Intensive Swimming Exercise-Induced Oxidative Stress and Reproductive Dysfunction in Male Wistar Rats: Protective Role of α-Tocopherol Succinate

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Abstract/Résumé

In the present study, 30 male rats (age 3 mos, Wt 128.6 ± 3.7 g) were randomly divided into Control group (CG), Experimental group (EG), and Supplemented group (SG), 10 per group. An exercise protocol (3 hrs swimming per day, 5 days a week for 4 weeks) was followed in EG and SG, with no exercise in CG. In SG, α-tocopherol succinate was injected subcutaneously at a dose of 50 mg·kg⁻¹ per body weight per day. After 4 weeks of exercise, significant diminutions (p < 0.05) were noted in somatic indices of testes and accessory sex organs; seminiferous tubular diameter (STD); testicular Δ⁵, 3β-hydroxysteroid dehydrogenase (Δ⁵, 3β-HSD), 17 β-hydroxysteroid dehydrogenase (17 β-HSD) activities; plasma levels of testosterone (T), luteinizing hormone (LH); preleptotene spermatocytes (pLSc), mid-pachytene spermatocytes (mPSc), and Stage 7 spermatids (7 Sd); testicular α-tocopherol and glutathione (GSH) content; superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione-s-transferase (GST) activities in EG when compared to CG. Moreover, a significant elevation (p < 0.05) in malondialdehyde (MDA) was found in testes of EG compared to CG. No significant alteration was noted in body weight among the

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groups. Co-administration of α-tocopherol succinate restored the above parameters. Intensive swimming exercise-induced oxidative stress causes dysfunction in the male reproductive system, which can be protected by α-tocopherol succinate.

Dans cette étude, 30 rats mâles (âge: 3 mois, masse: 128,6 ± 3,7 g) sont répartis aléatoirement dans 3 groupes: groupe de contrôle (CG); groupe expérimental (EG); et groupe avec supplément (SG), 10 dans chaque groupe. On a soumis les deux groupes expérimentaux (EG et SG) à un programme d’entraînement à la nage à raison de 3 h par jour, 5 jours par semaine, et ce, durant 4 semaines; le groupe de contrôle (CG) ne fit aucun entraînement. Le groupe SG reçut tous les jours une injection sous-cutanée de succinate d’α-tocophérol (50 mg par kg de masse corporelle). Après 4 semaines d’entraînement, on observa chez le groupe EG, comparativement au groupe CG, des diminutions significatives (p < 0,05) des indices somatiques des testicules et des organes sexuels accessoires: diamètre des tubes séminifères (STD); activités de la Δ5, 3β-hydroxystéroïde déshydrogénase testiculaire (Δ5, 3β-HSD) et de la 17β-hydroxystéroïde déshydrogénase (17β-HSD); niveaux plasmatiques de la testostérone (T), de l’hormone lutéinisante (LH), du contenu en spermocytes préleptotène (pLSc), en spermatozoïdes à mi-chemin du pachytène (mPSc), en spermatozoïdes au stade 7 (7Sd), de la concentration d’α-tocophérol et de glutathion testiculaires (GSH), de l’activité de la superoxyde dismutase (SOD), de la catalase (CAT), de la glutathion peroxydase (GPX), et de la glutathione-s-transférase (GST). De plus, on observa dans les testicules de EG, comparativement à CG, une augmentation significative (p < 0,05) de malondialdéhyde (MDA). On n’observa aucune variation de la masse corporelle dans tous les groupes. La coadministration de succinate d’α-tocophérol a permis de restaurer les variables prémentionnées. Un programme d’entraînement intense à la nage provoque un stress par oxydation et une dysfonction de l’appareil reproducteur mâle: le succinate d’α-tocophérol peut y remédier.

