Aminoguanidine prevented impairment of blood antioxidant system in insulin-dependent diabetic rats

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Abstract

Non-enzymatic glycation is implicated in the development of various diseases such as Alzheimer’s and diabetes mellitus. However, it is also observed during the physiologic process of aging. There is considerable interest in the contribution of oxidative stress to diabetes mellitus. An increase in the generation of reactive oxygen species can occur by non-enzymatic glycation and glucose autoxidation. Both of these processes lead to the formation of AGEs (Advanced glycation end-products) that contribute to the irreversible modification of enzymes, proteins, lipids and DNA. In this study, the effect of chronic hyperglycemia on the antioxidant system of diabetic rats was evaluated. The working hypothesis is that the loss of glucose homeostasis reduces the capacity to respond to oxidative damage. The enzymatic activities of CAT (catalase), GPx (glutathione peroxidase), GR (glutathione reductase) and GSH (reduced glutathione) were increased in the blood of healthy rats subjected to endurance training, whereas, in diabetic rats the activities of CAT, GPx and GR were unaltered by similar training. SOD showed low activity in endurance-trained rats. The administration of aminoguanidine (an inhibitor of glycation reactions) in the drinking water increased the activities of CAT, GPx and GR, suggesting that glycation may be responsible for the partial inactivation of these enzymes. These results indicate that the association of hyperglycemia with strenuous physical exercise may induce cellular damage by impairing the antioxidant defense system.

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Keywords: Antioxidant enzymes; Diabetic; Blood; Glycation; Free radicals

Introduction

Diabetes can cause tissue damage through non-enzymatic glycation via Schiff base formation between the carbonyl groups of sugar and the amino groups of proteins (Brownlee et al., 1984; Lapolla et al., 1995). In insulin-dependent diabetes, chronic hyperglycemia can damage cells through glucose autoxidation that promotes the generation of H2O2 and ketoaldehydes (Hunt and Wolff, 1990). These compounds have been associated with the development of diabetic complications related to the production and accumulation of AGEs (advanced glycation end-products) in tissues (Wolff and Dean, 1987). AGEs can be deposited in blood vessels producing free radicals, and can degrade vessel lipids and accelerate atherogenesis in hyperglycemic diabetic patients (Brownlee et al., 1984; Mullarkey et al., 1990).

In humans and animals, strenuous physical exercise may induce a state in which the antioxidant defenses of several tissues are overwhelmed by an excess of reactive oxygen intermediates (Jenkins, 1988; Ji, 1999; Ji et al., 1988). In tissues chronically exposed to elevated oxidative stress, there is an adaptation of the antioxidant defenses that involves the...
stimulation of enzymatic activity (Harris, 1992; Jornot and Junot, 1992; Venditti and Di Meo, 1997; Oztasan et al., 2004). The activities of glutathione peroxidase, glutathione reductase and superoxide dismutase are higher in exercised compared to sedentary rats (Cesquini et al., 1999). Senturk et al. (2001) showed that exercise-induced oxidative stress affected the erythrocytes of sedentary but not exercise-trained rats, thus indicating a role for antioxidant systems in cellular protection. Such systems provide cells with an important adaptive mechanism that may be useful to prevent oxidative stress in erythrocytes by up-regulating some of the antioxidant enzyme activities and may have implications in exercising humans.

Hyperglycemia may perturb cellular antioxidant defense systems and damage cells. Free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance. Methylglyoxal has been identified as an intermediate in non-enzymatic glycation, and increased levels have been reported in patients with diabetes. Methylglyoxal-mediated modification of Cu, Zn-SOD led to loss of enzymatic activity (Kang, 2003). Glyceraldehyde-3-phosphate dehydrogenase, glutathione reductase, and lactate dehydrogenase, three key cellular enzymes, were inhibited by carbonyl compounds (Morgan et al., 2002).

We hypothesized that diabetic rats have reduced capacity to answer to oxidative status and that this reduction is associated with hyperglycemia drives non-enzymatic glycation and oxidation of lipids, which enhances the formation of advanced glycation end products (AGEs), diminishing the enzyme activity. To test this hypothesis, we used red blood cells from diabetic rats subjected to regular training as a model to evaluate the effect of hyperglycemia on the antioxidant enzymes.

