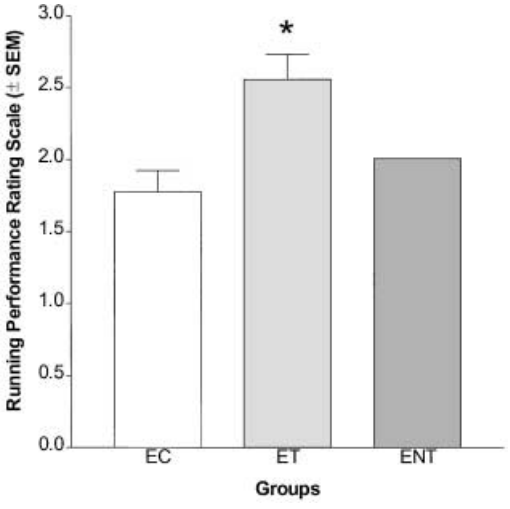

Fig. 3. Treadmill running performance and the effects of manipulating dietary taurine intake. Extensive notes were taken based on behavioral observation of the rats during the course of the treadmill exercise and running performance was rated as good (3), average (2) or poor (1). Taurine supplemented rats (*ET*) performed significantly (*$p < 0.05$) better than the other two groups

levels of taurine when compared to the EC or CON groups (Table 2). Exercise was associated with a significant reduction in plasma MET that was blocked by taurine supplementation. Glutamate concentrations were higher in ET rats than all the other groups. Valine concentrations declined due to exercise but only in the EC group. Other plasma amino acids were unaffected

Table 2. Effects of diets and exercise on plasma amino acids

Amino Acids	CON	EC	ET	ENT
TAU	254 ± 22	211 ± 16	554 ± 79[b]	109 ± 11
MET	46 ± 2	38 ± 2[c]	46 ± 2	39 ± 2[d]
GLU	145 ± 14	117 ± 5	181 ± 10[b]	131 ± 10
GLN	683 ± 19	671 ± 46	656 ± 20	742 ± 17
VAL	151 ± 9	119 ± 7[a]	135 ± 7	137 ± 7
TRP	53 ± 7	58 ± 9	47 ± 9	43 ± 6
LEU	109 ± 8	103 ± 9	102 ± 6	106 ± 2
ALA	379 ± 22	361 ± 13	486 ± 54	427 ± 52
ARG	136 ± 9	132 ± 9	132 ± 16	131 ± 10
GLY	310 ± 22	303 ± 8	317 ± 16	306 ± 10

All values expressed as nmoles/ml (μM) and N = 6 for all groups.
[a] $p < 0.05$ EC vs CON, [b] $p < 0.05$ ET vs all other groups, [c] $p < 0.05$ EC vs ET and CON, [d] $p < 0.05$ ENT vs ET and CON.

Table 3. Tissue taurine and β-alanine content

Tissues	CON	EC	ET	ENT	ENT (β-ALA)
EDL	14.11 ± 0.95	14.54 ± 0.50	19.92 ± 0.46[a]	7.32 ± 0.32[b]	1.35 ± 0.13
Soleus	19.29 ± 0.88	20.53 ± 1.52	21.92 ± 1.40	11.53 ± 1.11[b]	1.54 ± 0.12
GAST	17.10 ± 1.19	15.66 ± 0.71	25.47 ± 1.58[a]	9.33 ± 0.76[b]	2.31 ± 0.21
Triceps	14.46 ± 1.72	16.28 ± 0.88	23.25 ± 0.88[a]	8.12 ± 0.93[b]	2.30 ± 0.38
Heart	30.78 ± 0.95	29.25 ± 1.40	32.10 ± 1.19	20.65 ± 1.11[b]	ND
Liver	10.26 ± 1.32	8.96 ± 1.57	17.63 ± 1.13[a]	1.61 ± 0.43[b]	ND
Spleen	15.23 ± 1.50	14.93 ± 1.28	17.25 ± 1.14	10.08 ± 0.66[b]	3.73 ± 0.68

Data are expressed as μmoles/g wet tissue weight ± SEM. N = 4–6 rats per group. *ND* not determined. [a] $p < 0.05$ ET vs all other groups, [b] $p < 0.05$ ENT vs all other groups.

by exercise or dietary treatments. The effects of the dietary treatments on tissue taurine content are presented in Table 3. Dietary supplementation with taurine significantly elevated tissue taurine content in all skeletal muscles except the soleus. The heart and spleen also did not show significant increases in taurine content. Dietary treatment with 3% β-alanine reduced muscle taurine content about 40–50%. The liver had the greatest reduction (80% decline) while taurine content declined about 30% in the heart and spleen. Tissue content of β-alanine remained low relative to taurine content.

