doi:10.1111/j.1440-1746.2007.05263.x

GASTROENTEROLOGY

# Plasma lipid alterations after total splenectomy, subtotal splenectomy and splenic auto-implants in rats

Andy Petroianu, Denny FM Veloso, Luiz R Alberti and Leonardo de Souza Vasconcellos

Department of Surgery, Medicine School of the Federal University of Minas Gerais, Belo Horizonte, Brazil

**Key words**
cholesterol, partial splenectomy, spleen, splenectomy, splenic auto-implant, subtotal splenectomy, triglyceride.

Accepted for publication 17 November 2006.

**Correspondence**
Professor Andy Petroianu, Av. Afonso Pena, n° 1626, Apto 1901, Belo Horizonte, MG 30130-005, Brazil. Email: petroian@medicina.ufmg.br

## Abstract

**Background and Aim:** The relation between the spleen and lipid metabolism has not been properly studied. In order to contribute to the filling of this gap, in the present investigation we experimentally assessed the lipidogram of rats in the presence of the spleen, in asplenia, and after conservative spleen surgeries.

**Methods:** Fifty female Wistar rats of similar weight and age were divided into five groups submitted to the following procedures: group 1: control, with an intact spleen; group 2: sham operation; group 3: total splenectomy; group 4: subtotal splenectomy; and group 5: total splenectomy complemented with autogenous spleen tissue implants. Four months after the interventions, serum triglycerides, total cholesterol and fractions (very-low-density lipoprotein [VLDL]-cholesterol, low-density lipoprotein [LDL]-cholesterol, high-density lipoprotein [HDL]-cholesterol) were determined. The results for the four groups were compared by analysis of variance followed by the Tukey–Kramer test, with the level of significance set at $P < 0.05$.

**Results:** There were no differences between groups 1, 2, 4 and 5. In the animals submitted to total splenectomy, total cholesterol ($P = 0.0151$) and LDL-cholesterol fraction concentrations ($P < 0.0001$) were higher, whereas HDL-cholesterol fraction concentrations were lower ($P = 0.0026$) than those detected in the other groups. There was no difference in triglycerides ($P = 0.1571$) or VLDL-cholesterol ($P = 0.2527$) between groups.

**Conclusion:** The spleen probably plays an important role in the lipid metabolism of female rats and total splenectomy may be related to changes in cholesterol control. It is possible that preservation of spleen tissue prevents such metabolic disorders.

## Introduction

The functions of the spleen are part of the organic metabolism, and disorders of this organ are related to different diseases.[1–10] The lysosome deposits are considered to be the most frequent metabolic changes involving the spleen associated with an enzymatic defect. In Gaucher's disease, for example, the accumulation of glycocerebrosides in the lysosomes of cells of the mononuclear phagocytic system leads to the formation of lipid-loaded cells known as Gaucher cells. This accumulation of glycocerebrosides in spleen macrophages and liver Kupffer cells causes enlargement of these organs. In addition, other sphingolipidoses such as Niemann-Pick's disease, gangliosidoses and Fabry disease also present spleen manifestations.[10] The spleen participates in the metabolism of all metals, of albuminoids and of indirect bilirubin from phagocytized red blood cells.[11,12]

Patients with myeloproliferative diseases such as polycythemia vera and myelofibrosis usually course with reduced total cholesterol, high-density lipoprotein (HDL)-cholesterol, apolipoprotein B and apolipoprotein A-1 levels. In these diseases, splenectomy normalizes the values of these substances.[2,7,13]

There is a clear and confirmed relationship between dyslipidemia and vascular diseases, especially atherosclerosis, which is responsible for the elevated morbidity and mortality of the general population. Fatouros *et al*. observed that persons submitted to splenectomy due to trauma presented a higher incidence of coronary artery disease probably due to lipid disorders.[2] The changes in lipid metabolism caused by splenectomy due to trauma may eventually explain the high incidence of acute myocardial infarction detected in World War II veterans.[14]

Some experimental studies have reported the influence of the spleen on lipid metabolism. In 1914, King detected an increase in cholesterol in dogs after spleen removal.[5] Asai *et al*. observed that rabbits fed products containing high cholesterol levels showed an increase in cholesterol, triglycerides and phospholipids, and low HDL-cholesterol levels after removal of the spleen.[4] This fact was also reported in rats fed a cholesterol-enriched diet.[2]

Despite the extensive documentation about the dyslipidemias available in the literature, few and controversial studies have been reported about the correlation between the spleen and the effect of conservative splenic operations on lipid metabolism and about the efficacy of autogenous splenic tissue implants regarding the restoration of the metabolic function of the spleen.[15] In view of this gap in the literature, the objective of the present study was to assess the influence of the spleen and of different operations performed on it and of a spleen auto-implant on the lipidogram.