Introduction

The literature indicates that intensive exercise can result in dysfunction in the male reproductive system (Hackney, 1996). Some research findings in this area have shown that chronic exercise training lowers the levels of testosterone along with other reproductive hormonal abnormalities (Hackney, 2001; Raastand et al., 2000). However, testosterone plays a major role in the development and maturation of sperm during the process of spermatogenesis (Murono and Payne, 1979), and maintenance of testosterone levels within the Sertoli cells is essential for the development of adequate numbers of mature, viable sperms that are necessary for a male to be fertile. Moreover, many experiments involving either animals or humans have demonstrated that intensive exercise causes increased oxygen consumption which is accompanied by an increase in the production of reactive oxygen species as well as induced oxidative stress (Asami et al., 1998; Manna et al., 2003; Temiz et al., 2000).

Reactive oxygen species (ROS) are reported to damage almost all cellular macromolecules including membrane polyunsaturated fatty acids, some proteins and DNA, potentially causing impairment of cellular functions (Asami et al., 1998; Powers et al., 1999). Testicular membranes are rich in polyunsaturated fatty acids and therefore are susceptible to oxidative stress (Ghosh et al., 2002). Testicular steroidogenic and gametogenic activities are both sensitive to free radicals and
ROS (Georgion et al., 1987; Jana et al., 2002; Manna et al., 2003). A correlation was noted between free-radical production and gonadal steroidogenesis as well as spermatogenesis in our previous study (Manna et al., 2003).

Recent studies have drawn increasing attention to the potential for supplementary antioxidant \( \alpha \)-tocopherol succinate (vitamin E) to reduce free-radical-induced oxidative stress, as vitamin E has low toxicity. Between \( \alpha \)-, \( \beta \)-, and \( \gamma \)-tocopherol, \( \alpha \)-tocopherol has the greatest activity as an antioxidant (Jacobs, 1999). A protective effect of \( \alpha \)-tocopherol from oxidative stress depends not only on its chain-breaking capacity in lipid peroxidation but also on its role in the stabilization of membrane structure (Venditti et al., 1999). Apart from this, vitamin E has a stimulatory effect on gonadotrophin secretion and gonadal steroidogenesis (Barnes and Smith, 1975).

Therefore, the aim of the present study was to examine the protective effect of \( \alpha \)-tocopherol succinate on intensive swimming exercise-induced reproductive dysfunction in male rats and to determine whether the effects are associated with changes in oxidant stress and defense.

**Materials and Methods**

**ANIMALS**

The experiment was performed using a total of 30 sexually mature male Wistar strain albino rats, 3 months of age and weighing 127.2 \( \pm \) 5.06 g at the beginning of the experiment. The rats were randomly divided into three groups: Control group (CG, \( n = 10 \)), Experimental group (EG, \( n = 10 \)), and Supplemented group (SG, \( n = 10 \)) and housed in a temperature controlled room at 25 \( \pm \) 2 \( ^\circ \)C, 60\% relative humidity, with a 12:12 light-dark cycle, from 15 days prior to the experiment and thereafter throughout the experiment. Body weight of the animals was maintained among the groups by proper diet (dietary composition = egg albumin-420, corn starch-86, sucrose-240, cellulose-40, salt-80, coconut oil-70, oligoelements-16, and vitamin B complex-25 g per kg) and water ad libitum. All animals were pair fed and their body weight was recorded weekly. The principles of laboratory animal care (NIH Publ. No 85-23, revised 1985) were followed throughout the experiment. The experimental protocol was duly approved by the animal ethics committee of the institute.

**EXPERIMENTAL DESIGN**

*Exercise Protocol and Co-Administration of \( \alpha \)-Tocopherol Succinate.* Both the Experimental and Supplemented groups underwent an exercise protocol of 3 hours continuous swimming per day, 5 days per week for 4 weeks, whereas the Control group did not exercise. The EG and SG swam together in a water tank, with a calculated average 300 cm\(^2\) of water surface area for each rat and a depth of 60 cm at a water temperature of 35 \( \pm \) 1 \( ^\circ \)C. An electric hair dryer was used to dry the body immediately upon removal from the water. In the SG, \( \alpha \)-tocopherol succinate was given by subcutaneous injection at a dose of 50 mg per kg body weight per day from the acclimatization period throughout the 4-week exercise period. During this time, vitamin E was injected 4 hours after the end of each exercise
session. In the Control group and Experimental group, a placebo (5 ml sterile water per kg body weight per day) was given at the same time that \( \alpha \)-tocopherol succinate was co-administered to the Supplemented group.

