Effect of mild hyperlipidaemia on testicular cell population dynamics in albino rats

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Mild hyperlipidaemia induced by cholesterol feeding to male rats altered testicular histology. The sperm motility and density were significantly reduced in cauda epididymides and testes in mild hyperlipidaemic rats. The testicular cell population i.e. spermatocytes (primary and secondary) and spermatids were significantly reduced ($P \leq 0.01$ to $0.001$). However, the number of degenerating Leydig cells (interstitial cells) were increased significantly ($P \leq 0.001$). Serum biochemistry reveals significant rise in cholesterol and triglycerides. It is concluded that cholesterol feeding caused inhibition of spermogenesis.

There is a close association between ischaemic cardiovascular disease and hyperlipidaemia$^{1,2}$. Chronic hyperlipidaemia plays a key role in initiation and progression of atherosclerotic lesions$^3$. The relationship between the hyperlipidaemia and cardiovascular diseases has been extensively studied but the relationship between hyperlipidaemia and testicular function has remained relatively unexplored. Thus the present study deals with the effect of mild hyperlipidaemia on testicular cell population dynamics in albino rats.

Mature male albino rats (20) weighing 190-220 g maintained on a standard diet and water ad libitum were distributed into 2 groups of 10 animals each. Group I served as control and Group II received cholesterol powder (Loba) at the dose of 400 mg/kg body weight, orally along with 5% fat for 2 months. The animals were sacrificed on the 61th day by using light ether anaesthesia. The sperm motility and density were assessed in the testes and cauda epididymides$^5$. Testes and cauda epididymides were fixed in Bouin's solution. Paraffin section were prepared and examined. Serum was analysed for total cholesterol$^6$ and triglycerides$^7$.

The evaluation of testicular cell population dynamics was based on the calculations made for each cell types per across tubular section. All row counts were transformed to nuclear point by using Abercrombie's formula$^8$. Intertitial cell (Leydig cells) types such as fibroblast, immature, mature and degenerating Leydig cells were estimated applying a different count over 200 cells. This cell population was statistically verified by the binomial distribution$^9$.

Testicular cell population dynamics-sperm dynamics showed that motility as well as density was reduced significantly in mild hyperlipidaemic group ($P \leq 0.001$, Table 1). The number of spermatocytes reduced slightly ($P \leq 0.01$) whereas the number of secondary spermatocytes and spermatids was reduced significantly ($P \leq 0.001$). However the degenerating cell number were significantly increased (Table 2).

Serum biochemistry—Serum cholesterol and triglycerides were significantly increased as compared to control (Table 1).

Histology—The seminiferous tubules from the testes of animals fed on cholesterol for 2 months were wavy in outline and shrunken. As a result, the interstitium was enlarged. The Leydig cell nuclei were shrunken. The spermatogenesis was arrested at the primary spermatocyte stage. However, a few secondary spermatocytes with karyolytic nuclei were seen (Fig. 2). As the tubule had a large number of primary spermatocytes, it seems that the meiotic divisions were inhibited, when compared with the control (Fig. 1). Cauda epididymis from the experimental animals revealed reduced cell height and enlarged lumen without spermatozoa. Many of the epithelial cells were nacrotic, when compared with control (Figs 3 & 4).

The process of spermatogenesis is androgen dependent$^{10,11}$. Decreased androgen production reflects in reduced number of Leydig cells and there functional status. In the present study the number of degenerating Leydig cells increased significantly thereby reflecting the depletion of androgen level. It
was further supported by decreased number of germinal cells i.e. spermatocytes and spermatids since, these stages are completely androgen dependent. Plasma testosterone level inversely correlated with plasma cholesterol, triglycerides and beta lipoprotein level. The significant elevation in concentration of total cholesterol and triglycerides was noted in the present study, which proves indirectly the reduce level of circulating testosterone. Hence impairment of spermatogenesis takes place and also the decreased sperm density confirms the same.

It is noted that an oxidative phosphorylation is impaired in these rats, as evidenced by decreased sperm motility and density. The significant elevation in concentration of total cholesterol and triglycerides confirms this. Table 1 provides a summary of the changes in serum lipids and sperm dynamics in cholesterol-fed rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sperm motility (%)</th>
<th>Sperm density (mill/ml)</th>
<th>Serum cholesterol (mg/dl)</th>
<th>Serum triglycerides (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cauda epididymides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>74.12 ± 1.14</td>
<td>56.00 ± 2.80</td>
<td>104.2 ± 0.32</td>
<td>80.12 ± 0.71</td>
</tr>
<tr>
<td>(Gr-I)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol feeding for 60 days (Gr-II)</td>
<td>17.34 ± 3.74*</td>
<td>6.18 ± 2.32*</td>
<td>174.4 ± 0.71</td>
<td>136.1 ± 0.70</td>
</tr>
</tbody>
</table>

P ≤ 0.001

Fig. 1—Control rat testis showing normal spermatogenic activity and Leydig cell. HE × 200
Fig. 2—Testis of cholesterol-fed rat showing arrest of spermatogenesis at primary spermatocyte stage and enlarged interstitial space. HE × 200
Fig. 3—Cauda epididymis of control rat showing normal tubule with spermatozoa. HE × 200
Fig. 4—Cauda epididymis of cholesterol-fed rat showing reduced cell height and enlarged lumen without spermatozoa. HE × 200
Table 2—Testicular cell population Dynamics of cholesterol fed rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Germinal cell types</th>
<th>Interstitial cell type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spermato-gonia</td>
<td>Primary spermato</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cell</td>
</tr>
<tr>
<td>Intact (Gr-I)</td>
<td>24.06 ± 0.93</td>
<td>19.93 ± 0.80</td>
</tr>
<tr>
<td>Cholesterol feeding</td>
<td>17.05 ± 4.40</td>
<td>12.96 ± 2.41</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. from 10 animals

P values: *p ≤ 0.01; **p ≤ 0.001; 'non significant

required for ATP source which in turn is responsible for sperm motility. The slight reduction in ATP leads to motility reduction and thus causes infertility

It is possible that in the undertaken study high cholesterol level interferes with enzymatic reaction including oxidative phosphorylation uncoupling. Thus, increase of cholesterol level dysfunction the Leydig cells and as a result, there was a reduced level of testosterone, which was responsible for inhibition of spermatogenesis.

In conclusion mild hyperlipidaemia apart from specific predictory of myocardial infarction also leads to testicular dysfunction via affecting Leydig cell function.

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References