## Methods

The present study was conducted according to the recommendations of the International Norms for Animal Protection and the Brazilian Code of Animal Experimentation (1988) and was approved by the Ethics Committee of the Department of Surgery, Medicine School of the Federal University of Minas Gerais.[16,17]

Fifty female Wistar rats were housed in appropriate cages, up to five animals to a cage, at an ambient temperature of 25°C and on a 12 h light/12 h dark photoperiod, with free access to water. They were fed with regular rodent chow during the pre- and postoperative times of the experiment. Before the experiment, the rats were submitted to evaluation to rule out possible illness and were allowed to adapt for a period of 15 days. The animals were divided at random into the following groups.

Group 1 ($n = 10$)—Control: no surgical intervention.
Group 2 ($n = 10$)—Sham-operation: animals submitted to laparotomy and laparorrhaphy.
Group 3 ($n = 10$)—Total splenectomy: ligature of the spleen vascular pedicle performed with 5-0 chromic catgut and the spleen was completely removed.
Group 4 ($n = 10$)—Subtotal splenectomy: ligature of the vascular pedicle of the inferior pole of the spleen with 5-0 chromic catgut preserving the splenogastric vessels, and section of this segment, followed by running suture of the remnant upper pole with 5-0 chromic catgut.
Group 5 ($n = 10$)—Total splenectomy complemented with an autogenous spleen tissue implant in the omentum: ligature of the spleen vascular pedicle with 5-0 chromic catgut and full extraction of the organ. The spleen was sliced into five segments which were sutured to the anterior surface of the omentum with 5-0 chromic catgut.

The bodyweight of the animals of groups 2, 3, 4 and 5 was verified immediately before the surgical procedure. The day of the operation was considered as the first day of the experiment. The weight of the non-operated rats (group 1), were also assessed on the same day. The surgical procedures were carried out under conditions of asepsis and antisepsis and under general anesthesia with intraperitoneal association of ketamine (10 mg/kg) and xylazine (90 mg/kg). The median laparotomy was closed with continuous sutures in two layers using 5-0 cotton sutures.

The animals were monitored daily for 120 days. The same amount of food and water was offered to all groups. At the end of the experiment, the rats were anesthetized with the same drugs used for the first operation. The bodyweight of all animals was assessed once more on this day. Through a median laparotomy, the caudal vena cava was identified and punctured for vacuum collection of 5 mL blood into tubes containing gel and covered with aluminum foil for protection from light. The tubes were then immediately submitted to laboratory analysis. Serum concentrations of triglycerides, total cholesterol, very-low-density lipoprotein (VLDL-cholesterol) and (HDL-cholesterol) were determined by the enzymatic colorimetric method, after centrifugation at 3577 *g* for 10 min. The low-density lipoprotein (LDL) was measured by means of the Friedewald formula:

$$\text{LDL-cholesterol} = \text{total cholesterol} - \text{HDL-cholesterol} - (\text{triglycerides}/5).$$

**Table 1** Bodyweights (g) of the animals assessed at the beginning of the experiment and 4 months after the spleen operations

| Group | Bodyweight | |
|---|---|---|
| | 1st Day | 120th Day† |
| 1 | 209.52 ± 8.55 | 261.11 ± 7.67 |
| 2 | 211.20 ± 7.13 | 255.77 ± 4.95 |
| 3 | 210.98 ± 9.71 | 256.97 ± 9.80 |
| 4 | 210.40 ± 6.93 | 258.91 ± 6.27 |
| 5 | 209.38 ± 9.50 | 259.65 ± 9.83 |

Values are reported as means ± SD.
†Differences among the five groups are not significant ($P = 0.9897$, one-way ANOVA).
Group 1, control; group 2, sham operation; group 3, total splenectomy; group 4, subtotal splenectomy; group 5, total splenectomy + spleen auto-implant.

Then the animals were killed with an excess dose of intraperitoneal association of ketamine and xylazine.

Data regarding the bodyweight of the animals and the serum total cholesterol and its fractions and triglycerides were analyzed statistically in each group by the Kolmogorov-Smirnov (KS) test to determine Gaussian distribution of the data, and then by the Bartlett test to determine the variances. When the data presented Gaussian distribution and the same variance, they were submitted to one-way analysis of variance (one-way ANOVA) followed by the multiple comparison Tukey–Kramer test. The level of significance was set at $P < 0.05$ in all analyses.

## Results

No adverse events were observed in the experiment. The animals recovered rapidly from the surgical procedure during the immediate postoperative period. No difference in bodyweight was verified between the groups at the beginning or end of the experiment (Table 1).

