Effect of total splenectomy in the lipid profile in mice

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ABSTRACT

PURPOSE: To analyze total splenectomy effect on the lipid profile - total cholesterol, low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), very-low-density lipoprotein cholesterol (VLDL) and triglycerides levels, in Balb/c mice.

METHODS: Thirty Balb/c male mice, one (1) month old and average weight 26.2g ± 4.0 were used in the experiment. They were distributed into three groups of 10 animals each: a control group (non-operated), a simulation group (spleen manipulation) and the splenectomy group. The animals were subjected to blood sampling to measure plasma lipid levels, at three different times: before surgery, days 30 and 75 of the experiment.

RESULTS: Increased total cholesterol and LDL were observed in the control group from the start to end of the experiment. The simulation group showed increased rates of VLDL and triglycerides at the 30th and 75th days. Splenectomized animals showed no significant change.

CONCLUSION: Total splenectomy did not induce increased plasma lipids levels of in Balb/c mice.

Key words: Spleen. Splenectomy. Lipid Metabolism. Mice.
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Introduction

Spleen is responsible for numerous functions in the body such as blood clearance through hemocaterese and bacteria removal, the production of antibodies and lymphocytes, the regulation of the number of leukocytes and platelets as well as also participates in metabolic control1 and as a lipid reservoir2. The spleen influence over the lipid metabolism has been reported in studies with human beings3,4 and experimental animals5,6,8. There is increase in plasma cholesterol levels and LDL-cholesterol after splenectomy7 in humans with myeloproliferative disorders and splenomegaly. Such data suggest the important role of spleen in cholesterol metabolism, since the organ may be an important site of LDL-cholesterol catabolism8.

King9, observed increased cholesterol in dogs after spleen removal. Ham and Furneaux10, found no changes in lipoprotein lipase and plasma lipids in dogs two weeks after splenectomy.

Asai et al.5, studied rabbits fed with high cholesterol levels products. They observed significant increase in cholesterol, triglycerides and phospholipids as well as reduction in HDL levels in the splenectomized group if compared to simulation group. Petroianu et al.1 and Fatouros et al.1, showed increased triglycerides and low HDL in splenectomized rats fed with normal diet and with diet rich in cholesterol. Simões et al.11, suggested that total splenectomy alters lipids metabolism in rats fed with standard chow. A diet based on pork fat used as lipids source changed the animals’ lipid profile12.

The authors showed13, that rats submitted to splenic surgery and fed with a nutritionally balanced diet kept plasma lipid levels and hyperlipidemia occurred with the use of cholesterol-reducing chow and cholesterol-enriched diet in rats after splenectomy.

Study by Rezende et al.14 showed that splenectomy in BALB/c mice did not increase plasma lipid fractions. These authors suggested that spleen does not participate in plasma lipids levels regulation in the herein studied mice strain. Their work, however, did not assess the extent of cholesterol levels before splenectomy, it just compared results from the control and the splenectomized groups in the postoperative period14.

Although there are strong evidences of the spleen participation in lipid metabolism, the issue is still controversial. In face of such doubt, we performed the current study aiming at verifying the effect of total splenectomy in plasma lipids of BALB/c mice.

Methods

The study was approved by the Ethics Committee on Animal Use (Protocol No. 005/2008 - CEUA) of the College of Health Sciences (EMESCAM).

Thirty BALB/c male mice, one month old and weighing 26.2g ± 4.0 were used in the experiment. They were divided into three groups of 10 animals each: a control group (non-operated), a simulation group (spleen manipulation) and the splenectomy group. The distribution was carried out by specialized technician who didn’t know the group each animal belonged to. The animals were kept in proper and identified cages under adequate temperature, ventilation and lighting conditions to the studied species15. The mice were fed with normal diet (CR-1 Nuvilab autoclavable - Nuvital®) and with water ad libitum.