Only significant alterations in muscle amino acid content induced by either exercise or dietary treatments will be presented. Muscle glutamate values tended to be reduced in exercised rats but these reductions were only significant ($p < 0.05$) in the soleus of EC rats (1.00 ± 0.35μmoles/g) where basal glutamate content in the CON rats was higher (2.09 ± 0.18). Glutamate only declined about 15% in the ET and ENT rats in the soleus. Likewise, the EC group showed a larger drop in glutamate in both the EDL and GAST muscles compared to the ET and ENT groups but these effects were not statistically significant probably due to lower basal levels of glutamate in these

muscles (~0.5 μmoles/g). β-Alanine treatment showed a tendency to increase serine, glycine and alanine content in both the EDL and GAST muscles and no such trends were evident in the soleus or triceps. Thus, the ENT group had significantly ($p < 0.05$) higher serine in the GAST than all the other groups while both glycine and alanine were higher in the EDL. Finally, the ET group had significantly ($p < 0.05$) lower glutamine content in the EDL than either the sedentary controls (CON) or ENT groups.

Markers of cellular injury and oxidative stress

A two-way ANOVA (treatment group $\times$ muscle group) revealed a highly significant ($p < 0.0005$) effect for the experimental treatments on TBARS and as expected there was significant variation among muscle groups ($p < 0.0001$). Lipid peroxidation as indexed by TBARS was elevated by exercise in the EDL muscle, but this elevation was attenuated by both taurine and β-alanine supplementation (Table 4). There were no other significant alterations in the EDL muscle caused by either exercise or the dietary regimens (Table 4). Lipid peroxidation was also elevated in the gastrocnemius muscle but the increase was only significant in the EC group (Table 5). Ascorbic acid and uric acid

Table 4. Measures of cellular injury and oxidative stress in the EDL muscle

Markers	CON	EC	ET	ENT
TBARS (nmoles/g)	24.6 ± 1.8	38.5 ± 3.0*	25.7 ± 1.5	27.0 ± 2.4
Thiols (nmoles/g)	932 ± 151	827 ± 119	1,028 ± 128	1,011 ± 94
Protein thiols (μmoles/g)	8.33 ± 1.83	5.04 ± 0.36	5.34 ± 0.94	6.76 ± 1.05
Protein TRP (nmoles/g)	1,401 ± 241	973 ± 114	1,151 ± 245	1,427 ± 199
Ascorbic acid (μg/g)	26.6 ± 5.0	27.9 ± 3.6	24.4 ± 4.4	26.9 ± 3.2
Uric acid (μg/g)	7.82 ± 1.71	7.54 ± 0.98	6.26 ± 1.68	6.81 ± 1.30
LDH (U/g)	190 ± 20	216 ± 16	212 ± 12	198 ± 11
CK (U/g)	6,267 ± 588	7,756 ± 554	6,979 ± 628	7,340 ± 520

All values expressed as mean ± SEM (n = 5–6 per group). * $p < 0.05$ EC versus all other groups.

