Lipid Metabolism in Rats Fed Diets Containing Different Types of Lipids

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Objective - To assess the effect of different types of lipid diets on the lipid metabolism of aging rats.

Methods - Fifty male Wistar rats were studied from the time of weaning to 12 and 18 months of age. Their diets were supplemented as follows: with soybean oil (S), canola oil (CA), lard and egg yolk (LE), and canola oil + lard and egg yolk (CA + LE). Blood pressure (BP) was measured every month, and the heart/body ratio (H/BR) was determined. The rats were euthanized at the age of 12 and 18 months, and blood samples were collected for lipid analysis as follows: total cholesterol (TC), LDL-C, VLDL-C, HDL-C, triglycerides (TG), and glucose.

Results - The type of oil ingested by the animals significantly altered BP, H/BR, and serum lipid levels in rats at 12 and 18 months. No difference was observed in the survival curve of the animals in the different groups. The LE group had the highest BP; and the CA group was the only one in which BP did not change with aging. A reduction in the H/BR was observed in the LE and CA+LE animals. At the age of 12 months, differences in TC, HDL-C, LDL-C, VLDL-C, TG, and glucose were observed. At the age of 18 months, a significant difference in TC, HDL-C, and glucose was observed. The highest TC value was found in the CA group and the lowest in the S group.

Conclusion - No increase in BP occurred, and an improvement was evident in the lipid profile of rats fed a diet supplemented with CA, in which an elevation in HDL-C levels was observed, as compared with levels with the other types of diet.

Keywords: canola oil, lipoproteins, rats

Some experimental studies in animals have reported that an increase in cholesterol ingestion results in an elevation in the serum levels of cholesterol 1-3, with a resulting increase in the risk of cardiovascular diseases 4.

The n-3 fatty acids are long-chain polyunsaturated fatty acids naturally found in fish oils and in some plants. The fatty acids of the n-3 family are the following: eicosapentaenoic acid (EPA) (C20: 5n-3), docosahexaenoic acid (DHA) (C22: 6n-3), and α-linolenic acid (aLA) (C18: 3n-3) 5,6. The metabolic effects of EPA and DHA are already very well shown, but evidence of the metabolic effects of aLA is increasing. aLA is a fatty acid derived from vegetables and is found in soybeans, canola, saffron, and peanut oils 7. After being ingested, aLA may be desaturated and altered into other forms of long-chain polyunsaturated fatty acids, such as EPA and DHA 8,9. Canola oil is an important option for dietary sources of the n-3 fatty acid family, mainly for vegetarians and individuals who do not eat fish 10.

Connor 11 reported the possible effects of the ingestion of n-3 fatty acids on lipid metabolism: (1) inhibition of VLDL synthesis, (2) decrease in apolipoprotein B synthesis, (3) increase in VLDL catabolism, (4) decrease in LDL synthesis, and (5) decrease in postprandial lipemia.

Lipoproteins are closely related to the risk of cardiovascular diseases, as follows: low-density lipoproteins (LDL) indicate an increased risk, and high-density lipoproteins (HDL) are considered a protective factor 12,13. When the risk of cardiovascular diseases was considered a function of HDL-C and LDL-C, the incidence of cardiovascular diseases increased with the increase in the concentration of LDL-C and the decrease in the concentration of HDL-C 14. Both LDL-C and HDL-C are independent risk factors for cardiovascular diseases 15. This is important in establishing the conditions that influence the changes in LDL and HDL levels throughout life.

Due to the great genetic variability and the dietary habits of human populations, the experimental model with rats proved adequate for experimental studies 16-18. It is important to note that there is a general similarity between the cardiovascular system of rats and that of other mammals,
man inclusive 20. Despite the difficulty in producing hyperlipidemia and atherosclerosis in rats, special diets may induce an increase in the serum levels of cholesterol, and also induce arterial hypertension or renal diseases 31, such as those reported in our team’s previous studies 22-25.

The objective of this study is to investigate the possible influence of 4 experimental diets with different types of fat on the cardiovascular indicators and on the lipid metabolism of rats during aging.