Materials and methods

Animals

Male Wistar rats, 21 days old, were housed at 23 °C on a 12 h light–dark cycle with lights on 6:00 am and had access to standard rat chow and tap water ad libitum. The rats were allocated to one of two main groups, healthy containing (C) and diabetic (D). Each group consisted of three subgroups containing rats submitted to endurance training (CET and DET), rats treated with 0.1% (w/v) aminoguanidine solution ad libitum (CA and DA), and rats subjected to endurance training and treated with 0.1% aminoguanidine solution ad libitum (CETA and DETA).

Induction of diabetes

Rats, 21 days old, were fasted for 18 h and received a single dose of streptozotocin (STZ, 60 mg/kg, i.p.) diluted in 0.5 ml of 0.1 M sodium citrate buffer, pH 4.5.

Treatment with aminoguanidine

Rats, 21 days old, were offered a solution of 0.1% (w/v) aminoguanidine ad libitum for 54 days.

Training protocol

Rats, 54 days old, were subjected to swimming exercise for 21 days (temperature of water: 30 °C), using the following schedule (times in min):

<table>
<thead>
<tr>
<th></th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>5</td>
<td>15</td>
<td>25</td>
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<tr>
<td>Day 2</td>
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<td>Day 3</td>
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<td>Day 6</td>
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<tr>
<td>Day 7</td>
<td>15</td>
<td>25</td>
<td>30</td>
</tr>
</tbody>
</table>
**Procedure with the groups**

The treatment with streptozotocin, aminoguanidine, swimming exercise and cardiac puncture was performed according to the following scheme:

```
Birth   21 days old   54 days old   75 days old
```

- **Dose of STZ**
- **Cardiac puncture**
- **Aminoguanidine**
  - 54 days
- **Swimming exercise**
  - 21 days

**Blood**

Blood samples were obtained by cardiac puncture from rats anesthetized via intraperitoneal injection of pentobarbital sodium (6.5 mg/100 g body weight). The anticoagulants, sodium heparin (500 IU/ml) and EDTA (0.1%, w/v) were used, when plasma was required for the determination of fructosamine. The samples were centrifuged for 5 min at 1100 × g and the plasma separated to GSH determination. The red cells were then washed three times in 10 vol of 0.9% (w/v) NaCl.

**HbA1c, fructosamine and glucose concentration**

The plasma concentrations of HbA1c, fructosamine and glucose were determined using commercial kits (Labtest Diagnóstica).

**Determination of Hb concentration**

The Hb concentration of hemolysates was determined spectrophotometrically using the millimolar (mM) extinction coefficient for human Hb A, or cyanometHb using Drabkin reagent (100 mg NaCN and 300 mg K₃FeCN₆ dissolved in 1 l of water). The Hb solution was dissolved in Drabkin reagent to a concentration of 0.5–1.0 mg/ml and the $A_{540nm}$ read against an appropriate blank solution after 5 min. The Hb concentration was calculated using an $\epsilon_{540}$ of 11.5 mM⁻¹ cm⁻¹ (Winterbourn, 1990). The absorbances at 577 and 630 nm were determined and the methemoglobin concentration (mM) then calculated as follows:

$$\text{Methemoglobin} = \frac{279 \times A_{630}}{3.04 \times A_{577}}.$$  

**GSH determination**

The concentration of non-protein sulphydryl (NPSH) was assayed using 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) (Beutler, 1975). Two hundred microliters of a cell suspension (containing 2 mM Hb) was mixed with 2 ml of distilled water and 0.2 ml of this lysate was used to determine the Hb concentration. Three milliliters of precipitating solution (1.67 g of glacial metaphosphoric acid, 0.2 g of disodium ethylenediamine tetraacetic acid (EDTA) and 30 g of NaCl in 100 ml of distilled water) were added to 2 ml of hemolysate. After standing for 5 min, the mixture was centrifuged and NPSH was assayed in the clear supernatant. The reaction cuvette contained 0.2 M Na₂HPO₄, pH 8.0, 0.5 mM DTNB (2 mg in 10 ml of 1%, w/v, sodium citrate solution) and the sample, in
a final volume of 2 ml. The absorbance was read at 412 nm against a blank containing 2 ml of 0.2 M Na₂HPO₄. The concentration of NPSH was expressed as the number of SH per Hb tetramer. A $\varepsilon_{412}$ of 13.6 mM$^{-1}$ cm$^{-1}$ was used to calculate the NPSH concentration.