All total cholesterol values showed distribution within the normal curve ($P > 0.10$) (KS distance of 0.1572 for group 1, 0.1958 for group 2, 0.3355 for group 3, 0.1396 for group 4, and 0.1949 for group 5). The variances did not differ ($P = 0.9786$ by the Bartlett test). The animals submitted to total splenectomy (group 3) presented higher cholesterol concentrations ($P = 0.0151$ ANOVA). There were no differences among the other groups (Table 2).

The VLDL-cholesterol fraction of cholesterol showed values distributed within the normal curve ($P > 0.10$) (KS distance of 0.2140 for group 1, 0.2271 for group 2, 0.1353 for group 3, 0.1822 for group 4, and 0.2800 for group 5). The variances did not differ ($P = 0.4933$ by the Bartlett test). There were no significant differences among the other groups ($P = 0.2527$ ANOVA) (Table 2).

The LDL-cholesterol fraction of cholesterol showed values distributed within the normal curve ($P > 0.10$) (KS distance of 0.2142 for group 1, 0.1686 for group 2, 0.2065 for group 3, 0.2912 for group 4, and 0.1967 for group 5). The variances did not differ ($P = 0.5348$, Bartlett test). The animals submitted to total splenectomy (group 3) presented higher concentrations of LDL-cholesterol ($P < 0.0001$, ANOVA). There were no differences among the other groups (Table 2).

**Table 2** Cholesterol fractions and triglycerides of control and rats submitted to spleen operations

| Group | Cholesterol (mg/dL) | | | | Triglycerides (mg/dL) |
|---|---|---|---|---|---|
| | HDL* | VLDL | LDL** | Total*** | |
| 1 | 51.1 ± 12.8 | 10.1 ± 2.9 | 16.6 ± 9.0 | 77.9 ± 8.4 | 50.3 ± 14.0 |
| 2 | 47.0 ± 6.78 | 11.7 ± 3.0 | 17.4 ± 7.6 | 76.1 ± 9.46 | 54.8 ± 12.2 |
| 3 | 37.6 ± 11.0 | 13.9 ± 4.5 | 38.3 ± 13.7 | 89.7 ± 10.7 | 68.9 ± 21.8 |
| 4 | 55.1 ± 11.8 | 13.4 ± 3.8 | 7.8 ± 9.0 | 74.4 ± 9.2 | 67.7 ± 18.9 |
| 5 | 50.4 ± 14.7 | 12.4 ± 5.0 | 12.0 ± 11.7 | 74.6 ± 9.1 | 62.4 ± 25.3 |

Values are reported as means ± SD.

*$P = 0.0026$ (group 3 compared with the other groups).

**$P < 0.0001$ (group 3 compared with the other groups).

***$P = 0.0151$ (group 3 compared with the other groups).

Group 1, control; group 2, sham operation; group 3, total splenectomy; group 4, subtotal splenectomy; group 5, total splenectomy + spleen auto-implant.

HDL, high-density lipoprotein; LDL, Low-density lipoprotein; VLDL, very-low-density lipoprotein.

The determinations of the HDL-cholesterol fraction of cholesterol presented values distributed within the normal curve ($P > 0.10$) (KS distance of 0.1900 for group 1, 0.254 for group 2, 0.3416 for group 3, 0.2034 for group 4, and 0.1690 for group 5). The variances did not differ ($P = 0.2334$ by the Bartlett test). The HDL-cholesterol fraction of the group submitted to total splenectomy (group 3) was lower than that recorded for the remaining groups ($P = 0.0026$, ANOVA). There were no significant differences among the other groups (Table 2).

The triglyceride determinations also presented values distributed within the normal curve ($P > 0.10$) (KS distance of 0.2370 for group 1, 0.1413 for group 2, 0.2352 for group 3, 0.1776 for group 4, and 0.2559 for group 5). The variances did not differ ($P = 0.2486$ by the Bartlett test). There were no differences among the groups studied ($P = 0.1571$, ANOVA) (Table 2).