Methods

We studied 50 male Wistar rats obtained from colonies maintained by the Universidade do Estado do Rio de Janeiro. The research followed the guidelines established in the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23 revised in 1985).

The animals were placed in polypropylene boxes, in groups of 5, maintained at a controlled temperature (21±1°C) and humidity (60±10%). The environment underwent lightness-darkness cycles of 12 hours (artificial light from 7 AM to 7 PM) and cycles of air exhaustion (15 min/h). The groups received unrestricted water and the same baseline diet composed of casein, wheat flour, starch, egg white, and a mixture of vitamins and minerals. All diets had the same composition as follows: 47% carbohydrates, 29% lipids and 23% proteins with 11.5% dietary fibers, and 0.05% sodium (or 500 mg/kg of diet). The caloric values of the diets were practically the same. The diets were prepared once a week in the laboratory and stored at a temperature of +4°C. Depending on the group, the diet was supplemented with different types of lipids as follows: soybean oil (S group), canola oil (CA group), lard and egg yolk (LE group), and canola oil + lard and egg yolk (CA + LE group).

The animals received their diets beginning at the time of their weaning at the age of 21 days. For each group, at least 5 animals were euthanized at 12 and 18 months of life. Tail Blood pressure (BP) was measured with a pressure plethysmograph (RTBP1007, Kent Scientific Co., Litchfield, CT, USA) once a month. Total body mass was also assessed at the same frequency.

At the expected time, the animals were euthanized after they had fasted from 7 PM of the previous evening. They were anesthetized with diethyl ether. Their thorax was opened and a blood sample was collected from the right atrium. Then, 3 mL of 10% KCl was injected into the left ventricle until cardiac arrest in diastole occurred. The heart was withdrawn by sectioning the great vessels, as short as possible; cardiac volume and weight were determined by fluid displacement (Scherle’s method) 26. The heart/body ratio (H/BR) was determined by dividing the respective weights.

The following biochemical parameters were analyzed: total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), very-low-density lipoprotein cholesterol (VLDL-C), and glucose. Plasma was separated from total blood centrifugation at room temperature for 15 minutes with 120 grams of gravity, and, then, it was stored at a temperature of -80°C until being analyzed in the laboratory 1,27. Glucose was measured using the enzymatic method with i kit Art. 07.3757.7 (U.S.#42954); cholesterol was measured with the cholesterol kit 1.19738.0001 KgaA 64271, Multi-Test Calibration System, Merck Cat. n° 1.19720.0001 (Darmstadt, Germany); HDL was measured using the DIASYS kit Cat. n° 10.351.030; triglycerides were measured using the triglyceride kit 1.19706.0001 KgaA 64271, Multi-Test Calibration System, Merck Cat. n° 1.19729.0001 (Darmstadt, Germany). The LDL, VLDL, and chylomicron fractions were abundantly precipitated by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, HDL-C, which was maintained in suspension, was determined. LDL-C was calculated according to Friedwald’s formula 28.

The Mantel-Haenszel test (T) was used for comparing the survival curves of the animals in the different groups. If the null hypothesis were true, T was analyzed with χ² distribution (chi-square with 1 degree of freedom) 29. Descriptive statistics were calculated for all parameters. The differences between the groups were tested 2 by 2 using the nonparametric Mann-Whitney test with a significance level of 0.05 30.

Results

The results are summarized in table I and in figures 1 and 2. The type of oil ingested by the animals significantly altered BP, H/BR, and serum levels of lipids in rats at 12 and 18 months of age. No significant difference was observed in the survival curve of the animals considering the different groups.

Variations in BP are shown in figure 1. At the age of 12 months, the LE group had the highest. The differences were significant in the following group comparisons: S versus LE (+17%), CA versus LE (+16%), and LE versus CA+PY (-18%). At the age of 18 months, the increase in BP in the LE group was even more marked, and the CA+LE group also showed an increase in BP. The differences were significant in the following group comparisons: S versus LE (+29%), S versus CA+LE (+12%), CA versus LE (+32%), CA versus CA+LE (+15%), and LE versus CA+LE (-24%).

