Original / Investigación animal

Prolonged flaxseed flour intake decreased the thickness of the aorta and modulates some modifiable risk factors related to cardiovascular disease in rats

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Abstract

Cardiovascular diseases are a major public health problem. Different risk factors have been recognized as the main causes of the development and progression of cardiovascular diseases. Flaxseed is a source of dietary fiber, lignans, and alpha-linolenic acid. The aim of this study was to evaluate the effects of prolonged supplementation with flaxseed flour as preventive therapy on cardiovascular risk parameters in healthy Wistar rats.

Material and Methods: Female Wistar rats were divided into two groups after giving birth and during lactation period: the control group was fed with diet based on casein and the flaxseed group was fed with diet based on casein containing 25% of flaxseed flour. At weaning, 10 male offspring from each group continued to receive the same diets from their mothers during 250 days. The body weight, visceral fat mass, cholesterol, triglycerides, HDL, VLDL, glucose and thickness of the aorta were analyzed.

Results: The body weight, visceral fat mass, cholesterol, triglycerides, HDL, VLDL, glucose and thickness of the aorta values were statistically lower when compared to control group.

Conclusion: The data suggest that flaxseed flour supplementation in healthy wistar rats for a prolonged period may decrease the thickness of the aorta and may be used as a preventive measure in modulating some modifiable risk factors related to cardiovascular disease.

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Key words: Flaxseed. Rats. Cardiovascular disease. Lipid profile.

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List abbreviation

ALA: alpha-linolenic acid.
CG: Control Group.
FG: Flaxseed Group.
HDL: high density lipoprotein.
LA: linoleic acid.
LabNE: Experimental Nutrition Laboratory.
LDL: low density lipoprotein.
SDG: secoisolariciresinol diglycoside.
UFF: Federal Fluminense University.
VLDL: very low density lipoprotein.

Introduction

Cardiovascular diseases are a major public health problem and are responsible for 17.5 million deaths of people in the world, with 80% of these deaths occur in developing countries. It is estimated that in 2030 about 23.6 million people will die from this disease. Different risk factors have been recognized as the main causes of the development and progression of cardiovascular diseases. They are divided into modifiable risk factors such as hypercholesterolemia, overweight or obesity, hypertension, sedentary lifestyle, excessive alcohol intake, smoking and diabetes; and not modifiable, such as gender and heredity or a positive family history and age. Aging affects cardiovascular health, because with the increasing age there is an enlargement of the lumen area, wall thickening and loss of elasticity, thereby promoting changes in cardiac structure and function, and these changes were most evident in the aorta and major arteries. The vascular stiffening results in hyperplasia and elastin breakdown in the middle layer, responsible for the thickening of the arterial wall. This can contribute to endothelial dysfunction, cardiovascular events such as hypertension and atherosclerosis.

With respect to modifiable risk factors, researcher has observed that the control of these factors is an effective way to reduce the risk of these diseases. As an example, the reduction in saturated fat intake and/or supplementation of polyunsaturated fats. In this context, the consumption of functional foods is growing by population, and flaxseed (Linum usitatissimum) has been very popular due to its health benefits. This oilseed is a source of dietary fiber, lignans, protein, and contain 41% lipid, 50-55% being composed of alphalinolenic acid (ALA), 15-18% at linoleic acid (LA) and 18% by monounsaturated fatty acids omega-9.

Because of its composition, the seed has proved able to act as an effective factor of protection and reducing some of these cardiovascular risk factors such as glucose lowering, improved lipid profile, weight reduction and obesity, and reduction of visceral fat mass. Thus, the objective of this study was to evaluate the effects of prolonged consumption of flaxseed as preventive therapy on cardiovascular risk parameters in healthy Wistar rats with 250 days of life.

Materials and methods

Experimental design

Twenty female Wistar rats from the colony of the Experimental Nutrition Laboratory (LabNE) from Federal Fluminense University (UFF) with 90 days old, mated at a ratio of 3 females to 1 male, receiving commercial diet (23% of protein, Nuvilab*, Nuvital Ltda., Paraná State, Brazil).

