

# Plasma Lipid Levels of Rats Fed a Diet Containing Pork Fat as a Source of Lipids after Splenic Surgery

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**Abstract** Experimental studies have suggested an important role of the spleen in lipid metabolism, although with controversial results. Our purpose was to analyze the effect of a nutritionally balanced (NB) diet and a diet containing pork fat (PF) as source of lipids on the lipid profile of rats submitted to splenic surgery. Sixty adult male Wistar rats were divided into six groups of 10 animals each: 1 sham-operated, NB diet; 2 sham-operated, PF diet; 3 total splenectomy (TS), NB diet; 4 TS, PF diet; 5 TS followed by splenic autotransplantation (SA), NB diet; and 6 SA, PF diet. Blood samples were collected at the beginning (D0) and after 12 weeks of the experiment (D + 12) for plasma lipid determination. Morphologic regeneration of splenic tissues was observed, with no differences between groups 5 and 6. When D + 12 plasma lipid levels were compared to D0 levels there were no differences in groups 1, 3, and 5, while in groups 2, 4, and 6 total cholesterol (TC), low density lipoprotein (LDL), very low density lipoprotein (VLDL), and triacylglycerols (TAG) increased, and high density lipoprotein (HDL) decreased. At D + 12, groups 2, 4, and 6 had lower HDL than group 3. In conclusion, regardless of the surgical procedure applied to the spleen, an NB diet maintained

plasma lipid levels while a diet with PF as source of lipids changed the animals' lipid profile.

**Keywords** Splenectomy · Spleen · Autotransplants · Cholesterol · Dietary fats · Experimental surgery · Rats

## Abbreviations

AIN-93	American Institute of Nutrition (1993)
D0	Beginning of the experiment
D + 12	12 Weeks after the beginning of the experiment
HDL	High density lipoprotein
LDL	Low density lipoprotein
NB	Nutritionally balanced
PF	Pork fat
SA	Splenic autotransplantation
TC	Total cholesterol
TAG	Triacylglycerols
TS	Total splenectomy
VLDL	Very low density lipoprotein

## Introduction

It is well known that the spleen plays an important role in immunological processes and hemorheological homeostasis [1]. Since an increased risk of severe infection has been recognized to occur after splenectomy, even after many years, spleen-saving techniques, including autotransplantation of spleen fragments, have been performed when possible [2]. It has been demonstrated that splenic autotransplantation at an appropriate site successfully preserves splenic tissue after total splenectomy. Splenic autotransplants acquire the macro- and microscopic architecture of a

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normal spleen and are capable of restoring immune function [3–5].

A possible role of the spleen in plasma lipid level regulation was first suggested by Robinette and Fraumeni Jr (1977) who showed a high incidence of ischemic heart disease in splenectomized World War II veterans [6]. Both clinical and experimental studies have documented the spleen's influence on lipid metabolism in two different situations, i.e., hyperesplenism and asplenia [7–10].

Previous studies on patients with myeloproliferative disorders associated with splenomegaly have shown decreased plasma cholesterol levels, which normalize after total splenectomy [11–13]. Conversely, an increase in plasma cholesterol and triglyceride levels has been demonstrated in animals after total splenectomy [12, 14, 15]. Other studies have also suggested that the presence of splenic tissue, even in small amounts, can prevent such metabolic disorders [10, 16, 17].

Hypercholesterolemia is a cause of concern in our society since it constitutes one of the major risk factors for the development of cardiovascular diseases such as atherosclerosis and its complications, remaining the leading cause of death and disability in the world [18, 19]. Diet is the first line of therapy for the management of plasma lipid levels in the prevention and treatment of cardiovascular disease, with particular emphasis on fatty acid quality, much more than total fat content proper [20, 21]. Controlled feeding studies have consistently found that a reduction in the consumption of dietary saturated fatty acids decreases plasma total cholesterol and LDL levels, reducing the morbidity and mortality due to cardiovascular disease [22, 23].

Based on the possible correlation between the spleen and lipid metabolism and on the lack of studies considering the effect of different sources of fat on lipid profile after surgical procedures affecting the spleen, the purpose of the present study was to assess the impact of a nutritionally balanced diet and a diet containing pork fat as the source of lipids on plasma lipid levels of rats submitted to total splenectomy alone or in combination with splenic autotransplantation.

