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STUDIES IN HUMANS

Brown and golden flaxseed reduce intestinal permeability and endotoxemia, and improve the lipid profile in perimenopausal overweight women

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ABSTRACT

The effect of brown and golden flaxseeds on lipid profile, oxidative stress, intestinal permeability, endotoxemia, and fasting glycaemia of perimenopausal overweight women was investigated in this clinical trial. Thirty participants were divided into control (CG), brown flaxseed (BF), and golden flaxseed (GF) groups. BF and GF received 40 g of brown and golden flaxseed for 12 weeks. Venous blood samples were collected at the beginning and at the end. Intestinal permeability analysis was performed by urinary excretion of lactulose and mannitol. There was significant reduction in intestinal permeability in flaxseed groups, with delta of lactulose/mannitol ratio smaller ($p \leq 0.05$). LPS levels were reduced in the flaxseed groups, whereas low-density lipoproteins (LDL) was decreased in the GF group ($p \leq 0.05$). Flaxseed consumption did not change oxidative stress markers and glycaemia. Flaxseed consumption, especially golden flaxseed, reduced intestinal permeability and improved the lipid profile, showing positive effects on metabolic changes caused by menopausal transition.

HIGHLIGHTS

- Brown and golden flaxseeds show a high content of insoluble fibre and alpha-linolenic acid, and brown flaxseed presented higher antioxidant activity.
- Golden flaxseed improved the lipid profile.
- Brown and golden flaxseeds reduced intestinal permeability and endotoxemia.
- Brown and golden flaxseed can be a promising alternative for the prevention of metabolic changes caused by menopausal transition, and for the improvement of the intestinal health.

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Flaxseed; menopause; overweight; intestinal permeability; endotoxemia

Introduction

Perimenopause or menopausal transition is defined as the period in which menstrual cycle changes occur, generating irregular cycles, and this phase begins between 47 and 48 years of age, and lasts for two to five years (Mendoza et al. 2013). As a consequence, there is a reduction in the production of ovarian hormones, such as oestrogen and progesterone (Siobán et al. 2012; Mendoza et al. 2013). Menopause takes place after 12 consecutive months of amenorrhoea, with no other pathological or physiological established cause (Mansfield et al. 2004).

The reduced oestrogen production increases the risk of metabolic changes, resulting in bone disease, diabetes, altered body fat distribution, and abnormal plasma lipid profile (Tremollieres et al. 1996; Garrido et al. 2015). Reduced oestrogen production is also associated with increased oxidative stress, which in turn may lead to endothelial dysfunction, vascular inflammation and, consequently, increased risk of cardiovascular diseases (Mittal and Kant 2009; Al-Anazi et al. 2011). In animal models, chemical or surgical menopause leads to downregulation of epithelial junction proteins, gut barrier dysfunction, and increased gut permeability. This, in turn, permits the translocation of microbes from the intestinal lumen into the subepithelial space, triggering immune cells to produce proinflammatory cytokines (Moreira et al. 2012; Shieh et al. 2020). Whether an increase in gut permeability accompanies the menopause transition in humans is uncertain. If gut permeability does increase,

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whether it is associated with inflammation and end-organ manifestations is also unknown.

Functional foods, such as flaxseed (*Linum usitatissimum* L.), have been studied aiming to minimise the oxidative stress caused by oestrogenic rate reduction (Nuernberg et al. 2005). Indeed, we have recently shown the positive effect of flaxseed consumption, especially golden flaxseed, in reducing waist circumference, body weight, and fat mass in perimenopausal women (Aguilar et al. 2017). Still, brown or golden flaxseed consumption may be capable of decreasing intestinal permeability and serum LPS levels, modulating inflammatory markers and contributing to glycemic and blood lipid profiles control Mehta et al. 2010).

Flaxseed contains considerable amounts of soluble and insoluble fibres, phenolic compound such as lignans, and polyunsaturated lipids, such as α-linolenic acid (ALA) and linoleic acid (LA) (NEPA-UNICAMP 2011). Some studies have indicated that the supplementation with n–3 fatty acids has the capacity to change the membrane phospholipids profile and, in response, decrease serum oxidative stress, contributing to decrease endothelial dysfunction associated with menopause (Gianluca 2013). Moreover, lignans may work as oestrogens, proving to be beneficial against the menopause effects (Arango et al. 2011; Machado et al. 2015). Due to the bioactive compounds present, flaxseed may be associated with reduction of total cholesterol (TC), LDL-c (low-density lipoprotein) and glucose concentrations, endothelial oxidative stress, as well as endothelial inflammation progression, and decreasing the risk of developing cardiovascular diseases (Zhao et al. 2004; Dodin et al. 2005; Zhang et al. 2008).

Among the existing flaxseed varieties, the most well-known are brown and golden flaxseeds (Moetazza et al. 2015; Tavarini et al. 2019). Golden flaxseed is cultivated in cold-climate countries, including Canada and the Northern United States. Brown flaxseed, in turn, is mainly cultivated in hot- and humid-climate regions, such as the South region of Brazil (Tavarini et al. 2019). It is unknown, however, if the physiological effects of brown flaxseed are comparable to those of its golden analogue, due the few studies on this variety.

In the current study, we tested the hypothesis that consumption of brown flaxseed would develop results better or similar to golden flaxseed in relation to improving clinical manifestations related to menopausal transition. Therefore, the aim of this study was to compare the effects of brown and golden flaxseeds on lipid and glucose metabolisms, oxidative stress, intestinal permeability, and endotoxemia in perimenopausal women.