A comparison of the results at 12 and 18 months revealed that the CA group was the only one in which a change in BP was not observed with aging. The other 3 groups showed an increase in BP. The S group showed a 10% increase, the LE group a 23% increase, and the CA+LE group an 18% increase.

Variations in H/BR are shown in figure 2. At the age of 12 months, no significant difference in H/BR was observed in the following groups: S versus LE (+43%) and CA versus LE (+31%).

At the age of 18 months, no difference in H/BR was observed in the groups studied.

Comparing the results at 12 and 18 months in the S and
CA groups, no alteration in H/BR was observed with aging. A reduction in H/BR was observed in the LE and in the CA+LE groups. The LE group had an 86% reduction and the CA+LE group a 37% reduction in H/BR.

Variations in body weight are shown in table I. At the age of 12 months, a significant difference in body weight occurred in the S versus CA (-20%) groups and in the S versus LE (-34%) groups. At the age of 18 months, no difference in body weight occurred in the groups studied. Comparing the results at 12 and 18 months, no difference was observed.

Glucose and serum lipid levels are shown in table I. At the age of 12 months, differences in TC, HDL-C, LDL-C, VLDL-C, TG, and glucose were observed. Differences were significant among the following groups: for TC, S versus CA groups, CA versus CA+LE groups (-22%), CA versus CA+LE groups (-27%), and LE versus CA+LE groups (-37%); for HDL-C, the highest value was in the CA group (+25% than in the S group, +103% than in the LE group, and +65% than in the CA+LE group), the lowest HDL-C value was found in the LE group, and the differences between S versus LE groups (-39%) and S versus CA+LE groups (-25%) were also significant. At the age of 12 months, all groups, except the CA+LE group, had increased levels of LDL-C. LDL-C levels were higher in the LE group and lower in the CA+LE group, but statistically significant differences occurred in the following comparisons: S versus CA groups (+45%), S versus LE groups (+46%), CA versus CA+LE groups (-58%), and LE versus CA+LE groups (-59%). VLDL-C and TG also had the same pattern of variation; the highest values of VLDL-C and TG were found in the S group, and the lowest in the LE group. At the age of 12 months, the S group had the heaviest animals. Significant differences occurred in the following comparisons: S versus LE groups (-47% for VLDL-C and -46% for TG), CA versus LE groups (-45% both for VLDL-C and TG), and LE versus CA+LE groups (+77% for

## Table I - Results (mean±SD) of the concentration of glucose and serum lipids at 12 and 18 months of age in rats fed diets supplemented with different types of lipids. Differences between ages and the groups were tested with the nonparametric Mann-Whitney test.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Glucose (mg/dL)</th>
<th>Cholesterol (mg/dL)</th>
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<tbody>
<tr>
<td></td>
<td>12</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>S</td>
<td>560.6±46.7</td>
<td>540.5±93.0</td>
<td>171.17±20.72</td>
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<tr>
<td>CA</td>
<td>450.2±70.7</td>
<td>456.8±190.1</td>
<td>129.54±11.89</td>
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<tr>
<td>LE</td>
<td>369.9±76.9</td>
<td>499.9±135.8</td>
<td>120.36±27.93</td>
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<tr>
<td>CA+LE</td>
<td>426.5±160.0</td>
<td>538.4±98.5</td>
<td>144.32±16.40</td>
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</table>

Comparisons between ages

<table>
<thead>
<tr>
<th>S</th>
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<th>0.008</th>
<th>NS</th>
<th>0.02</th>
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<tr>
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Comparisons between groups