The animals were weighed (electronic scale Filizola - sensitivity 1g), and identified with markings on the tail. They underwent blood sampling after six hours, and approximately 10% of their blood volume was removed. By considering that each animal has an average of 80 ml of blood per body kilogram and that its weight of 20 to 30 grams on average, approximately 0.16 to 0.24 ml of blood was removed by a tail vein. The tail-bleeding technique was done after the animal’s tail was cleaned with 70% alcohol. The animal was placed into a wide-mouth funnel and immobilized on a support. The mouse's tail was tilted to vertical position and heated in hot water for 30 to 60 seconds. Next, a cross-section of the tail was done to removal a 5 mm distal. A collection tube was placed in the position and the blood was collected in order to get the aforementioned amount. Finally, each mouse was taken back to the cage and kept under observation.

We used the semi-automated enzymatic colorimetric laboratory method for the quantification of plasma lipids (total cholesterol, HDL and triglycerides) and concentrations of LDL and VLDL fractions of cholesterol were calculated by using the Friedewald formula (LDL-C = TC - HDLc - TG/5). The lipids dosage was done in the Siemens Dimension RxLMax equipment.

Fifteen days after blood collection, animals from groups 2 and 3 were anesthetized with xylazine, at a dose of 10mg/ kg, associated with intraperitoneally administered ketamine hydrochloride at 100 mg/kg. The trichotomy and antisepsis of the abdominal and thoracic walls with topic PVPI, the definition of the local of the laparotomy and of the median longitudinal incision in the skin and subcutaneous tissue and the opening in the linea alba and peritoneum were done. Group 2 was submitted to spleen manipulation. Group 3, underwent total splenectomy to mobilize the spleen in the abdominal cavity. The vessels near to the spleen...
underwent ligatures and section, then the organ was removed. The abdominal wall was sutured with nylon 6.0 in two planes. After recovering from anesthesia, the animals were fed with standard diet, water and pain reliever. The new blood collection for plasma lipids dosage was done in all the animals on the 30th day after the start of the experiment. It corresponds to the 15th postoperative day in animals from groups 2 and 3. On the 75th day of the experiment, which corresponds to the 60th postoperative day, the collections were performed in three groups of animals. The sequence of events in the experiment is shown in Figure 1.

![FIGURE 1](image.png)

**FIGURE 1** - Chronological sequence of procedures performed in three groups (control, simulation, and splenectomy) of Balb/c mice analyzed for lipid profile.

The descriptive statistics was used to calculate arithmetic mean and standard deviation of mice’s weight and plasma lipid levels; the Student t-test was used in related samples to compare weight and the amounts of preoperative and postoperative cholesterol total and cholesterol fractions (HDL, LDL, VLDL and triglycerides) of animals in the same group. The “p” values were considered significant when they were lower than 0.05 (p<0.05).

**RESULTS**

There was no significant weight change in animals since the beginning of the experiment till its 30th day in the three groups, as shown in Table 1. However, the control group showed a significant increase in weight if comparing the initial weight and the weight in the 75th day of the experiment and if comparing that of the 30th day with the weight in the 75th day of the experiment. In simulation groups and in the total splenectomy group, the changes were not significant.

<table>
<thead>
<tr>
<th>Initial weight</th>
<th>Weight in the 30th day</th>
<th>Weight in the 75th day</th>
<th>p</th>
<th>p1</th>
<th>p2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AM</td>
<td>SD</td>
<td>AM</td>
<td>SD</td>
<td>AM</td>
</tr>
<tr>
<td>Control</td>
<td>26.40 ± 4.11</td>
<td>27.40 ± 5.08</td>
<td>28.70 ± 5.33</td>
<td>0.11</td>
<td>0.004*</td>
</tr>
<tr>
<td>Simulation</td>
<td>27.50 ± 3.65</td>
<td>27.90 ± 7.72</td>
<td>28.00 ± 1.87</td>
<td>0.30</td>
<td>0.43</td>
</tr>
<tr>
<td>Splenectomy</td>
<td>24.60 ± 4.16</td>
<td>24.62 ± 5.26</td>
<td>26.00 ± 4.40</td>
<td>0.63</td>
<td>0.24</td>
</tr>
</tbody>
</table>

**TABLE 1** - The Weight (grams) of the mice in the control, simulation and total splenectomy groups, immediately before each blood collection.

