

Original

Impact of dietary flaxseed (*linum usitatissimum*) supplementation on biochemical profile in healthy rats

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Abstract

Flaxseed has been suggested play preventive and therapeutic roles in cardiovascular disease. The aim of this study was to evaluate the influence of flaxseed-supplemented dietary in healthy rats. We used 30 rats divided in three groups (n = 10): Control Group (C) was fed with a casein-based chow (10% protein; 5% fiber; 7% lipid); Flaxseed Group (F) was fed with the casein-based chow supplemented with 25% flaxseed (10% protein; 7% fiber; 11% lipid); Internal Control Group (IC) was fed with the casein-based chow plus soybean oil and fiber (10% protein; 7% fiber; 11% lipid). The blood was obtained by cardiac puncture (after 180 days) and the serum was separated for lipid profile, glucose and uric acid analyses by commercial kit. Although all groups fed the same amount of ration, F group presented low (p < 0.05) body mass than C and IC groups. Total cholesterol and triacylglycerol were similar between all groups. F group presented HDL-C (High-density lipoprotein cholesterol) increase (p < 0.05) in 47% when compared C group. The LDL-C (Low-density lipoprotein cholesterol), glucose and uric acid were reduced (p < 0.05) 22%, 78% 64%, respectively, in F compared to C group. All results together suggest that the supplementation with 20% o flaxseed might be important to prevent cardiovascular disorders.

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Key words: *Cardiovascular risk factors. Flaxseed. Lipid profile. Prevention. Supplementation. Uric acid.*

IMPACTO DE LA SUPLEMENTACIÓN DIETÉTICA DE LA LINAZA (*LINUM USITATISSIMUM*) EN PERFIL BIOQUÍMICO EN RATAS SANAS

Resumen

Ha sido sugeridos papeles preventivos y terapéuticos de la linaza en enfermedad cardiovascular. Le objetivo de este estudio era evaluar la influencia de la complementación de la linaza en ratas sanas. Utilizamos 30 ratas divididas en tres grupos (n = 10): El grupo Controle (C) fue alimentado con ración basado en caseína (10% del proteína; 5% del fibra; 7% del lípido); El grupo de la linaza (F) fue alimentado con ración basado en caseína con complementación del 25% de la linaza (10% del proteína; 7% del fibra; 11% del lípido); El grupo Controle Interno (IC) fue alimentado con ración basado en caseína con el aceite de soja y la fibra 10% del proteína; 7% del fibra; 11% del lípido). La sangre fue obtenido por puntura cardiaca (después de 180 días) y el suero fue separado para el perfil del lípido, la glucosa y los análisis del ácido úrico por el kit comercial. Aunque todos los grupos alimentaran la misma cantidad de ración, el grupo F presentó bajo (p < 0,05) peso que grupos C y IC. El colesterol y el triacylglycerol totales eran similares entre todos los grupos. El grupo de F presentó el aumento del HDL-C (colesterol de la lipoproteína de alta densidad) (p < 0,05) en 47% cuando comparado con grupo C. Le LDL-C (colesterol de la lipoproteína de baja densidad), la glucosa y el ácido úrico del grupo F fueron reducidos el 22%, el 78% el 64%, respectivamente, en compararon al grupo C. Todos los resultados juntos sugieren que la suplementación del 20% con la linaza pudiera ser importante a prevenir desordenes cardiovasculares.

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Palabras clave: *Desórdenes cardiovasculares. Linaza. Perfil del lípido. Prevención. Suplementación. Ácido úrico.*

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Abbreviations

C: Control group.
F: Flaxseed group.
IC: Internal control group.
HDL-c: High-density lipoprotein cholesterol.
LDL-c: Low-density lipoprotein cholesterol.
CVD: Cardiovascular disease.
LabNE: Laboratory of Experimental Nutrition.
RJ: Rio de Janeiro.
Ltda: Limitada.
PE: Pernambuco.
SP: São Paulo.
i.p.: intra-peritoneal.
EDTA: Ethylenediaminetetraacetate.
TC: Total cholesterol.
TAG: Triacylglycerols.
BW: Body weight.