Materials and methods

Raw materials and composition analysis

Brown and golden flaxseeds were obtained from the Cerealista São José Company, São Paulo, Brazil. These were ground and packed in polyethylene bags, stored at 2–8 °C, and provided to the study participants.

Soluble and insoluble fibres were quantified via the enzymatic-gravimetric method, and total fibre was calculated by the sun of soluble and insoluble fibres (AOAC 1998). Antioxidant activity content was determined by the ABTS radical assay method (2,2′-azino-bis(3-etilethylbenzo-thiazoline-6-sulfonic acid) diammoninum salt) (Re et al. 1999). Total phenolic content from flaxseed extracts was determined by spectrophotometric assay with the Folin-Ciocaulteau reagent (Rufino et al. 2010; Singleton and Rossi 1965).

Fatty acids were extracted using the direct transesterification. The fatty acids profile was determined by gas chromatography (Shimadzu Model 17 A), by using capillary column (SP-2560) of 100 m length and 0.25 mm diameter. The initial temperature was 100 °C, heating at 10 °C per minute until reaching 180 °C and then heating at 1 °C per minute until 240 °C, remaining at that temperature for 10 min. The injector temperature was 250 °C and the detector 270 °C. The fatty acids were identified by comparison between the retention times of the sample peaks with the standard mixture of 37 fatty acids methyl esters containing C4:0 to C22:6 (Supelco®, Bellefonte, PA) (Lepage and Roy 1986).

Population

The intervention study is a non-randomized, prospective, parallel clinical trial, and included women overweight (body mass index (BMI) between 25 and 34.9 kg/m^2) ranging in age from 40 to 55 years (Rohrig et al. 2010; WHO 2000). The sample size and statistical power were calculated according to Rohrig et al. (2010). A total of 122 women were recruited to participate in the study, of which 77 attended the pre-selection survey, after the pre-selection consultation, in which a questionnaire was applied to obtain general information, anthropometric parameters, blood pressure was measured, and capillary blood and urine samples were collected. Out of these 77 women, 46 did not meet one or more of the inclusion criteria or

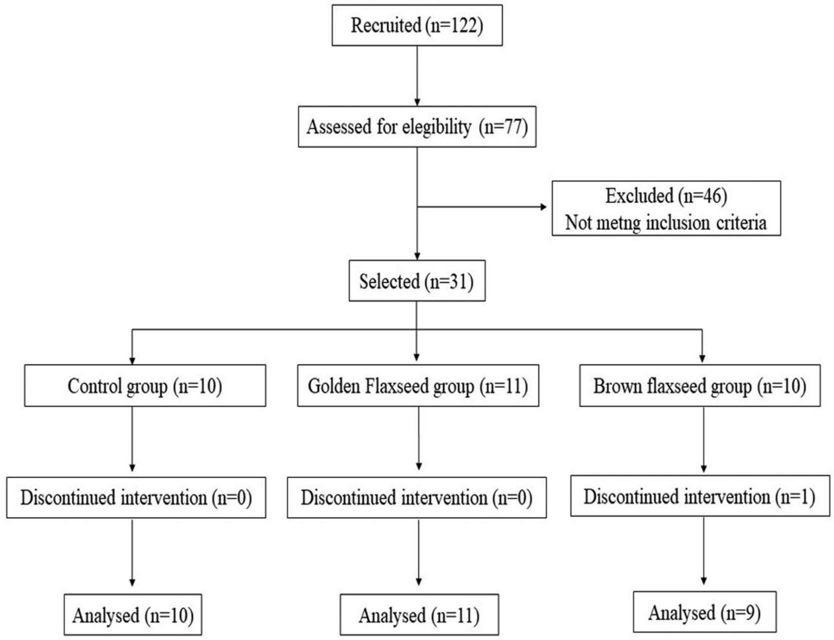

Figure 1. Flow diagram with the selection and diet group distribution of the subjects.

met at least one of the exclusion criteria. At the end, 31 women were selected, and one missed the last survey. Therefore, a total of 30 women concluded the study. The participants were divided into three groups: Control group (CG $n = 10$), Brown flaxseed (BF $n = 9$), and Golden flaxseed (GF $n = 11$), according to their body mass index (BMI) and the presence of amenorrhoea to ensure sample homogeneity (Figure 1).

Inclusion/exclusion criteria and ethical approval

The following inclusion criteria were considered: women with ages between 40 to 55 years and body mass index (BMI) between 25 and 34.9 kg/m^2. The exclusion criteria were women who were taking medicines that could interfere with the results (hormone replacement therapy, anti-inflammatory drugs, antibiotics, antacids, cholesterol-lowering drugs or hypoglycaemic agents); dietary supplements (calcium, vitamin D); allergic to flaxseed; presented cancer, endometriosis, osteopenia, osteoporosis, cardiovascular diseases, liver or kidney disease, diabetes mellitus, or dyslipidemia; used pacemakers; smoker; and had total cholesterol $\geq$240 mg/dL, triglycerides $\geq$140 mg/dL, and fasting glucose $\geq$127 mg/dL.

All participants signed an informed consent form. This study complied with the guidelines provided by the Declaration of Helsinki. All procedures were approved by the Human Research Ethics Committee of the Centre of Health Sciences of the Federal University of Espírito Santo (#632.531 of 04/30/2014).