Female Wistar rats were randomly divided into two groups after giving birth. During the lactation period the Control Group (CG, n = 10) was fed with a diet based on casein containing approximately 18% of protein, and Flaxseed Group (FG, n = 10) with a diet based on casein, containing approximately 18% of protein with addition of 25% of flaxseed flour.

At weaning, 10 male offspring from each group continued on their origin groups, however to receiving diets with approximately 11% of protein (AIN-93M) until 250 days old when they were killed. The weight and diet consumption were registered three times a week. All animals were kept in an animal house with controlled temperature (21-23°C) and light/dark cycle (12/12 hours), receiving water and food ad libitum.

This research project was approved by the Animal Research Ethics Committee of the Faculty Office of Research and Postgraduate Studies at the Federal Fluminense University, Niterói, Brazil, under n. 00105-09. All procedures followed the norms of the National Research Council (US) Institute for Laboratory Animal Research.

Experimental diets

The diets used during lactation were prepared in the Experimental Nutrition Laboratory of the UFF according to the American Institute of Nutrition (AIN 93G) recommendation for rodent diets (table I). The diet offered to the FG had a concentration of 25% of flaxseed flour that aimed to meet the entire recommended fiber intake. Moreover, this amount of flaxseed flour lipid supplies the needs of animals and provides a diet with a high content of ALA and low LA, whereas the addition of soy oil in the control diet provides an inverse proportion.

Blood collection and sample processing

The rats were anesthetized with an intraperitoneal injection of Thiopentax® (Sodium Thiopental 1G, Cristália Chemicals Pharmaceuticals LTDA, Brazil) at 5% (0.15 ml/100 g pc, ip) for blood collection by cardiac
puncture and the blood was placed in tubes without anticoagulant. The collected blood was centrifuged (Sigma centrifuge) at 3500 rpm for 15 minutes to obtain serum, which was stored at -20ºC. Analyses of cholesterol, triglycerides and high density lipoprotein (HDL) were performed with the use of the kits BIOCLIN® (Quibasa Industry - Basic Chemicals LTDA / Brazil). The concentration of very low density lipoprotein (VLDL) cholesterol was calculated according to Friedewald et al13. The glucose was measured by using the test strips glucometer brand Accu-Chek Active (Roche Diagnostics, Germany).

Visceral fat mass

Fat mass was removed and weighed when the animals were sacrificed. We considered all fats retroperitoneal, epididymal and mesenteric.

Histological Methods

The thoracic aorta fragments were fixed in buffered formaldehyde (formalin of Millonig) for 24 h, and processed according to standard histological technique for paraffin as used by Pereira et al14. After inclusion, the paraffin blocks containing the fragments of aorta were cut in microtome CUT 4050 (Microtec®), in 5 m sections and mounted on glass slides for optical microscopy.

The slides were stained by hematoxylin - eosin (overall), Weigert's resorcin fuchsin (elastic fiber staining). After, capturing images through a microscope Olympus BX40® coupled to a digital camera Lumenera®, using 20X objective. Histomorphometry scan was used to measure the thickness of the aorta through software Image J® (U.S. National Institutes of Health, Bethesda, Maryland, USA).

Statistical analysis

Data are reported as means and standard deviations. The results were tested for normal distribution using the Shapiro-Wilk test. For results with normal distribution, comparisons between groups were carried out using Student’s t test for independent data. Where results did not have normal distribution, the Mann-Whitney nonparametric test was employed. In all tests, significance was set at p ≤ 0.05. SPSS for Windows, version 10.0, was used for statistical analysis.