**Sacrificing, Collection of Blood, and Reproductive Organs.** Animals of all groups were sacrificed by light ether anesthesia 24 hours after the last day of exercise in order to avoid the acute effect of exercise. No food was provided for 2 hours before they were sacrificed. Body weight of the animals was recorded. An initial 5 ml of blood was collected from the dorsal aorta using a heparinized syringe with a 21-gauge needle and centrifuged at 2,000 \( \times \) g for 15 min at room temperature. The plasma samples were separated and stored at –20 °C prior to hormone assay. The testes and accessory sex organs were dissected out and weighed. One testis from each animal was used for histology and the other was used for the study of steroidogenic and scavenger enzyme activities along with the levels of glutathione, \( \alpha \)-tocopherol, and lipid peroxidation. Seminiferous tubule diameter was also measured using an ocular micrometer at \( \times 400 \).

**Quantitative Study of Spermatogenesis.** Quantitative study of spermatogenesis was carried out at Stage 7 of the spermatogenic cycle according to the method of Clermont and Morgentaler (1955). Characteristic cellular association present in this stage is spermatogonia-A (Asg), preleptotine spermatocytes (pLSc), midpachytene spermatocytes (mPSc), Stage 7 spermatids (7Sd), and most mature Step 19 spermatids (19Sd). The different nuclei of the germ cells were counted, except for Step 19 spermatids which cannot be enumerated precisely.

**Radioimmunoassay of Testosterone and LH.** Radioimmunoassay of testosterone was carried out (Jacobs, 1974) using a double-antibody (\(^{125}\)I) RIA (ICN, Biochemical Inc., Costa Mesa, CA). Since chromatographic purification of the sample was not performed, each testosterone value was the sum of testosterone and dihydrotestosterone. The intra-assay coefficient of variation was 6.5%. Plasma level of LH was measured (Moudgal and MadhawaRaj, 1974) using reagents supplied by the rat pituitary distribution program and the National Institute of Diabetes & Digestive & Kidney Diseases (NIDDK, Bethesda, MD). Carrier-free \(^{125}\)I for hormone iodination was obtained from Bhaba Atomic Research Center (Mumbai, India). Pure rat LH (NIDDK-rLH-I-5) was iodinated using chloramine-T (Sigma Chemical, St. Louis, MO). NIDDK anti-rat LH-S-5 was used as antisera at final dilution of 1:10,000. Goat anti-rabbit \( \gamma \)-globulin was used as a second antibody (Indo-Medicine, Friendswood, TX). The intra-assay variation was 6% for LH. All samples were run in duplicate in a single assay to avoid inter-assay variation.

**Assay of Testicular \( \Delta^5 \), 3\( \beta \)-HSD, and 17\( \beta \)-HSD Activities.** Testicular \( \Delta^5 \), 3\( \beta \)-HSD (Talalay, 1962), and 17\( \beta \)-HSD (Jarabak et al., 1962) activities were measured spectrophotometrically at 340 nm. One unit of enzyme activity is equivalent to a change in absorbancy of 0.001 per min at 340 nm.

**Assessment of Antioxidant Status and Lipid Peroxidation.** Testicular superoxide dismutase (SOD) activity was measured according to the method of Paoletti and Mocali (1999). One unit of SOD activity was defined as the amount of enzyme required to inhibit the rate of NAD(P)H oxidation by 50% and was expressed as unit per mg protein. An activity of catalase (CAT) was measured biochemically (Beers and Sizer, 1952). One unit of CAT activity was defined as the
amount of H₂O₂ consumption per minute. Assay of glutathione peroxidase (GPX) activity was determined by the modified procedure of Paglia and Valentine (1967). GPX activity was expressed as nmol NAD(P)H oxidized per min per mg of protein. Testicular glutathione-s-transferase (GST) activity was measured spectrophotometrically according to the method of Habig et al. (1974).

