Effect of different intensities of swimming exercise on testicular oxidative stress and reproductive dysfunction in mature male albino Wistar rats

I Manna, K Jana & P K Samanta*
Dept. of Surgery & Radiology, West Bengal University of Animal & Fishery Sciences, Kolkata 700 037, India

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Swimming exercise for 1, 2 and 3 hr for 5 days/week for consecutive 4 weeks, results in a significant reduction in testicular, epididymal, prostetic, seminal vesicle somatic indices; epididymal sperm count, sperm motility; preleptotid spermatocytes, mid pachytyene spermatocytes and stage 7 spermatids; plasma levels of testosterone, luteinizing hormone; testicular Δ, \( \Delta \)-hydroxysteroid dehydrogenase, \( \Delta \)-hydroxysteroid dehydrogenase; testicular superoxide dismutase, catalase, glutathione peroxidase, glutathione-s-transferase and glutathione along with significant elevation in malondialdehyde in male albino rats. However, no significant change was noted in final body weight, spermatagonia-A and plasma level of follicle stimulating hormone. The results that oxidative stress develops with the increasing of exercise intensity, which may interfere in male reproductive activities.

Keywords: Exercise, Oxidative stress, Spermatogenesis, Steroidogenesis, Rat

Physical activity has been recognized as an important lifestyle factor, which contributes to good health and delays the onset of many diseases later in life. It is well established that mitochondria generate reactive oxygen species (ROS) and that the extent of this production is a direct function of the rate of oxygen utilization. Because oxygen utilization increases 10 to 15 fold during exercise, it seems likely that free radical production will also be enhanced. Numerous studies have been published investigating this concept. In addition, there have been several studies that examined antioxidant defense systems, which appear to increase during regular exercise.

Intensive exercise can result dysfunction in the male reproductive system. Some research findings in this area have shown that chronic exercise training lowers the levels of testosterone along with other reproductive hormonal abnormalities. However, testosterone plays a major role in the development and maturation of sperm during the process of spermatogenesis and maintenance of testosterone levels within the Sertoli cells is essential for the development of adequate numbers of mature, viable sperm that are necessary for a male to be fertile. ROS are reported to damage almost all cellular macromolecules including membrane polyunsaturated fatty acids (PUFA), containing impairment of cellular function. Testicular membrane is rich in polyunsaturated fatty acids and is highly susceptible to oxidative stress.

The present study has been to find out the effect of chronic intensive exercise on male reproductive system in rats with testicular oxidative stress and antioxidant defense systems as parameters.

Materials and Methods

Animals—Sexually mature 3-month old male Wistar strain albino rats (24), weighing 127.95±4.05 g at the beginning of the experiment were used. They were housed in a temperature controlled room at 25°C±2°C, 60% RH with a 12:12 L:D cycle 15 days prior to the experiment and thereafter during the whole period of 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, vitamin B complex-1.5) g kg\(^{-1}\)] and water ad libitum. All animals had their body weight recorded weekly. The principles of Laboratory Animal care (NIH publication No 85-23, revised 1985) were followed throughout the experimental
schedule. The experiment was duly approved by the Animal Ethics Committee of the Institute.

Exercise protocol—The rats were randomly divided into following 4 groups of 6 each: (1) Control (CG; no exercise); (2) exercise group I (EGI; exercise for 1 hr/day, 5 days/week for 4 weeks); (3) exercise group II (EGII; exercise for 2 hr/day, 5 days/week for 4 weeks) and (4) exercise group III (EGIII; exercise for 3 hr/day, 5 days/week for 4 weeks). All the rats of the exercise groups swam at the same time in separate water tanks with a calculated average 300 cm² of water surface area for each rat and a depth of 60 cm at 35° ± 1° C. An electric hair dryer was used to dry the body immediately on removal from water.

Collection of blood and reproductive organs—Animals of both the groups were killed by light ether anesthesia 24 hr after the last day of exercise in order to avoid the acute effect of exercise. No food was provided for 2 hr before the decapitation. Body weight of the animals was recorded. An initial 5 ml of blood from the dorsal aorta was collected and allowed to clot for 3 hr at 4°C and then centrifuged at 3000 rpm (2000 g) for 15 min at room temperature. The plasma samples were separated and stored at −20°C prior to the hormone assay. The testes and the accessory sex organs were dissected out and weighed for the study of steroidogenesis, and the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-s-transferase (GST) along with the levels of glutathione (GSH) and malondialdehyde (MDA) were performed.