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<td>S</td>
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VLDL-C and TG also had the same pattern of variation; the highest values of VLDL-C and TG were found in the S group, and the lowest in the LE group. At the age of 12 months, the S group had the heaviest animals. Significant differences occurred in the following comparisons: S versus LE groups (-47% for VLDL-C and -46% for TG), CA versus LE groups (-45% both for VLDL-C and TG), and LE versus CA+LE groups (+77% for
VLDL-C and +75% for TG). At the age of 12 months, glycemia was altered in the different groups. The highest glucose level was in the S group, and the lowest in the LE group; significant differences, however, occurred only in the S versus CA (-24%) and in S versus LE (-30%) groups. At the age of 18 months, a significant difference was found in the levels of TC, HDL-C, and glucose. The highest TC value was found in the CA group, and the lowest in the S group; significant differences occurred in S versus CA groups (+29%) and S versus CA+LE groups (+11%). For HDL-C, the highest value was in the CA group, and the lowest in the LE group; significant differences occurred in S versus CA groups (+57%), CA versus LE groups (-44%), CA versus CA+LE groups (-34%), and in LE versus CA+LE groups (+19%). Glucose was different only in S versus CA+LE groups (-37%). LDL-C, VLDL-C, and TG showed no significant differences.

Comparing the results obtained at 12 and 18 months, in regard to TC, no variation was found in the S and LE groups. The CA and CA+LE groups showed the following increases: +35% in the CA group and +57% in the CA+LE group. In regard to HDL-C values, the S group showed no variation. In the remaining groups, HDL-C levels increased as follows: +37% in the CA group, +54% in the LE group, and +49% in the CA+LE group. LDL-C levels decreased in all groups, except in the CA+LE group, as follows: -48% in the S group, -73% in the CA group, -55% in the LE group. The CA+LE group was the only one to show a different pattern; LDL-C levels did not change in the ages studied. VLDL-C and TG levels did not change in the groups studied. For glycemia, the S, CA, and LE groups showed no variation; in the CA+LE group, a reduction in glycemia was observed at the age of 18 months (-31%).

### Discussion

We observed that the diets supplemented with different dietary lipids influenced serum lipid levels. The experimental diets in our study contained 3 to 10 times the amount of lipids recommended for the normal diet of rats 31, which were required for determining alterations in the lipoproteins of rats, which are animals resistant to hyperlipidemia 21.

Aging by itself affects the metabolism of lipids. In rats, no difference was reported in total cholesterol levels between 2 and 12 months of age, but a significant increase in total cholesterol was observed at the age of 24 months 32, which is in accordance with our study that showed an elevation in serum levels of TC and HDL-C at the age of 18 months. Despite the great variations in the blood biochemistry of the animals of the different groups at different ages, the values found were within the 95% confidence interval of the values considered normal for male Wistar rats 31.

Increased LDL-C levels and decreased HDL-C levels in
the animals receiving pork fat and yolk were not unexpected when compared with those in the other groups, and this result is in accordance with a previous study on this subject. The differences in the serum lipid levels of the rats in the S and CA groups may result from the composition of the fatty acids of these 2 oils. The soybean oil has approximately 50% linoleic oil (LN) (18:2n-6), and the canola oil has a mixture of oleic and alpha-linolenic acid (aLA). The oleic acid does not compete with aLA in the conversion of long-chain polyunsaturated fatty acids (n-3). McLennan and Dallimore reported that the elevated content of LN found in the soybean oil may reduce the efficiency of aLA and they suggested that the LN/aLA ratio might determine the efficacy of the conversion into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

Baba et al. suggest that the presence of aLA in canola oil may contribute to its effect in reducing TG and TC levels. Glycemia does not usually change over time. In our study, the alterations in glycemia comprised an increase in the glucose levels in the S group at the age of 12 months, probably due to body mass gain that occurs at this age, and a reduction in the glucose levels in the CA+LE group at the age of 18 months. This group had no alteration in body mass during aging. The above-cited alterations in glycemia had no relation to the type of lipid ingested or to time.

Total cholesterol seems to be more influenced by time than by the type of lipid ingested. HDL-C, however, was the lipoprotein most influenced by the type of dietary lipid. The CA group had the highest levels of HDL-C both at 12 and 18 months of age. This finding is supported by the study carried out by Kobutake et al., who assessed the lipid metabolism of rats ingesting a mixture of polyunsaturated fatty acids (PFA), n-3 FA inclusive. The other groups studied also had an increase in HDL-C levels over time; this increase, however, was not as marked as that in the CA group.

