L-Arginine attenuates xanthine oxidase and myeloperoxidase activities in hearts of rats during exhaustive exercise

Wan-Teng Lin¹, ⁴, Suh-Ching Yang², Shiow-Chwen Tsai³, Chi-Chang Huang² and Ning-Yuean Lee¹*

¹Department of Nutrition and Food Sciences, Fu-Jen Catholic University, Taipei, Taiwan, Republic of China
²School of Nutrition and Health Sciences, Taipei Medical University, Taipei, Taiwan, Republic of China
³Central Laboratory, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan, Republic of China
⁴De Lin Institute of Technology, Taipei, Taiwan, Republic of China

(Received 21 January 2005 – Revised 23 June 2005 – Accepted 12 August 2005)

The present study was to investigate the effects of L-arginine (L-Arg) supplementation on cardiac oxidative stress and the inflammatory response in rats following acute exhaustive exercise on a treadmill. Rats were randomly divided into four groups: sedentary control (SC); SC with L-Arg treatment (SC + Arg); exhaustive exercise (E); exhaustive exercise with L-Arg treatment (E + Arg). Rats in groups SC + Arg and E + Arg received a 2% L-Arg diet. Rats in groups E and E + Arg performed an exhaustive running test on a treadmill at a final speed of 30 m/min, 10% grade, at approximately 70–75% VO₂max. The results showed a significant increase in cardiac xanthine oxidase (XO) and myeloperoxidase activities and membrane lipid peroxidation endproduct (malondialdehyde; MDA) levels of exercised rats compared with SC rats. The increased cardiac XO activity and MDA levels in exercised rats were significantly decreased in exercised rats supplemented with L-Arg. Myocardial GSSG content increased whereas the GSH:GSSG ratio was depressed in exercised rats compared with SC rats. Cardiac GSSG levels significantly decreased, whereas total glutathione, GSH and the GSH:GSSG ratio increased in exercised rats supplemented with L-Arg compared with exercised rats. The activities of creatinine kinase (CK) and lactate dehydrogenase (LDH), and lactate, uric acid, and nitrite and nitrate levels in the plasma significantly increased in exercised rats compared with SC rats. The activities of plasma CK and LDH were significantly decreased in L-Arg-supplemented plus exercised rats compared with exercised rats. These findings suggest that L-Arg supplementation reduces the oxidative damage and inflammatory response on the myocardium caused by exhaustive exercise in rats.

L-Arginine: Xanthine oxidase: Myeloperoxidase: Oxidative stress: Exhaustive exercise

The heart tissues are mostly composed of muscles that contain plenty of mitochondria, which need more O₂ compared with other organs. Basically, the heart needs lots of O₂ to work well. Nowadays, O₂ is sometimes apt to be dealt with as a negative factor in oxidative stress instead of as an indispensable factor for life activity. Although increased O₂ flux through the mitochondrial electron transport chain is considered the main source of reactive oxygen species (ROS), other pathways of ROS generation, such as xanthine oxidase (XO) and polymorphonuclear neutrophils, may also be activated during or after strenuous exercise (Ji, 1999).

In previous studies, exhaustive physical exercise has been known to induce free radicals in vivo and lead to oxidative damage in several tissues such as muscle, liver, lung, and heart (Kumar et al. 1992; Frankiewicz-Jozko et al. 1996; Ashto et al. 1998; Radak et al. 1998). During exhaustive exercise, XO activity was significantly increased in the circulation and tissues (Radak et al. 1995; Hellsten et al. 1997; Vina et al. 2000). XO, a metalloflavoprotein, has a major role for oxygen-derived free radicals in post-ischaemic tissue injury (McCord, 1985). Vina et al. (2000) further demonstrated that XO is responsible for free radical production and tissue damage during exhaustive exercise. Recently, it was proposed that XO-derived oxidants are chemotactic to neutrophils (Judge & Dodd, 2004). In human and animal studies, neutrophil infiltration in tissues is associated with strenuous exercise-induced tissue damage (Fielding et al. 1993; Belcastro et al. 1996). Neutrophils are capable of further generation of free radicals via NADPH oxidase and producing HOCl from H₂O₂ via myeloperoxidase (MPO), a marker for neutrophil infiltration in tissues, in the initiation of exercise-induced muscle damage (Tiidus, 1998). These studies indicate that both XO and MPO are the two main sources of extracellular free radicals during strenuous exercise. These two enzymes are also responsible for exhaustive exercise-induced oxidative stress in several tissues including muscle, liver, and heart.

