Original
Flaxseed energy and macronutrients balance

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Abstract

Background/objectives: Flaxseed has functional properties in the reduction of the risk of chronic non-communicable diseases such as cardiovascular disease, diabetes and cancer. Regardless of its high energy density, the consumption of flaxseed tends to promote body weight maintenance. The purpose of this study was to evaluate energy and macronutrient balance after flaxseed consumption.

Subjects/methods: Twenty four healthy volunteers were allocated into 3 experimental groups, when they consumed flaxseed (FS), defatted flaxseed flour (FF), or flaxseed oil (FO). During the control period they were provided a diet without flaxseed products for 7-9 days. Following that diets containing 70 g of one of the flaxseed products were consumed for another 7-9 day-period. Test foods were consumed exclusively in the laboratory and fecal excretion was collected during the study. There was a higher energy excretion (P < 0.05) in the FF and FS groups, compared to their control and FO group.

Results: The excretions of total lipid and the PUFA α-linolenic acid were higher in FS group (P < 0.05).

Conclusions: The intake of 70 g/day of FS and FF raised lipid and energy excretion, which may mitigated the effect of flaxseed consumption on body weight.

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Key words: Flaxseed. Flaxseed oil. Flaxseed flour. Energy balance.

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Introduction

The Latin American Consensus on Obesity reported an increase of 53% in the prevalence of overweight and obesity in Brazil between 1975 and 1989, especially among lower income groups. Furthermore, the World Health Organization reported that in 2001, 60% of 56.5 million deaths worldwide were caused by cardiovascular diseases (CVD) and that in 2020 the proportion will be 57% higher. These trends support efforts to prevent and manage such diseases.

Flaxseed has a high fat content (≈ 40%). This makes flaxseed an energy dense food, supplying 534 kcal/100 g, and a threat for promoting weight gain. However, weight maintenance has been observed in flaxseed consumers.

The objective of this work was to evaluate energy and macronutrient balance after flaxseed (rich in ω-3 fatty acids and fiber), flaxseed defatted flour (without ω-3 fatty acids and rich in fiber) and flaxseed oil (only ω-3 fatty acids) consumption. It is possible that the differences compositions can intervene in digestibility, resulting increased or decreased excretion of nutrients.

Subjects and methods

Subjects

The sample size was calculated to have 24 participants (8 per group), based on Mera Thompson and Prasad, using the energy excretion as the principal variable. It was considered the difference of 15% in relation to the mean ± SD of the energy excretion of 6.925 ± 0.65%. The statistical power of 90% and the significance level of 5% were adopted.

Intervention survey

The study consisted of two sessions of controlled feeding: control and one of three test diets. Body weight, height, blood pressure, heart rate, blood glucose, cholesterol levels, waist and hip circumferences, total body fat, lean mass, body water and energy metabolism were measured during screening. Participants were instructed to keep the same level of activity during the study. During the control session (baseline period), they were provided a diet without flaxseed products for 6-10 days. The study duration was based on individual variability in bowel habits. Following the baseline period, participants were randomly assigned to receive diets containing 70 g of one of flaxseed products (flaxseed, defatted flaxseed flour or flaxseed oil) for a seven- to nine- day period, depending on their bowel function. Dietary compliance was assessed by random finger stick blood glucose tests prior to meals. Additionally, upon arrival each day a small saliva sample of each participant was collected, and they were informed that the sample would be used for compliance analysis. However, such test was not conducted. Fasting blood glucose values between 65 and 110 mg/dL was considered as indicative of subject compliance with the study protocol.