Epididymal sperm count and sperm motility—The epididymal sperm were collected by cutting epididymis into small pieces and flushing the sperm in normal saline. The sperm collected was centrifuged at 250 g for 10 min. The pellet was resuspended in 2 ml of normal saline. An aliquot of sperm suspension was homogenized for a few seconds, thereafter centrifuged at 800 g for 10 min and used for biochemical assays. Sperm motility and count were noted. The activities of antioxidant enzymes, the levels of non-enzymatic antioxidants and levels of lipid peroxidation were measured in epididymis and epididymal sperm of both the groups. Epididymal sperm counts and evaluation of the motility of epididymal sperm were done by the standard method. The epididymal sperm was obtained as described above and incubated at 32°C, which is the optimum temperature of rat epididymal sperm. The epididymal fluid was diluted to a volume of 5 ml of pre-washed (32°C) normal saline. An aliquot of this solution was placed in Neubauer hemocytometer and motile sperm were counted under compound microscope. Non-motile sperm numbers were first determined, followed by counting of total sperm. Sperm motility was expressed as a per cent of motile sperm of the total sperm counted.

Quantitative study of spermatogenesis—Quantitative study of spermatogenesis was carried out at stage 7th of seminiferous epithelial cycle according to the method of Clermont and Margutealer. Characteristic cellular association present in this stage is spermatogonia-A (ASg), preleptotene spermatocytes (pLSc), midleptotene spermatocytes (mPSc), stage 5 spermatids (5Sd) and most mature step 19 spermatids (19Sd). The different nuclei of the germ cells (except step 19 spermatids, which can not be enumerated precisely) were counted.

Assay of hormones by RIA—Testosterone concentration in plasma samples was analyzed following the method of Jacobs using a double-antibody (125I) RIA (ICN, Biochemical Inc, Diagnostic Division, Costa Mesa, CA, USA). Plasma level of luteinizing hormone (LH) and follicle stimulating hormone (FSH) were measured by RIA using reagents supplied by the rat pituitary distribution program and NIDDK (Bethesda, MD, USA). The hormone assays were carried out in duplicate.

Assay of testicular Δ, 3β-HSD and 17β-HSD activities—The levels of Δ, 3β-hydroxysteroid dehydrogenase (Δ, 3β-HSD) and 17β-hydroxysteroid dehydrogenase (17β-HSD) were estimated using tissue homogenate taken from the left testis of each animal.

Assessment of testicular oxidative stress—Oxidative stress as indicated by the measurement of enzymatic and non-enzymatic antioxidants and the products of free radicals were estimated in testes. Antioxidant enzymes viz. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-s-transferase (GST) were estimated using tissue homogenate taken from the left testis of each animal. The amount of protein present in the tissue was measured by the Lowry’s method for the determination of oxidative stress parameters.

Statistical analysis—The data were first converted into mean and standard error of mean (SE). To find out the difference between the variables among the
groups ANOVA followed by multiple comparison t-test with Bonferroni modification was employed. Accordingly, statistical software package (SPSS) was used.

**Results**

**Effects of different intensities of swimming exercise on body weights, reproductive organs weights, sperm count and sperm motility (Table 1)**—The effect of chronic intensive exercise has shown no significant alteration in body weight among the groups. In contrast testicular, epididymal, prostatic and seminal vesicle somatic indices were decreased significantly \( (P<0.05) \) in all the exercise groups. Sperm count and sperm motility were declined significantly \( (P<0.05) \) in all exercise groups compared to control. Moreover, severe effects were noted in EGIII compared to other exercise groups (EGI and EGII).

**Effects of different intensities of swimming exercise on quantitative analysis of spermatogenesis (Table 2)**—A significant reduction \( (P<0.05) \) in the number of pLS, mPSc and 7sd were noted in all the exercise groups. However, no significant alteration was noted in the number of Asg among the groups.

**Effects of different intensities of swimming exercise on plasma levels of testosterone, LH and FSH (Fig. 1)**—The levels of plasma testosterone and LH were found to reduce significantly \( (P<0.05) \) in all the exercise groups when compared to control, whereas no significant change was observed in plasma levels of FSH among the groups.

**Effects of different intensities of swimming exercise on testicular \( \Delta^3 \), 3\( \beta \)-HSD and 17\( \beta \)-HSD activities (Fig. 2)**—After prolonged intensive exercise, the activities of both testicular \( \Delta^3 \), 3\( \beta \)-HSD and 17\( \beta \)-HSD decreased significantly \( (P<0.05) \) in all the exercise groups.