Enzyme activity was expressed as nmol of product formed per min per mg of protein. Estimation of malondialdehyde (MDA) was performed by the method of Ohkawa et al. (1979). The MDA level was expressed as nmol per mg of protein. Testicular glutathione (GSH) was measured following the standard method (Ellman, 1959). The amount of GSH in the tissue was expressed as nmol per mg of protein. The amount of proteins present in the tissue was measured by the method of Lowry et al. (1951) for the determination of oxidative stress parameters. Biochemical analysis of testicular α-tocopherol was performed by the HPLC method (Ciora and Marina, 1998) and expressed as micrograms per mg of tissue. Plasma level of α-tocopherol was also measured following the same procedure, taking plasma samples in order to determine the fate of supplemented α-tocopherol succinate.

STATISTICAL ANALYSIS

For statistical analysis of the data, we employed analysis of variance followed by a multiple two-tailed t-test (Das, 1998). We analyzed our data by one-way ANOVA, which is used to examine the effects of a single independent variable on a dependent variable. We employed multiple two-tailed t-tests with Bonferroni modification to find out whether the differences of mean values in each parameter between groups were significant. Differences were considered significant at p < 0.05. Accordingly, statistical software (SPSS) was used.

Results

EFFECTS OF α-TOCOPHEROL SUCCINATE ON EXERCISE-INDUCED OXIDATIVE STRESS

Growth, Reproductive Organ Weight, and Seminiferous Tubules. Intensive chronic swimming exercise as well as α-tocopherol co-administration did not exert any significant change on general body growth with respect to control animals (see Table 1). In contrast, however, the relative wet weights of testis and accessory sex organs as well as seminiferous tubule diameter were decreased significantly (p < 0.05) in the Experimental group compared to the Control group. But co-treatment with α-tocopherol succinate restored the relative wet weights of testis and accessory sex organs as well as seminiferous tubule diameter to the Control level.

Spermatogenesis. A quantitative study of spermatogenesis at Stage 7 revealed a detrimental effect of intensive swimming exercise on testicular germ cell populations (see Table 2). The numbers of pLSc, mPSc, and 7Sd were decreased significantly (p < 0.05) in the Experimental group compared to the Control group, with no significant alteration in the number of Asg. After co-administration of α-tocopherol succinate, the number of pLSc, mPSc, and 7Sd had significantly recovered to the Control level.
Table 1  Effect of α-Tocopherol Succinate Co-administration (M ± SEM) in Rats Exposed to Intensive Swimming Exercise

<table>
<thead>
<tr>
<th>Group</th>
<th>IBW (g)</th>
<th>FBW (g)</th>
<th>TSI (g%)</th>
<th>ESI (g%)</th>
<th>PSI (g%)</th>
<th>SVSI (g%)</th>
<th>STD (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>127.1a</td>
<td>145.5a</td>
<td>1.55a</td>
<td>0.61a</td>
<td>0.47a</td>
<td>0.42a</td>
<td>266.2a</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>±5.41</td>
<td>±6.81</td>
<td>±0.06</td>
<td>±0.03</td>
<td>±0.06</td>
<td>±0.02</td>
<td>±10.2</td>
</tr>
<tr>
<td>Experimental</td>
<td>127.2a</td>
<td>138.1a</td>
<td>1.02b</td>
<td>0.24b</td>
<td>0.25b</td>
<td>0.32b</td>
<td>228.6b</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>±4.15</td>
<td>±8.44</td>
<td>±0.03</td>
<td>±0.02</td>
<td>±0.05</td>
<td>±0.03</td>
<td>±11.4</td>
</tr>
<tr>
<td>Supplemented</td>
<td>127.4a</td>
<td>143.7a</td>
<td>1.54a</td>
<td>0.61a</td>
<td>0.46a</td>
<td>0.44a</td>
<td>258.3a</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>±5.62</td>
<td>±6.61</td>
<td>±0.08</td>
<td>±0.06</td>
<td>±0.06</td>
<td>±0.05</td>
<td>±11.1</td>
</tr>
</tbody>
</table>

Note: ANOVA followed by multiple two-tailed t-tests with Bonferroni modification. In each column the mean with different superscript (a, b) differ from each other significantly, p < 0.05. IBW = initial body weight; FBW = final body weight; TSI = testicular somatic index; ESI = epididymal somatic index; PSI = prostatic somatic index; SVSI = seminal vesicle index; STD = seminiferous tubule diameter.

