doi: 10.13037/rbcs.vol13n43.2433 ISSN 2359-4330
Rev. de Atenção à Saúde, v. 13, nº 43, jan./mar. 2015, p. 11-17

THE INFLUENCE OF FLAXSEED ON THE BODY WEIGHT OF WISTAR OVARIOCTOMIZED RATS

INFLUÊNCIA DA SEMENTE DE LINHAÇA SOBRE O PESO RELATIVO CORPORAL DE RATAS WISTAR OOFORECTOMIZADAS

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Data de entrega do artigo: 06/11/2013
Data de aceite do artigo: 17/04/2014

ABSTRACT

Objectives: To evaluate the influence of flaxseed for a long period of time and its hormonal effects of weight loss in castrated rats. **Methods**: We used 21 *Wistar* rats that underwent bilateral oophorectomy for induction of menopause, randomly divided into three groups: control group (CG) (n = 7), which received a diet based on casein 10 percent; flaxseed group, that received a diet based on casein plus 25 percent of flaxseed (FG) (n = 7); and modified control group, that received a diet based on modified casein 10 percent and with addition of lipid and fibers (CGM) (n = 7). All diets were prepared according to the recommendations of the AIN 93, the animals were kept under controlled temperature (22ºC ± 2ºC) and adequate lighting (light/dark cycle of 12 to 12 hours). The animals received food and water (*ad libitum*). The rats were anesthetized with Thiopentax® for blood collection by cardiac puncture for the determination of 17β-estradiol and collection of tissues (liver, bladder and uterus). Data was applied to statistical analysis using the nonparametric Kruskal-Wallis test and applied post hoc Mann-Whitney U for two by two comparison through Software S-Plus version 8.0, with the level of 0.05 for the significant differences. **Results**: The results show that the concentration of 17β-estradiol of FG was 40.2 percent higher than the CG and 25.77 percent higher than CGM, suggesting the estrogenic action of the flaxseed. In the body weight of the animals there were significant differences, suggesting a greater weight of the GC. There were no significant differences in the relative liver (p=0.2405) and bladder (p=0.7734) weight between the groups, andt there was no significant difference in the relative weight of the uterus (p=0.7922) between the casein and flaxseed groups. **Conclusion**: The consumption of 25 percent of flaxseed during menopause influenced on body weight, suggesting a phytoestrogen action in the consumption of seeds during the physiological stage.

Keywords: flaxseed, hormonal effects, body weight, ovariotomy.

RESUMO

Objetivos: Avaliar a influência da semente de linhaça por um longo período de tempo e seus efeitos hormonais sobre o peso relativo corporal em ratas ooforectomizadas. **Material e métodos:** Foram utilizadas 21 ratas *Wistar*, submetidas à ooforectomia bilateral para indução da menopausa, sorteadas aleatoriamente em três grupos: Grupo Controle (GC) (n=7), que receberam ração à base de caseína 10%; o Grupo Linhaça (GL) (n=7), que recebeu ração à base de linhaça 25%; e o Grupo Controle Modificado (GCM) (n=7), que recebeu ração à base de caseína modificada 10% e adição de lipídio e fibras. Todas as rações foram elaboradas segundo as recomendações da AIN 93, os animais mantidos em ambiente com temperatura controlada (22ºC ± 2ºC) e iluminação adequada (ciclo claro/escuro de 12 em 12 horas). Os animais receberam ração e água (*ad libitum*). As ratas foram anestesiadas com Thiopentax® para a coleta do sangue por punção cardíaca, para determinação do *17β*–Estradiol. Foi realizada coleta dos tecidos: fígado, bexiga e útero para determinação do peso relativo corporal. Aos dados foram aplicados a análise estatística, utilizando o teste não paramétrico de *Kruskal-Wallis* e aplicado teste post *hoc U de Mann-Whitney* para comparação dois a dois por meio do Software S-Plus versão 8.0, com o nível de 0,05 para as diferenças significativas. **Resultados:** Os resultados mostram que a concentração do *17β*-estradiol do GL foi 40,2% superior ao GC e 25,77% maior que GCM, sugerindo a ação estrogênica da semente de linhaça. No peso relativo corporal dos animais houve diferença significativa, sugerindo maior peso para o GC. Não houve diferença significante no peso relativo do fígado (p=0.2405) e bexiga (p=0.7734) entre os grupos. Já no peso relativo do útero (p=0.7922), não houve diferença significativa entre o grupo caseína e linhaça. **Conclusão:** Conclui-se que o consumo de 25% de semente de linhaça durante a menopausa influenciou no peso relativo corporal, sugerindo ação fitoestrógena da semente durante a fase fisiológica.