### Table I

Control and flaxseed diets composition of each 100 g of diet

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Casein (g)</th>
<th>Flaxseed (g)</th>
<th>Casein (g)</th>
<th>Flaxseed (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein1</td>
<td>20</td>
<td>14.11</td>
<td>11.8</td>
<td>5.9</td>
</tr>
<tr>
<td>Flaxseed2</td>
<td>0</td>
<td>25</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Starch3</td>
<td>52.95</td>
<td>45.84</td>
<td>61.2</td>
<td>54.1</td>
</tr>
<tr>
<td>Refined Sugar4</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Minerals Mix AIN 93G1</td>
<td>3.50</td>
<td>3.50</td>
<td>3.50</td>
<td>3.50</td>
</tr>
<tr>
<td>Vitamins Mix5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Soy Oil6</td>
<td>7</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Cellulose7</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Choline Bitartrate1</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Cystine1</td>
<td>0.30</td>
<td>0.30</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>Tert-Butyl hydroquinone</td>
<td>0.0014</td>
<td>0.0014</td>
<td>0.0008</td>
<td>0.0008</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Macronutrient Composition (100 g/diet)

| Protein         | 19.53  | 18.36     | 11.68     | 11.00        |
| Fat             | 19.46  | 22.28     | 19.75     | 22.54        |
| Carbohydrate    | 61.01  | 59.36     | 68.57     | 66.46        |
| Total energy (kJ/100 g) | 1488.83 | 1581.1 | 1485.9 | 1579.47 |

Ingredients used in diets preparation were provided by 1M. Cassab Comércio e Indústria Ltda (São Paulo, SP, Brazil); 2Arma Zen Produtos Naturais Ltda. (Rio de Janeiro City, Rio de Janeiro State, Brazil); 3Maisena da Unilever Bestfoods Brazil Ltda (Mogi Guaçu City, São Paulo State, Brazil); 4União (Rio de Janeiro, RJ, Brazil); 5Liza da Cargill Agricultura Ltda. (Mairinque City, SP, Brazil); and 6Microcel da Blanver Ltda (Cotia City, SP, Brazil).
Results

At 250 days of life, FG had 20% less body weight when compared to the CG (table II). The FG had lower values in visceral fat mass (-44%, FG = 40.3 ± 10.9 g, CG = 71.9 ± 22.2 g, p = 0.0016) and these were maintained even after the calculation of relative fat mass (-29%, FG = 7.6 ± 1.7 g/100 g body weight, CG = 10.8 ± 2.3 g/100 g body weight, p = 0.0016).

Table II shows that the flaxseed group showed changes in biochemistry parameters. As regards the lipid profile of the FG showed changes in all parameters. The FG lower values for cholesterol (p <0.000), triglycerides (p <0.000), HDL (p = 0.046) and VLDL (p = 0.028). The concentration of serum glucose was lower in the FG (p = 0.004).

At 250 days it was observed in the flaxseed group a smaller thickness of the aorta (GL = 0.13 ± 0.01 mm; GC = 0.15 ± 0.02 mm; p < 0.005) (fig. 1).

Discussion

Supplementation with flaxseed flour or food source of ALA in the cardiovascular system has been investigated by many researchers, but studies only evaluate this short-term consumption. The differential of this research was the study of the effects of this prolonged consumption in healthy mice as a preventive measure in parameters related to cardiovascular risk. The flaxseed flour shown to reduce body weight gain and visceral fat accumulation, improve lipid profiles, lower blood glucose and in protect against the thickening of artery.

The reduction in body weight can be attributed to the fact that the flaxseed contains 28% fibers, and these can promote the control of energy intake, increase satiety and reduce the risk of developing obesity. These functions may occur due to the physicochemical properties of the fibers by forming a gel indicate a feeling of early satiety and prolonged15,16.

In the present study it was observed lower accumulation of visceral fat. Few studies describe the possible protective effect of this oilseed on adiposity. The administration of secoisolariciresinol diglycoside (SDG), which is the main lignan found in flaxseed reduced the accumulation of visceral fat mass in mice and rats10,17. No statistical differences were found in visceral fat mass of rats that received the seed components only during lactation, although there was, in FG, a numerical reduction of 21.43%18. The same tendency was shown in mice that received a diet containing flaxseed oil compared to mice fed a diet rich in saturated fats19.