L-Arginine (L-Arg) is used in current basic and clinical research for its important therapeutic qualities. Most

Abbreviations: AST, aspartate aminotransferase; CAT, catalase; CK, creatinine kinase; E, exhaustive exercise; GPX, glutathione peroxidase; GR, glutathione reductase; L-Arg, L-arginine; LDH, lactate dehydrogenase; MDA, malondialdehyde; MPO, myeloperoxidase; NOx, nitrite and nitrate; ROS, reactive oxygen species; SC, sedentary control; SOD, superoxide dismutase; UA, uric acid; XO, xanthine oxidase.

* Corresponding author: Dr Ning-Yuean Lee, fax +886 2 2904 3234, email als2046@mails.fju.edu.tw
pharmacological actions of L-Arg are attributed to NO, which is able to act on a wide range of tissues and is a multipurpose messenger molecule implicated in a wide variety of biological processes (Wu & Morris, 1998). For example, in patients with CVD, intravenous and oral L-Arg administration has been shown to support endothelial function by enhancing vasodilatation and reducing monocyte adhesion (Wang et al., 1994). Increasing NO bioactivity through L-Arg supplementation has been shown to reduce blood lactate and NH₃ after maximal or submaximal exercise (Schaefer et al. 2002). Recent findings suggest that L-Arg supplementation could significantly enhance the exercise-induced increases of NO production and changes of Fe metabolism (Xiao et al., 2003). Several studies have also demonstrated that L-Arg has a protective role against ROS attack. This was possible due to its direct chemical interaction with superoxide anions (O₂⁻) in vitro (Wascher et al., 1997; Lass et al. 2002). However, so far as we know, there is no study investigating the effect of L-Arg supplementation on cardiac XO activity, the inflammatory response and antioxidant defense systems during exhaustive exercise. We therefore studied whether dietary L-Arg may protect heart tissue and have beneficial effects on ameliorating exercise-induced oxidative stress and inflammation in the myocardium of rats.

Materials and methods

Thirty-two male Sprague–Dawley rats weighing 280–300 g (8 weeks old) were purchased from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). All rats were given free access to water and standard pellet rat food (no. 5001; PMI Nutrition International, Brentwood, MO, USA) and individually housed in a room maintained at 23 ± 2°C with a 12 h light–dark cycle. In order to adapt to the experimental standard diet, rats were fed an AIN-93 diet in the second week. Afterward, rats were randomly divided into four groups: a sedentary control group with AIN-93 diet (SC; n 8); sedentary control with AIN-93 containing 2 % L-Arg diet (SC + Arg; n 8); exhaustive exercise with AIN-93 diet (E; n 8); exhaustive exercise with AIN-93 containing 2 % L-Arg diet (E + Arg; n 8). Rats were fed diets for 30 d beginning in the third week. Both diets were identical in energy content (Table 1) (Reeves et al. 1993; Lewis & Langkamp-Henken, 2000). The experiment was approved by the Fu-Jen Catholic University Animal Care and Usage Committee and followed the guidelines established by the National Laboratory Animal Breeding and Research Center in Taiwan. It is well known that exercise performance is limited by increase of body temperature during exercise. In order to eliminate this complicating factor, all running tests were conducted in an environmental chamber at 10–12°C to reduce the undue rise in body temperature due to sustained exercise. Rats in groups E and E + Arg were introduced to treadmill running with 15–20 min exercise bouts at 15–30 m/min for 6 d to accustom them to running by a modification of the method of Ji et al. (1991). The treadmill was equipped with an electric shocking grid on the rear barrier to provide the animal with exercise motivation (T510E treadmill device; DR instrument, Taipei, Taiwan). On the day of the exercise test, they were required to run to exhaustion on a six-lane inclined (10°) treadmill at a final speed of 30 m/min, which was approximately 70–75 % of V̇O₂max (Brooks & White, 1978). The measurement of maximal O₂ consumption (100 % V̇O₂max) was considered valid only if the animal ran until it could no longer maintain pace with the treadmill (Somani et al., 1995). Therefore, exhaustion was determined as the rat being unable to upright itself when placed on its back (Ji & Mitchell, 1994; de Oliveira et al. 2003). To eliminate diurnal effects, the experiments were performed at the same time (09.00 hours to 12.00 hours).

All animals were anaesthetised with ethyl ether and killed immediately after exhaustive exercise. After anaesthesia, body temperature was measured by use of a rectal thermometer. Then, the heparinised blood sample was collected from the abdominal aorta and the heart tissue was carefully removed, rinsed in ice-cold normal saline, blotted dry and stored at − 80°C for further analysis.

