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Effect of the ingestion of vegetable oils associated with energy-restricted normofat diet on intestinal microbiota and permeability in overweight women

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ABSTRACT

Previous studies suggest that the type of dietary fatty acid may modulate the intestinal bacterial ecosystem. However, this effect is still inconclusive. Thus, the aim of this study was to investigate the effect of the intake of vegetable oils rich in different types of fatty acids, associated with energy-restricted normofat diets, on the composition of intestinal microbiota and permeability, on LPS concentrations, and fecal short chain fatty acids and pH. This was a 9 consecutive weeks (±5 days), randomized, parallel, double-blind clinical trial. Overweight women received daily breakfast containing 25 mL of one of the test oils: soybean oil (n = 17), extra virgin olive oil (n = 19) or coconut oil (n = 16). Blood, fecal and urine samples were collected on the first and last day of the experiment for the analysis of the variables of interest. The consumption of the three oils did not affect the diversity and relative abundance of intestinal bacteria. We observed an increase in bacterial richness estimated by the Chao 1 index, and a reduction in the concentration of isovaleric fatty acid in the group that ingested soybean oil. Paracellular and transcellular permeability increased after the ingestion of extra virgin olive oil and coconut oil. However, LPS concentrations remained unchanged. The intake of different types of fatty acids associated with the energy-restricted normofat diet modestly affected the intestinal microbiota and permeability, without resulting in metabolic endotoxemia in overweight women.

1. Introduction

Abnormal or excessive fat accumulation, characteristics of overweight and obesity, are important risk factors for non-communicable diseases such as cardiovascular diseases, diabetes and some types of cancer (World Health Organization, 2018). According to WHO, adults presenting a BMI greater than or equal to 25 are overweight; and those with a BMI greater than or equal to 30 are obese (World Health Organization, 2018). Several authors suggest the association of intestinal microbiota in the development of overweight and its comorbidities (Bouter, Van Raalte, Groen, & Nieuwdorp, 2017; Chávez-Carbajal et al., 2019; Isolauri, Sherman, & Walker, 2017).

Diet is one of the major determinants of intestinal microbial composition (Alou, Lagier, & Raoult, 2016; Cuevas-sierra, Ramos-lopez, Riezu-boj, Milagro, & Martinez, 2019). The effect of the quantity and quality of fats ingested on the microbiota has been increasingly investigated by researchers (Wolters et al., 2018). The intake of high-fat diet (40% fat and 46% carbohydrate total energy), containing soybean oil, rich in polyunsaturated fatty acids (PUFAs) for 6 months increased relative abundance of Bacteroidetes, and reduced the Firmicutes in eutrophic individuals (Wan, Wang, Yuan, Li, Jiang, Zhang, & Li, 2019). Nevertheless, in a study with obese individuals at risk of metabolic syndrome, the consumption of diets rich in monounsaturated fat fatty acids (MUFAs) and PUFAs for 30 days almost did not affect the

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composition of the microbiota at the phylum level (Pu, Khazanehei, Jones, & Khafipour, 2016). The predominant phylum in the normal human gut microbiota are Bacteroidetes and Firmicutes. A balanced intestinal microbial ecosystem favors homeostasis and intestinal protection, while an imbalance in the proportion of these main phyla is related to obesity and associated metabolic conditions (Candido et al., 2017).

In addition, increased intestinal permeability due to changes in the gut microbiota can lead to endotoxemia, with the onset of inflammation and metabolic diseases (Hersoug, Møller, & Loft, 2018). Some authors have verified the increase in paracellular permeability in obese women compared with lean women (Teixeira et al., 2012). However, others studies did not identify difference in intestinal permeability (Brignardello et al., 2010; Machado et al., 2020).

In a longitudinal study involving eutrophic, overweight and obese subjects, while the higher consumption of MUFAs and PUFAs n-6 was negatively associated with lower Bifidobacterium abundance, the consumption of PUFAs n-3 was positively associated with higher Lactobacillus abundance. However, the abundance and diversity of the bacterial groups did not differ between normal-weight, overweight, and obese individuals (Simoes et al., 2013). On the other hand, the consumption of saturated fatty acids (SFAs) was positively associated with Blautia abundance in overweight and metabolic syndrome men (Org et al., 2017). In humans, the modulation of the microbiota by dietary fatty acids and as a function of the BMI presented is still inconclusive and controversial. Conversely, in animals, the increase in the Firmicutes/Bacteroidetes ratio after ingestion of high-fat diets is well consolidated (Abulizi et al., 2019; Murphy et al., 2010).

Despite evidence that the type of dietary fatty acids acts on the intestinal microbial ecosystem, further studies should be conducted to address the role of quality and quantity of the ingested fat on the microbiota (Wolters et al., 2018). Since the intake of a high fat diets favor obesity occurrence, a reduction in fat intake is recommended to prevent and control overweight and obesity. We did not identify in the literature any study in which the effect of oils sources of different types of fatty acids, associated with normofat diet, on intestinal microbiota and permeability in overweight individuals was evaluated.