Keywords: flaxseed, hormonal effects, body weight, ovariotomy.

INTRODUCTION

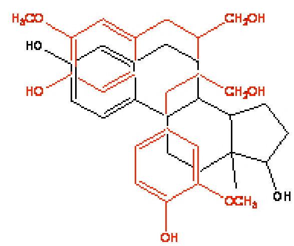
The flaxseed (*Linum usitatissinum*) is an oilseed often used as a food supplement, and it is a source of soluble and insoluble fiber, protein (providing 20% of its composition), 41% fat, 6% carbohydrate and 4% of waste (1). This seed is considered a phytoestrogen, which produces metabolic and physiological benefits (2). The consumption of flaxseed has been beneficial to health in different conditions, such as cardiovascular disease prevention, reduction of cholesterol and triglycerides (4), minimizing the symptoms of menopause, exerting a beneficial effect on obesity (5), preventing osteoporosis (6) and certain types of breast and uterus cancer that are developed under the influence of hormones (7).

The flaxseed is recognized as a major food source of lignans (52.7 mg/100 g seed flour) on its diglucosidic (8,9) compounds with similar chemical structure to estrogen (10), specifically the Secoisolariciresinol Diglucoside (SDG) (figure 1) that after been absorbed through the gastrointestinal tract and distributed to the tissues have the ability to bind to estrogen receptors (ER α and β) (11,12) acting as agonists or antagonists, depending on the amount of it in the body (13). In premenopausal stages these lignans act as antagonists, reducing the action of this hormone (14). In post-menopause and menopause stages, when estrogen levels are naturally ceased, the lignans act as agonists, similar to the effects of estrogens, although less potent (15).

Among the estrogens, estradiol is the most important and abundant in the blood circulation (16), but when bound to albumin it has the feature of decoupling quickly as it is released in the active form in various tissues with estrogen receptors (17,18)

Estrogen receptors have high sensitivity and affinity for a specific hormone estrogen produced in the body. Therefore, extremely low concentrations of hormone produce a particular effect, inducing a natural response. However, these hormone receptors bind to other substances such as phytoestrogens, even at very low concentrations, and are capable of generating purposes, thus provoking responses agonists or antagonists (19).

Figure 1: Chemical structural similarity between Estradiol and Secoisolariciresinol Diglucoside



Hu et al., 2007.

Despite the benefits attributed to the consumption of flaxseed, its use in stages of menopause and post menopause has sparked interest in the scientific community. The association of flaxseed and menopause remains uncertain because of the prevalence of central obesity and overweight (20,21) among female population in this physiological stage (22), due to the complex physiological and psychological factors involved (23), such as the reduction of lean mass (22), a reduction in the metabolism that alters the hormonal balance and energy expenditure (24).

Little is known about consumption of seed exclusively at menopause, because the results of studies on the subject were very contradictory, varying greatly depending on the concentration of flaxseed that was used. This study aims to observe the effects of consuming 25 percent of flaxseed at menopause for a long period of time and its hormonal effects on weight loss of ovariectomized female rats.

Materials and methods

Ethical aspects

This project was approved by the Ethics Committee in Research of Hospital Antônio Pedro/UFF, Protocol

188/06. In order to be able to develop the assay, this project was approved by the Ethics Committee for Animal Research (NAL)/UFF, under the Protocol 00103-09 and followed the protocol standards set out in Guide for Care and Use of Laboratory Animals published by U.S. National Institutes of Walt. This project was conducted within the ethical principles of Good Practices of the Brazilian College of Animal Experimentation (COBEA).