Table 2  Effect of α-Tocopherol Succinate Co-administration (M ± SEM) on Quantitative Analysis of Spermatogenesis at Stage 7 in Rats Exposed to Intensive Swimming Exercise

<table>
<thead>
<tr>
<th>Group</th>
<th>ASg</th>
<th>pLSc</th>
<th>mPSc</th>
<th>7Sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 10)</td>
<td>1.54a</td>
<td>14.11a</td>
<td>18.42a</td>
<td>42.67a</td>
</tr>
<tr>
<td></td>
<td>±0.22</td>
<td>±2.24</td>
<td>±3.61</td>
<td>±4.51</td>
</tr>
<tr>
<td>Experimental (n = 10)</td>
<td>1.51a</td>
<td>9.24b</td>
<td>11.59b</td>
<td>30.26b</td>
</tr>
<tr>
<td></td>
<td>±0.21</td>
<td>±1.56</td>
<td>±2.46</td>
<td>±3.32</td>
</tr>
<tr>
<td>Supplemented (n = 10)</td>
<td>1.52a</td>
<td>13.71a</td>
<td>17.51a</td>
<td>38.21a</td>
</tr>
<tr>
<td></td>
<td>±0.21</td>
<td>±3.41</td>
<td>±3.21</td>
<td>±4.66</td>
</tr>
</tbody>
</table>

Note: ANOVA followed by multiple two-tailed t-tests with Bonferroni modification. In each column the mean with different superscript (a, b) differ from each other significantly, p < 0.05. ASg = spermatogonia-A; pLSc = preleptotin spermatocytes; mPSc = midpachytene spermatocytes; 7Sd = Stage 7 spermatids.
Testosterone and LH. The levels of plasma testosterone and LH were significantly decreased \((p < 0.05)\) in the Experimental group when compared to Control group after 4 weeks of intensive swimming exercise (see Figure 1). Co-administration of \(\alpha\)-tocopherol succinate restored the plasma levels of testosterone and LH toward the Control level.

Testicular \(\Delta^5\), 3\(\beta\)-HSD, and 17\(\beta\)-HSD Activities. The activities of \(\Delta^5\), 3\(\beta\)-HSD, and 17\(\beta\)-HSD were significantly decreased \((p < 0.05)\) in the Experimental group when compared to the Control group after 4 weeks of intensive swimming exercise (see Figure 2). Co-administration of \(\alpha\)-tocopherol succinate restored the levels of the above parameters to the Control level.

Antioxidant Enzymes, MDA, GSH, and \(\alpha\)-Tocopherol. To assess testicular oxidative stress, we measured enzymatic and nonenzymatic antioxidants along with the MDA product of lipid peroxidation. In the Experimental group we noted a significant reduction \((p < 0.05)\) in the activities of testicular antioxidant scavenger enzymes SOD, CAT, GPX, and GST (Figure 3) after 4 weeks of intensive swimming exercise. Testicular lipid peroxidation, as estimated by the levels of MDA (Table 3), was elevated significantly in the Experimental group vs. the Control group. On the other hand, there was a significant reduction in the level of nonenzymatic antioxidants, GSH, and \(\alpha\)-tocopherol (Table 3) in the Experimental vs. the Control group. Co-administration of \(\alpha\)-tocopherol succinate restored the
Figure 2. Effect of α-tocopherol succinate on testicular Δ⁵, 3β-HSD, and 17β-HSD activity in rats exposed to intensive swimming exercise. Each bar represents mean ± SEM, n = 10 (ANOVA followed by multiple two-tailed t-tests with Bonferroni modification). Bar with different superscript (a, b) in a parameter differ from each other significantly, p < 0.05. CG = Control group, EG = Experimental group, SG = Supplemented group.