Dietary intervention

The test diet provided during the control sessions differed from the treatments by the absence of flaxseed (FS), defatted flaxseed flour (FF) or flaxseed oil (FO). These last three isonertetic diets provided a mean of 2495 ± 23 kcal per day, 55/30/14% of energy provided as carbohydrate, fat and protein, respectively, for all volunteers, woman and man, as determined by the soft-

Abbreviations

CVD: Cardiovascular Diseases.
FS: Flaxseed.
FF: Defatted Flaxseed Flour.
FO: Flaxseed Oil.
ANOVA: Analysis of Variance.
REE: Resting Energy Expenditure.
EER: Estimated Energy Requirements.
AMDR: Acceptable Macronutrient Distribution Ranges.
SFA: Saturated Fatty Acids.
MUFA: Monounsaturated Fatty Acids.
PUFA: Polyunsaturated Fatty Acids.
F: Female.
M: Male.
BMI: Body Mass Index.
WC: Waist Circumference.
HC: Hip circumference.
WHR: Waist and Hip ratio.
Participants were served a four-day menu, which was repeated during each treatment period. They were required to eat all the foods served in the laboratory. Small fat-free evening snacks were provided to each participant and its consumption was optional. Participants were instructed to return any uneaten snack the next day. Dietary energy and macronutrient intakes during the study are presented in Table I.

**Test products and meals analysis**

The nutritional composition and caloric content of test products and daily menus served to volunteers were analyzed. Daily meals (breakfast, lunch and dinner) were homogenized in a Simer® LS-10L industrial blender prior to the analysis. The optional night snacks were analyzed separately.

The moisture content of the meals was determined in a Super Modulyo, Edwards® freeze-dryer at -60°C, during 24 to 48 h, until a stable weight was obtained. For total lipids, a modified method of Folch 11 was used. A lipid extract of 0.1 g of lyophilized sample was obtained by adding 1.9 mL of chloroform-methanol mixture 2:1, by homogenizing it in a model AP56 Phoenix® vortex-type tube shaker for 3 minutes, by adding 0.4 mL of methanol, and centrifuging it at 150 g. After transferring the supernatant to another tube, 0.8 mL of chloroform and 0.64 mL of solution of NaCl in water (0.73%) was added. After homogenization, centrifugation and discarding of the supernatant the tube inner wall was washed 3 times with Folch solution (made of 3% of chloroform, 48% of methanol, 47% of water and 2% of NaCl in water at 0.29%), and the tube containing the lipid extract was kept in a Quimis® open heater at 60°C. Then, they were cooled in desiccator and weighed. The analysis was done in duplicate.

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The total caloric value was measured in a model NSI 13 Parr Instruments® oxygen calorimeter bomb by direct calorimetry.

**Anthropometric assessment**

Volunteers were weighed in the morning on a Filizola® electronic scale, capacity of 150 kg accuracy of 100 g, wearing minimal clothing. Height was measured with an anthropometer of 200 cm, with 1 mm precision (Seca®).

Waist-hip ratio was measured using an inelastic measuring tape.14,15

The percentage of total body fat, percentage of lean mass and percentage of body water were measured by the method of bioelectrical impedance analysis, Biodynamics model 31016.

**Biochemical assessment**

Cholesterol (Roche® Accutrend GCT device) and glucose (Johnson & Johnson’s® One Touch Basic equipment) concentrations at baseline were determined by finger prick analyses.

**Blood pressure and heart rate**

Blood pressure and heart rate were measured using the Model HEM-711AC Automatic Blood Pressure Monitor with IntelliSense™ at the beginning and at the end of each study period.

**Basal metabolism assessment**

Energy expenditure was assessed by indirect calorimetry, with Datex Engstrom® Deltatrac II metabolic monitor, for 60 continuous minutes. Nitrogen excretion was standardized at 13 mg N/day.

The volunteers were driven to the Laboratory of Energy Metabolism, after 12 h fasting, 8 hours of sleep and remained at rest for 30 minutes after arrival.

**Table I**

<table>
<thead>
<tr>
<th>Composition of the daily menu planned for the volunteers in the control and test periods (mean ± standard deviation)</th>
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</thead>
<tbody>
<tr>
<td>Carbohydrate* (%)</td>
</tr>
<tr>
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<tr>
<td>C (n = 24)</td>
</tr>
<tr>
<td>FS (n = 8)</td>
</tr>
<tr>
<td>FF (n = 8)</td>
</tr>
<tr>
<td>FO (n = 8)</td>
</tr>
</tbody>
</table>

*Means did not differ by analysis of variance at 5% probability.