AM-Arithmetic Mean. SD-Standard Deviation. Student t-test for related samples. p<0.05 significant*. p-comparison between the initial weight and the weight of animals in the 30th day in the same group. p1-comparison between the initial weight and the weight of animals in the 75th day in the same group. p2-comparison between the weight in 30th day and in 75th day in the same group.

Two deaths were recorded in the total splenectomy group and one in the simulation group. The cause of death was not found after necropsy.

The total splenectomy surgical technique was feasible in all cases and it lasted for approximately 40 minutes. Surgery time started to be counted from incision and the ending point was the suture procedure applied to the skin. No hemorrhages, suture dehiscence or infections on the walls were observed within the operated groups.

It was not possible to perform the plasma lipids measurement in three of the blood samples from the simulation group and in a
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We observe significant increase of total cholesterol in the control group in the beginning of the experiment if compared with the 75th day of experiment. The other changes in cholesterol values were not significant (Table 2).

**TABLE 2** - Values of total cholesterol of the mice in the control, simulation and total splenectomy groups.

<table>
<thead>
<tr>
<th></th>
<th>Initial collection</th>
<th>Collection at the 30th day</th>
<th>Collection at the 75th day</th>
<th>p</th>
<th>p₁</th>
<th>p₂</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AM</strong></td>
<td><strong>SD</strong></td>
<td><strong>AM</strong></td>
<td><strong>SD</strong></td>
<td><strong>p</strong></td>
<td><strong>p₁</strong></td>
<td><strong>p₂</strong></td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>86.50 ± 17.95</td>
<td>95.62 ± 27.37</td>
<td>106.10 ± 15.70</td>
<td>0.61</td>
<td>0.003*</td>
<td>0.52</td>
</tr>
<tr>
<td><strong>Simulation</strong></td>
<td>106.12 ± 14.69</td>
<td>106.85 ± 23.03</td>
<td>98.87 ± 6.51</td>
<td>0.91</td>
<td>0.17</td>
<td>0.51</td>
</tr>
<tr>
<td><strong>Splenectomy</strong></td>
<td>94.60 ± 16.94</td>
<td>86.37 ± 15.76</td>
<td>98.66 ± 19.14</td>
<td>0.38</td>
<td>0.97</td>
<td>0.38</td>
</tr>
</tbody>
</table>

AM-Arithmetic Mean. SD-Standard Deviation. Student t-test for related samples. p<0.05 significant*. p₁-comparison between the initial collection and the blood collection in the 30th day in the same group. p₂-comparison between the initial collection and the blood collection in the 75th day in the same group.

Table 3 shows the non-significant HDL changes in the three groups throughout the experiment.

Table 4 shows LDL increase in the control group after 75 days of the experiment when it is compared to the initial value. In the simulation group, there was a decrease in the amount of LDL when the value from the 30th day and the 75th day of the study were compared.

**TABLE 3** - Values of HDL-cholesterol in the mice in the control, simulation and total splenectomy groups.

<table>
<thead>
<tr>
<th></th>
<th>Initial collection</th>
<th>Collection at the 30th day</th>
<th>Collection at the 75th day</th>
<th>p</th>
<th>p₁</th>
<th>p₂</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AM</strong></td>
<td><strong>SD</strong></td>
<td><strong>AM</strong></td>
<td><strong>SD</strong></td>
<td><strong>p</strong></td>
<td><strong>p₁</strong></td>
<td><strong>p₂</strong></td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>54.10 ± 16.86</td>
<td>60.62 ± 14.25</td>
<td>60.33 ± 13.75</td>
<td>0.85</td>
<td>0.40</td>
<td>0.93</td>
</tr>
<tr>
<td><strong>Simulation</strong></td>
<td>55.00 ± 15.82</td>
<td>50.14 ± 9.51</td>
<td>56.00 ± 5.01</td>
<td>0.67</td>
<td>0.86</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>Total splenectomy</strong></td>
<td>59.10 ± 9.76</td>
<td>50.75 ± 13.33</td>
<td>57.16 ± 13.52</td>
<td>0.23</td>
<td>0.46</td>
<td>0.43</td>
</tr>
</tbody>
</table>