Figure 3. Effect of α-tocopherol succinate on testicular SOD, CAT, GPX, and GST activity in rats exposed to intensive swimming exercise. Each bar represents mean ± SEM, n = 10 (ANOVA followed by multiple two-tailed t-tests with Bonferroni modification). Bar with different superscript (a, b) in a parameter differ from each other significantly, p < 0.05. CG = Control group, EG = Experimental group, SG = Supplemented group.
activities of SOD, CAT, GPX, GST, and content of GSH and α-tocopherol to the Control level. The level of MDA was reduced significantly after co-administration of α-tocopherol succinate toward Control level. The plasma level of α-tocopherol [(CG-7.4 ± 0.52 a, EG-4.1 ± 0.664 b, SG-9.2 ± 0.94 a) µg mg⁻¹] was also found to be significantly reduced in the Experimental vs. the Control group. Co-administration of α-tocopherol succinate significantly increased the level of α-tocopherol in plasma and testicular tissues.

**Table 3** Effect of α-Tocopherol Succinate Co-administration on Testicular MDA, GSH, and α-Tocopherol Levels in Rats Exposed to Intensive Swimming Exercise

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA (nmol mg⁻¹ protein)</th>
<th>GSH (nmol mg⁻¹ protein)</th>
<th>α-tocopherol (µg mg⁻¹ tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 10)</td>
<td>3.06 a</td>
<td>312.4 a</td>
<td>4.6 a</td>
</tr>
<tr>
<td></td>
<td>±0.66</td>
<td>±14.81</td>
<td>±0.61</td>
</tr>
<tr>
<td>Experimental (n = 10)</td>
<td>6.22 b</td>
<td>246.4 b</td>
<td>3.2 b</td>
</tr>
<tr>
<td></td>
<td>±0.58</td>
<td>±12.24</td>
<td>±0.46</td>
</tr>
<tr>
<td>Supplemented (n = 10)</td>
<td>3.01 a</td>
<td>301.2 a</td>
<td>6.2 c</td>
</tr>
<tr>
<td></td>
<td>±0.55</td>
<td>±17.23</td>
<td>±0.41</td>
</tr>
</tbody>
</table>

Note: ANOVA followed by multiple two-tailed *t*-tests with Bonferroni modification. In each column the mean with different superscripts (a, b, c) differ from each other significantly, *p* < 0.05. MDA = malondialdehyde, GSH = glutathione.

Discussion

The present study shows induction of oxidative stress on the male reproductive system in rats after intensive swimming exercise with an associated heat stress. In testicular steroidogenic events, Δ⁵, 3β-HSD, and 17β-HSD play a key regulatory role (Murono and Payne, 1979). The inhibition of these steroidogenic enzyme activities in the EG after chronic intensive swimming exercise is in agreement with our previous findings where testicular Δ⁵, 3β-HSD, and 17β-HSD activities were decreased by intensive swimming exercise (Manna et al., 2003). The inhibition in these testicular steroidogenic enzyme activities in the Experimental group may be a result of low plasma LH levels, as this is a prime regulator of testicular steroidogenic enzyme activities (Shaw et al., 1979).

The low activities of testicular Δ⁵, 3β-HSD, and 17β-HSD have been corroborated by the diminution in plasma testosterone levels in this experiment, as testosterone is the prime androgen in male gonads (Rivier and Vale, 1985). Moreover, the inhibition in testicular steroidogenesis is again confirmed here by the significant diminution in epididymal somatic index, prostatic somatic index, and
Swimming Exercise and Oxidative Stress • 181

seminal vesicle somatic index in the Experimental group, as the growth of these accessory sex organs is controlled by plasma testosterone (Edman, 1983). In addition, a significant inhibition in testicular somatic index in the Experimental group also confirmed the low level of plasma LH, as testicular growth is purely dependent on plasma levels of LH (Debnath and Mandal, 2000). Indeed, the reason for the diminution in plasma LH in this experiment is not clear but might be due to hyperactivation of the hypophysical-adrenocortical axis, as it has been well established that high stress conditions also stimulate this axis (Rivier and Vale, 1985). It is well known that forced exercise is more stressful than spontaneous exercise (Asami et al., 1998).