Heart tissues (100 g/l) were homogenised in ice-cold buffer (sucrose (0.25 mol/l), tri(hydroxymethyl)-aminomethane-HCl (10 mmol/l), and phenylmethylsulfonyl fluoride (0.25 mmol/l); pH 7.4) using a Polytron homogeniser (Kinematica GmbH, Lucerne, Switzerland) with a Teflon pestle and glass tube (Glas-Col, Terre Haute, IN, USA). The homogenates were centrifuged at 10 000 g for 20 min at 4°C. The supernatant fractions

<table>
<thead>
<tr>
<th>Ingredient (g/kg)</th>
<th>Group SC</th>
<th>Group SC + Arg</th>
<th>Group E</th>
<th>Group E + Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>0</td>
<td>16</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Maize starch</td>
<td>397·5</td>
<td>381</td>
<td>397·5</td>
<td>381</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>132</td>
<td>132</td>
<td>132</td>
<td>132</td>
</tr>
<tr>
<td>Sucrose</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Soyabean oil</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>AIN-93 mineral mix</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>AIN-93 vitamin mix</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2·5</td>
<td>2·5</td>
<td>2·5</td>
<td>2·5</td>
</tr>
<tr>
<td>tert-Butylhydroquinone</td>
<td>0·014</td>
<td>0·014</td>
<td>0·014</td>
<td>0·014</td>
</tr>
</tbody>
</table>

SC, sedentary control; Arg, L-arginine; E, exhaustive exercise.

* The components for the diets were purchased from ICN biochemicals, Inc. (Costa Mesa, CA, USA), with the exception of L-arginine, sucrose and soyabean oil, which were obtained from Sigma-Aldrich-Fluka (St Louis, MO, USA). For details of diets and procedures, see p. 68.
were transferred to a new Eppendorff tube and used for determination of XO activity, antioxidant enzyme activities and protein levels. The pellets were washed twice in an ice-cold buffer (phosphate buffer; 20 mmol/l; pH 6-0) and sonicated in MPO homogenisation buffer containing 0.5 % hexadecyltrimethylammonium bromide, EDTA (10 mmol/l) and phosphate buffer (50 mmol/l) (pH 6-0) at 25°C for 1 min with an ultrasonic processor (Vibra Cell model VCX-500; Sonics and Materials Inc., Danbury, CT, USA). The homogenates were then centrifuged at 17000g at 4°C for 15 min. Supernatant fractions were aspirated into new tubes and stored on ice until the assay was performed. In addition, all samples were diluted in deionised water for analysis, if necessary. All chemicals used in the present study were purchased from Sigma-Aldrich-Fluka (St Louis, MO, USA) unless stated otherwise.

**Plasma parameters**

Blood samples were centrifuged at 1400g at 4°C for 10 min. The supernatant fractions (plasma) were used for the determination of creatinine kinase (CK), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and uric acid (UA) with an automatic analyser (Hitachi 7170; Hitachi, Tokyo, Japan). In addition, nitrate and nitrite (NOx) levels in plasma were quantified by using the Griess reagent to measure nitrite ion concentration (Green et al. 1982). NOx concentrations in plasma were measured spectrophotometrically at 550 nm according to a previous study (Schmidt et al. 1992).

**Assay of xanthine oxidase activity in the heart tissue**

XO activity of heart tissue was determined by the method of Westerfeld et al. (1959). A diluted sample was added to xanthine (0.1 mmol/l) (dissolved in sodium phosphate buffer, 50 mmol/l; pH 7.5). XO activity was measured at 25°C on a Hitachi U-2000 spectrophotometer at 290 nm for 3 min. One unit of XO activity was defined as 1 mmol urate formation/min at 25°C. XO activity was expressed as U/g protein for specific activity.

**Assay of myeloperoxidase activity in the heart tissue**

MPO activity of heart tissue was determined as a marker enzyme for measuring neutrophils accumulating in tissue samples, because it is closely correlated with the number of neutrophils present in the tissue (Mullane et al. 1985). MPO activity was determined by the method of Schierwagen et al. (1990). A portion of diluted sample (50 μl) was added to 1 ml mixed substrate containing H2O2 (3 mmol/l) dissolved in 3,3′,5′,5′-tetrathylbenzidine (R&F Systems Inc., Minneapolis, MN, USA). MPO activity was measured at 37°C on a Hitachi U-2000 spectrophotometer at 655 nm for 3 min. One unit of MPO activity was defined arbitrarily as the amount of enzyme necessary to catalyse an increase in absorbance of 1.0 at 655 nm/min at 37°C. MPO activity was expressed as U/mg protein for specific activity.