**Catalase (CAT) activity**

The catalase activity was determined by monitoring the absorbance of H₂O₂ at 230 nm. The reaction cuvette contained 9 mM H₂O₂, 50 mM Tris–HCl, pH 7.4, and 0.25 mM EDTA. The mixture was incubated at 37 °C for 10 min before the addition of different concentrations of hemolysate (Beutler, 1975). A $\varepsilon_{230}$ of 0.071 mM$^{-1}$ cm$^{-1}$ was used to calculate the enzyme activity.

**Glutathione reductase (GR) activity**

The activity was measured by following the oxidation of NADPH spectrophotometrically at 340 nm (5) in 50 mM Tris–HCl, pH 8.0, containing 0.25 mM EDTA, 9 μM FAD, 3.3 μM GSSG, 0.1 mM NADPH and hemolysate (50 μM Hb) at 37 °C. No GSSG was present in the blank cuvette. The activity of the enzyme was expressed as micromoles of NADPH consumed/min/g of Hb.

**Glutathione peroxidase (GPx) activity**

The enzyme activity was measured by following the reduction of GSSG by glutathione reductase using NADPH. The reaction cuvette contained 2 mM GSH, 0.6 mM NADPH, 0.1 mM Tris–HCl, pH 8.0, 0.5 mM EDTA, hemolysate (50 μM Hb) and 1 IU GR/ml. The mixture was incubated at 37 °C for 10 min prior to the addition of 0.07 mM t-BOOH. The oxidation of NADPH was followed, and a $\varepsilon_{340}$ of 6.22 mM$^{-1}$ cm$^{-1}$ was used to calculate the enzyme activity (Beutler, 1975).

**Superoxide dismutase (SOD) activity**

The assay of SOD activity was based on the reduction of NBT (nitro blue tetrazolium) by superoxide anion generated through the hypoxanthine (HPX)/xanthine oxidase (XO) system at 37 °C. The inhibition of the above reaction by SOD was followed spectrophotometrically by monitoring the formation of formazan at 560 nm. The following reagents were added to the reaction cuvette: 0.1 M phosphate buffer, pH 7.4, 0.07 U of XO/ml, 100 μM HPX, 600 μM NBT and varying concentrations of hemolysate (Winterbourn et al., 1975).

**Lipid peroxidation (LP)**

Lipid peroxidation was determined using a spectrophotometric assay (kit Calbiochem-EMD Biosciences) for lipid hydroperoxides in blood.

**Statistical analysis**

The data were analyzed by one-way ANOVA followed by the Scheffe test. A $p$ value <0.05 was considered to indicate significance.

**Results**

**Plasma glucose and fructosamine concentrations and blood HbA₁c levels**

Table 1 summarizes the glucose and fructosamine concentrations and the HbA₁c level of the different groups. The plasma glucose concentration of rats treated with STZ (group D) was significantly higher (496.4±8.05 mg/dL) than in control (C) rats (137.2±2.61 mg/dL). Regular training significantly decreased the glucose concentration of the rats in groups CET (115.17±1.04 mg/dL) and DET (468.5±7.12 mg/dL), when compared with their respective controls (C and D, respectively). The treatment with aminoguanidine (CA, CETA, DA and DETA) did not alter the glucose concentrations.

The fructosamine concentrations were significantly higher in the diabetic groups (D and DET) than in the healthy groups (C and CTE). Treatment with aminoguanidine decreased the fructosamine concentrations in groups DA and DETA compared to groups D and DET, respectively (Table 1).

The HbA₁c levels in group D were significantly higher than in group C. Exercise training did not alter the HbA₁c levels in group CET but increased them in group DET compared to group D. Treatment with aminoguanidine (CA, DA, CETA and
DETA did not affect the blood HbA1c levels when compared to the non-treated groups (C, D, CET and DET) (Table 1).

Glutathione reductase (GR)

In rats treated with STZ (group D), GR activity was not significantly different from that of control (C) rats (Fig. 1). In healthy rats, regular training (CET) significantly increased GR activity whereas in diabetic rats (DET), regular training did not significantly affect this enzyme (Fig. 1). In diabetic rats (DETA and DA), the aminoguanidine treatment increased GR activity (Fig. 1). Aminoguanidine had no significant effect on GR activity in groups CA and CETA, compared to groups C and CET.

GSH concentrations in erythrocytes and blood

Erythrocytes

There was a significant increase in the GSH concentration of erythrocytes from rats treated with STZ when compared to control rats (Fig. 2). The level of GSH in CET rats was similar to that of control rats. Similarly the DET group had a GSH concentration similar to that of group D. Treatment with aminoguanidine did not alter the GSH levels in any of the experimental groups (CA; CETA; DA; DETA) (Fig. 2).