A significant increase in lipoprotein levels usually occurs during aging, particularly, in LDL-C levels. The progressive increase in the concentration of LDL-C during aging represents a higher risk for cardiovascular diseases, which was observed in this study with rats. In our study, the experimental diets with pork fat and yolk (LE group) and with canola oil + pork fat and yolk (CA+LE group) contained 3% and 1.5% dietary cholesterol, respectively; however, the most affected were LDL-C and HDL-C levels in the different groups and at both ages. Different from that which happens in humans, in the rats in the present study, a reduction in LDL-C and an increase in HDL-C occurred with age.

Another important aspect of the analysis of the influence of dietary oil on the lipid metabolism of rats is the polyunsaturated/saturated fatty acid ratio (PFA/SFA). An elevated PFA/SFA ratio is believed to reduce serum lipid levels. Monounsaturated fatty acids (MFA) have also been reported to reduce serum lipid levels as effectively as does PFA. The PFA+MFA/SFA ratio, however, has been con-

![Fig. 2 - Variations in the heart/body ratio at 12 and 18 months of age in rats fed diets supplemented with different types of lipids. NS- nonsignificant; TG-triglycerides. Groups: S-soybean group; CA-canola group; LE-lard and egg yolk; CA+LE-canola + group. The lower case letters on the bars indicate, when different, statistically significant differences (P<0.05).](image-url)
considered an even better indicator of the effect of dietary oil on serum lipids. Canola oil, pork fat, and yolk contain a higher amount of MFA than does soybean oil. In this study, the diets in the CA, LE, and CA+LE groups contained 58%, 50%, and 53% MFA, respectively. According to the PFA/MFA/SFA hypothesis, the canola oil, which contains 92% MFA+PFA, may favor a significant increase in HDL-C when compared with that of other groups. Soybean oil has a small amount of MFA (26%), a high amount of PFA (61%), and a relatively high amount of MFA+PFA, which, however, is smaller than that in canola oil.

Huang and Chang studied the effects of different types of dietary oils containing fixed ratios of fatty acids (PFA/SFA = 1, PFA+MFA/SFA = 5.7 and 4) in rats and observed that MFA may increase, instead of reduce, TC, TG, LDL-C, and hepatic cholesterol. A higher PFA/SFA ratio was reported to be possibly better. In the present study, however, the animals in the CA and S groups had differences in serum lipids, despite the similar PFA/SFA ratios of their diets. A more detailed discussion about the composition of the diets was reported in a previous study by this team.

Of the lipids used in this study, the PFA+MFA/SFA ratio was higher in the CA group (11%) and lower in the LE group (2%), which may indicate the existence of a possible relation between PFA+MFA/SFA and HDL-C. The PFA content was lower in the LE (15%) and CA+LE (21%) groups, relatively high in the CA group (33%), and higher in the S group (61%).

The PFA/SFA ratio was 4.4 in soybean oil and 4.1 in canola oil, indicating that no relation exists between PFA/SFA and serum lipids in rats. The PFA/SFA ratio in the S and CA groups was 5 times higher than that in the CA+LE (0.8) and LE (0.4) groups.

In regard to the biometry of the animals, diets supplemented with different dietary lipids influenced BP and H/BR in rats at 12 and 18 months of age. The type of dietary lipid is known to affect BP. Diets with a great amount of saturated fat may increase BP, while diets with higher amounts of polyunsaturated and monounsaturated fat may reduce BP.

A cholesterol-rich diet primarily causes pressure overload, which may explain the BP increase observed in animals fed a diet supplemented with lard and egg yolk. In our study, even though the sodium content was the same in the 4 experimental diets, the animals in the LE group had higher BP levels at the age of 12 months, and this increase was even more marked at the age of 18 months. The animals in the CA+LE group, which had no BP alteration at the age of 12 months, had an increase in BP at the age of 18 months.

The animals’ body mass had great variations over time; the animals in the LE group were lighter at the age of 12 months than the rest of the animals, and those in the S group were the heaviest. However, at the age of 18 months, this difference in body weight no longer existed.

Finally, we may suggest that no increase in BP occurs, but the lipid profile of rats fed a diet supplemented with canola oil for a long time improves and the rats experience an elevation in the HDL-C levels as compared with rats fed the other types of diets.

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References