Dietary interventions

The intervention lasted 12 weeks, each participant meeting with the researchers once a week to receive the flaxseed enough for intake in the following week and to verify the participants adherence to the protocol. Each volunteer received a customised diet plan. The CG participants received the diet plan consisting of a calorie-restricted diet of 250 kcal/day, as well as nutritional guidance, and were instructed not to eat flaxseed during the intervention period. The other experimental groups (BF and GF) received 40 g/day of either ground brown or golden flaxseed, the diet plan consisting of a calorie-restricted diet of 250 kcal/day (considering the flaxseed calories) and nutritional guidance and were instructed not to eat foods rich in fibre, such as oatmeal, granola, flaxseeds (beyond that provided in the study), wheat bran and oil seeds, due to possible interference with the results. In addition, they received suggestions about flaxseed use in food preparations.

The participants were also instructed to consume the entire package content (40 g/day) and to return any leftovers. The amount of flaxseed provided and the 12 weeks intervention period was based on prior studies (Lucas et al. 2002; Dodin et al. 2005; Rhee and Brunt 2011).

Biochemical assessment

For blood collection, the volunteers were asked to fast for 12 h. At T1 period and after 12 weeks of intervention period (T12), a 10-mL blood sample was intravenously collected from the median antecubital vein using gel clot activator tubes for serum separation (BD Vacutainer®, Curitiba, PR, Brazil). Blood samples were centrifuged (Thermo Scientific™ Heraeus™ Megafuge™ 16 R, Ostedore, DE) for 15 min at 3500 rpm, and the serum samples were transferred to properly labelled 2.0-mL polypropylene tubes (Eppendorf®, São Paulo, SP, Brazil), and then stored in an ultra-freezer (Thermo Scientific™ Revco™ Exf 8924, Marietta, OH) at −80 °C.

Lipid profile

Total cholesterol (TC), high-density lipoproteins (HDL), and triacylglycerols (TAG) were analysed in triplicate by routine techniques using colorimetric enzyme kits (Bioclin®). Low-density lipoprotein (LDL) content was estimated using the equation (Friedewald et al. 1972).

Blood oxidative stress

Oxidative stress was analysed by serum malondialdehyde (MDA), using a commercial kit – TBARS (Thiobarbituric Acid Reactive Substances), following the instructions of the manufacturer (BioAssad Systems®), at wavelength of 535 nm, and expressed as μM MDA.

Plasma oxidised Low-Density Lipoprotein (LDL-ox) was estimated by Enzyme Linked Immunosorbent Assay – ELISA, using a specific analysis kit (Cloud Clone Corp®) and expressed as pg/mL.

Plasma total antioxidant capacity

Plasma total antioxidant capacity (TAC) was measured by colorimetric assay using a specific analysis kit, which was based on the reduction of Cu^{2+} into Cu^{+}, at wavelength of 570 nm. The analysis was conducted following the manufacturer's instructions (BioAssad Systems®) and was expressed as μM Trolox.

Glucose assessment

Fasting glucose was measured using the Glucose Monoreagent Bioclin® kit. Glucose was classified as proposed by the guidelines of the Brazilian Society of Diabetes (SBD 2015). Such evaluations were performed at T1 and T12 through the collection of intravenous blood.

Endotoxemia assessment

Endotoxemia was assessed at T1 and T12, aiming to compare flaxseed effect under the LPS concentration as well as to associate it with the inflammation markers. Plasma aliquots (10 μL) were diluted in endotoxin-free water at 1:10 proportion. LPS levels were measured in serum by the Limulus Amoebocyte Lysate (LAL) test with chromogenic endpoint determination, using the commercial kit (HIT302 HycultBiotech®). The results were expressed as EU/mL.

Intestinal permeability

Intestinal permeability was analysed at T1 and T12. For the test, the volunteers fasted for 5 h. At the time, they were instructed to eliminate any urinary residue, and then 120 mL of isosmolar solution containing 6.25 g of lactulose and 3.0 g of mannitol diluted in water was supplied. All urinary volume was collected during the 5-h fasting period. Then, the obtained content was homogenised, and the total urinary volume was recorded. Aliquots of 10 mL were stored in labelled and sealed falcon tube and added with 0.24 mg of thimerosal in order to prevent bacterial growth. After this procedure, the material was duly identified and stored in a freezer at −80 °C (Vilela et al. 2008).

For analyses of mannitol and lactulose, urine was filtered through a 0.22-μm membrane filter (Millex®) and analysed by high-performance liquid chromatography (HPLC). Mannitol and lactulose (Sigma Aldrich®, São Paulo, SP, Brazil) were used as internal standards. The conditions used for the intestinal permeability analysis by determination of urinary concentration of mannitol and lactulose were: HPLC Shimadzu® equipment, model ACCELLA IR; coupled to the refraction index detector RID-10A and a 300-cm-long, 8.7-mm-diameter HPX-87 H column (Biorad®); oven column temperature of 55 °C, flow of 1.0 mL/min, pressure of *ca.* 120 psi, and run time for each sample of 34 minutes. The injected volume was 50 μL, and the mobile water phase in 0.005 mmol/L of sulphuric acid (Vasconcelos de Sá et al., 2011).

For test standardisation purposes, values were measured in mmol/L and subsequently converted into g/L. Known amounts of lactulose and mannitol were separately and repetitively evaluated through HPLC by following concentration points in a decreasing manner and building analytical curves. The mannitol and lactulose standards were purchased from Sigma Aldrich®. After obtaining the respective areas, the concentrations of the two substances in urine (g/L) were determined.