Delineament Experiment

In the biological assay of 180 days, we used 21 female animals, newly weaned *Wistar* (*Rattus norvegicus*, *Albinus* variety, order *Rodentia mammalia*, family *Muridae*) from the colony of the Laboratory of Experimental Nutrition of the Department of Nutrition and Dietetics in the School of Nutrition, at the Fluminense Federal University, in Niterói, RJ, Brazil. After the weaning in approximately 21 days of life, the animals received a commercial diet (Cr-1 Nuvilab Autoclavable-Nuvital®) until they could be considered adults. In 90 days, the necessary period of time for a normal animal to reach adulthood after his birth, the rats underwent bilateral ovariectomy to induce an early menopause, according to (25). After surgery, the animals were fed commercial for 30 days. After this period, which was the time required in order to achieve a status of menopause, the animals were randomly selected and divided into groups, which will be detailed below.

At the end of 180 days of experiments, the animals were anesthetized with thiopental (0.15ml/100g, intraperitoneal) in order to allow the researchers to perform the blood collection by cardiac puncture for determination of 17 β-estradiol. For this procedure it was used a hypodermic needle into Vacutainer tubes with capacity of 10 ml. The tubes containing blood were centrifuged (Sigma) for 30 minutes at 3500 revolutions per minute (RPM) (26) to obtain the serum and after it the tubes were stored in the freezer-20 for determination of the serum 17 β-estradiol in animals. The tissues were collected, weighed on a precision scale and stored in nitrogen at -80 °C.

Throughout the test, the animals were kept in plastic cages in an environment with constant temperature (22 ºC ± 2 ºC) and adequate lighting (light/dark cycle of 12 to 12 hours). Water was offered (*ad libitum*), and the diet, weight and feed intake were recorded every two days.

Experimental groups and diets

The animals were divided into groups (n=7): a control group (CG), which was fed with a casein diet with 10 percent of protein, 5 percent of fiber, and 7 percent of oil; the Flaxseed Group (FG), which was fed with a casein diet with 10 percent of protein, plus 25 percent of flaxseed meal, 7 percent of fiber and 11 percent of lipid; and Modified Control Group (MCG), which was fed with a casein diet, with 10 percent of protein, 7 percent of fiber and 11 percent of oil.

The insertion of the Modified Control Group was made by the chemical composition present in flaxseed, which is a good source of lipids and has high fiber content. In order to obtain parameters for comparison of diets, we aimed to increase the concentrations of their constituents so that they would present similar chemical compositions to their compositions.

To obtain the flour milling of flaxseed the process was performed on fresh seed mill Fritsch Pulverisette®, that held the weight of flaxseed meal resulting from this process they were bagged, sealed and stored in refrigerator (4 °C) until used for the manufacture of ration. All diets were prepared according to the density (from lightest to heaviest) of the ingredients in pet food. Then they were mixed in the container, and subsequently taken to the mixer for homogenisation. After that, the pellets were placed in stainless steel trays and placed in an oven (55-60 °C) for 24 hours and after identification, the ration resulting from this process was refrigerated until use.

All diets were added to the mixture of minerals and vitamins, according to rules of the Laboratory of Animal Diets on Committee, of 1979, modified in accordance with the recommendations of the American Institute of Nutrition-AIN 93G.

Methods

Surgical: Bilateral Oophorectomy

This procedure followed the standards of vivisection of animals set by the Brazilian College of Animal Experimentation (COBEA) under the Law nº 6638 of May 8, 1979. The surgical procedures were performed in the morning. Before the procedure, the rats underwent eight hours of fasting. We used intramuscular anesthesia with ketamine (Cristalia ® -100mg/kg the animal intramuscule) and xylazine (Anasedanâ Vetbrands® - 20 mg/kg of animal intramuscule). Moreover, the animals received analgesia with tramadol (TramaLive Teuto ® - 5mg/Kg the animal intramuscule), which was injected 1-2 minutes after surgical incision in order to minimize the suffering of rats in the postoperative period (Laboratory Animals published by U.S. National Institutes of Walt).

The animals were placed in a supine. After trichotomy, antisepsis was performed with iodine alcohol. A bilateral oophorectomy was performed through a midline incision of approximately 4 cm in length. After opening the abdominal cavity, the ovaries were identified and their heel ends were clipped and ligated using catgut 3/0 simple.