AM-Arithmetic mean. SD-Standard deviation. Student t-test for related samples. p<0.05 significant*. p₁-comparison between the initial collection and the blood collection in the 30th day in the same group. p₂-comparison between the initial collection and the blood collection in the 75th day in the same group.

**TABLE 4** - Values of LDL-cholesterol in the mice in the control, simulation and total splenectomy groups.

<table>
<thead>
<tr>
<th></th>
<th>Initial collection</th>
<th>Collection at the 30th day</th>
<th>Collection at the 75th day</th>
<th>p</th>
<th>p₁</th>
<th>p₂</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AM</strong></td>
<td><strong>SD</strong></td>
<td><strong>AM</strong></td>
<td><strong>SD</strong></td>
<td><strong>p</strong></td>
<td><strong>p₁</strong></td>
<td><strong>p₂</strong></td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>20.00 ± 13.11</td>
<td>17.97 ± 10.56</td>
<td>29.80 ± 10.77</td>
<td>0.94</td>
<td>0.001*</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Simulation</strong></td>
<td>33.77 ± 11.52</td>
<td>34.14 ± 14.13</td>
<td>19.07 ± 11.67</td>
<td>0.97</td>
<td>0.07</td>
<td>0.04*</td>
</tr>
<tr>
<td><strong>Splenectomy</strong></td>
<td>21.00 ± 7.41</td>
<td>22.25 ± 9.67</td>
<td>27.10 ± 10.04</td>
<td>0.64</td>
<td>0.27</td>
<td>0.61</td>
</tr>
</tbody>
</table>

AM-Arithmetic Mean. SD-Standard Deviation. Student t-test for related samples. p<0.05 significant*. p₁-comparison between the initial collection and the blood collection in the 30th day in the same group. p₂-comparison between the initial collection and the blood collection in the 75th day in the same group.

Table 5 shows that VLDL increased in the simulation group in the 30th and 75th days of the experiment if compared to the initial value.

Table 6 shows that triglycerides increased in the 30th and 75th days of the experiment in animals subjected to splenic manipulation when their values were compared with the initial value.
TABLE 5 - Values of VLDL-cholesterol in the mice in the control, simulation and total splenectomy groups.

<table>
<thead>
<tr>
<th></th>
<th>Initial collection</th>
<th>Collection at the 30th day</th>
<th>Collection at the 75th day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AM  SD</td>
<td>AM  SD</td>
<td>AM  SD</td>
</tr>
<tr>
<td>Control</td>
<td>12.40 ± 3.26</td>
<td>17.02 ± 11.24</td>
<td>16.00 ± 3.40</td>
</tr>
<tr>
<td>Simulation</td>
<td>16.10 ± 4.61</td>
<td>22.57 ± 5.30</td>
<td>24.67 ± 9.69</td>
</tr>
<tr>
<td>Splenectomy</td>
<td>14.50 ± 4.05</td>
<td>13.27 ± 1.90</td>
<td>14.40 ± 3.95</td>
</tr>
</tbody>
</table>

AM-Arithmetic Mean. SD-Standard Deviation. Student t-test for related samples. p<0.05 significant*. p-comparison between the initial collection and the blood collection in the 30th day in the same group. p<comparison between the initial collection and the blood collection in the 75th day in the same group. p<comparison between the blood collection in 30th day and in 75th day in the same group.

TABLE 6 - Values of tryglicerides in the mice in the control, simulation and total splenectomy groups.