**Lipid peroxidation in the heart tissue**

The malondialdehyde (MDA) concentration of the heart tissue homogenate was assessed colorimetrically at 586 nm using a commercial kit (Calbiochem 437634; Calbiochem-Novabiochem, La Jolla, CA, USA).

**Assay of superoxide dismutase activity in the heart tissue**

Superoxide dismutase (SOD) activity of heart tissue was measured with a commercial kit (SD 125; Randox Laboratories, Antrim, UK). A portion of diluted standard or sample (50 μl) was added to 1.7 ml mixed substrate (xanthine (50 mmol/l) and 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (25 mmol/l)). XO (250 μl; 80 U/l) was added to the mixture and SOD activity was measured by the degree of inhibition of this reaction at 37°C on a Hitachi U-2000 spectrophotometer at 505 nm for 3 min. Activity was expressed as U/mg protein for specific activity.

**Assay of catalase activity in the heart tissue**

Catalase (CAT) activity of heart tissue was determined at 25°C with a Hitachi U-2000 spectrophotometer UV-VIS spectrophotometer by the method of Beers & Sizer (1952). A diluted sample was added to H2O2 (59 mmol/l) (dissolved in potassium phosphate buffer; 50 mmol/l; pH 7.0) and CAT activity was measured at 240 nm for 3 min. One unit of CAT activity was defined as 1 mmol H2O2 degraded/min. Activity was expressed as U/mg protein for specific activity.

**Assay of glutathione peroxidase activity in the heart tissue**

Glutathione peroxidase (GPX) activity of heart tissue was determined with a commercial kit (RS 504; Randox Laboratories, Antrim, UK). A portion of diluted sample (20 μl) was added to 1 ml mixed substrate (glutathione (4 mmol/l), glutathione reductase (GR; 0.5 U/l) and NADPH (0.34 mmol/l) dissolved in phosphate buffer (50 mmol/l); pH 7.2; EDTA, 4.3 mmol/l). Cumene hydroperoxide (40 μl; diluted in deionised water) was added to the mixture and GPX activity was measured at 37°C on a Hitachi U-2000 spectrophotometer at 340 nm for 3 min. One unit of GPX activity was defined as the amount of enzyme that catalysed the reduction of 1 mmol NADPH/min. Activity was expressed as U/mg protein for specific activity.

**Assay of glutathione reductase activity in the heart tissue**

GR activity of heart tissue was measured with a commercial kit (Calbiochem 359962; Calbiochem-Novabiochem). A portion of diluted sample (200 μl) was added to 400 μl GSSG buffer (2.4 mmol/l; dissolved in potassium phosphate buffer (125 mmol/l); pH 7.5; EDTA, 2.5 mmol/l). NADPH (400 μl; 0.55 mmol/l; dissolved in deionised water) was added to the mixture and GR activity was measured at 340 nm for 5 min on a Hitachi U-2000 spectrophotometer. One unit of GR activity was equal to 1 mmol NADPH oxidised/min. Activity was expressed as mU/g protein for specific activity.
**Assay of total and oxidised glutathione in the heart tissue**

Total glutathione was determined using a modification of the method of Tietze (1969). Total glutathione was assayed by the addition of 190 μl freshly prepared assay buffer (NADPH (100 μmol/l), 5,5'-dithiobis-(2-nitrobenzoic acid) (5 mmol/l), GR (1 unit/ml), EDTA (1 mmol/l) and phosphate buffer (50 mmol/l); pH 7.2) to 10 μl diluted sample. The change in absorbance was measured after 3 min at 405 nm using a microplate reader (Labsystem Multiskan RC, Helsinki, Finland) and compared with a standard curve, from 0 to 100 μmol/l.

GSSG was determined by the method of Griffith (1980). Diluted samples or standards (70 μl) were derivatised with 1-methyl-2-vinylpyridinium trifluoromethane sulfonate to remove GSH by adding 4 μl 1-methyl-2-vinylpyridinium trifluoromethane sulfonate and 3.2 μl triethanolamine. Samples were left at room temperature for 1 h to allow the reaction to occur. The GSSG that remained was then assayed in the same manner as for total glutathione.

**Total protein concentrations in samples**

Total protein concentrations of samples were spectrophotometrically estimated according to the method of Lowry et al. (1951) using a Bio-Rad DC protein assay kit (catalogue no. 500-0116; Bio-Rad Laboratories, Hercules, CA, USA).

**Statistical analysis**

Values are expressed as means with their standard errors. To evaluate differences among the groups studied, two-way ANOVA with Fisher’s post hoc test was used. SAS software (version 8.2; SAS Institute Inc., Cary, NC, USA) was used to analyse all data. Differences were considered statistically significant when P<0.05.