Table 5. Measures of cellular injury and oxidative stress in the gastrocnemius muscle

Markers	CON	EC	ET	ENT
TBARS (nmoles/g)	14.4 ± 1.6	20.3 ± 1.4[a]	19.2 ± 1.6	17.9 ± 0.8
Thiols (nmoles/g)	662 ± 101	509 ± 59	610 ± 131	711 ± 133
Protein thiols (μmoles/g)	10.56 ± 1.76	10.82 ± 1.10	12.70 ± 1.79	12.35 ± 1.09
Protein TRP (nmoles/g)	4,496 ± 613	3,252 ± 637	4,008 ± 478	3,910 ± 166
Ascorbic acid (μg/g)	13.7 ± 3.1	14.3 ± 1.1	10.9 ± 1.4[b]	17.5 ± 2.7
Uric acid (μg/g)	4.12 ± 1.13	4.76 ± 0.30	3.30 ± 0.23[b]	4.55 ± 0.76
LDH (U/g)	230 ± 31	138 ± 21[a]	196 ± 22	210 ± 13
CK (U/g)	9,041 ± 899	7,297 ± 497	7,860 ± 585	8,224 ± 818

All values expressed as mean ± SEM (n = 5–6 per group). [a] $p < 0.05$ EC vs. CON; [b] $p < 0.05$ ET vs. ENT.

Table 6. Measures of cellular injury and oxidative stress in the soleus muscle

Markers	CON	EC	ET	ENT
TBARS (nmoles/g)	32.7 ± 4.4	38.7 ± 3.8	33.9 ± 5.0	33.3 ± 1.2
Thiols (nmoles/g)	1,919 ± 129	1,372 ± 139	1,535 ± 177	1,638 ± 224
Protein thiols (μmoles/g)	6.67 ± 1.99	4.06 ± 1.27	5.56 ± 1.53	5.34 ± 1.22
Protein TRP (nmoles/g)	502 ± 65	580 ± 163	499 ± 93	729 ± 159
Ascorbic acid (μg/g)	ND	ND	ND	ND
Uric Acid (μg/g)	3.86 ± 0.66	9.50 ± 1.56[a]	7.67 ± 0.94	6.47 ± 1.08
LDH (U/g)	43 ± 6	41 ± 11	48 ± 4	50 ± 4
CK (U/g)	2,038 ± 145	1,681 ± 267	2,219 ± 149	1,800 ± 134

All values expressed as mean ± SEM (n = 5–6 per group). [a]$p < 0.05$ EC versus CON group. *ND* not determined.

Table 7. Measures of cellular injury and oxidative stress in the triceps muscle

Markers	CON	EC	ET	ENT
TBARS (nmoles/g)	14.2 ± 0.9	19.0 ± 1.5	18.6 ± 2.8	15.8 ± 0.7
Thiols (nmoles/g)	519 ± 17	568 ± 93	700 ± 68	508 ± 67
Protein thiols (μmoles/g)	8.77 ± 1.83	8.36 ± 1.28	8.83 ± 1.91	6.33 ± 1.30
Protein TRP (nmoles/g)	2,818 ± 497	2,589 ± 395	2,890 ± 633	3,253 ± 437
LDH (U/g)	151 ± 34	218 ± 39	230 ± 14	251 ± 16
CK (U/g)	4,962 ± 940	7,509 ± 1,305	7,752 ± 638	8,115 ± 474

All values expressed as mean ± SEM (n = 5–6 per group). The triceps samples were not assayed for ascorbic acid or uric acid.

content of the gastrocnemius muscle was lower in the ET group compared to the ENT group (Table 5). LDH activity of the gastrocnemius muscle was reduced in the EC group but the ET and ENT groups did not differ from the CON group (Table 5). There were no significant effects of exercise or diet on any markers in the soleus muscle except uric acid content (Table 6). Exercise significantly elevated uric acid content in the soleus muscle of EC rats (Table 6). There were no major alterations in markers of cellular injury or oxidative stress in the triceps muscle (Table 7). Protein thiols and acid soluble thiols showed significant ($p < 0.001$) variation among muscle groups but no main effect of the treatment conditions was found.

Serum creatinine was not altered by exercise or diets (data not shown) and lactic acid levels (n = 5–6 per group) were unaffected in the EDL and gastrocnemius muscle by either exercise or the dietary treatments (data not shown).