Intensive swimming exercise-induced spermatogenic disorders have been reflected by the diminution in the numbers of different generation of germ cells at Stage 7 in the spermatogenic cycle, which corroborates our previous studies (Manna et al., 2003). Stage 7 of the seminiferous epithelial cycle was selected as the quantitative study of spermatogenesis because all varieties of germ cells are present at this stage (Clermont and Morgeutaler, 1955). Moreover, the decrease in diameter of the seminiferous tubule indicates an inhibition of spermatogenesis (Kocak et al., 2002), as found in the present study.

This inhibition in spermatogenesis may be due to low levels of LH and testosterone (Chowdhury, 1979), which is corroborated by the low plasma levels of testosterone and LH observed in the present study. Moreover, low plasma levels of testosterone in the Experimental rats also endorse the idea about the inhibitory effect of intensive swimming exercise on testicular androgenesis. Testosterone secretion is affected mainly by testicular blood flow through the testicular area, since testosterone is lipid soluble and thus freely diffusible. Furthermore, the testicles apparently have little or no storage capacity for testosterone. Testicular blood flow, moreover, is a function of the levels of vascular vasoconstriction or vasodilation (Hackney, 2001). Therefore, anything that influences vascular tone can affect the rate of testosterone secretion by increasing sympathetic nervous system activity (Hackney, 2001).

It has been observed that endurance exercise reduces blood flow and increases temperature to the testicles, in turn causing low levels of testosterone secretion and affecting some degree of spermatogenesis (Hackney, 2001), which closely resembles the diminution of spermatogenesis found in the present study. In this study we chose swimming exercise so as to minimize any changes in core and testicular temperature in these animals. Hence the changes that have been noted in testicular functions following intensive swimming exercise are less likely to be due to abnormally high testicular temperature.

Moreover, the testicular steroidogenic enzyme activity inhibition in the Experimental rats may be due to the generation of large amounts of free radicals or reactive oxygen species (ROS) in testicular tissue, as steroidogenic enzyme activity in testis is reduced in the presence of ROS (Georgion et al., 1987; Jana et al., 2002). The extent of ROS generation, lipid peroxidation, and consequently the tissue damage from free radicals can be assayed by the measurement of MDA, which results from the breakdown of polyunsaturated fatty acids (Halliwell and Gutteridge, 1989). Free radicals cause cytotoxicity, one of the manifestations of which is lipid peroxidation (Debnath and Mandal, 2000). The elevation in testicu-
lar free radicals in the Experimental rats has been supported by a diminution in the activity of testicular SOD, CAT, GPX, and GST, as these are the important scavenger enzymes against free radicals (Chainy et al., 1997; Jana et al., 2002; Manna et al., 2003). SOD protects the cell against spontaneous lipid peroxidation and inhibits ROS generation. SOD and CAT act synergistically to remove superoxide anion ($O_2^{•−}$) generated by NADPH-oxidase in the cell. They play an important role in decreasing oxidative stress and membrane lipid peroxidation (Powers et al., 1999). GPX, a selenium containing antioxidant enzyme with glutathione as the electron donor, removes peroxyl (ROO•) radicals from various peroxides including $H_2O_2$. GPX has a low specificity for hydroperoxides. In this regard, it reduces a variety of hydroperoxides ranging from $H_2O_2$ to numerous complex organic hydroxides. This characteristic makes GPX an important cellular protectant against ROS-mediated damage to membrane lipids, proteins, and nucleic acids (Powers et al., 1999).

Antioxidant enzymes such as glutathione-s-transferases (GSTs), a family of enzymes, can detoxify electrophilic compounds by catalyzing the formation of glutathione conjugates (Habig et al., 1974). Mammal GSTs are also involved in the intracellular transport of a variety of endogenous metabolites and hormones by their ability to bind these substances. Particularly, GSTs are glucocorticoid-binding proteins and thereby may influence the transport, metabolism, and action of steroids (Hemachand et al., 2002). Thus GSTs are also involved in the transport of sex steroids and could play a key role in the physiological action of these steroids. GST and GSH are important regulators for proliferation and differentiation of germ cells, and they protect the germ cells against the harmful effects of free radicals (Chainy et al., 1997; Jana et al., 2002).