The ovaries were then removed and the ligature were reviewed. A raffia in the peritoneum was performed and also a with a 3/0 chromed catgut. The skin was closed with separated mononylon 4 / 0 according to the methodology described by Biondo-Simões *et al.* (25).

Biochemical Analysis: Hormonal

The animals were anesthetized with intraperitoneal thiopental (Thiopentax® ml/100g 0.15 pc, intraperitoneal) and the blood was collected by cardiac puncture using a hypodermic needle and vacuum tubes (Vacueete) with capacity of 10ml. The blood collected in tubes was centrifuged in a centrifuge (Sigma®) for 30 minutes at 3500 rpm (revolutions per minute) (26) to obtain the serum. This was separated and fractionated in eppendorffs, one of them for determining concentrations of *17 β*-estradiol, and stored in freezer at -20°C.

The hormonal assays were determined by specific radioimmunoassay for each hormone, using the biochemical kit from Diagnostic Products Corporation – CPS ®, Tortelly Carlos Municipal Hospital, in Niterói, in a VITALAB Selectra® apparatus.

Biological Analysis: Determination of the relative weight and body tissues (liver, bladder and uterus).

Before all animals were weighed, we made sure they were unfed, in order to remove the organs. We used forceps and scissors for the procedures, and they were weighed on an analytical Bosch brand balance, model S2000, accurate to 0.0001 g.

This procedure was performed to calculate the relative weight of tissues and relative body weight, which calculated the weight of each organ and divided it by the body weight. The result was multiplied by 100, being indicated in percentage (%).

Statistical Analysis

The results displayed below include mean and standard deviation (SD). The obtained results were submitted to the Kruskal-Wallis test for an analysis of variance. When a significant difference was detected, the *Mann-Whitney U* test for comparison of two by two was applied *post hoc*.

These statistical analysis were performed by the software S-Plus version 8.0, with a level of significance of 0.05.

Results

Table 1: Composition of experimental diets (g/100g).

Groups Ingredients	CG	CGM	FG
Flaxseed[1]	–	–	25,00
Casein[2]	10,87	10,87	5,43
Starch[3]	62,08	59,08	54,52
Sugar[4]	10,00	10,00	10,00
Minerals mix[1]	3,50	3,50	3,50
Viitamins mix[1]	1,00	1,00	1,00
Oil[5]	7,00	11,00	–
Celullose[6]	5,00	7,00	–
B-Choline[1]	0,25	0,25	0,25
Cystine[1]	0,30	0,30	0,30
Total	100,00	100,00	100,00

Note: The CG was fed with a casein diet with 10 percent of protein, 5 percent of fiber and 7 percent of oil, the MCG was fed with a casein diet with 10 percent of protein, 7 percent of fiber and 11 percent of oil, and the FG was fed with a casein diet with 10 percent of protein supplemented with 25 percent of flaxseed meal, 7 percent of fiber and 11 percent of oil.

Table 2: Approximate chemical composition of experimental diets

Chemical composition (g/100g)	Casein	Casein modification	Flaxseed
Moisture %	2,23 ± 0,04	2,35 ± 0,04	1,95 ± 0,08
Lipids %	7,79 ± 0,30	11,79 ± 0,12	11,70 ± 0,12
ash %	4,27 ± 0,02	4,47 ± 0,07	4,42 ± 0,07
Protein %	9,77 ± 0,45	9,75 ± 0,45	10,18 ± 1,06
Fibers %	5,00 ± 0,34	6,70 ± 0,52	6,98 ± 0,47
Carboydrate %	70,91	64,92	64,77
BEV*	392,83	379,8	379,8

* BEV= Baseline Energy Value

In order to study the use of flaxseed, it is supplemented in experimental diets. There is still a consensus in the literature regarding the daily recommendation of this seed, therefore, we used about 25 percent of it in the ration. We chose to use the seed in the form of flour because it allows a better use of its functional properties.

Determination of 17β-estradiol

The concentration of 17β-estradiol FG (48.7 ± 12.31 pg/dl) was 40.2 percent higher than in CG (29.0 ± 5.4 pg/dl) and 25.77 % higher than in MCG (36,0 ± 5.60 pg/dl), suggesting the estrogenic action of flaxseed (P<0.0012).