The following equations were used to determine mannitol and lactulose excretion rates:

$$\%\ \text{mannitol excretion} = [(\text{concentration in g/L} \times 5\ \text{h urinary volume})/1000\ \text{mL} \times 100]/3\ \text{g of mannitol}$$

$$\%\ \text{lactulose excretion} = [(\text{concentration in g/L} \times 5\ \text{h urinary volume})/1000\ \text{mL} \times 100]/6.25\ \text{g of lactulose}$$

The values of 3 g of mannitol and 6.25 g of lactulose represent the total intake of each woman in the study, diluted in a solution of 120 mL of water, for urine collection in 5 h of fasting. The lactulose/mannitol ratio was determined from the %lactulose excretion divided by %mannitol excretion.

Statistical analysis

Data normality was assessed by the Kolmogorov-Smirnov test. The unpaired *t*-test was used to compare variables and the paired *t*-test was used to compare variables in the same group, using the initial and final data (T1 and T12). Analysis of variance (ANOVA) was used to compare the three groups (CG, BF, and GF) and the variations (T12–T1). ANOVA was complemented with Tukey's test for multiple comparisons at $p \leq 0.05$. GraphPad Prisma® (La Jolla, CA), version 6, was used to analyse the data.

Results

Population

Thirty individuals with a BMI 29.76 ± 0.48 kg/m^2 were included in the data analyses. All individuals showed elevated body fat and waist circumference. Participants from both groups started the intervention under the same conditions (Table 1).

Table 1. Baseline characteristics of participants.

Variables	CG ($n = 10$)	BF ($n = 9$)	GF ($n = 11$)
Age (years)	49.02 ± 1.11^a	49.11 ± 1.24^a	49.23 ± 1.03^a
Weight (kg)	75.84 ± 4.78^a	78.21 ± 2.67^a	76.16 ± 2.68^a
Body mass index (kg/m^2)	29.21 ± 2.30^a	30.00 ± 2.40^a	30.09 ± 3.28^a
Body fat (%)	37.78 ± 2.62^a	37.82 ± 2.53^a	38.43 ± 3.32^a
Waist circumference (cm)	89.3 ± 3.97^a	94.55 ± 6.15^a	96.39 ± 11.43^a
SP (mmHg)	136 ± 3.12^a	125 ± 2.63^a	132 ± 3.64^a
DP (mmHg)	85 ± 4.01^a	84 ± 2.42^a	80 ± 3.21^a

Data are expressed as the mean ± SD. Different letters mean statistical difference by Tukey test at $p \leq 0.05$. CG: control group; BF: brown flaxseed group; GF: golden flaxseed group; SP: systolic pressure; DP: diastolic pressure.

Dietary fibre, fatty acids, antioxidant activity and total phenolics in the flaxseeds

Brown and golden flaxseed show a high content of insoluble fibre and alpha-linolenic acid. Flaxseeds differed for antioxidant activity, but not for phenolics' concentration. Brown flaxseed presented higher antioxidant activity ($p < 0.05$; Table 2).

Table 2. Dietary fibre, fatty acids, antioxidant activity and total phenolics of the brown and golden flaxseeds (wet base).

	Brown flaxseed	Golden flaxseed
Total dietary fibre (g 100 g^{-1})	27.88 ± 1.88^a	28.56 ± 1.39^a
Insoluble fibre (g 100 g^{-1})	23.67 ± 1.82^a	27.18 ± 0.18^a
Soluble fibre (g 100 g^{-1})	4.20 ± 0.05^a	1.31 ± 1.21^a
Palmitic acid (C16:0)	1.95 ± 0.01^a	1.48 ± 0.01^b
Stearic acid (C18:0)	1.56 ± 0.01^a	1.18 ± 0.01^b
Oleic acid (C18:1 n9)	6.03 ± 0.02^b	7.63 ± 0.02^a
Linoleic acid (C18:2 n6)	4.06 ± 0.02^b	4.73 ± 0.03^a
Alpha-linolenic acid (C18:3 n3)	16.48 ± 0.03^a	13.66 ± 0.02^b
Antioxidant activity (µM Trolox/g)	29.66 ± 3.05^a	20.33 ± 1.52^b
Total phenolic (g AGE/g)	1.54 ± 0.12^a	1.40 ± 0.14^a

Data are expressed as the mean ± SD ($n = 3$). Different letters mean statistical difference by Tukey test at $p \leq 0.05$. AGE: Gallic acid equivalent.

Effect of brown and golden flaxseed consumption on lipid profile and glucose

No significant difference in lipid profile was observed neither among the tested groups nor between final and initial times within each group ($p > 0.05$; Figure 2(A–D)). However, a significant difference for the delta (T12 – T1) of golden flaxseed group was observed regarding the LDL-c: −10.66 mg/dL ($p < 0.05$; Figure 2(C)). No significant intergroup and intragroup differences were observed for the fasting glucose levels at the beginning and at the end of the study ($p > 0.05$; Figure 2(E)).

Effect of brown and golden flaxseed consumption on blood oxidative stress and plasma total antioxidant capacity

There was no statistically significant difference in the biomarkers of oxidative stress between initial and final times as well as between experimental groups ($p > 0.05$; Figure 3).