MPO activity

MPO activity was evaluated in the EDL and gastrocnemius muscles since these muscles showed evidence of oxidative injury. MPO activity was elevated

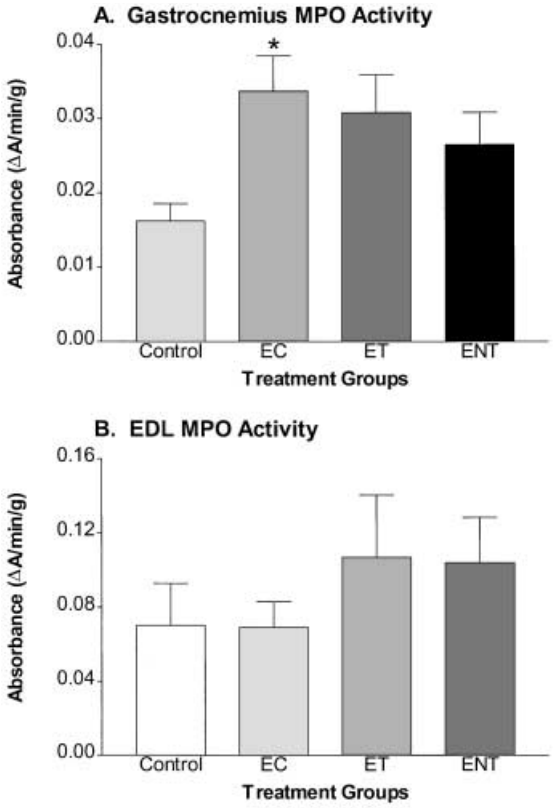

Fig. 4. Myeloperoxidase (*MPO*) activity in gastrocnemius and EDL muscle. Exercise produced an increase in MPO activity in gastrocnemius muscle that was significant (*$p < 0.05$) in the EC group but not the ET or ENT groups. No significant changes in MPO activity were noted in the EDL muscle

by exercise in the gastrocnemius muscle, but the increase was only significant in the EC group (Fig. 4A). There was only a slight nonsignificant elevation in MPO activity in the EDL muscle of ET and ENT rats (Fig. 4B). MPO activity was elevated by exercise in the liver of the EC group, but dietary supplementation with either taurine or β-alanine attenuated the rise in MPO activity (Fig. 5A). There was no effect of exercise or dietary treatments on MPO activity in the lung (Fig. 5B) or the spleen (data not shown).

Discussion

Taurine had modest actions to reduce some markers of oxidative stress caused by downhill treadmill running. Interestingly, the depletion of muscle taurine stores with β-alanine also appeared to confer some mild degree of protection against exercise-induced muscle injury. There was an apparent confound in the experimental design since taurine-supplemented rats appeared to exercise with more vigor and consequently their muscles may have been subjected to a greater degree of oxidative stress. In contrast, the β-alanine supplemented

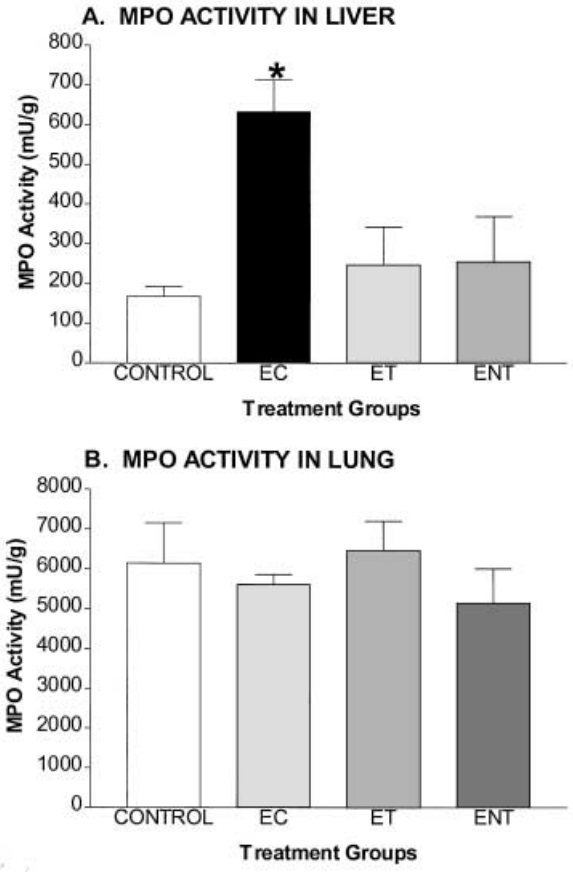

Fig. 5. Myeloperoxidase (*MPO*) activity as determined in liver (**A**) and lung (**B**) tissue 24 hours after treadmill exercise. Exercise was associated with a signficant (*$p < 0.05$) increase in liver MPO activity and this effect was attenuated by both taurine (*ET*) and β-alanine (*ENT*) supplementation. No effect of diet or exercise was noted in the lung