**Results**

**Body weight and endurance time**

At the end of 30 d, body weight in the groups SC, SC + Arg, E and E + Arg were 446 (SEM 7), 446 (SEM 10), 447 (SEM 6) and 451 (SEM 8) g, respectively. There were no significant differences in body weight among the four groups. The mean endurance time of treadmill running to exhaustion was 81 (SEM 4) min for group E and 87 (SEM 5) min for group E + Arg, which was not significantly different between the two groups.

**Xanthine oxidase activity in the heart tissue**

As shown in Fig. 1, XO activity of heart in group E was significantly increased by 87 % compared with that in group SC. However, XO activity was significantly decreased by 44 % in group E + Arg compared with group E (P<0.05).

**Myeloperoxidase activity in the heart tissue**

The degree of neutrophil infiltration in the myocardium is shown in Fig. 2. In comparison with group SC, MPO activity of heart in group E was significantly increased by 18 % (P<0.05).

**Lipid peroxidation in the heart tissue**

MDA, a quantitative marker of lipid peroxidation, was measured in the heart tissue as shown in Fig. 3 (P<0.05). The content of MDA in group E was significantly elevated by 44 % compared with group SC. When compared with group E, the elevation of MDA content was significantly decreased in group E + Arg (P<0.05).

**Plasma creatinine kinase, aspartate aminotransferase, lactate dehydrogenase, uric acid, and nitrite and nitrate levels**

Plasma CK, AST, LDH, UA, and NOx levels in group E were all significantly elevated by 453, 81, 363, 268 and 93 %, respectively, compared with those in group SC (P<0.05) (Table 2). In contrast, the CK and LDH activities of plasma were significantly lowered by 46 and 33 %, respectively, in
group E + Arg compared with group E (P<0.05). Elevations of muscle enzymes such as CK, AST, and LDH in plasma are characteristic responses to strenuous exercise and often used as indicators of muscle damage. Especially, changes in activities of AST and LDH have been used previously as indicators of myocardial damage.

Antioxidant enzymes activities in the heart tissue

Table 3 shows the antioxidant enzyme activities. The antioxidant enzyme activities of SOD and CAT in heart tissue were significantly increased by 58 and 62 %, respectively, in group E compared with in group SC (P<0.05). In contrast, antioxidant enzyme activities of SOD, CAT, GPX, and GR in group E + Arg were all significantly lower than those in group E (P<0.05). It is possible that the higher activity of antioxidant enzymes as a result of exercise might be indicative of a compensatory measure to counteract the possible detrimental effects associated with oxidative stress.

**Discussion**

Acute physical exercise increases cardiac contractility and heart rate. Strenuous exercise may be associated with a fourfold increase in blood flow through and O2 consumption by the myocardium. A number of studies have shown free radical-mediated damage during exercise-induced oxidant stress (Ji et al. 1991; Ji, 1993; Ji & Mitchell, 1994; Sen et al. 1994; Somani et al. 1995). The present study assessed the effects of dietary L-Arg supplementation on acute strenuous exercise-induced oxidative damage of myocardium and modulations of cardiac antioxidant enzymes activities. It is the first study to examine the antioxidant potential of L-Arg using an in vivo animal exercise model.

The present results demonstrate that in exhaustive exercise with higher anaerobic components, the lactate concentration in plasma of group E (750 (SEM 60) mg/l) was significantly higher than that of group SC (550 (SEM 40) mg/l) (P<0.05), but not of group E + Arg (460 (SEM 20) mg/l). This result indicates that the rats are not able to obtain a sufficient amount of O2 during running; hence, hypoxia metabolism supports a significant part of the exhaustive exercise. This situation is in accordance with some references in the literature (Radak et al. 1995; Vina et al. 2000). The production of radicals in the heart tissue after acute exhaustive exercise caused some degree of oxidative damage. The importance of this pathway is associated primarily with the consumption of ATP. An increase in the ADP:ATP ratio, followed by AMP

**Table 2. Effects of L-arginine (Arg) supplementation on plasma creatinine kinase (CK), lactate dehydrogenase (LDH), uric acid (UA), and nitrite and nitrate (NOx) levels after exhaustive exercise (E)**

(Mean values with their standard errors for eight rats)