C = Control; FS = Flaxseed; FF = Defeated Flaxseed flour; FO = Flaxseed oil.

**e.g:** Breakfast Menu 1 – Muffins Control or with flaxseed oil or flaxseed or flaxseed flour with cashew juice and cereal with milk.
Measurements were made in a calm and temperature-controlled room.

Fecal collection

Two food dye markers were given in the form of pills (Red Carmine, and FD&C 13% blue aluminum lake, Sensient Technologies Corporation, St Louis, MI, USA) on day one of the control period, and again 3 days after the first marker was passed. Participants were instructed to collect all stools after the appearance of the first dye marker (red carmine) in the feces until the second marker (blue lake) was passed (typically a 4-day collection). They were provided with plastic urine hats, lidded cups and thermal boxes to assist them with quantitative collections. In addition, a privative facility with the materials needed for fecal collection was provided on campus for convenience. Samples were immediately frozen (at -24°C) after collection.

Fecal analysis

Fecal samples from control and intervention periods were weighed and homogenized for 3 minutes with water (1:2 w:w). Samples of the homogenate were dried in a lyophilizer (Freeze Dry System / Freezezone 4.5, Labconcor®), at about -50°C, and stored in a conventional freezer (-24°C). The composition of the homogenate was analyzed using the same methodology as used for the analysis of foods, except the lipid profile that was determined by gas chromatography. Fatty acid profile was determined using CG-17A Shimadzu/Class® chromatographer equipped with SP-2560 smelt silicon chromatographic column (100 m x 0.25 mm) and nitrogen carrier gas (linear velocity of 20 cm/s). Initial temperature was programmed at 140°C for 1 minute, at 2.5°C/minute addition until reaching 230°C; 1°C/minute addition until reaching 235°C; and 0.5°C/minute addition until reaching 240°C, remaining at 240°C for 15 minutes. Injection temperature was 250°C and detection temperature was 260°C. Peaks were identified by comparing the standard retention time periods (fatty acid methyl ester mixture from Sigma-Aldrich Ltd, St Louis, USA).

Statistical analysis

Descriptive statistics, including mean and SD (standard deviation) were used to describe the distributions of all variables. Parametric repeated measures analysis of variance (ANOVA) was used to examine treatment effects of the flaxseed products. The criterion for statistical significance was $P < 0.05$, two-tailed. Within treatment comparisons were conducted using paired t-tests. Statistical analyses were performed with Sigma Stat 3.0 software.

Results

There was no statistical difference on the biochemical, anthropometric or body composition parameters presented by the participants of the experimental groups (FF, FS and FO) (table II). The energy supply provided during the study met the needs of all volunteers, since each participant had a REE (Resting Energy Expenditure) equal or below 75% of the calories offered and they also maintained a constant physical activity level (average ± 30 min/day) during the study. The mean calculated EER (Estimated Energy Requirements) was 2,043.7 ± 125.8 and 2,573.6 ± 167.0 kcal for women and men, respectively. Thus, the caloric intake of the volunteers, 2,846.03 ± 111.31 kcal, was higher than their EER throughout the experiment. There was no change of weight for men or women. There was also no difference among the sessions concerning the anthropometric measurements taken (waist circumference, hip circumference, lean and fat mass) ($P > 0.05$) (table II).

There was a lower fat and protein intake in the FF and FO groups, respectively, during the control compared to treatment period (table III). However, the macronutrient of the food ingested during the study was within the acceptable distribution ranges (Acceptable Macronutrient Distribution Ranges-AMDR); 56% ± 2.1, 14% ± 1.96 and 30% ± 0.6.