rats lost more weight after exercise and this group had a lower overall fluid intake and slightly reduced body weights. Therefore, the exact level of exercise-induced muscle injury was probably not equated across all groups. With this caveat stated, it was apparent that taurine supplementation prevented an exercise-induced drop in plasma methionine and attenuated lipid peroxidation in the EDL muscle.

Downhill running did cause a number of significant changes in muscle amino acids and markers of oxidative stress. The muscle groups we examined where selected based on fiber composition and potential for injury using the downhill running model. The soleus muscle contains predominately slow twitch oxidative fibers, the gastrocnemius and triceps are mixed fiber type muscles and the extensor digitorium longus (EDL) is primarily composed of fast twitch fibers. Exercise produced a general trend for TBARS to be elevated (20–50%) in all muscle groups and acid soluble thiols (11–29% $\downarrow$), and protein tryptophan (30% $\downarrow$) tended to decline in the other muscles excluding the triceps. Protein thiols also declined (40% $\downarrow$) after exercise in the

EDL and soleus muscles. Unfortunately, having 4 experimental groups and small sample sizes precluded some of these effects from being statistically significant. Therefore, it is informative to examine the overall pattern of changes in markers of oxidative stress and injury. These alterations in markers of free radical damage are consistent with what would be predicted from the literature on exercise-induced muscle injury. In most cases, taurine supplementation appeared to blunt the exercise-induced fall in acid soluble thiols. The exact mechanisms involved in this apparent trend require further study.

Previously, Allo et al. (1997) had shown that taurine depletion with β-alanine could provide cardioprotection from ischemic injury. These authors postulated a number of potential mechanisms for taurine depletion to cause an attenuation of cardiac muscle injury. In addition recent evidence also suggests that a reduction in intracellular taurine could facilitate the activation of ATP-sensitive potassium channels (Tricarico et al., 2000) that may aid in the recovery from ischemia. It can also be speculated that chronic taurine depletion would also evoke compensatory increases in osmolytes and other cytoprotective factors. β-Alanine treatment could also facilitate taurine efflux and attenuate its reuptake potentially resulting in transient elevations of taurine at extracellular sites of action. Few studies have directly examined the myriad of potential adaptive cellular changes that could be evoked by taurine depletion. The cytoprotective effects of taurine depletion are somewhat paradoxical given the well-characterized cardiomyopathy that develops in cats deprived of an adequate dietary source taurine (Tenaglia and Cody, 1988). Our present study confirms that β-alanine-induced taurine depletion can provide a degree of protection against exercise-induced muscle injury, but does not shed light on a likely specific mechanism of action for this effect.

While many studies have employed 3% taurine in the drinking water to produce beneficial effects against various insults, we noted that tissue and blood levels of taurine were in fact lower than those where we employed 1.5% taurine (Dawson et al., 1999). The 3% taurine also caused diarrhea in a majority of subjects and was likely related to a downregulation of the intestinal taurine transporter (Satsu et al., 1997) with a consequent reduction in taurine, sodium and water uptake in the intestine. In addition, previous studies have suggested that taurine can evoke diuresis (Meldrum et al., 1994; Mozaffari et al., 1997) and under certain circumstances promote natriuresis. These actions may have accounted for the increase fluid consumption seen in the taurine supplemented group. Likewise, 3% β-alanine has been reported to reduce fluid and sodium excretion (Mozaffari et al., 1997) and probably contributed to the lower levels of fluid intake seen in that group. We used 3% taurine to match the amount of β-alanine reported to produce maximal taurine depletion (Schaffer and Kocsis, 1981). It is possible that lower levels of taurine supplementation would have resulted in improved protection against exercise-induced muscle injury. Future studies will be needed to determine the optimal level of supplementation to maximize cytoprotective actions in muscle tissue.