## Experimental Procedure

### Animals

Sixty adult male Wistar rats weighing 240–270 g were housed in individual cages under conditions of controlled temperature and humidity and on a 12-h light/12-h dark photoperiod. The animals were randomly divided into six groups of 10 animals each: group 1 – Control – sham-operated, fed a nutritionally balanced (NB) diet; group

2 – Control – sham-operated, fed a diet containing pork fat (PF) as a source of lipid; group 3 – total splenectomy (TS), fed the NB diet; group 4 – TS, fed the PF diet; group 5 – splenic autotransplantation (SA), fed the NB diet; and group 6 – SA, fed the PF diet.

After animals were submitted to different surgical procedures at the beginning of the experiment, they were fed two types of diet, according to the groups, and were monitored daily over a period of 12 weeks. Then, animals were killed with an anesthetic overdose at the end of the experiment. The study was approved by the Ethics Committee on Animal Research of the Biology Institute Roberto Alcantara Gomes, Rio de Janeiro State University Brazil. All procedures rigorously followed current regulations on animal experimentation [24, 25].

### Surgical Procedures

After a 12-h fast, the surgical procedures were carried out under conditions of asepsis and antisepsis and under general anesthesia with a combination of ketamine hydrochloride (50 mg/kg) (Ketamina Agener®, Agener União, Saúde Animal) and xylazine (5 mg/kg) (Calmium®, Agener União, Saúde Animal) administered intraperitoneally. The day of the operation was considered to be the first day of the experiment (D0).

A supraumbilical midline laparotomy was performed. In the sham-operated groups (1 and 2), the animals were submitted to mobilization of the spleen to the wound surface followed by replacement to its original position in the abdomen. In groups 3 and 4, total splenectomy was performed, and in groups 5 and 6, the removed spleen was weighed and cut transversely into five slices, each about 2 mm thick. The splenic sections were implanted into the greater omentum using continuous 4-0 polyglycolic acid (Dexon II®, Brasmédica S/A, São Paulo, SP, Brazil) sutures. Slices were introduced alternately between the omentum and splenic tissue to allow interposition of omental tissue between them. Laparorrhaphy was carried out on two planes (peritoneal-aponeurotic plane and skin) using continuous 3-0 polyglycolic acid (Dexon II®, Brasmédica S/A, São Paulo, SP, Brazil) sutures.

### Blood Analysis

Blood samples were first collected from the animals immediately before the surgical procedure by cardiac puncture under anesthesia and then 12 weeks later at the end of the experiment. The following biochemical parameters were analyzed: total cholesterol (TC), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), high-density lipoprotein (HDL), and triacylglycerols (TAG). Plasma concentrations of TC, HDL, and TAG were

measured by the enzymatic colorimetric method after centrifugation using an automatic analyzer A15 (BioSystems®). LDL and VLDL levels were determined by the Friedewald formula, as follows:

$$\text{VLDL(mg/dL)} = \text{TAG}/5$$

$$\text{LDL(mg/dL)} = \text{TC} - \text{VLDL} - \text{HDL}$$

#### Animal Feeding

The animals received commercial rat chow (Focus 1722 Roedores, Agrocere®) and had unrestricted access to water until reaching the ideal weight for the experiment. Starting during the immediate postoperative period, all animals received a manipulated rat chow based on the recommendations of the American Institute of Nutrition Rodents Diets (AIN-1993) [26]. Groups 1, 3, and 5 were fed a nutritionally balanced diet with soybean oil as the source of fat, as recommended by AIN-93M. Groups 2, 4, and 6 were fed a diet containing the same amount of fat, but with pork fat as the main source, which contains a high percentage of saturated fatty acids compared to soybean oil. The composition of the two diets is presented in Table 1. In these groups, 18% of the total fat was soybean oil in order to guarantee essential fatty acid content [27]. Animal's intake of chow as well as body weight were controlled weekly, always at the same time of day.

#### Statistical Analysis

Descriptive analysis was applied to calculate the mean and standard deviation for the following variables: chow intake, weight gain, percentage of splenic mass regeneration, and TAG, TC, HDL, LDL, and VLDL levels. The Wilcoxon test was applied to compare preoperative and postoperative plasma lipid levels. The Kruskal–Wallis test and Dunn's post-test were used to compare plasma lipid levels between groups at the beginning and at the end of the experiment, as well as data regarding body weight variation and chow

**Table 1** Composition of the nutritionally balanced (NB) and pork fat (PF) diets used during the experiment (g/100 g)

Composition	NB diet (g)	PF diet
Carbohydrates	62.10	62.10 g
Protein	14	14 g
Fat	4	4 g
Saturated fat	0.67	1.56 g
Monounsaturated fat	0.97	1.65 g
Polyunsaturated fat	2.37	0.79 g
Trans fat	0	0 g
Cholesterol	0	2.36 mg
Fiber	5	5 g

intake. The Student *t*-test for independent samples was applied to compare splenic mass between groups 5 and 6. The level of significance was set at  $P \leq 0.05$  in all analyses. Statistical analysis was performed with the Graph Pad Prism 4® software for Windows version 4.0 (2003) and the SAS Statistical Software Package® for Windows 2007.