Introduction

Substantial evidence from epidemiological and experimental studies indicate that a Western-style diet, high in fat and red meat as well as diet low in fibers and vegetables, increases the risk of cardiovascular disease (CVD).¹ Hence, identification of dietary constituents that prevent CVD is important and a major focus of research in recent years. Recently, there has been a keen interest in the protective and therapeutic effects of certain plant chemicals on chronic diseases including CVD. Especially, dietary phytochemicals that consist of a wide variety of biologically active compounds have drawn a great deal of attention from both the scientific community and the general public owing to their demonstrated ability to prevent CVD.^{1,2}

CVD is a preventable chronic disease condition for most and is closely associated with a poor diet. Research has indicated an inverse relationship between fruits, vegetables, and fiber consumption, and the risk for heart disease.² A potential interest in the health benefits of functional foods is growing, such as flaxseed. Flaxseed content high amount of polyunsaturated fatty acids (particularly linolenic acid), vegetable protein, soluble fiber, and flavonoids. These related compounds that may possess cholesterol-lowering antioxidant and sex hormone agonistic and antagonistic activities.^{3,4}

There is evidence that whole flaxseed may lower serum cholesterol in hyperlipidemic subjects^{5,6}. On the other hand there is no considerable data available concerned the effect of flaxseed in non-hyperlipidemic subjects. Based on these considerations the aim of this study was to explore the effect of bioactive constituents of flaxseed in healthy animal model.

Materials and methods

Animals and experimental groups

We used 30 male Wistar rats, aged 21 days (after lactation), from the Laboratory of Experimental Nutrition (LabNE) of the Department of Nutrition and Dietetics, Nutrition College, Federal Fluminense University, Niteroi, RJ, Brazil.

The rats were divided into 3 groups (n = 10) as follows: the Control Group (C) was fed with a casein-based chow (10% protein; 5% fiber; 7% lipid); the Flaxseed Group (F) was fed with the casein-based chow supplemented with 20% flaxseed (10% protein; 7% fiber; 11% lipid); and the Internal Control Group (IC) was fed with the casein-based chow plus soybean oil and cellulose fiber (10% protein; 7% fiber; 11% lipid). The IC was created to provide an internal control for the enhanced lipid and fiber load in the F group diet. In this study, the percentage of flaxseed was based on previous experimental studies, which used dietary concentrations of 20-25%.^{7,8} Animals were fed exclusively with the diets specified above, from weaning until they were 180 days old. They were kept in polypropylene cages under a controlled temperature at 22°C and a 12 h light/dark period. Water and diets were provided ad libitum; food consumption (g) and body mass (g) were recorded daily.

The study was approved by the Ethics Committee in Clinical Research of Antonio Pedro Hospital, Federal Fluminense University (188/06 protocol), following the norms of the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication N 85-23, revised in 1996).

Experimental ration

Flaxseed was supplied by Armazem[®] Ltda (Rio de Janeiro, RJ, Brazil). The suppliers of the other dietary components were as follows: Maizena corn starch by Refinements of Maize[®] Ltda (Granhuns, Recife, PE, Brazil); refined sugar by União[®] (Rio de Janeiro, RJ, Brazil); Liza[®] soy oil by Cargill Agricultural[®] Ltda (Mairinque, SP, Brazil); Microcell cellulose by Blanver[®] Ltda (Cotia, SP, Brazil); and cysteine, choline bitartrate, casein, and mixtures of vitamins and minerals by Rhoster[®] Indústria e Comércio Ltda (Vargem Grande Paulista, SP, Brazil).

All diets were prepared at LabNE and contained 10% protein (1.75% nitrogen)/100 g. The mixtures of vitamins and minerals were added following the rules of the Committee on Laboratory Animal Diets, 1979, modified according to the recommendations of the American Institute of Nutrition-93⁹. The ingredients of the diets (table I) were homogenized in an industrial mixer with boiling water. The obtained mass was transformed into tablets, which were dried in a ventilated oven at 60°C for 24 h, properly identified, and stored under refrigeration (4 ± 2°C) until the time of use.¹⁰⁻¹²

Ingredients	Groups		
	C	IC	F
Flaxseed	–	–	25
Casein	10.87	10.87	10.87
Cornstarch	62.08	56.08	62.08
Sucrose	10	10	10
Mineral mix*	3.5	3.5	3.5
Vitamin mix*	1	1	1
Soybean oil	7	11	7
Cellulose power	5	7	5
Choline bitartrate	0.25	0.25	0.25
L-cystine	0.3	0.3	0.3

C, Control group; IC, Internal Control group; F, Flaxseed group; *According to AIN-93G, see Reeves et al. (1993) for more details.