<table>
<thead>
<tr>
<th></th>
<th>Group SC</th>
<th>Group SC + Arg</th>
<th>Group E</th>
<th>Group E + Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>CK (UI)</td>
<td>158a</td>
<td>7</td>
<td>231a</td>
<td>26</td>
</tr>
<tr>
<td>AST (UI)</td>
<td>79a</td>
<td>3</td>
<td>90a</td>
<td>6</td>
</tr>
<tr>
<td>LDH (UI)</td>
<td>169a</td>
<td>18</td>
<td>377ab</td>
<td>76</td>
</tr>
<tr>
<td>UA (mg/l)</td>
<td>9.4a</td>
<td>1-0</td>
<td>19.9b</td>
<td>2.8</td>
</tr>
<tr>
<td>NOx (µmol/l)</td>
<td>1.38a</td>
<td>0.14</td>
<td>1.48a</td>
<td>0.20</td>
</tr>
</tbody>
</table>

SC: sedentary control; AST: aspartate aminotransferase.

a,bMean values within a row with unlike superscript letters were significantly different (P<0.05).

For details of diets and procedures, see p. 68.

**Total and oxidised glutathione in the heart tissue**

The levels of total glutathione, GSH and GSSG, and the GSH:GSSG ratio in the heart tissue are shown in Table 4. The levels of total glutathione and GSH showed no difference between groups E and SC. However, the content of GSSG in group E was significantly higher by 63 % compared with that of group SC (P<0.05). However, the GSH:GSSG ratio in group E was significantly lower by 55 % than that in group SC (P<0.05). When compared with group E, the content of GSSG was significantly lower by 31 % in group E + Arg (P<0.05). However, the levels of total glutathione and GSH, and the GSH:GSSG ratio were all significantly increased by 55, 93 and 171 %, respectively, in group E + Arg compared with those in group E (P<0.05).
increase in XO was observed in the L-Arg-supplemented group in response to exercise. Numerous studies have shown NO, a biologically important molecule, to have many modula-
tory functions for cells and tissues. NO has been shown to be pro-
ductive in ischaemia and reperfusion (Zweier et al. 1995; Vina et al.
2000). Our data support the find-
ing of neutrophils after exhaustive exercise. This finding was similar to that of a previous report (Vina et al. 2000). Hence, the protective role of NO could probably be due to its property of scavenging free radicals and inhibiting XO; this is in accordance with the present results in the E + Arg group.

MPO has been shown to provide an index of neutrophil infiltration in tissues, and its activity has been reported to be elevated with strenuous running in several tissues of rats, including heart tissue (Belcastro et al. 1996). Our data also showed that heart MPO activity in group E was markedly increased after exhaustive exercise. Therefore, it can be con-
cluded that XO-derived oxidants are important in the accumu-
lation of neutrophils after exhaustive exercise. This chemotaxic potential of oxidants from XO is in agreement with previous investigators (Kubes et al. 1990, 1991; Nonami, 1997). However, exhaustive exercise-induced cardiac MPO activity was not significantly reduced by L-Arg supplementation.

As described earlier, lipid peroxidation, measured in the form of MDA, significantly increased in the heart tissue of the E group, also supporting the possibility of increased ROS production leading to higher rate of lipid peroxidation (Frankiewicz-Jozko et al. 1996; Venditti & Di Meo, 1996). L-Arg supplementation, however, significantly protects heart tissue from ROS-mediated oxidative damage during exhaust-
ive exercise.

Vina et al. (2000) have demonstrated that administration of allopurinol, an inhibitor of XO, prevents the increase of the activities of cytosolic enzymes CK and LDH in plasma after exhaustive exercise. Our data also showed that L-Arg sup-
plementation could inhibit the elevation of XO activity and resulted in a significant decrease in plasma levels of CK and LDH in group E + Arg compared with that in group E. This finding was similar to that of a previous report (Vina et al. 2000).

Furthermore, Qian et al. (2001) and Reid (1998) have suggested that long-term strenuous exercise may stimulate the activity of NO synthase and hence increase NO synthesis, also causing a significant increase in NOx production in plasma. In the present study, the results indicate that in both the E and E + Arg groups there was a significant increase in plasma NOx after exhaustive exercise compared with controls. NO, however, is also a free radical and hence the toxicity of NO (excess production of NO) is markedly enhanced by its reaction with O2· from peroxynitrite (ONOO·), which is

### Table 3. Effects of L-arginine (Arg) supplementation on antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione reductase (GR) activities of heart tissue after exhaustive exercise (E)

<table>
<thead>
<tr>
<th></th>
<th>Group SC</th>
<th>Group SC + Arg</th>
<th>Group E</th>
<th>Group E + Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/mg protein)</td>
<td>15·41b</td>
<td>2·97</td>
<td>6·40a</td>
<td>2·10</td>
</tr>
<tr>
<td>CAT (U/mg protein)</td>
<td>30a</td>
<td>3</td>
<td>38a</td>
<td>4</td>
</tr>
<tr>
<td>GPX (U/mg protein)</td>
<td>161a,b</td>
<td>6</td>
<td>150a,b</td>
<td>9</td>
</tr>
<tr>
<td>GR (mU/g protein)</td>
<td>7·25a,b</td>
<td>1·14</td>
<td>7·60a,b</td>
<td>0·62</td>
</tr>
</tbody>
</table>