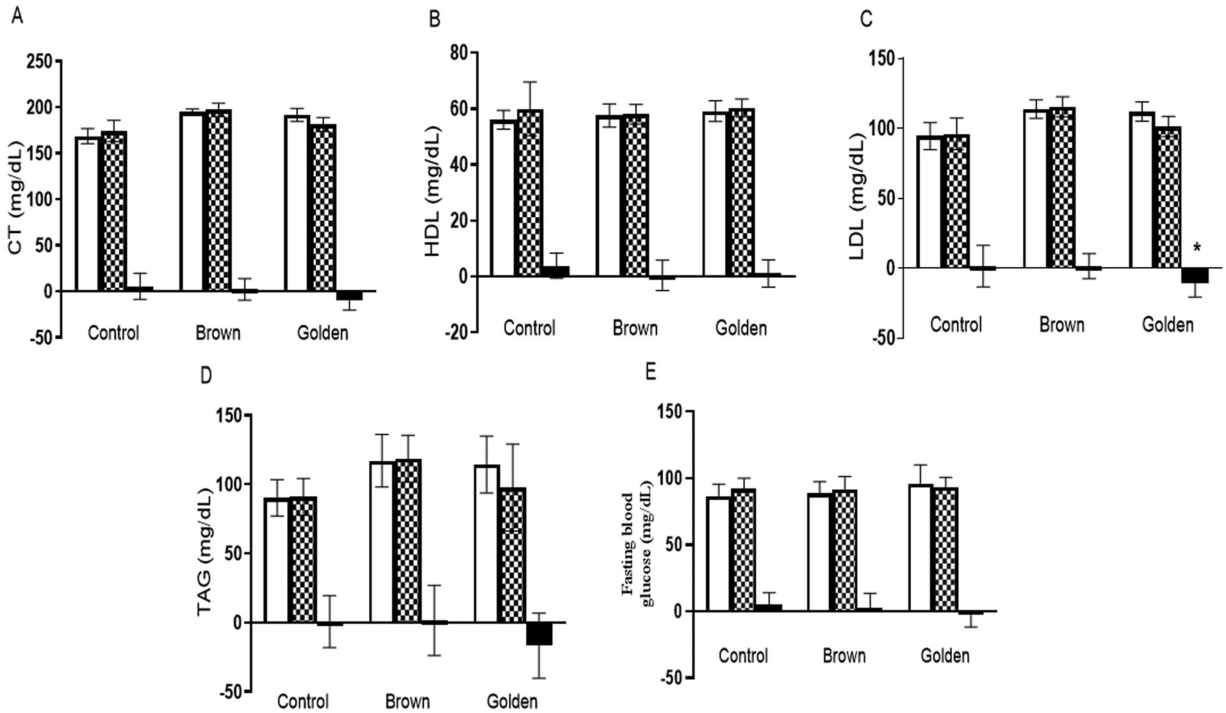

Figure 2. Lipid profile: Total cholesterol (A); High-density lipoproteins (B); Low-density lipoprotein (C); Triacylglycerols (D), and fasting blood glucose (E) at the beginning (T1) and end (T12) of the intervention. Values expressed as mean ± SD. White bars = T1, Hatched bars = T12, Black bars = Delta (T12–T1). * = Statistically significant difference in Delta between groups (intergroup difference between delta values) by Tukey test at $p \leq 0.05$. No significant difference was observed intragroup and between groups at T1 and T12 by Tukey test at $p > 0.05$. TC: Total cholesterol; HDL: high-density lipoproteins; LDL: Low-density lipoprotein; TAG: triacylglycerols.

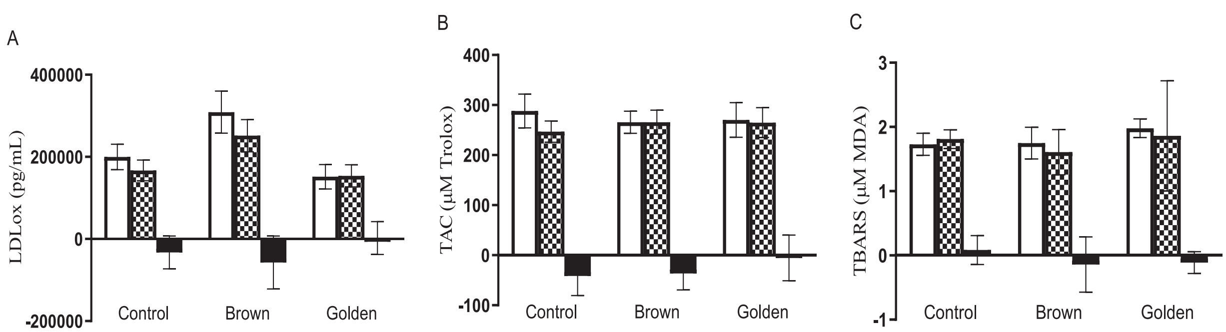

Figure 3. Markers of oxidative stress: Oxidised Low-Density Lipoprotein (A); Plasma Total Antioxidant Capacity (B); Thiobarbituric Acid Reactive Substances (C). Values expressed as mean ± SD. White bars = T1 (initial), Hatched bars = T12 (final), Black bars = Delta (T12–T1). No significant difference was observed intragroup and between groups at T1 and T12 by Tukey test at $p > 0.05$. LDLox: Oxidised Low-Density Lipoprotein; TAC: Plasma Total Antioxidant Capacity; TBARS: Thiobarbituric Acid Reactive Substances.