## Discussion

In medical practice, particular emphasis is currently being placed on the spleen because of its attributions, partially well known, such as its immune, filtering and hematopoietic roles.[18] In addition to these functions, the participation of the spleen in metabolic control is receiving increasing attention.[1–9]

The respect due to this organ because of its importance in the physiopathology of many diseases has favored the development of multiple operations aimed at preservation of the spleen, with a consequent reduction of sepsis rates and postoperative hematological disorders.[19] Total splenectomy, which used to be extensively indicated in the past, is being progressively replaced with spleen-conserving procedures such as spleen suture, partial splenectomy, subtotal splenectomy or autogenous spleen tissue implant after spleen removal.[18,20–23] More recently, the non-surgical approach to spleen trauma has started to be well accepted, with better results than those obtained by surgery.[24]

Some clinical and experimental studies have evaluated the possible relation between the spleen and lipid metabolism in two antagonistic situations (i.e. hypersplenism[1–3,6–8] and after asplenia).[1,2,5,7,9] Gilbert *et al*. noted a reduction of serum levels of total cholesterol and its LDL and HDL fractions in patients with myeloproliferative diseases associated with hypersplenism.[8] Cholesterol levels were even more reduced during periods of worsening of the base disease. After total splenectomy or when the disease was controlled with chemotherapeutic agents, hypocholesterolemia was reversed.[8] Other authors also analyzed this relation in patients with type 1 Gaucher's disease[6] and type B thalassemia major[3] and obtained similar results.

However, in experimental studies, total splenectomy was accompanied by elevated cholesterol and triglyceride levels.[2,4,5] Aviram *et al*. noted that the LDL fraction of cholesterol was elevated after splenectomy carried out in order to treat myeloproliferative disorders.[7]

According to Fatouros *et al*., cholesterol levels do not change after splenectomy.[2] However, the level of triglyceride increases in asplenic rats. All these findings are different from our results. These differences may be related to the species of the rat or to their gender. Even without literature supporting any hypothesis related to these aspects, we do not believe these findings are related to the period of follow up. In contrast, we did not find any data suggesting that weight and age may influence the lipidogram findings after splenectomy.

Dyslipidemia secondary to total splenectomy may result in a higher incidence of atherosclerotic disorders.[25–29] In an attempt to establish this relation, Asai *et al*.[5] observed that rabbits submitted to total splenectomy and receiving a cholesterol-rich diet presented atherosclerotic plaques in the aorta after four months.

Some theories have been proposed to explain the possible mechanisms implicated in the regulation of plasma lipids by the spleen.[1,3,5–7] Schmidt *et al*.[1] compared the spleen to a lipid reservoir that increases in situations of hypersplenism. By an increase in phagocytosis, spleen macrophages may accumulate large quantities of fat, with consequent hypolipidemia.[1] Another explanation for lipid reduction could be the autoimmune effect of the mononuclear phagocytic system against the structures found in the HDL-cholesterol and LDL-cholesterol lipoproteins, resulting in their plasma clearance.[3,6]

On the basis of these theories, after total splenectomy, the inverse effect may provoke an increase in the serum levels of plasma lipids. According to Asai *et al*., a 'splenic factor' is related to hypocholesterolemia by decreasing the serum cholesterol level.[5] Thus, in an asplenic condition, this factor is missed and metabolism reactions enhance serum cholesterol levels. However, there is no evidence of lipid metabolism in spleen cells, even knowing the spleen storage

of phospholipids in dislipidemias such as Gaucher's disease.[1,2,4,6,13,25,30,31] Further investigation of this phenomena is needed to understand the relation between spleen and lipid metabolism.

The present study also detected elevation of total cholesterol and its LDL fraction, whereas the concentration of the HDL fraction was lower. Different from Fatouros *et al*.[2] and Asai *et al*.[4] who described increasing serum triglyceride levels, in our study, both triglyceride and VLDL-cholesterol levels were unchanged, as previously reported by Aviram *et al*.[7] None of these authors, nor other studies in the literature define the mechanism by which the spleen and splenectomy interfere with lipid metabolism.

It is important to point out that conservative surgeries of the spleen kept the lipidogram at normal levels. Thus, the presence of spleen tissue, even in smaller amounts, can maintain the functions of the organ related to the regulation of lipid metabolism. In addition, considering the hypothesis that blood irrigation of the omentum may be less favorable than irrigation of the spleen itself, reducing the functionality of the organ, there was no difference in lipidograms between group 4 and groups 1 and 3.

The catabolic effect of surgical trauma on the organism is well known, resulting in bodyweight reduction. However, as the bodyweights in the present study were verified after 120 days, the operated animals had enough time to recover from the surgical trauma and to increase their bodyweight. No association was observed between the serum lipids and bodyweight. Thus, it is worth considering that the differences verified in the serum lipids were not related to bodyweight, but to the spleen surgeries.

## Conclusion

The spleen probably plays an important role in the lipid metabolism of female rats and total splenectomy may be related to changes in cholesterol control. It is possible that preservation of spleen tissue prevents such metabolic disorders.

## Acknowledgments

The authors gratefully thank Renata Figueiredo Rocha for her assistance with the presentation of this paper. Financial support was provided by The National Council of Science and Technology (CNPq) and the Foundation for Assistance to Research of Minas Gerais State (FAPEMIG).

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