**Effects of different intensities of swimming exercise on testicular oxidative stress (Table 3)**—For the assessment of testicular oxidative stress, enzymatic and non-enzymatic antioxidants were measured along with products of lipid peroxidation. In all the exercise groups ANOVA followed by multiple comparison t test with Bonferroni modification. In each vertical column the mean with different superscript \( (a, b, c) \) differ from each other significantly, \( P<0.05 \). Asg=spermatogonia-A, pLS=preleptotene spermatocytes, mPSc =midpachytene spermatocytes, 75d=stage 7 spermatids, CG=control group, EGI=exercise group I, EGII= exercise group II, EGIII= exercise group III.

**Table 1—Effect of different intensities of swimming exercise on body weight, testicular, epididymal, prostatic and seminal vesicle somatic indices, epididymis, epididymal sperm count and sperm motility in rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>IBW*</th>
<th>FBW*</th>
<th>TSI*</th>
<th>EST*</th>
<th>PSI*</th>
<th>SVSI*</th>
<th>ESC**</th>
<th>SM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>127.5a</td>
<td>139.9a</td>
<td>1.41a</td>
<td>0.606a</td>
<td>0.475a</td>
<td>0.472a</td>
<td>4.09a</td>
<td>81.66a</td>
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<td>±4.1</td>
<td>±3.4</td>
<td>±0.06</td>
<td>±0.04</td>
<td>±0.03</td>
<td>±0.02</td>
<td>±0.13</td>
<td>±9.43</td>
<td></td>
</tr>
<tr>
<td>EGI</td>
<td>128.1b</td>
<td>136.7b</td>
<td>1.25b</td>
<td>0.39b</td>
<td>0.37b</td>
<td>0.33b</td>
<td>3.5b</td>
<td>66.9b</td>
</tr>
<tr>
<td>±4.2</td>
<td>±2.1</td>
<td>±0.05</td>
<td>±0.04</td>
<td>±0.03</td>
<td>±0.03</td>
<td>±0.25</td>
<td>±8.6</td>
<td></td>
</tr>
<tr>
<td>EGII</td>
<td>127.9a</td>
<td>138.2a</td>
<td>1.19b</td>
<td>0.33b</td>
<td>0.34a</td>
<td>0.34b</td>
<td>3.34b</td>
<td>56.7b</td>
</tr>
<tr>
<td>±3.8</td>
<td>±2.4</td>
<td>±0.06</td>
<td>±0.03</td>
<td>±0.04</td>
<td>±0.03</td>
<td>±0.19</td>
<td>±9.2</td>
<td></td>
</tr>
<tr>
<td>EGIII</td>
<td>128.3a</td>
<td>136.1a</td>
<td>1.08c</td>
<td>0.24c</td>
<td>0.25b</td>
<td>0.32b</td>
<td>3.12c</td>
<td>42.34a</td>
</tr>
<tr>
<td>±4.1</td>
<td>±2.2</td>
<td>±0.04</td>
<td>±0.04</td>
<td>±0.03</td>
<td>±0.02</td>
<td>±0.21</td>
<td>±8.67</td>
<td></td>
</tr>
</tbody>
</table>

ANOVA followed by multiple comparison t test with Bonferroni modification. In each vertical column the mean with different superscript \( (a, b, c) \) differ from each other significantly, \( P<0.05 \). IBW=initial body weight, FBW=final body weight, TSI=testicular somatic index, EST=epididymal somatic index, PSI=prostatic somatic index, SVSI=seminal vesicle somatic index, ESC=epididymal sperm count, SM=sperm motility, CG=control group, EGI=exercise group I, EGII=exercise group II, EGIII=exercise group III, *: g; \( ^*: g^%; **: \text{count} \times 10^6 \text{epididymis}^{\cdot;} 1 \); : %.
groups, a significant reduction \((P<0.05)\) in the activities of testicular antioxidant scavenger enzymes like SOD, CAT, GPx and GST as well as non enzymatic antioxidant GSH were noted. Testicular lipid peroxidation, as estimated by the levels of MDA was elevated significantly \((P<0.05)\) in all the exercise groups. However, significant \((P<0.05)\) inter group variations were noted in CAT, GST, GSH and MDA levels among the exercise groups.