It has been suggested that flaxseed and its components can reduce the risk of cardiovascular diseases20, but the mechanisms of this reduction are not yet well established. Some authors attribute the decrease in risk with improved lipid profile6. The effect of flaxseed on the lipid profile has been investigated extensively and assigned not only to seed flour, but also to SDG, its oil and its protein. It has been found in the present study a 36% reduction in total cholesterol, 29% in triglycerides

| Table II
| Effect of diets on weight and biochemical parameters of the animals at the end of the experiment |
| FG (n = 10) | CG (n = 10) | P |
| Final body weight (g) | 522.4 ± 54.3 | 654.2 ± 77.2 | p < 0.000 |
| Cholesterol (mg/dL) | 68.9 ± 10.6 | 108.5 ± 21.5 | p < 0.000 |
| Triglycerides (mg/dL) | 46.4 ± 8.9 | 66.2 ± 8.5 | p < 0.000 |
| HDL (mg/dL) | 57.9 ± 11.7 | 84.3 ± 37.3 | p = 0.046 |
| VLDL (mg/dL) | 8.4 ± 3.3 | 12.1 ± 4.2 | p = 0.028 |
| Glucose (mg/dL) | 95.7 ± 5.3 | 103.2 ± 6.6 | p = 0.004 |

Data are presented as mean ± standard deviation of 10 rats per group.
CG: Control group; FG: Flaxseed group.

Fig. 1.—Photomicrography of the rat’s aorta. The flaxseed group (A) showed a smaller thickness compared to the control group (B).
and 30% in VLDL in the FG when compared to the values of the group that did not consume the seed. The use of 20 g of flaxseed for two months in hyperlipidemic patients resulted in modification of cardiovascular risk factors, with significant reduction of cholesterol, low density lipoprotein (LDL) and triglycerides. Flaxseed oil reduced total cholesterol and low density lipoprotein in mice that consumed a diet rich in fat and protect against renal lesions associated with hypercholesterolemia.

Despite the improvement in lipid profile, the FG had lower HDL in the present study. A survey conducted by Bloedon et al showed negative effect of flaxseed consumption on the concentration of HDL, when flaxseed was supplied at a dose of 40 g/day in hypercholesterolemic humans. A similar result was found when women consumed 25 g of defatted flaxseed for 12 weeks. There was no improvement in HDL after use of the linseed oil associated with a diet high in fat. A contrary result was found in hamsters that fed 15% flaxseed for twenty weeks and showed 91% increase in HDL compared to the control group.

In this study, adult rats fed flaxseed showed, when compared to the control group, a 7% reduction in blood glucose. These results suggest a beneficial effect of flaxseed flour consumption on the regulation and maintenance of glycemic homeostasis. As well as Abuegassim who also noted that the flaxseed extract caused a reduction in glucose concentrations of diabetic and nondiabetic mice.

Aging is generally associated with changes in the cardiovascular system, particularly in the structure and function of the arteries. Histological analysis revealed a smaller thickness of the aorta in rats fed with a diet rich in flaxseed. This result suggests that a diet with 25% of this oilseed has cardioprotective effect against this thickening of artery.

In another study, the animals that consumed a diet rich in phytoestrogens for a prolonged period had a lower thickness of the aorta when compared to the control group. Faintuch et al., noted that obese patients who consumed the golden flaxseed for 12 weeks showed no changes in carotid diameter. Prim et al. demonstrated that flaxseed supplementation in rabbits fed with hypercholesterolemic diet for one month did not alter the thickness of the intima and the ratio of the area of aorta intimamedia. Winnik et al., using a rat model of developing atherosclerosis, demonstrated that supplementation with ALA decreased the formation of atheroma plaques.

A limiting factor to be considered in this study is that the imbalance observed between u-6 and u-3 cannot be extrapolated to humans, because flaxseed was the only lipid source in the diet. Human food has a greater variety of lipid sources. Additionally, the highest percentage of u-6 soybean oil may modulate to a greater inflammatory response in the control group. However, the u-6/u-3 ratio must be observed by respecting the needs of the organism.

Conclusions

So the extrapolation of results from animal studies to humans must be made with caution, but this study in healthy rats showed that the flaxseed flour consumed for a prolonged period decreased the thickness of the aorta and may be used as a preventive measure in modulating some modifiable risk factors related to cardiovascular disease.

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