**SC, sedentary control.**

**Mean SEM Mean SEM Mean SEM Mean SEM**

### Table 4. Effects of L-arginine (Arg) supplementation on total glutathione, GSH and GSSG concentrations, and GSH:GSSG ratio of heart tissue after exhaustive exercise (E)

<table>
<thead>
<tr>
<th></th>
<th>Group SC</th>
<th>Group SC + Arg</th>
<th>Group E</th>
<th>Group E + Arg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total glutathione (mg/mg protein)</td>
<td>0·047a</td>
<td>0·003</td>
<td>0·060b</td>
<td>0·006</td>
</tr>
<tr>
<td>GSH (mg/mg protein)</td>
<td>0·039a,b</td>
<td>0·003</td>
<td>0·05b,c</td>
<td>0·006</td>
</tr>
<tr>
<td>GSSG (mg/mg protein)</td>
<td>0·008a</td>
<td>0·001</td>
<td>0·009a</td>
<td>0·001</td>
</tr>
<tr>
<td>GSH:GSSG</td>
<td>5·33b</td>
<td>0·63</td>
<td>6·19b</td>
<td>1·14</td>
</tr>
</tbody>
</table>

**SC, sedentary control.**

**Mean SEM Mean SEM Mean SEM Mean SEM**

**Mean values within a row with unlike superscript letters were significantly different (P<0·05).**

For details of diets and procedures, see p. 68.

and its degradation to hypoxanthine, culminates in UA increasing and O2·− production by XO (Westing et al. 1989; Radak et al. 1995; Vina et al. 2000). Our data support the findings of the present study in which the XO activity and the UA and MDA concentrations in the heart show similar marked increases immediately following the exhaustive running. This may suggest that the XO-derived free radicals exert some deleterious effect on heart tissue. However, no such increase in XO was observed in the L-Arg-supplemented group in response to exercise. Numerous studies have shown NO, a biologically important molecule, to have many modulatory functions for cells and tissues. NO has been shown to be cardio protective in ischaemia and reperfusion (Zweier et al. 1995; Au et al. 2003). Furthermore, NO may exert direct effects on cardiac myocytes (Shiono et al. 2002). The inhibition of XO by NO may be mediated through direct binding of NO to the enzyme Fe–S moiety (Hassoun 1996). Hence, the protective role of NO could probably be due to its property of scavenging free radicals and inhibiting XO; this is in accordance with the present results in the E + Arg group.
L-Arginine and exhaustive exercise

extremely cytotoxic (Beckman & Koppenol, 1996). Numerous studies have shown that infusion of ONOO\(^-\) into working rat hearts impairs cardiac contractile function by decreasing cardiac efficiency (Liu et al. 1997; Schulz et al. 1997; Yasmin et al. 1997). However, Rubbo et al. (1994) reported that NO actually inhibits peroxynitrite-induced lipid peroxidation. The data demonstrated that NO will significantly enhance lipid peroxidation only when rates of NO production approach or are equivalent to rates of O\(_2^-\) production by XO. In the present study, we measured the effect of exhaustive exercise at the rate of NOx synthesis in plasma in both exercised groups. Obviously, rats in group E had significant increases in XO activity, MPO activity, MDA levels and GSSG release that will cause oxidative stress in heart tissue. It means that the enhanced production of O\(_2^-\) by XO may be possibly as a result of synergy between higher-level NO and O\(_2^-\) to form cytotoxic ONOO\(^-\) after exhaustive exercise. This is in contrast with the E + Arg group; when NO concentrations are increased, the protective role of NO and/or l-Arg itself may probably be due to the property of scavenging free radicals and possibly inhibiting XO as well as inflammation of neutrophils. Therefore, the present study suggests that the E + Arg group has higher levels of NO to inhibit ONOO\(^-\) formation and it is unlikely that ONOO\(^-\) may have a role in this process. The present results are in agreement with the findings of Rubbo et al. (1994); that NO alone does not induce lipid peroxidation. Thus, we conclude that NO can exacerbate oxidant injury via the production of the potent oxidant ONOO\(^-\) or exert a protective role via scavenging O\(_2^-\) by termination of free radical-dependent chain-propagation reactions by diverse initiating species.