One of our initial hypotheses was that muscle injury would be correlated to MPO activity and free radical damage associated with inflammatory reactions. Taurine is known to be an excellent scavenger for HOCI produced by MPO (Prutz, 1996). There was a clear elevation in MPO activity in the gastrocnemius muscle, but no great loss of protein thiols or ascorbic acid that are both known targets for HOCI-mediated oxidant effects (Prutz, 1996). Neither taurine nor β-alanine altered MPO activity in the gastrocnemius or EDL muscles. We noted an increase in MPO activity in both the gastrocnemius muscle and liver after treadmill running, as previously reported (Belcastro et al., 1996), however, these authors looked immediately after exercise rather than 24 hours later. Both taurine and β-alanine supplementation attenuated the exercise-induced increase in MPO activity in the liver. This was unexpected since we anticipated that taurine would simply act as a scavenger for HOCI and reduce oxidative damage. Son et al. (1998) demonstrated that oral administration of taurine could attenuate elevated colonic MPO activity caused by toxin-induced colitis. This effect was mimicked by the NFκB blocker, sulfasalazine, and taurine is known to inhibit the actions of NFκB (Gurujeyalakshi et al., 2000). This effect of taurine on NFκB appears to be mediated by taurine chloramine (Barua et al., 2001). Taurine chloramine has also been reported to inhibit chemokine production in alveolar macrophages that are involved in the recruitment of neutrophils to sites of inflammation (Liu and Quinn, 2002) which could explain the reduction in MPO activity in the liver. It is unclear why this effect on MPO was restricted to the liver but taurine can blunt the immunological activation of Kupffer cells, the resident macrophages in the liver (Seabra et al., 1998).

Skeletal muscle contains high concentrations of taurine, but its function is poorly understood. Osmoregulation appears to be a basic function of taurine and taurine concentrations appear to fluctuate with the contractile state of the muscle (Quesada et al., 1993; Lobo et al., 2000). Denervation can increase taurine in the EDL and gastrocnemius muscle but not the soleus (Iwata et al., 1986), although Turinsky and Long (1990) failed to see an effect of denervation on taurine in the soleus, plantaris or gastrocnemius muscles. In our study the soleus muscle had the highest concentration of taurine and its storage pool was not increased by supplementation. The soleus thus appears to maintain high levels of taurine and it apparently regulates intracellular taurine levels differently than other muscle groups. The soleus appeared to have a higher basal level of lipid peroxidation than either the triceps or gastrocnemius muscles. The soleus muscle had a prominent exercise-induced increase in uric acid which would be consistent with an enhanced generation of superoxide radical via a xanthine oxidase dependent pathway (Jackson, 2000). Uric acid levels were not affected by dietary treatments in any of the muscle groups examined. Thus, the soleus, which contains predominately slow twitch oxidative fibers, appears to have a higher level of basal oxidative stress and may require high intracellular taurine concentrations to function efficiently.

There is credible evidence that taurine supplementation can attenuate calcium-dependent and/or free radical mediated myocardial injury (Milei et

al., 1992; Raschke et al., 1995; Schaffer et al., 2000). The role of taurine in augmenting skeletal muscle contractile mechanisms or mitigating oxidative damage associated with exercise has received limited attention. McIntosh et al. (1998) report that taurine levels decline in the *mdx* mouse, a genetic homologue to human Duchenne muscular dystrophy. De Luca et al. (2001) found that *in vitro* application of high extracellular concentrations of taurine could enhance excitation-contraction coupling in *mdx* mice. Peirno et al. (1996) found that dietary taurine supplementation could improve indices of muscle function in aged rats. Thus, it appears taurine supplementation can reverse certain types of functional deficits in skeletal muscle. Our results suggest that taurine supplementation may facilitate exercise performance and reduce some limited aspects of muscle injury caused by exercise. Interestingly, taurine depletion caused by β-alanine administration had cytoprotective actions as well.

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