Blood

The levels of GSH in D and CET groups were higher than in group C. In the diabetic group, with regular training (DET), there was a decrease in the levels of GSH (Fig. 3). Treatment with aminoguanidine did not alter the GSH levels in any of the experimental groups (Fig. 3).

Glutathione peroxidase (GPx)

The GPx activity was similar in groups C and D (Fig. 4). Regular training in healthy (control) rats (CET) significantly increased the GPx activity. As with GR activity, there was no change in the GPx activity of diabetic rats subjected to endurance training (DTE) (Fig. 4). However, treatment with

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>( \text{HbA}_1c ) (%)</th>
<th>Glucose (mg/dL)</th>
<th>Fructosamine (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>2.19 ± 0.13</td>
<td>137.2 ± 2.61</td>
<td>2.27 ± 0.49</td>
</tr>
<tr>
<td>CA</td>
<td>2.24 ± 0.26</td>
<td>137.22 ± 5.9</td>
<td>2.33 ± 0.17</td>
</tr>
<tr>
<td>CET</td>
<td>1.98 ± 0.10</td>
<td>115.17 ± 1.04</td>
<td>2.75 ± 0.54</td>
</tr>
<tr>
<td>CETA</td>
<td>2.19 ± 0.35</td>
<td>115.0 ± 0.91*</td>
<td>2.42 ± 0.49</td>
</tr>
<tr>
<td>D</td>
<td>6.15 ± 0.20**</td>
<td>496.40 ± 8.05**</td>
<td>3.12 ± 0.17*</td>
</tr>
<tr>
<td>DA</td>
<td>6.36 ± 0.22**</td>
<td>495.60 ± 6.59**</td>
<td>2.0 ± 0.13*</td>
</tr>
<tr>
<td>DET</td>
<td>7.05 ± 0.52**</td>
<td>468.42 ± 7.12**</td>
<td>3.92 ± 0.19*</td>
</tr>
<tr>
<td>DETA</td>
<td>7.08 ± 0.63**</td>
<td>468.7 ± 6.67**</td>
<td>3.0 ± 0.32</td>
</tr>
</tbody>
</table>

The values are the mean±SD of 8 rats per group. (C) Healthy (controls) rats; (CET) healthy (control) rats subjected to endurance training; (D) diabetic rats; (DET) diabetic rats subjected to endurance training; (CA) control group treated with aminoguanidine; (CETA) control group treated with aminoguanidine and subjected to exercise; (DA) diabetic rats treated with aminoguanidine; (DETA) diabetic rats treated with aminoguanidine and subjected to exercise. For HbA1c: **p < 0.01 for all diabetics groups vs. healthy groups, and p < 0.05 for DET vs. D. For Glucose: **p < 0.01 for all diabetic groups (D, DA, DET and DETA) compared to health groups (C, CA, CET and CETA) and *p < 0.05 for CET and CETA vs. C and CA. For Fructosamine: *p < 0.05 for D, DET and DETA compared to C, DETA vs. DET and DA vs. D.

![Fig. 1. Glutathione reductase (GR) activity in the red blood cells of rats; (C) healthy (controls) rats, (CET) healthy (control) rats subjected to endurance training, (D) diabetic rats, (DET) diabetic rats subjected to endurance training, (CA) healthy (controls) rats treated with 0.1% aminoguanidine, (CETA) healthy rats subjected to exercise, (DA) diabetic rats treated with aminoguanidine, (DETA) diabetic rats treated with aminoguanidine and subjected to exercise. Each column represents the mean±SD of four independent experiments. *p < 0.05 for CET vs. C; CETA vs. CA; DA vs. CA; DA vs. D; DETA vs. DET.](image1)

![Fig. 2. Glutathione levels in red blood cells from untreated and treated rats with 0.1% aminoguanidine: (C) healthy rats (controls), (CET) healthy rats subjected to endurance training, (D) diabetic rats, (DET) diabetic rats subjected to endurance training (CA) healthy (controls) rats treated with 0.1% aminoguanidine, (CETA) healthy rats treated with 0.1% aminoguanidine, (DA) diabetic rats treated with 0.1% aminoguanidine, (DETA) diabetic rats treated with 0.1% aminoguanidine subjected to endurance training. Each column represents the mean±SD of four independent experiments. *p < 0.05 for D vs. C and DA vs. CA.](image2)
aminoguanidine increased the GR activity in groups DA and DETA (Fig. 4).