There was no significant difference in energy intake between the groups during the control and treatment periods. However, comparing the intake between sessions for the same group, there was a significant increase in carbohydrate and reduction in protein intake in the FF group, while in the FO group, a significant increase was observed in all macronutrients and energy during the treatment period. There was a higher excretion of carbohydrate, protein and energy in the FF group, fat and energy in the FS group, and lower fat excretion in the FO group during the treatment period (table III).

In the treatment period, there was a lower excretion of carbohydrate by the FO compared to the FF group, lower excretion of protein in the FO group compared to the test groups, greater excretion of fat by the FS group compared to the other groups and lower caloric excretion in the FO group compared to FS and FF (table III).

The analysis of fecal fat revealed a significantly higher excretion of -linolenic acid in the FS group treatment period, with consequently higher excretion of PUFA compared to the FO and FF groups (table IV).

Discussion

Body weight is maintained constant when energy intake is equivalent to equals energy loss and expenditure. A positive energy balance was expected after the consumption the high fat, energy- dense flaxseed. Therefore, weight gain was expected especially in the
<table>
<thead>
<tr>
<th>Anthropometric parameters</th>
<th>FS (n = 8)</th>
<th>FF (n = 8)</th>
<th>FO (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treatment</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>52.5 ± 3.7</td>
<td>64.9 ± 6.0</td>
<td>52.7 ± 3.4</td>
</tr>
<tr>
<td>BMI (kg.m⁻²)</td>
<td>20.3 ± 1.3</td>
<td>21.6 ± 1.6</td>
<td>20.6 ± 1.2</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>65.0 ± 3.5</td>
<td>74.9 ± 3.2</td>
<td>65.7 ± 3.7</td>
</tr>
<tr>
<td>HC (cm)</td>
<td>93.9 ± 3.6</td>
<td>92.8 ± 4.2</td>
<td>94.1 ± 4.1</td>
</tr>
<tr>
<td>WHR</td>
<td>0.7 ± 0.0</td>
<td>0.8 ± 0.0</td>
<td>0.7 ± 0.0</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>39.7 ± 3.4</td>
<td>55.9 ± 4.7</td>
<td>39.8 ± 3.0</td>
</tr>
<tr>
<td>Lean mass (%)</td>
<td>70.6 ± 1.1</td>
<td>69.3 ± 0.7</td>
<td>70.5 ± 1.3</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>12.4 ± 2.3</td>
<td>9.4 ± 1.7</td>
<td>12.9 ± 1.9</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>23.7 ± 3.2</td>
<td>14.4 ± 1.7</td>
<td>24.4 ± 2.9</td>
</tr>
</tbody>
</table>

FS: Flaxseed; FF: Defatted flaxseed flour; FO: Flaxseed Oil; F: Female; M: Male; BMI: Body Mass Index; WC: Waist Circumference; HC: Hip circumference; WHR: Waist and Hip ratio. Means followed by different letters differ from each other at 5% probability.
treatment period (FF and FO), because there energy density of the diet provided for this groups was a significantly higher than one provide for the control group. However, this was not observed in the current study. This should be due to the short period of intervention. Besides, a lower fat bioaccessibility in the FF and FS groups compared to FO group, suggests the occurrence of a lower body weight gain than the expected when flaxseed is consumed. Indeed, body weight maintenance was reported in animal and human studies having a duration of 3 to 12 months.

There was no difference in nutrient excretion among the groups in the control sessions, which characterizes the sample homogeneity. A higher excretion of carbohydrates and proteins was observed in the FF treatment compared to the control session, leading to a higher fecal energy excretion. However, it is important to report that a significant increase in daily intake of these macronutrients was also noticed in the test period, which could explain this result.

It should be noted that there was about 4 times more fat in the feces of the FS group compared to the FO group.