**Discussion**

The present experiment enlightens the effect of oxidative stress on male reproductive system imposed by chronic heavy exercise in albino rats. In testicular steroidogenic events, \(\Delta^5\), 3\(\beta\)-HSD and 17\(\beta\)-HSD play a key regulatory role\(^{23}\). The inhibition in steroidogenic enzyme activities in the experimental rats after chronic exercise may be a result of low plasma levels of LH as this is a prime regulator of testicular steroidogenic enzyme activities\(^{24}\). However, no significant change was noted in plasma level of FSH. Moreover, inhibition of testicular steroidogenic enzyme activities in experimental rats after intensive exercise may be due to elevation in testicular MDA products of lipid peroxidation, as mitochondrial and microsomal steroidogenic enzyme activities in testis.

![Graph](image1)

**Fig. 1**—Effect of different intensities of swimming exercise on plasma levels of testosterone, LH and FSH in rats. [Each value represents mean ± SE from 6 rats in each group. ANOVA followed by multiple comparison t test with Bonferroni modification. In each vertical column the mean with different superscript \((a, b)\) differ from each other significantly, \(P<0.05\). CG= control group, EGI= exercise group I, EGII= exercise group II, EGIII= exercise group III.]

![Graph](image2)

**Fig. 2**—Effect of different intensities of swimming exercise on testicular \(\Delta^5\), 3\(\beta\)-HSD and 17\(\beta\)-HSD activities in rats. [Each value represents mean ± SE for 6 rats in each group. ANOVA followed by multiple comparison t test with Bonferroni modification. In each vertical column the mean with different superscript \((a, b)\) differ from each other significantly, \(P<0.05\). CG= control group, EGI= exercise group I, EGII= exercise group II, EGIII= exercise group III.]

<table>
<thead>
<tr>
<th>Group</th>
<th>SOD*</th>
<th>CAT*</th>
<th>GPx*</th>
<th>GST*</th>
<th>GSH*</th>
<th>MDA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>0.54±0.09</td>
<td>4.41±0.4</td>
<td>37.2±6.6</td>
<td>4.7±0.2</td>
<td>311.7±12.4</td>
<td>3.08±0.43</td>
</tr>
<tr>
<td>EGI</td>
<td>0.37±0.08</td>
<td>3.72±0.6</td>
<td>29.1±8.5</td>
<td>3.4±0.3</td>
<td>271.5±10.2</td>
<td>4.67±0.3</td>
</tr>
<tr>
<td>EGII</td>
<td>0.32±0.08</td>
<td>2.65±0.7</td>
<td>27.7±7.6</td>
<td>2.8±0.3</td>
<td>268.2±10.3</td>
<td>5.7±0.4</td>
</tr>
<tr>
<td>EGIII</td>
<td>0.35±0.07</td>
<td>2.21±0.5</td>
<td>24.4±8.8</td>
<td>2.6±0.2</td>
<td>244.8±10.4</td>
<td>6.24±0.3</td>
</tr>
</tbody>
</table>