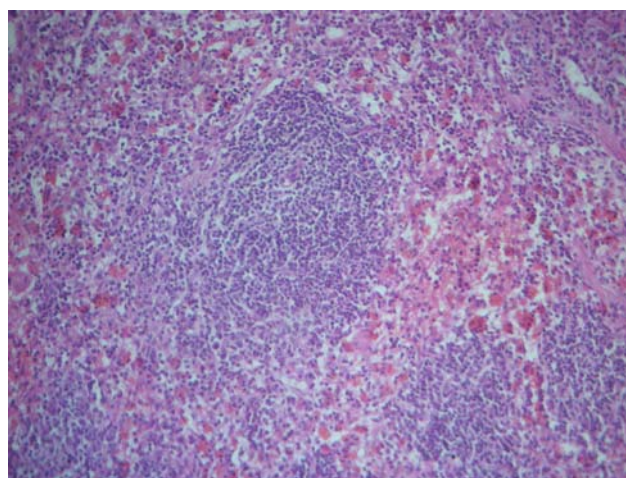
#### Morphologic Study

Removed splenic implants were placed in a solution containing 10% buffered formalin. The tissue was processed with increasing concentrations of alcohol and xylol, cut into 4-mm-thick slices and embedded in paraffin. Slides prepared from these slices were stained with hematoxylin and eosin and analyzed by light microscopy.

#### Results

During the immediate postoperative period the animals recovered rapidly from the surgical procedure. No complications resulting from anesthesia or surgery were observed. Only one animal (group 4) died during the 6th week of the experiment. Examination of the abdominal and thoracic cavity of the dead rat did not reveal the possible cause of death.

Regeneration of the splenic implants was observed in all animals of groups 5 and 6, with no difference in percent regeneration between them ( $P = 0.7716$ ). Microscopic examination revealed a normal morphological appearance in all animals submitted to total splenectomy combined with splenic autotransplantation, with the presence of red and white pulps, besides lymphoid follicles (Fig. 1).



**Fig. 1** Microscopic morphology of a regenerated splenic autotransplant showing the presence of white pulp containing lymphoid follicles and of red pulp with pigments of hemosiderin phagocytized by macrophages ( $\times 40$ )

**Table 2** Comparison of TC, HDL, LDL, VLDL, and TAG levels at weeks 0 and 12 of the experiment in each group of animals—*P* values

Variable	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
TC	0.8457	0.0273*	0.1934	0.0234*	0.1289	0.0039*
HDL	0.6406	0.0039*	0.2031	0.0234*	0.9219	0.0039*
LDL	0.4316	0.0391*	0.8457	0.0234*	0.3750	0.0039*
VLDL	0.2969	0.0020*	0.0840	0.0156*	1.0	0.0039*
TAG	0.4961	0.0020*	0.0645	0.0039*	0.1309	0.0039*

TC total cholesterol, HDL high density lipoprotein, LDL low density lipoprotein, VLDL very low density lipoprotein, TAG triacylglycerols