Biochemical analysis

At the end of the feeding period, after an overnight fast, the animals were euthanized under thiopental anesthesia (0.10 mL/100 g body mass, i.p.) and blood was drawn by cardiac puncture. Blood was collected in tubes containing ethylenediaminetetraacetate (EDTA; 1.4 g/L) and Trasylol (100 kU/L), and serum was separated for subsequent biochemical analyses. The levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triacylglycerols (TAG), glucose and uric acid were determined using commercial kit from LABTEST® (Rio de Janeiro, RJ, Brazil).

Statistical analysis

Descriptive data are reported as means \pm standard deviation, and the results were analyzed statistically by a multiple comparison one-way analysis of variance, with the level of significance set at $p < 0.05$. When a statistically significant difference was detected between variables, the Scheffé test was applied using the Bonferroni coefficient for multiple comparisons. All statistical analyses were performed using SPSS for Windows 10.0.

Results

Body weight (g), ration intake (g), protein intake (g) and caloric intake (kcal/g/day).

There was no difference in ration intake, protein intake and average daily energy intake (kcal/g/day) between the experimental groups. However F showed lower ($p < 0.05$) body weight at the end of the experimental period in relation to the C and the IC, which reflected a less variation in weight of F group ($p < 0.05$).

Table II
The initial and final body mass, diet intake, growth index, and biochemical parameters of the different groups

Variables	Groups		
	C	IC	F
Initial BM	48.1 \pm 5.3	47.1 \pm 3.2	47.8 \pm 2.9
Final BM	438.6 \pm 35.2 ^a	464.6 \pm 43.2 ^a	322.1 \pm 24.0 ^b
BM variation	390.5 \pm 12.2 ^b	376.2 \pm 15.3 ^b	274.3 \pm 8.5 ^a
Ration intake (g/day)	10.2 \pm 1.2	9.5 \pm 2.6	9.3 \pm 2.3
Calorie intake (kcal/g/day)	32.44 \pm 8.3	40.44 \pm 6.2	38.3 \pm 8.5
Triacylglycerols (mg/dL)	66.2 \pm 4.7	68.7 \pm 5.3	65.5 \pm 7.8
Total cholesterol (mg/dL)	69.3 \pm 3.2	66.9 \pm 4.7	67.6 \pm 2.9
HDL-C (mg/dL)	17.3 \pm 0.9 ^a	16.5 \pm 1.2 ^a	25.2 \pm 3.5 ^b
LDL-C (mg/dL)	37.3 \pm 1.2 ^a	37.5 \pm 2.0 ^a	29.7 \pm 1.3 ^b
Glucose (mg/dL)	142.7 \pm 5.9 ^a	149.8 \pm 8.7 ^b	112.5 \pm 4.6 ^c
Uric acid (mg/dL)	1.82 \pm 0.1 ^a	1.89 \pm 0.2 ^a	1.13 \pm 0.2 ^a

BM, body mass; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; C, Control group; IC, Internal Control group; F, Flaxseed group; Results are presented as means \pm SD. Numbers followed by different superscript letters are statistically significant ($p \leq 0.05$).

Biochemical profile

To evaluate the flaxseed supplementation on biochemical profile we collect blood samples of the rats and some biochemical parameters were analyzed that were present in table II. The values of TC and TAG were similar in all groups. F group showed increase of 47% and 53% in plasma HDL-C in relation to the C and IC groups, respectively. Additionally the average of LDL-C was 20% less in F group than in C and IC groups. The values of serum glucose decreased ($p < 0.05$) in F group, as the uric acid levels were 34% and 44% less in F group when compared to C and IC, respectively.

Discussion

Renewed interest in flaxseed, as an important source of human nutrition has arisen both out of a shift to “natural foods” in the diets of developed countries and out of growing evidence of direct effects on health. We demonstrated that a supplementation with 20% of flaxseed is able to diminish cardiovascular risks improving the biochemical profile as the body weight.

Our findings showed that the all groups fed the same amount of ration but the supplemented group with 20% flaxseed (F) presented lower BW (body weight) compared to non - supplemented groups. Freedland and Aronson¹³ reported that the consumption of flaxseed has favorable effects on the BW and fat distribution of experimental animals. A part of these benefits could be attributed not only to the type but also to the amount of insoluble fiber present in flaxseed. Therefore, although the IC group had the same amount of fiber and oil, the F had a lower BW than IC. This result suggests that the

presences of natural fibers from flaxseed as their bioactive compounds are able to reduce the BW.