Determination of relative body weight

There were significant differences in the body weight in the groups on flaxseed and casein, that had a higher value (p<0.0013). In the Modified Casein Group the higher value (p< 0.002) had no significant difference in comparison to the other groups. (Table 3).

Determining the relative weight of tissues

There were significant differences in relative liver weight (p<0.2) and in the relative weight of the bladder (p<0.5) between the groups; the casein group presented heavier weights (Table 4 and Chart 2). The weight of the uterus (p<0.9) did not differ between the casein and flaxseed group, but the modified casein and flaxseed group showed significant differences (Table 4 and Chart 3).

Table 3: Relative body weight of the bladder, uterus and liver and serum estradiol values.

	CG	MCG	FG
Relative Weight body (g)	294 ± 33.1	339.5 ± 24.6	275.75 ± 33.9
Relative Weight of the bladder (mg)	0.01 ± 0.003	0.01 ± 0.002	0.01 ± 0.013
Relative Weight if the uterus (mg)	0.01 ± 0.01	0.02 ± 0.06	0.02 ± 0.06
Relative Weight of the liver (mg)	0.06 ± 0.01	0.07 ± 0.025	0.08 ± 0.01
Estradiol (pg/dl)	29,0 ± 5,4	36,0 ± 5,60	48,7 ± 12,31

Values are expressed as ± SD. There are statistical differences in the groups, measured by the Mann-Whitney test (p>0.05). FG: Flaxseed Group, CG: Control Group, CMCG: Modified Control Group.

Discussion

The dietary fibers are able to accelerate intestinal transit, and promote satiety, helping to reduce the body weight (26,27,28,29,30), among other functions. In this study, we observed that flaxseed has a high concentration of soluble and insoluble fibers, suggesting that variation of weight in the FG is presented below the CG, since the fibers are able to help in the reduction of the body weight (31,32,33). Moreover, the presence of phytates in this seed can affect the bio-utilization of nutrients in the ration to which was added, which may be diminishing the weight of animals (34). Animal studies that used flaxseed also found lower weight gain in those who consumed the seed. (35).

Several authors (36) conducted studies with estradiol-treated ovariectomized rats that gained less weight, while animals receiving progesterone hormone replacement therapy lost weight. Moreover, similar results to the ones in our study (37), did not find weight alterations in the tissues after feeding rats with flaxseed or defatted flaxseed for eight weeks.

There is a close relation between the action of phytoestrogens in the flaxseed and lower relative body weight gain and menopause, suggesting that the influence of seed increased energy consumption and consequently lowered the body weight. The estrogenic action, specifically the SDG (Figure 1), in this seed significantly increased serum levels of 17β-estradiol, influenced by an interaction of hormone receptors in animal organism (13). This is an extremely important observation, since the majority of circulating estradiol binds to estrogen receptors, including the phytoestrogens.

It was found that an increase in hormone levels of FG could be associated with high concentration of lipid present in this diet, which stimulates the synthesis of this hormone in other ways besides in the ovaries. However, several papers reported that the influence of phytoestrogens on serum hormones is still uncertain (15). In one of these studies, conducted by seven weeks with menopausal rats, the consumption of 5 to 10 g/day of flaxseed significantly reduced the concentration of estrogen. Lucas *et al.*, 2003 (3) conducted a study for 3 months with menopausal rats fed with 40g/day of flaxseed has found a slight increase in estrogen levels, which confirms our results, since the rate was higher and there was a hormonal influence of the seed.

According to literature, flaxseed may produce different results depending on the concentration used and the period of consumption. To date, this is the first study to examine the effects of 25 percent of flaxseed in a diet during menopause and the results that were found corroborate with an earlier study that demonstrated the absence of effects on hormonal levels and on body weight, using different concentrations of flaxseed. This observation is very important, since the benefits of the consumption of phytoestrogen are potential for the health of women and also for the general population.

Flaxseed is a good option when compared to other phytoestrogens because it presents the largest source of lignans found in nature and its consumption during menopause does not cause damage to health.

Conclusion

The obtained results show that the intake of 25 percent of flaxseed during menopause influences positively on the reduction of the body weight and serum levels of 17 ß-estradiol, suggesting a positive action in the consumption of seeds during this physiological stage.

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