\* Statistically significant

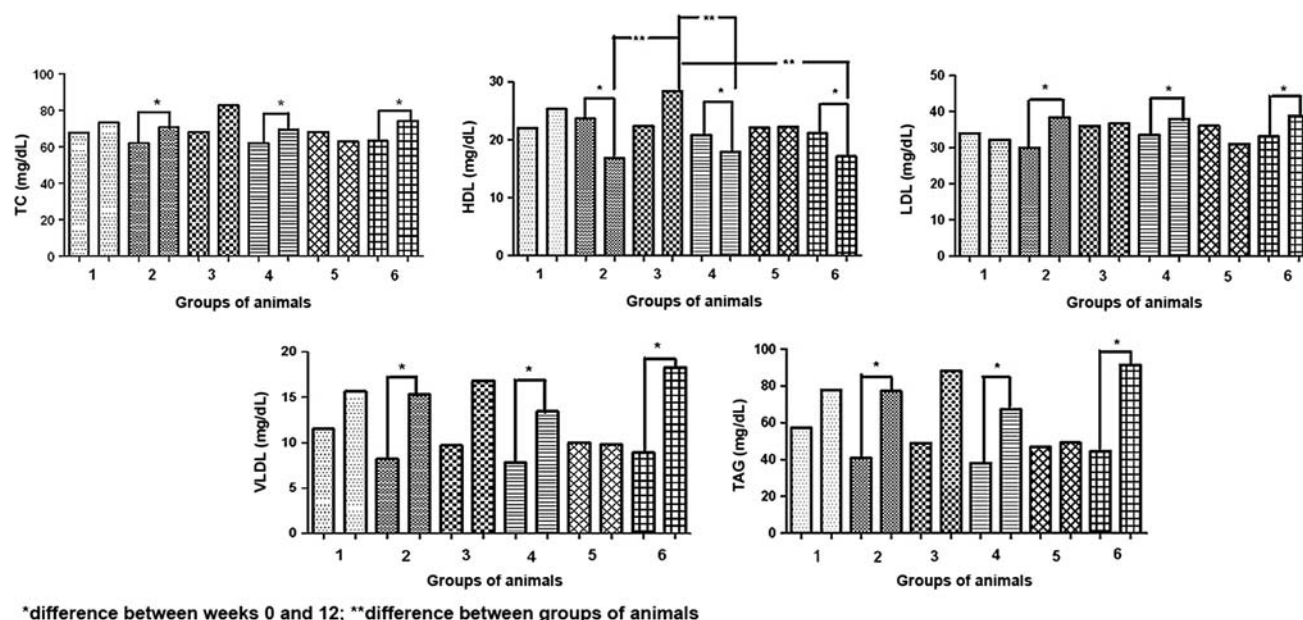
No difference was observed in body weight gain ( $P = 0.8542$ ) or in chow intake ( $P = 0.1846$ ) between the various animal groups during the 12 weeks of the experiment. All groups had similar plasma lipid levels at the beginning of the experiment. When plasma lipids were compared at weeks 0 and 12, no significant difference in levels of TC, LDL, HDL, VLDL, and TAG was observed in groups 1, 3, and 5, while in groups 2, 4, and 6 there was an increase in TC, LDL, VLDL, and TAG levels, and a decrease in HDL levels (Table 2; Fig. 2).

When all animal groups were compared at the end of the experiment, only the HDL levels of groups 2, 4, and 6 were lower than those of group 3 (Fig. 2). Additionally, when groups 1 versus 3 versus 5 and 2 versus 4 versus 6 were compared in order to identify the separate effect of the surgical procedures on the spleen, no difference was observed.

## Discussion

For many years, total splenectomy has been usually performed due to trauma, some diseases, and for diagnostic purposes, since there was insufficient knowledge about its consequences [2, 15]. Once the importance of the spleen in host defense was recognized, the immunological consequences of total splenectomy were given more attention and the development of new conservative spleen procedures was recommended, in an attempt to avoid the complications of asplenism [28, 29]. Subtotal and partial splenectomies have been recommended in many situations. Nevertheless, when total splenectomy is unavoidable, splenic autotransplantation seems to be the only alternative [30, 31].

A variety of studies have confirmed morphological regeneration of autotransplanted splenic tissue [32–35]. However, splenic regeneration does not necessarily mean recovery of organ function. Several authors have shown that splenic autotransplantation is a simple, effective procedure [30, 36] and that, when performed at appropriate sites, it successfully maintains some spleen functions after total splenectomy, as demonstrated by indirect laboratory methods and scintigraphic techniques [4, 37]. Due to their blood supply and venous drainage into the portal vein, as is the case for the spleen in situ, splenic implants in the greater omentum seem to develop and function better than at other sites [38, 39]. Although the regeneration sequence begins hours after autotransplantation, the time needed for the occurrence of morphological and functional



**Fig. 2** Comparison over time of the experiment (*Wilcoxon test*) and between groups of animals at the end of the experiment (*Kruskal–Wallis test*) of TC, HDL, LDL, VLDL, and TAG levels at weeks 0

(left column in each group) and 12 (right column in each group) in all groups of animals

regeneration has been reported to range from 8 to 12 weeks [40, 41].