Effect of brown and golden flaxseed consumption on endotoxemia and intestinal permeability

It was found in T1 a higher urinary excretion of mannitol than in T12 in all the groups evaluated. At the baseline (T1), the lowest levels of mannitol excretion were found in women in the control group and the highest levels in flaxseed groups. The same pattern was observed in T12. The delta (T12 – T1) was higher in flaxseed groups ($p < 0.05$; Figure 4(A)).

The lactulose excretion was higher in T1 when compared to T12. In T12, the levels of lactulose excretion in the control group were smaller than that found in brown and golden flaxseed group. The delta (T12 – T1) was higher in flaxseed groups ($p < 0.05$; Figure 4(B)).

The ratio was higher in T1 when compared to T2 in control group. In T12, the lowest level was found in the control group. The delta (T12 – T1) was higher

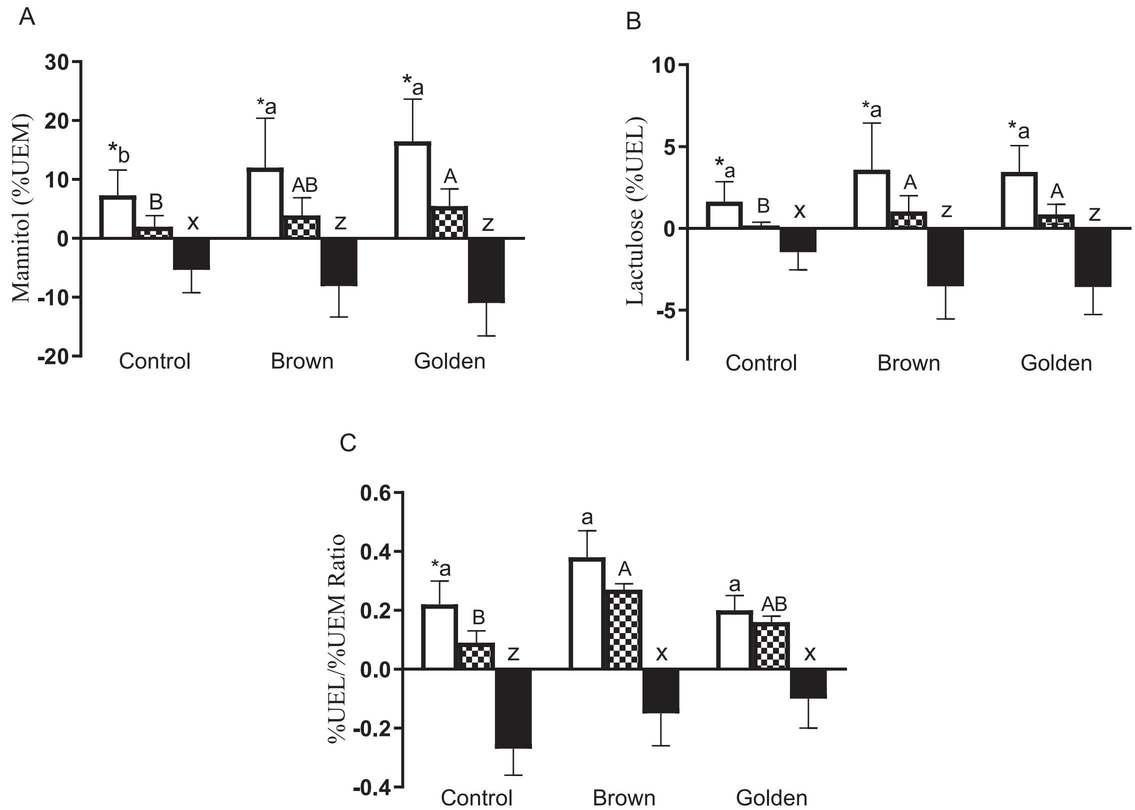

Figure 4. Percentage of urinary excretion of mannitol (A), lactulose (B), and lactulose/mannitol ratio (C). Values expressed as mean ± SD. White bars = T1 (initial), Hatched bars = T12 (final), Black bars = Delta (T12–T1). * = Statistically significant difference between T1 and T12 (intragroup difference). *a*, *b* = Statistically significant difference between groups at T1 (intergroup difference at baseline); A, B = Statistically significant difference between groups at T12 (intergroup difference at the end of the experimental period); *x*, *z* = Statistically significant difference in Delta between groups (intergroup difference between Delta values) by Tukey test at $p \le 0.05$. UEM: urinary excretion of mannitol; UEL: urinary excretion of lactulose.

in control group and similar in flaxseed groups ($p < 0.05$; Figure 4(C)).

Compared to T1, BF and GF groups showed in T12 reductions in blood LPS, to levels similar to those found in control group. Moreover, the delta (T12 – T1) for the BF and GF groups was higher than that of the control group ($p < 0.05$; Figure 5).

Discussion

This is the first study comparing the effect of brown and golden flaxseed on intestinal permeability and endotoxemia in perimenopausal women. The potential benefit of flaxseeds is based on their bioactive compounds, such fibres, phenolic compounds and $n-3$ polyunsaturated fatty acids (PUFA) (da Costa et al. 2016; Dali et al. 2019). These compounds may prevent metabolic disorders generated by menopause. Fibres help to reduce weight gain and improve lipid profile, phenolic compounds have antioxidant, antilipidemic and anti-inflammatory activities, and $n-3$ PUFA act by reducing the inflammatory process (Zhang et al. 2008; Tavarini et al. 2019).