ANOVA followed by multiple comparison t test with Bonferroni modification. In each vertical column the mean with different superscript \((a, b, c, d)\) differ from each other significantly, \(P<0.05\). SOD=superoxide dismutase, CAT= catalase, GPx= glutathione peroxidase (GPx), GST=glutathione-s-transferase, GSH= glutathione, MDA= malondialdehyde, CG= control group, EGI= exercise group I, EGII= exercise group II, EGIII= exercise group III. *: Unit/mg protein; #: nmol/mg protein
are reduced in the presence of these products\textsuperscript{23,24}. Free radicals cause cytotoxicity, one of the manifestations of which can be observed through lipid peroxidations\textsuperscript{25}. Induction of membrane damage through lipid peroxidation by UV irradiation has been observed\textsuperscript{25}. In the present study, the chronic intensive exercise elevated the levels of MDA. The elevation in testicular free radicals in the experimental rats has been supported by the diminution in the activities of testicular SOD, CAT and GPx as these are important scavenger enzymes against free radicals in male gonad\textsuperscript{28}. Moreover, low plasma levels of testosterone in the experimental rats also strengthen the idea about the inhibitory effect of chronic intensive exercise on testicular steroidogenesis. Further, the low activities of testicular $\Delta^5$, $\Delta^3$-HSD and 17$\beta$-HSD has been supported by diminution in plasma testosterone levels in this experiment as testosterone is the prime androgen in male testis\textsuperscript{29}. Moreover, inhibition in testicular steroidogenesis is again confirmed in the present study by the significant diminution in epididymal somatic index, prostatic somatic index and seminal vesical somatic index in experimental group as the growth of the accessory sex organs are purely under the control of plasma testosterone\textsuperscript{29}. The inhibition in gonadal steroidogenesis in stress condition is in consistence to others, where gonadal steroid synthesis significantly interferes in mammals in stress condition\textsuperscript{25,31,32}. In addition to that, a significant inhibition in testicular somatic index in experimental animals also supported the low level of plasma testosterone and LH in stress condition as testicular growth is dependant upon the plasma level of LH\textsuperscript{25,26}. Indeed, the actual causes for the diminution in plasma testosterone and LH levels in this experiment are not clear but this could occur due to hyper activation of hypophysical-adreno-cortical axis\textsuperscript{31}. As it has been well established that the stress full condition also stimulates this axis\textsuperscript{32}. Low level of LH and testosterone may interfere in spermatogenesis process\textsuperscript{33}. The concentration of testosterone in the circulation is a function of the amount of hormone entering (testicular production and secretion) and the amount leaving (metabolic clearance) the blood pool. This process is affected by any change in the physiological state that alters metabolic turnover of the hormone\textsuperscript{34}. In fact, testosterone secretion is affected mainly by testicular blood flow through the testicular area, since testosterone is lipid soluble and thus freely diffusible. Further, the testicules apparently have little or no storage capacity for testosterone. Testicular blood flow moreover is a function of the levels of vascular vasoconstriction or vasodilatation\textsuperscript{35}. Therefore, anything that influences vascular tone can affect the rates of testosterone secretion by increasing sympathetic nervous system activity\textsuperscript{6}. Chronic intensive swimming exercise-induced spermatogenic disorders has been reflected by the diminution in the number of different generation of germ cells at stage VII in spermatogenic cycle. Stage VII of seminiferous epithelial cycle was selected as quantitative study of spermatogenesis, because all varieties of germ cells are present at this stage\textsuperscript{10}. This inhibition in spermatogenesis may be due to low level of LH and testosterone\textsuperscript{33}. It has been observed that endurance exercise reduces the blood flow to the testicles and causes low level of testosterone secretion thus affecting some degree of spermatogenesis\textsuperscript{36}. In the present study, swimming exercise was employed in order to minimize the changes in core and testicular temperature in these animals (compared to running exercise). Hence, the changes that were noted in testicular functions following the exercise are less likely to be due to abnormally high testicular temperatures. Beside this hormonal alterations, the spermatogenic inhibition in experimental rats (may be due to lipideroxidation, indicated by the elevated levels of MDA as it is a product of lipid peroxidation) exert detrimental effect on steroidogenesis and spermatogenesis\textsuperscript{25}. Diminution in testicular somatic index and relative wet weights of accessory sex glands in the experimental rats also supports the inhibition in testicular steroidogenesis\textsuperscript{25}. As body growth was not altered significantly in the experimental rats with respect to control, these adverse effects of intensive exercise on male reproductive organs may be due to oxidative stress caused by the increased production of free radicals. The elevation in the levels of MDA further supports the diminution in the activities of oxidant scavenger enzymes like SOD, CAT, GPx and GST in testicular tissues of rats\textsuperscript{25,28}. The microsomal steroidogenic enzyme activities in testis were reduced as the level of MDA (breakdown products of lipid peroxidation) elevated\textsuperscript{36}. SOD protects the cell against spontaneous lipid peroxidation and ROS generation. SOD and CAT act synergistically to remove superoxide anion ($O_2^-$) generated by NADPH-oxidase in the cell. These play
an important role in decreasing oxidative stress and membrane lipid peroxidation. GPX, selenium containing antioxidant enzyme with glutathione as the electron donor, removes peroxyl radicals (ROO') from various peroxides including H2O2 (ref. 38). Antioxidant enzymes such as GSTs, a family of enzymes, are able to detoxify electrophilic compounds by catalyzing the formation of glutathione conjugates. Mammal GSTs are also involved in the transport of sexual steroids and could play a key role in the physiological action of sex steroids. GSH is the most abundant non-protein thiol found virtually in all mammalian cells. GSH serves multiple roles in cellular antioxidant defenses. The most important antioxidant function of GSH is to remove hydrogen peroxide and organic peroxides. Therefore, any decline in the level of GSH indicates the increased production of free radicals, as found in the present study.

The overall results of the study indicate that oxidative stress is imposed on male reproductive system by chronic intensive exercise. It is suggested that with the increasing intensity of exercise the detrimental effect of oxidative stress elevated gradually. Long-term exposure to such types of stress may interfere in testicular steroidogenesis and spermatogenesis, which may lead to disorders in male reproductive systems.

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