Table III

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 8)</th>
<th>Treatment (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>2002.4 ± 527.0</td>
<td>1977.1 ± 258.6</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>478.6 ± 65.9</td>
<td>422.1 ± 55.2</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>184.9 ± 48.6</td>
<td>162.6 ± 21.2</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>12,094.0 ± 1,511.7</td>
<td>11,557.0 ± 3,183.3</td>
</tr>
<tr>
<td>Excretion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>73.1 ± 22.7</td>
<td>88.2 ± 33.5</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>50.4 ± 15.0</td>
<td>56.7 ± 12.1</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>21.8 ± 10.2</td>
<td>48.9 ± 14.3</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>685.3 ± 19.6</td>
<td>1033.6 ± 294.1</td>
</tr>
</tbody>
</table>

Means followed by the same small letter in the line are not significantly different.
*Means of Control and Treatment in the line differ statistically from each other at 5% of significance level.
**Balance period (between the intake of red dye and the appearance of feces stained with blue dye).
FS: Flaxseed; FF: Defatted flaxseed flour; FO: Flaxseed oil.

Table IV

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Control (n = 8)</th>
<th>Treatment (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>c13:0</td>
<td>5.6 ± 0.8*</td>
<td>1.9 ± 1.2*</td>
</tr>
<tr>
<td>palmitic</td>
<td>22.9 ± 8.6*</td>
<td>7.5 ± 1.8*</td>
</tr>
<tr>
<td>stearic</td>
<td>21.1 ± 9.5</td>
<td>11.5 ± 9.2</td>
</tr>
<tr>
<td>oleic</td>
<td>16.5 ± 5.2</td>
<td>27.2 ± 5.5</td>
</tr>
<tr>
<td>linoleic</td>
<td>8.1 ± 6.5</td>
<td>9.7 ± 2.9</td>
</tr>
<tr>
<td>α-linolenic</td>
<td>0.0</td>
<td>28.6 ± 13.1*</td>
</tr>
<tr>
<td>Σ SFA</td>
<td>49.6 ± 15.7</td>
<td>20.9 ± 12.2</td>
</tr>
<tr>
<td>Σ MUFA</td>
<td>16.5 ± 5.2</td>
<td>27.2 ± 5.5</td>
</tr>
<tr>
<td>Σ PUFA</td>
<td>8.1 ± 6.5</td>
<td>38.3 ± 15.7*</td>
</tr>
</tbody>
</table>

Means followed by the same small letter in the line are not significantly different.
*Means of Control and Treatment in the line differ statistically at 5% of significance level.
**Balance period (between the intake of red dye and the appearance of feces stained with blue dye).
FS: Flaxseed; FF: Defatted flaxseed flour; FO: Flaxseed oil; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.
group and about 3 times more compared to the FF group. PUFA and α-linolenic acid were the main fat types excreted. There was difference in fat intake between groups during the treatment sessions. Similar results were presented in our pervious study with peanuts.17,18,19

According to Ellis,4 the ingestion of seeds lead to a lower fat bioavailability, due to inefficient chewing and processing in the gastrointestinal tract. The cell wall protects the intracellular fat and consequently decreases its bioaccessibility. Although flaxseed was ground in a blender, the presence of intact seeds was observed in the feces of the volunteers in the FS group, favoring a higher fat excretion in this group.

The low bioavailability of α-linolenic acid from flaxseed should be studied further since this fatty acid leads to a reduction in the CVD risk.6,20

Conclusion

Flaxseed and flaxseed flour causes an increase in fat, mainly PUFA and α-linolenic acid, and energy fecal excretion.

Although the short duration of the intervention was not designed with the objective of verifying the effect of flaxseed consumption on body weight, the results of this study suggest that despite its high energy density, flaxseed may favor body weight maintenance because it leads to a higher fecal fat and energy excretion.

These data should be better investigated in future chronic feeding studies to evaluate the effects of flaxseed on body weight and non-communicable diseases manifestation.

Acknowledgements

We thank the volunteers, the students engaged in the study, prof Benedito Vital for bomb calorimetry, prof Raimundo Barros and prof Maria Aparecida Moreira for the freeze-dryer loan.

References