In the present study, rats were submitted to total splenectomy for the induction of an asplenic condition in order to identify a possible role of the spleen in plasma lipid levels 12 weeks after the beginning of the experiment. Splenic autotransplantation following total splenectomy was performed to determine if such a simple procedure would protect the animals from these changes. Additionally, rats were fed two diets with different sources of lipids in order to identify the effect of such diets on the lipid profile of the animals after these surgical procedures.

The participation of the spleen in lipid metabolism has been investigated in clinical and experimental studies [42–44]. Studies performed in rats have demonstrated increased levels of cholesterol after TS, although with controversial results. Animals fed standard chow have been reported to show an increase in TAG and a decrease in HDL levels after TS compared with sham-operated animals [14], in contrast to the results from other studies showing an increase in TC and LDL and a decrease in HDL levels after the same procedure, with no alterations in TAG or VLDL levels [10], or elevation of all of these parameters [17].

Some theories have been proposed to explain the possible role of the spleen in plasma lipid levels. Increased macrophage activity in myeloproliferative disorders would lead to hypocholesterolemia through an increase in LDL catabolism [45–48]. Schmidt et al. [49] compared the spleen to a lipid reservoir that accumulates a great part of the fat in states of hypersplenism. Another explanation for the participation of the spleen in lipid metabolism would be the production of anti-oxidized LDL antibody, with spleen-associated immune activity protecting against the development of atherosclerosis [50].

Most of the studies that showed a correlation between the spleen and plasma lipid level regulation did not consider chow intake or weight gain during the experiment. In the present study, all animal groups showed similar weight gain and chow intake. There is evidence that excess body adiposity can lead to a worsening of cardiovascular risk factors, including dyslipidemia [51, 52], making the control of such variables extremely relevant in order to guarantee a correct interpretation of the results, avoiding, for example, an erroneous attribution of lipid profile alteration to a surgical procedure on the spleen.

Some authors have increased fat content in rat chow during the post-splenectomy period in order to induce hyperlipidemia and emphasize possible alterations in the lipid profile by adding cholesterol to the commercial chow [9, 17, 43]. Others have shown that increasing dietary

cholesterol results in higher plasma cholesterol concentrations [53, 54], with the addition of cholesterol itself being expected to alter plasma lipid levels. However, most studies have also demonstrated that dietary cholesterol is a less potent regulator of plasma cholesterol concentrations than dietary fatty acids [18, 55].

Recent evidence suggests that a consistent reduction in the risk of cardiovascular disease due to changes in the plasma lipoprotein profile can be achieved through changes in fat source consumption, such as replacing *trans* and saturated fat with mono- and polyunsaturated fatty acids [56–58]. In the present study, we changed the fat source instead of increasing fat content in an attempt to identify the impact of fat quality on lipid profile, using pork fat due to its high saturated fatty acid content.

In contrast to other experimental studies [12, 14–16], we did not observe any changes in plasma lipid levels after any surgical procedure on the spleen when rats were fed a nutritionally balanced diet. Thus, surgical trauma and total splenectomy alone or in combination with splenic autotransplantation did not influence the lipid profile of the animals. Rezende et al. [44] also showed similar plasma levels of cholesterol and its fractions in groups of mice fed a similar diet and submitted to the same surgical procedures. Comparable results were obtained by Caligiuri et al. [59], who did not detect changes in cholesterol levels in splenectomized mice, although atherosclerosis had drastically worsened after the surgical procedure.

When animals were fed a chow containing pork fat as a source of lipids, a worsening of all parameters was observed. Again, these changes occurred in all animals regardless of the surgical procedure on the spleen. Our findings confirm the results obtained in other studies that showed an increase in TC and LDL levels with the use of a high saturated fat diet [60–62]. Alterations in HDL, VLDL, and TAG levels may be due to changes in the fat content or fatty acid profile with the chow used during the postoperative period compared to the commercial chow used during the preoperative period.

Despite the possible role of the spleen in myeloproliferative diseases and in protective immunity against atherosclerosis, we did not find any effect of the spleen on plasma lipid levels. It was the quality of the fat, rich in saturated fatty acids, that significantly worsened the lipid profile of rats in contrast to a nutritionally balanced diet. This result suggests that the spleen, in the absence of diseases, does not seem to have an important role in the regulation of plasma lipid levels. Further studies dealing with other animal models and different contents and sources of fat in the diet are necessary to better elucidate the correlation between the spleen and the lipid profile.

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