2 - ORIGINAL ARTICLE
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Is there a relationship between lipids metabolism and splenic surgeries?[1]

Existe relação entre o metabolismo lipídico e as cirurgias esplênicas?

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ABSTRACT

PURPOSE: To assess the influence of spleen surgeries (splenectomy, presence of spleen and after conservative surgeries) on lipids metabolism.

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 (VLDL-cholesterol, LDL-cholesterol, 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: Splenectomy is related to changes in the lipid metabolism that are reverted by autogenous spleen tissue implants.

Key words: Spleen. Splenectomy. Cholesterol. Triglycerides. Rats.

RESUMO

OBJETIVO: Avaliar a influência das cirurgias esplênicas (esplenectomia, presença do baço e cirurgias conservadoras) sobre o metabolismo de lipídios.

MÉTODOS: Foram utilizados 50 ratos fêmea Wistar de peso e idades semelhantes distribuídas em quatro grupos: Grupo 1 - controle, com baço íntegro; Grupo 2 – laparotomia e laparorrafia, Grupo 3 - esplenectomia total; Grupo 4 - esplenectomia subtotal e Grupo 5 - esplenectomia total complementada por implantes de tecido esplênico autógeno. Após quatro meses, foram dosados os níveis séricos de triglicérides, colesterol total e suas frações VLDL, LDL, HDL. Os resultados dos quatro grupos foram comparados entre si pela análise de variância, seguido pelo teste de Tukey-Kramer, com significância para $p<0,05$.

RESULTADOS: Não houve diferença entre os grupos 1, 2, 3 e 4. Nos animais submetidos à esplenectomia total, as concentrações de colesterol total ($p=0,0151$) e de sua fração LDL ($p<0,0001$) foram maiores, enquanto a fração HDL foi menor ($p=0,0026$) do que as encontradas nos demais grupos. Não houve diferença entre os grupos com relação aos triglicérides ($p=0,1571$) e VLDL ($p=0,2527$).

CONCLUSÃO: A esplenectomia leva a alterações do metabolismo lipídico que são revertidas pelo implante de tecido esplênico autógeno.

Descritores: Baço. Esplenectomia. Colesterol. Triglicerídeos. Ratos.

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, 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*.[2] observed that persons submitted to splenectomy due to trauma presented a higher incidence of coronary artery disease probably due to lipid disorders. 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. King[13] in 1914, detected an increase in cholesterol in dogs after spleen removal. Asai *et al*.[4] 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. 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. 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 of 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 ambient temperature of 25°C and on a 12 hour light/12 hour dark photoperiod, with free access to water. They were fed with regular rodent chow during the pre and postoperative time 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 in 5 segments which were sutured to the anterior surface of the omentum with 5-0 chromic catgut.

The body weight 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 not 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 (10mg/kg) and xylazine (90mg/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. 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 body weight 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 5ml of 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), high density lipoprotein (HDL-cholesterol) were determined by the enzymatic colorimetric method, after centrifugation at 4000 rpm for 10 minutes. The low density lipoprotein (LDL) was measured by mean of the Friedewald formula: LDL-cholesterol = total cholesterol– HDL-cholesterol – (triglycerides/5). Then the animals were killed with an excess dose of intraperitoneal association of ketamine and xylazine.

Data concerning about the body weight 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 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 experiment. The animals recovered rapidly from the surgical procedure during the immediate postoperative period. No difference in body weight was verified between the groups at the beginning or end of the experiment (Table 1).

TABLE 1 - The body weight (g) of the animals assessed at the beginning of the experiment and four months after the spleen operations. Values are reported as means ± SD.

Group	Body weight(g)	
	1st Day	120th Day [1]
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

Group 1: Control
Group 2: Sham operation
Group 3: Total splenectomy
Group 4: Subtotal splenectomy
Group 5: Total splenectomy + spleen auto-implant
[1]Differences among the five groups are not significant (p=0.9897 - one-way ANOVA)

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 was no difference 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 was no significant difference among 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 was no difference among the other groups (Table 2).

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 was no significant difference among 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 was no difference among the groups studied (p=0.1571, ANOVA) (Table 2).

TABLE 2 - Cholesterol fractions and triglycerides of control and rats submitted to spleen operations. Values are reported as means ± SD.

Group	Cholesterol (mg/dl)				Triglycerides (mg/dl)
	HDL [1]	VLDL	LDL [2]	Total [3]	
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

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
VLDL - Very low density lipoprotein
LDL - Low density lipoprotein
[1]: $p = 0.0026$ (Group 3 compared with the other groups)
[2]: $p < 0.0001$ (Group 3 compared with the other groups)
[3]: $p = 0.0151$ (Group 3 compared with the other groups)

Discussion

Particular emphasis is being currently placed on the spleen in medical practice 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 aiming at the preservation of the spleen, with a consequent reduction of sepsis rates and of postoperative hematologic 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[18,20,21]. More recently, the non-surgical approach to spleen trauma started to be well accepted, with better results than those obtained by surgery[22-24.]

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*.[8] noted a reduction of serum levels of total cholesterol and its LDL and HDL fractions in patients with myeloproliferative diseases associated with hypersplenism. 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.

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

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

Dyslipidemia secondary to total splenectomy may result in a higher incidence of atherosclerotic disorders[25]. 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*.[5], a "splenic factor" is related to hypocholesterolemia by decreasing the serum cholesterol level. 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] 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 of its LDL fraction, whereas the concentration of the HDL fraction was lower. Differently from Fatouros *et al*.[2] and Asai *et al*.[4] who described increasing in serum triglyceride, in our study both triglyceride and VLDL-cholesterol levels were unchanged, as it was previously reported by Aviram *et al*.[7]. None of these authors neither 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 the 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 lipidogram between group 4 and groups 1 and 3.

It is well known the catabolic effect of surgical trauma on the organism, resulting in body weight reduction. However, as the weights in the present work were verified after 120 days, the operated animals had enough time to recover from the surgical trauma and to increase their weight. No association was observed between the serum lipids and the body weight. Thus it is worth to suppose the differences verified in the serum lipids are not related to the body weight, but to the spleen surgeries.

Conclusion

Splenectomy is related to changes in the lipid metabolism that are reverted by autogenous spleen tissue implants.

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