<table>
<thead>
<tr>
<th></th>
<th>Initial collection</th>
<th>Collection at the 30th day</th>
<th>Collection at the 75th day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AM  SD</td>
<td>AM  SD</td>
<td>AM  SD</td>
</tr>
<tr>
<td>Control</td>
<td>62.00 ± 16.32</td>
<td>85.12 ± 56.22</td>
<td>79.88 ± 17.13</td>
</tr>
<tr>
<td>Simulation</td>
<td>80.50 ± 23.08</td>
<td>112.87 ± 26.52</td>
<td>123.37+48.47</td>
</tr>
<tr>
<td>Total splenectomy</td>
<td>72.50 ± 20.29</td>
<td>66.25 ± 9.46</td>
<td>72.00 ± 19.79</td>
</tr>
</tbody>
</table>

AM-Arithmetic Mean. SD-Standard Deviation. Student t-test for related samples. p<0.05 significant*. p-comparison between the initial collection and the blood collection in the 30th day in the same group. p<comparison between the initial collection and the blood collection in the 75th day in the same group. p<comparison between the blood collection in 30th day and in 75th day in the same group.

Discussion

By comparing the weights of animals in the 30th day and those in the 75th day of the experiment and the weights from the beginning of the experiment to the weights at the end of it, we observed that animals in the control group showed significant weight gain. Such result may be associated with the fact that these animals were not subjected to surgical stress and it may have favored the regular feeding throughout the experiment time. The animals that underwent surgical procedures showed no significant weight changes.

The survival rate was of 90% and it included two deaths in the postoperative total splenectomy period and one in the simulation group, both death causes not found in necropsy.

The handling of the animals during the experiment was conducted without difficulties. The total splenectomy surgical technique and the spleen manipulation were feasible in all cases. Postoperative complications weren’t observed. The blood sampling technique was effective and the sample volume was sufficient to the required dosages of lipid fractions. Hemolysis occurred in two samples and it may be justified by possible failures during blood collection: was it caused by the contact of the sample with the water used to heat the mouse’s tail and/or was lysis caused by excessive manipulation of the animal’s tail. The results that did not allow the calculation of LDL raised two hypotheses: error in laboratory determinations and the unsuitability of the Friedewald formula for the experimental test model.

When analyzing lipid levels in the three groups of animals, we observed that the mice in the control and in the simulation groups showed significant lipid changes. There was increase in total and LDL cholesterol in the control group and of triglycerides, LDL and VLDL in the simulation group. These findings may be related to greater weight gain in the control group. However, so far, there are no explanations for the aforementioned changes within the simulation group. Importantly, as in humans, other factors may be associated with increased dyslipidemia risks, among them there are genetic predisposition, poor diet, obesity and sedentary lifestyle.

The group which underwent total splenectomy showed no significant changes in lipid fractions at any time of the experiment. These results are consistent with those reported by Rezende et al., who suggested that the spleen does not seem to participate in the regulation of plasma lipid levels in BALB/c mice. A recent study also showed that total cholesterol, LDL, HDL and triglycerides levels after splenectomy did not show an abnormal lipid profile on children. However, they differ from results found in splenectomized dogs, described by Paulo and Silva. It was the first work published in Brazil on this subject. The authors demonstrated significant increase in total cholesterol, LDL and HDL, whereas triglycerides levels and the VLDL fraction remained unchanged. Cray et al. reported reduced total cholesterol and LDL levels in patients with hereditary spherocytosis and intact
spleen. They found higher cholesterol levels in splenectomized patients, although the high cholesterol levels were normal limits.

Spleen’s participation in lipid metabolism is still controversial. Studies with different experimental models showed significant changes in the lipid profile of the splenectomized animals. However, when part of the spleen is preserved, its viability is maintained and changes in lipid profile are not observed. Clinical and experimental studies seek to determine what the actual functions of the spleen in lipid metabolism are. It is necessary to perform further research in experimental models closer to the metabolic profile of humans due to the strong controversies and high morbidity and mortality rates caused by complications from the atherosclerotic disease.

**Conclusion**

Total splenectomy in BALB/c mice does not lead to significant increase in total cholesterol, HDL, LDL, VLDL and triglycerides.

**References**


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