Glutathione (GSH) is the most abundant nonprotein thiol found in virtually all mammalian cells. GSH serves multiple roles in cellular antioxidant defenses (Powers et al., 1999). The most important antioxidant function of GSH is to remove hydrogen peroxide and organic peroxides. These toxic oxygen species may be detoxified via reduction by glutathione peroxidase (GPX), which is converted to oxidized glutathione (GSSG) in the process. In turn, oxidized GSH is reduced by glutathione reductase (GR), in the presence of NADPH. Therefore, any decline in the level of GSH indicates the increased production of free radicals (Chainy et al., 1997), as found in the present study.

The spermatogenic inhibition in the Experimental rats may be due to increased production of ROS as well as lipid peroxidation, indicated by elevated MDA levels, a product of lipid peroxidation which exerts detrimental effects on spermatogenesis (Ghosh et al., 2002). It has been reported that in the course of physical exercise, the increase in oxygen consumption is accompanied by an increased production of ROS and causes oxidative DNA damage (Asami et al., 1998). Diminution in testicular somatic index and relative wet weights of accessory sex glands in the Experimental rats also supports the notion of inhibition in testicular steroidogenesis (Trasler et al., 1986). Since body growth was not altered significantly in the Experimental rats vs. the Control group, these adverse effects of intensive swimming exercise on male reproductive organs were not due to its general toxic effects but to its toxicity on the target organs. The elevation in MDA levels is further corroborated here by the diminution in the activity of antioxidant scavenger enzymes SOD, CAT, GPX, and GST as well as in the levels of GSH and
α-tocopherol in testicular tissues in rat (Chainy et al., 1997; Debnath and Mandal, 2000).

For the management of antigonal effects and oxidative stress on the testis induced by intensive swimming exercise, α-tocopherol succinate is co-administered because it has the greatest tocopherol activity of all the tocopherols (Jacobs, 1999). In the present study, low levels of α-tocopherol were noted in testicular tissues of the Experimental rats, indicating the decline of in vivo antioxidant scavenger systems apart from the scavenger enzymes and GSH. Administration of α-tocopherol succinate in Supplemented rats had a protective effect on intensive swimming exercise-induced testicular steroidogenic and gametogenic dysfunctions. These results may be due to a direct stimulatory effect of α-tocopherol on enzymes of gonadal steroid biosynthesis (Barnes and Smith, 1975) and may also have some modulatory action on gonadotrophin synthesis and secretion (Das and Chowdhury, 1999). In addition, α-tocopherol is an important nonenzymatic antioxidant that elevates scavenger enzyme activity (Das and Chowdhury, 1999). This is in agreement with the present study, in which we noted significant restoration of testicular SOD, CAT, GPX, and GST activities and glutathione content.

The protection in testicular steroidogenesis and gametogenesis after α-tocopherol co-administration may be due to less production of free radicals as well as lipid peroxidation (Ghosh et al., 2002); this is in agreement with our results whereby α-tocopherol co-administration restored MDA levels in testes to the Control levels. Restoration of testicular steroidogenic and gametogenic function may be due to intracellular free radical scavenging, which increased due to the incorporation of vitamin E (Gerold and Combs, 1992).

Regarding the fate of α-tocopherol succinate, it may be assumed that α-tocopherol is associated with circulatory lipoproteins and is cleared through liver where it is hydrolyzed and at least partly secreted into circulation as α-tocopherol (Ghosh et al., 2002). To verify this, we also measured the plasma levels of α-tocopherol in all groups and found that in the co-administrated group, the α-tocopherol level in plasma was greater than in the Control group. Thus, recovery of the above parameters by α-tocopherol in chronic intensive swimming exercise-induced testicular steroidogenic and gametogenic dysfunction may be explained to some extent. The actual mechanism of α-tocopherol on intensive exercise-induced dysfunction in the male reproductive system would be revealed in future studies along these lines.

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References


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