**Catalase (CAT)**

There was a significant increase in CAT activity in group D compared to group C (Fig. 5). Regular training in group CET significantly increased the CAT activity when compared to group C. In diabetic rats subjected to training (DET), the CAT activity was similar to group C. Endurance training increased the CAT activity of diabetic rats treated with aminoguanidine (DETA) (Fig. 5), but had no effect on the CAT activity of groups CA and CETA (Fig. 5).

**Superoxide dismutase (SOD)**

Rats treated with streptozotocin (group D) had less SOD activity than control (C) (Fig. 6). Regular training decreased the SOD activity in both healthy and diabetic rat groups. Aminoguanidine had no effect on the SOD activity in any of the groups studied when compared to non-treated rats (Fig. 6).

**Lipid peroxidation (LP)**

LP was significantly increased in rats treated with streptozotocin (group D), compared to the healthy rats (group C) (3.8 and 2.3 μM, respectively), but regular training did not induce any significant increase in lipid hydroperoxides level. Diabetic
rats subject to training (DET—1.4 µM) showed a small increase in lipid hydroperoxides level compared to group D. The treatment with aminoguanidine (group DA and DETA) significantly diminished the LP (Fig. 7).

Body weight

In diabetic rats (D), the body weight was 21% lower than that of group C at the end of the experiment. Regular training did not alter the body weight of the healthy rats, but reduced the body weight of diabetic rats (13%). Aminoguanidine did not affect the body weight in any of the groups (Fig. 8).

Discussion

Hyperglycemia is the primary clinical manifestation of diabetes and is associated with the development of several clinical complications (Brownlee et al., 1984; Jornot and Junot, 1992; Collins, 2002). Some blood components, such as HbA1c, fructosamine and glucose levels, are used as indicators of the metabolic status of diabetic individuals. As shown here, there was an increase in the HbA1c, fructosamine, and glucose concentrations in diabetic rats (D) compared to healthy animals (C). These results confirmed that streptozotocin caused hyperglycemia that resulted in the glycation of proteins. Exercise increased the levels of HbA1c and fructosamine in the diabetic group (Table 1), despite a decrease in the plasma glucose concentrations, probably via an insulin-independent mechanism. Recent studies have shown that the activation of AMP-dependent kinase protein (AMPK) is responsible for the increase in glucose uptake in muscle (Itani et al., 2003; Merrill et al., 1997).

Regular exercise is recommended to diminish the fat reserves and to control the glucose levels in diabetic individuals (Peirce, 1999; Peltoniemi et al., 2001). Hyperglycemia increases the production of reactive oxygen species through protein glycation (Elgawish et al., 1996; Zhao et al., 2000) and patients with type 1 diabetes mellitus have higher...
plasma concentrations of free radicals than healthy individuals (Davison et al., 2002). In this way, increased glucose level is an important factor implicated with the development of the diabetes-associated complications.

Diabetes and endurance training increased GSH and lipid hydroperoxide levels in the plasma. As shown here, there was a significant increase in the plasma GSH levels of trained (CET and CETA) and diabetic rats (D and DA). The increase in the GSH level of the plasma may reflect the oxidative condition in other tissues, such as the muscle and endothelial cells. Tissues exposed to oxidative condition uptake GSH from blood by liver exportation (Bray and Taylor, 1993). Our results are in agreement with data obtained from humans submitted to physical activity (Karolkiewicz et al., 2003). In this study, elderly men submitted to physical activity presented an increase in the blood GSH level. The GSH may attack directly or indirectly the oxidant molecules decreasing cellular oxidative damage by improving the cellular and blood antioxidant power.