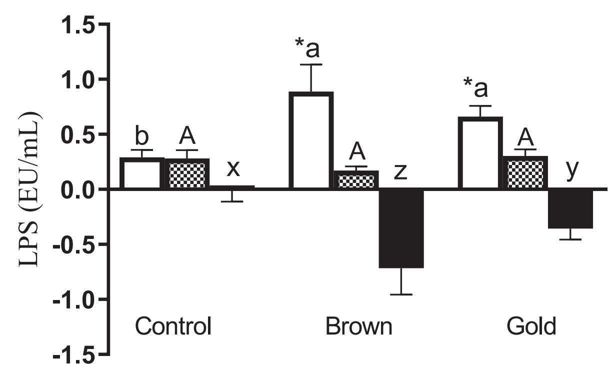

Figure 5. Plasma LPS concentration. Values expressed as mean ± SD. White bars = T1 (initial), Hatched bars = T12 (final), Black bars = Delta (T12–T1). * = Statistically significant difference between T1 and T12 (intragroup difference). *a*, *b* = Statistically significant difference between groups at T1 (intergroup difference at baseline); *X*, *Z* = Statistically significant difference in Delta between groups (intergroup difference between Delta values) by Tukey test at $p \le 0.05$. A = No significant difference was observed in T12 between groups (intergroup difference at the end of the experimental period).

Although the flaxseeds have similar phenolic content, the antioxidant activity of the brown flaxseed was greater than the golden, indicating that the antioxidant activity is not related only to the phenolic content, and that other compounds present in the brown flaxseed favour the greater antioxidant activity. The differences related to the antioxidant activities of brown and golden flaxseeds observed in this study differ from those obtained by Wanderley et al. who observed greater antioxidant activity for golden flaxseed (Wang et al. 2017). The environmental conditions of cultivation, such as soil and climate, as well as origin-related characteristics, genetic factors, and the stage of seed development may cause variations in the centesimal composition of flaxseed (Tavarini et al. 2019). Also, both flaxseeds had a high content of dietary fibre, especially the insoluble fraction. Fibres are related to reduced weight gain, improved lipid profile and inflammation, disorders related to the menopause phase (Fuller et al. 2016). Associated with this, linseeds are sources of $n-3$ PUFA that have potential anti-inflammatory action (Siriwardhana et al. 2012).

Regarding the blood lipid profile, we found significant reduction in the delta for LDL-c in GF group, which demonstrates that golden flaxseed contributes to reduce this risk factor of atheromatous plaque formation, and consequently may prevent cardiovascular problems. After all, the excess of blood cholesterol, especially the LDL-c fraction, is directly related to increased LDL-c deposition in the intimal layer of the artery (Kobiyama and Ley 2018). Similar results were found by Dodin et al, who incorporated 40 g of golden flaxseed in the diet plan of menopausal women for 12 weeks and observed significant reduction of TC and LDL-c fractions at the end (Dodin et al. 2005). Regarding brown flaxseed, no change was observed, however, given the scarcity of studies with brown flaxseed, the influence of that flaxseed should be better studied.

The reduction in LDL-c observed may be attributed to fibres and phenolic compounds present in flaxseeds. A phenolic compound present in flaxseed, diglucosídeo secoisolariciresinol (SGD) demonstrate contributes to the regulation of cholesterol metabolism by acting on the expression of the enzyme that regulates the synthesis of bile salts. Therefore, there are evidences that a higher excretion of hepatic cholesterol in the bile may occur upon increased intake of flaxseed (Machado et al. 2015). Moreover, soluble fibres act by reducing intestinal absorption of cholesterol by adsorbing water and forming gels, hence contributing to the delay of gastrointestinal emptying and, as a result, to the decrease of glucose and cholesterol absorptions in the small intestine (Sposito et al. 2007). Consequently, lipid uptake by enterocytes is lowered. In addition, fibres may bind bile acids and cholesterol during the formation of intestine micelles, resulting in lower cholesterol absorption and to regulate the increase of LDL receptors expression in the liver (Sposito et al. 2007; Cherem and Bramosrki 2008).

In the present study, no significant changes were observed in blood glucose levels between the control group and the groups that consumed brown and golden flaxseeds, but no dramatic reductions of glycaemia were expected in this present study, given that the volunteers were normoglycaemics (i.e. glucose levels lower than 126 mg/dL). In the menopausal transition, hormonal changes can trigger metabolic alterations, such as weight gain, that can consequently result in insulin sensitivity alterations and lead to the development of diabetes (Tremollieres et al. 1996; Garrido et al. 2015; Machado et al. 2015). In this context, flaxseeds can prevent changes in glucose levels, due to inhibition glucosidase and pancreatic α-amylase, leading to the reduction in starch hydrolysis and eventually reduced glucose levels (Bhat et al. 2011; Sudha et al. 2011). On the other hand, the study by Gok et al. concluded that flaxseed can by modulating glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and glutathione reductase activities; develop antihyperglycemic effect (Gök et al. 2016). Pilar et al. supplemented 40 g of flaxseed for 28 days in subjects with metabolic syndrome, and observed significant reduction in fasting glycaemia (Pilar 2014). However, a possible effect of flaxseed on glycaemia is not ruled out, which may suggest the development of other studies with flaxseed by varying intervention time and experimental groups.