It is well established that alterations on lipid profile is a common pattern of cardiovascular diseases, mainly elevated levels of triacylglycerols, total and LDL cholesterol, besides decreased HDL-cholesterol.¹⁴⁻¹⁶ In our study we used healthy rats to evaluate the influence of flaxseed as a supplement (20%) in a balance diet. The analysis of lipid profile revealed that the flaxseed had no influence in TC and TAG. On the other hand diminished the LDL-C and elevated HDL-C concentrations. Our findings corroborate with previous reports that demonstrated modulation of lipid metabolism with supplementation with flaxseed. Whether the hypolipidemic effects of whole flaxseed are due to a single component or the interactions among its components remains unclear. Kuroda et al.¹⁷ evaluated the hypolipidemic properties of a series of diesters of aryl-naphthalene lignans. They reported that these synthetic lignans effectively lower serum total cholesterol and LDL-C while increasing HDL-C. Lignans have also been shown to modulate activities of 7-hydroxylase and acyl CoA cholesterol transferase¹⁸, two of the key enzymes involved in cholesterol metabolism. Prasad et al.¹⁹ concluded that reduction in hypercholesterolemic atherosclerosis by flaxseed is due to a decrease in serum total cholesterol and LDL cholesterol and that the antiatherogenic activity of flaxseed is independent of its α -linolenic acid content. Soluble fiber mucilage present in flaxseed may also contribute to the observed hypocholesterolemic properties.^{20,21} Hence, the mode of action of flaxseed is unclear and needs to be investigated in future studies. Flaxseed is also a rich source of lignans, with potential weakestrogenic and antiestrogenic activity similar to that of the isoflavones found in soy.²² These plant-derived sex hormone analogues have attracted attention as possible anti-atherogenic agent. In addition to their estrogenic activity, if lignans block androgen or progesterone receptors, they may alter the cardiovascular disease risk profile by changing HDL-cholesterol metabolism.²²⁻²⁴

Not only lipid profile, but also others marks, as glucose levels are important to control a good health. In our study, the flaxseed supplementation reduces the glucose levels in 78% when compared to control. The mechanism by which bioactive compounds from flaxseed may influence blood glucose levels remains unclear.

Some studies with Omega-3 fatty acids from flaxseed have been found to alter whole body insulin sensitivity in non-diabetic animals. Changes in insulin response also have been observed in healthy men fed omega-3 fatty acids from flaxseed for a 2- to 3-month period.²⁵ In contrast, insulin sensitivity was not affected when administered to men with coronary heart disease²⁶ or hypertension²⁷ showing that flaxseed supplementation is effective when administrated as a preventive therapy.

In the present study, the consumption of 20% of flaxseed was able to decrease the plasmatic concentration of uric acid. A similar reduction of serum uric acid levels has been observed in rats fed pectin-enriched diets,²⁸ olive heat,²⁹ polyphenols,³⁰ cinnamon,³¹ apple³² and green tea.³³

Various observations suggesting that uric acid may actually increase the risk of cardiovascular diseases.³⁴⁻³⁶ A higher serum uric acid has been associated with increased cardiovascular risk disease in a prospective cohort case-control study.^{37,38} A higher level of uric acid in the plasma could result from an increased xanthine oxidase activity, a known source of superoxide free radicals, resulting in an impaired vascular function as observed in hypercholesterolemic rabbits.³⁹ A higher plasma antioxidant capacity, often linked to an increase of uric acid level, could therefore rather be regarded as a risk factor of hypercholesterolemia rather than a protective factor as commonly considered in the field of antioxidants.⁴⁰

The meaning of a relatively high uric acid level in unstressed conditions or in the general population is less clear, as well as that of variations induced by dietary antioxidants. As already stressed above, uric acid level and antioxidant capacity measured in plasma and serum are often unaffected in polyphenol intervention studies.³² Furthermore, this raises concerns about the value of such serum biomarkers, and interpretations should be made with caution when evaluating the potential health effects of bioactive compounds present in food. The strong reduction of uric acid in plasma observed here after supplementation with flaxseed may actually indicate a reduction of oxidative stress and an improved vascular function, and explains the reduction of LDL-C and increased of HDL-C. The exact mechanisms are not known. These effects could be explained by an inhibition of uric acid renal reabsorption or an inhibition of xanthine oxidase as has been shown in the rat with various functional foods.³²

Taken all results together we might conclude that 20% of flaxseed supplementation in balanced diet may play a role in preventing cardiovascular disease, notably by increase of HDL-C and decreasing HDL-C, glucose, uric acid plasma level. However, precise mechanisms implicated in these processes have to be established.

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