Additionally, lipid hydroperoxide levels were higher in diabetic rats (group D) and diabetic rats subjected to endurance training (DET) than in healthy rats. These results suggest that chronic hyperglycemia associated with diabetes produced cellular damage, probably through the attack imposed by oxidant compounds to the lipids in the membrane. The aminoguanidine diminished the lipid hydroperoxide level in these groups. In the groups DA (2.7 μM) and DETA (2.9 μM), the lipid hydroperoxide levels were significantly lower than those of the D (3.8 μM) and DET (4.4 μM) groups and similar to those of the healthy rats (2.3 μM) (Fig. 7). The low levels of the lipid hydroperoxides in the groups treated with aminoguanidine (DA and DETA) may reflect the antioxidant activity of aminoguanidine. Nitric oxide formation was observed in men subjected to acute submaximal exercise test (Bode-Boger et al., 1994) and the aminoguanidine may prevent oxidative damage to cells and tissues inhibiting iNOS and/or attacking hydroxyl radicals (Giardine et al., 1998). However, glycation process of the antioxidant enzymes could not be discharged since in rats that received aminoguanidine the levels of fructosamine diminished significantly. This data suggests that protein glycation is an important component of the damage imposed by hyperglycemia to the antioxidant system. Therefore, in addition to the cellular oxidative damage caused by free radical production, the capacity of the cell to prevent the attack of free radicals may be impaired by the glycation process. Using rats with streptozotocin-induced diabetes Limaye et al. (2003) showed an increase in CAT gene expression in contrast to a decrease in enzyme activity. This observation suggested a role for post-translation modification in altering the activity of this enzyme with the glycation process being the strongest candidate. In our results the involvement of the glycation in impairing antioxidant defenses was suggested by the results obtained with rats treated with aminoguanidine, a compound with antioxidant activity, and that can prevent the complications of diabetes (Giardine et al., 1998). In this study model the high levels of HbA1c and fructosamine found in rats treated with streptozotocin suggest that the glycation process is active, nevertheless the treatment with aminoguanidine reduced (35%) the levels of fructosamine (D vs. DA) (Table 1). Furthermore, when the diabetic rats were submitted to endurance training (DET group) the activities of antioxidant enzymes did not alter, as expected (Cesquini et al., 1999). Conversely, diabetic rats, treated with aminoguanidine, subjected to endurance training presented an increase in the CAT, GR and GPx activities in the DET vs. DETA. Our results suggest that aminoguanidine prevented the glycation process. Two points support this hypothesis: first, the groups treated with aminoguanidine (DA and DETA) presented lower values of fructosamine (2.0 ± 0.13 and 3.0 ± 0.32, respectively), than groups D and DET (3.12 ± 0.17 and 3.92 ± 0.19, respectively) (Table 1); and groups DA and DETA, in spite of hyperglycemia, showed higher activity of antioxidant enzymes than D and DET groups. In this study the direct evidence of antioxidant enzymes glycation was not showed. However, we believe that an increase in free radicals production should cause an increase in antioxidant enzymes activities. Aminoguanidine can act as a scavenger of reactive dicarbonyl, thereby preventing the formation of AGES (Hirsh et al., 1992). Several studies have demonstrated that aminoguanidine diminished the progression of complication in diabetes through their ability to reduce AGE accumulation (Cantini et al., 2001; Forbes et al., 2004; Thomas et al., 2004). However, the diabetic rats lost weight and the treatment with aminoguanidine did not diminish the significant difference observed between diabetic and healthy animals from day 55 to day 75.

Endurance training increases the energy supply to tissues in activity, resulting in oxidative stress conditions (Atalay et al., 2004; Kinnunen et al., 2004). In healthy rats, swimming enhanced the activity of most blood antioxidant enzymes (Cesquini et al., 1999). Other studies have reported a similar behavior in different tissues (Inal et al., 2001; Senturk et al., 2001; Oztasan et al., 2004). Thus, an efficient antioxidant system is necessary to attack the free radicals produced during exercise. However, as shown here, exercise did not increase the antioxidant enzyme activity in diabetic rats, while swimming increased the activity of antioxidant enzymes in diabetic rats treated with aminoguanidine (D vs. DA and DET vs. DETA). Thus, we believe that diabetic hyperglycemia is an important factor modulating oxidative stress in the blood.

In aging and diabetes, an accelerated formation of free radicals accompanies protein glycation (Baynes, 1991; Kristal and Yu, 1992). Products such as glyoxal, methylglyoxal and 3-deoxyglucosones are produced by intracellular glycation (Abordo et al., 1999). These compounds are more reactive than the parent sugars and have been associated with cytotoxicity and damage to tissues exposed to hyperglycemia (Okado et al., 1996). Additional studies are required to clarify the role of hyperglycemia in diabetic rats previously adapted to exercise.

In conclusion, the current findings provide strong evidence that antioxidant enzymes of red blood cells are affected by chronic hyperglycemia. The red blood cells from diabetic rats showed lower capacity to up-regulate some of the antioxidant enzyme activities than healthy cells. The changes in enzyme activity correlate with the hyperglycemia and may be atten-
ipated by an AGE-inhibitor, implicating a role for AGE in the impairment of the antioxidant defenses of the blood in diabetics. Thus, strenuous physical activity may cause greater cellular and tissue damage in diabetic individuals without glycemic control.

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