Regarding oxidative stress markers, no changes were observed with the consumption of flaxseeds. Studies suggest that flaxseed intake contributes to reducing the oxidative stress and the risk of cardiovascular diseases, being associated mainly with content phenolics and ALA that have antioxidant properties, results which were not observed in the present work (Shahidi et al. 2007; Yang et al. 2012). Phenolics reduce free radicals and increase antioxidant enzymes activity, which contribute to reduce the oxidation of LDL-c native, being a protection factor for endothelial damages and inducing cardio-protective effect, and also acting may work as antioxidants, inactivating free radicals and reactive oxygen species, and may even influence enzymes (e.g. glutathione) of the endogenous antioxidant system (Yuan et al. 1999; Deng et al. 2017). In their study, Matusiewicz et al. demonstrated that flaxseed increased the antioxidant capacity and decreased the malondialdehyde concentration (Matusiewicz et al. 2015). Other authors observed that the ALA present in flaxseed may reduce the serum oxidative stress and membrane dysfunction as well as prevent lipid peroxidation (Yang et al. 2012). This effect is suggested to be associated with the reduction of endothelial dysfunction through reversal of the changes in the membrane lipid composition and vascular oxidative stress reduction, and also influence inflammatory biomarkers (e.g. TNF-α) in which, after a consumption of flaxseed, a significant reduction on TNF-α levels was reported (Zhao et al. 2007; Gianluca 2013).

In the current study, we found that the use of brown and golden flaxseed reduced permeability to mannitol and lactulose, and LPL levels in flaxseed groups after 12 weeks of intervention. Thus, the daily administration of 40 g of brown and golden flaxseed was proved to be capable of improving the intestinal barrier function and integrity by reducing the translocation of endotoxins responsible for the increase of inflammation. These results demonstrated a beneficial effect of both flaxseeds on intestinal health. The intestinal barrier is composed of epithelial cells linked by cell junctions known as tight, adhesion, and gap junctions, which are responsible for controlling the amount of luminal antigens that cross the epithelium (Mehta et al. 2010). In this case, intestinal barrier dysfunction leads to an increase in intestinal permeability and bacterial translocation, which has the potential to release inflammatory mediators and immunological cells, and to exacerbate the development of inflammation and consequent translocation of microbes from the intestinal lumen into the subepithelial space, triggering immune cells to produce proinflammatory cytokines (Verediano et al. 2020). Shieh and collaborators in a study with 65 women in the menopause transition, demonstrated for the first time in a human study, that gut permeability increases during this period (Shieh et al. 2020). These findings show the importance of research with potential therapeutic target for preventing the inflammation caused by the barrier rupture.

The improvement in intestinal barrier function in the present study is positively related with the flaxseed consumption, related to antioxidants and fibres content, capable of decreasing fat absorption, such as saturated fatty acids, which also induces the production of pro-inflammatory cytokines (Fuller et al. 2016; Wang et al. 2017). Dietary fibres are capable of stimulating the increase in the number of the L cells in intestinal epithelium, cells which secrete glucagon-like 2 peptide that restores the expression of proteins related to intestinal barrier function by increasing its integrity. Therefore, the intestinal permeability reduction and the concomitant reduction in plasma LPS levels are consequences of the decreased activation of pathways involved in the synthesis of pro-inflammatory cytokines (Moreira et al. 2012). In the study of Dehghan and collaborators with overweight/obese women with type 2 diabetes, significant reductions were observed in LPS plasma levels (26.0 ± 7.6 to 20.3 ± 6.4 EU/mL) in participants allocated in the group that received oligofructose-enriched syrup, whereas in the control group no significant difference was observed (Dehghan et al. 2014). Thus, this study reinforces fibre consumption in lowering LPS blood levels.

Additionally, the reduction in LPS levels in groups that consumed flaxseed may be explained by the fact that this is a food rich in *n*–3 PUFA. Studies have suggested that the *n*–3 PUFA prevents the physical connection between LPS and toll-like receptors (TLRs), reducing the phosphorylation of the enzyme complex IKK, blocking and attenuating inflammation, both by reducing the expression of genes of pro-inflammatory cytokines and by decreasing the activation of NF-κB stimulated by LPS (Novak et al. 2003; Zhao et al. 2004). Mani et al. showed that LPS concentration was increased in a meal rich in saturated fatty acids (SFA). Contrastingly, LPS concentrations were reduced after a meal rich in *n*–3 PUFA (Mani et al. 2013).

Despite the benefits found in the present study, the limitations of the study with human beings should be considered, such as the variation that exists and the inability to have total control over individuals, in addition to the form of consumption of flaxseeds. Thus, further studies are suggested to verify the mechanism by which flaxseeds can affect intestinal permeability and lipid profile in overweight perimenopausal women, as well as other forms of flaxseed consumption and different intervention times.

Conclusion

This study was the first to compare the relation of brown and golden flaxseed consumption with intestinal permeability. Both flaxseed supplementations were efficient to reduce intestinal permeability and endotoxemia, resulting in improved intestinal health. Golden flaxseed reduced the LDL-c fraction, indicating a cardio-protective effect. On the other hand, supplementation with 40 grams of both brown and golden flaxseed, showed no significant effect on oxidative stress biomarkers and glycaemia. Therefore, brown and golden flaxseed showed positive effects on metabolic changes caused by menopausal transition, which indicates the importance and the need for more human studies.

Disclosure statement

The authors report there are no competing interests to declare.

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