It is well known that SOD, CAT, GPX and GR are regarded as the first line of the antioxidant enzyme system against ROS generated during exhaustive exercise. The present study showed that these enzymes are increased as a compensatory mechanism in response to the increase of oxidative stress due to exhaustive exercise. It means that the increased XO observed in the present study denotes the increased generation of O\(_2^-\), which will have triggered the activation of SOD by dismutating O\(_2^-\) to H\(_2\)O\(_2\). This result is supported by previous studies (Ji, 1993; Somani et al. 1995; Leeuwenburgh et al. 1996). The increased CAT activity levels in heart (162 %) observed in the present study were also presented in Somani et al. (1995), who observed CAT activity increase by 358 % in the heart of rats subjected to acute strenuous exercise. Our data show that SOD and CAT activities were significantly lower or were not different in the E + Arg group compared with the control groups. These results indicate that l-Arg supplementation has beneficial effects on attenuating the oxidative stress induced by exhaustive exercise. The increased activity of GPX in heart after acute strenuous exercise has been reported in a previous study; GPX converts H\(_2\)O\(_2\) to water and O\(_2\) to cope with abrupt exercise-induced oxidant stress (Somani et al. 1995). In addition, we found significantly increased heart GR activity in the E group. However, it is different from the results of a few reports showing no change (Ji, 1993; Leeuwenburgh et al. 1996). It may be possible that myocardium shows a marked GR adaptation to GSH depletion and increased GSSG levels in the present study. However, growing evidence (Ji, 1993; Somani et al. 1995; Leeuwenburgh et al. 1996) suggests that some antioxidant enzymes can be increased in myocardium actively involved in exhaustive exercise. The results of the present study indicate that myocardium of the E group may be more susceptible to oxidative stress than that of the SC group. These disparate results may be due to differences in tissues or organs, exercise intensity, duration and mode of exercise.

GSH is a major non-enzymic antioxidant and has been reported to play an important role in protecting the myocardium from ischaemia and reperfusion-induced oxidative damage (Ji, 2002). Previous studies have shown a decrease in the myocardial GSH of rats after a vigorous swimming exercise and exercise running (Kihlstrom et al. 1989; Seward et al. 1995; Leichtweis et al. 1997). Also, the GSH/GSSG ratio decreases in the myocardium of exhausted rats (Sen et al. 1994). Subsequent studies also reported that levels of total glutathione fell in exhaustive-exercise rats (Duarte et al. 1993). In the present study, we found that GSSG was significantly increased and that the GSH/GSSG ratio was significantly decreased in the E group, but GSH did not reach statistical significance (P = 0.0687). Also, total glutathione did not change significantly in the E group compared with the SC group. However, l-Arg supplementation significantly increased total glutathione and GSH in both the SC + Arg and E + Arg groups. As a result of the protective effect of l-Arg, GSSG level significantly decreased in comparison with the E group. It means that healthy non-stressed cells maintain notably a high intracellular GSH/GSSG ratio to ensure the availability of GSH, and thereby promote active reduction of H\(_2\)O\(_2\) through the GSH redox cycle. The effects of l-Arg supplementation in exhaustively exercised animals on enzymic and non-enzymic antioxidant systems cannot be clearly explained at present. They are probably related to the following two mechanisms. First, l-Arg serves as a precursor of the synthesis of NO that may have an indirect antioxidant role to scavenger O\(_2^-\), prevent cellular damage; but, in the presence of NO synthase inhibitor l-NAME, this protection is abolished (Wascher et al. 1997). Second, l-Arg has some protective roles against ROS attack by its direct chemical property interaction with O\(_2^-\) (Wascher et al. 1997; Lass et al. 2002). Hence, it may be possible to maintain normal enzymic and non-enzymic antioxidant systems of the redox cycle after exhaustive exercise. By contrast, non-Arg supplementation of rats significantly increases these antioxidant enzymes and decreases the GSH/GSSG ratio, possibly as a compensatory mechanism to cope with the enhanced production of ROS during exhaustive exercise.

In conclusion, the present results support the mechanism that the increase of XO and MPO activities is the main pathway involved in the production of free radicals during exhaustive exercise. Our data also indicate that the administration of l-Arg could prevent exhaustive exercise-induced production of lipid peroxide levels, elevation of XO activity, and imbalance of antioxidant enzymes activities. Our findings may have important implications in the development of therapeutic strategies aimed at manipulating l-Arg supplementation for NO production and/or l-Arg itself in exercise-induced oxidative stress.
Acknowledgements

This project was supported by the Shin Kong Wu Ho-Su Memorial Hospital of Taiwan, SKH-FIU—92—13.

References