The increase in fat intake usually leads to concomitant variation in the intake of other nutrients, such as reduced carbohydrate intake. In our study, the test diets differed in the type of fat, but presented similar macronutrients content. Vegetable oils are sources of fatty acids and vary greatly in fatty acid composition. For example, soybean oil, extra virgin olive oil and coconut oil are commonly used by the population, and are recommended for exhibiting antimicrobial and antioxidant activity (Coelho, Cândido, & Alfenas, 2018; George et al., 2018; Silva et al., 2020). Coconut oil is classified as a source of saturated fat (92%), whose medium chain fatty acids (AGCM), represent about 64%, with lauric acid (C12: 0) as its greatest representative (Boateng, Ansong, Owusu, & Steiner-asiedu, 2016; USDA, 2010). Olive oil, on the other hand, has a predominance of monounsaturated fatty acids (MUFA) (80%) and only 8–26% of these are saturated fatty acids (SFAs) (Quintero-flórez, Nieva, Sánchez-Ortíz, Beltrán, & Perona, 2015). While soybean oil is comprised of primarily polyunsaturated fatty acids (PUFAs), particularly linoleic acid (LA, C18:2), an omega-6 (ω6) fatty acid (Deol et al., 2017).

The identification of the predominant fatty acid effect in each of these oils on intestinal microbiota and permeability can contribute to identify strategies capable of preventing dysbiosis and associated diseases. Consequently, in the present study we evaluated if intake of oils containing different types of fatty acids (soybean oil, extra virgin olive oil and coconut oil), associated with an energy-restricted normofat diet, on intestinal microbiota and permeability, on LPS (Lipopolysaccharide) concentrations, and on fecal short chain fatty acids and pH.

2. Material and methods

2.1. Participants

Seven hundred and fifty-three (753) women were recruited in the local community and evaluated for eligibility criteria. Only women were selected because we understand that adherence to the prescribed diet would be greater, given that they are more concerned about their health. In addition, this eliminates possible biases in physiological differences between men and women when comparing treatments. The main inclusion criteria were: body mass index between 26 and 35 kg/m^2, body fat percentage >30%, age from 19 to 41 years, non-smokers, non-pregnant, non-lactating and with stable physical activity level (last 3 months). Exclusion criteria were: alcohol consumption (>15 g ethanol/d); elite athletes (>10 h of exercise/week); use of medications or supplements; presence of cardiovascular diseases, diabetes, hypertension, liver and/or gastrointestinal diseases; presence of food intolerance or allergy; recent changes (<3months) of eating habits. Eighty-five women were considered eligible and randomized in one of three experimental groups: soybean oil, extra virgin olive oil and coconut oil, using the block randomization technique (Zelen, 1974), with concealment for the researchers. Of these, 72 went to the intervention. Twenty participants were excluded from the analyses due to factors such as secondary pathologic events (n = 5), personal reasons (n = 3), pregnancy (n = 2) and for not collecting fecal samples (n = 10). After the 9 weeks of intervention, 52 women completed the study protocol (Fig. 1).

The study was conducted according to guidelines established in the Declaration of Helsinki, and all procedures involving subjects were approved by the Ethics Committee of the Federal University of Viçosa (protocol number: 892.467/2014). The trial was registered in the Brazilian Registry of Clinical Trials (ReBEC) (identifier: RBR-7z358j).

2.2. Study design

This is a 9 consecutive weeks (±5 days), randomized, parallel, double-blind clinical trial in which participants were randomly allocated to soybean oil (SO), extra virgin olive oil (EVOO) and coconut oil (CO) groups. During the study, participants daily attended the Food Intake Study Laboratory to consume a breakfast consisting of sweet or savory biscuits and a drink (300 mL) containing 25 mL of one of the tested oil types, as part of an energy-restricted (−500 kcal/day) and normofat (32% of calories from fats) individually prescribed diet. The drinks were served in colored glasses, to avoid visual identification of the type of oil tested. On weekends, participants received the ingredients and instructions for preparation and intake of the drink at home, accompanied by biscuits, in the same quantities provided in the laboratory. Breakfast intake was monitored through the return of empty packages to researchers and by questions asked to participants about consumption. The other meals of the day were consumed under free living conditions.

On the first and last day of the study (9th week), participants attended the laboratory to undergo anthropometric assessment, blood and urine samples collection, and delivery of the collected feces. To reduce the influence of different nutrients consumed in the evaluations performed on these test days, participants received a standardized meal to be ingested the night before the test days. This standard meal was composed of spaghetti, grated cheese and juice (600 kcal, carbohydrate: 62% Energy, fat: 29.4% Energy, protein: 8.5% Energy). After eating this meal, participants fasted for approximately 12 h.

On the test days (first and last day of the experiment) blood samples were collected in fasting to analyse the metabolic biomarkers and LPS concentrations. Urine and feces samples were collected for intestinal permeability and microbiota, besides fecal short chain fatty acid and pH analyses. On the first day of the study (baseline) anthropometrics, body composition and level of physical activity (International Physical Activity Questionnaire - IPAQ) (Hagstromer, Oja, & Sjo, 2006) were

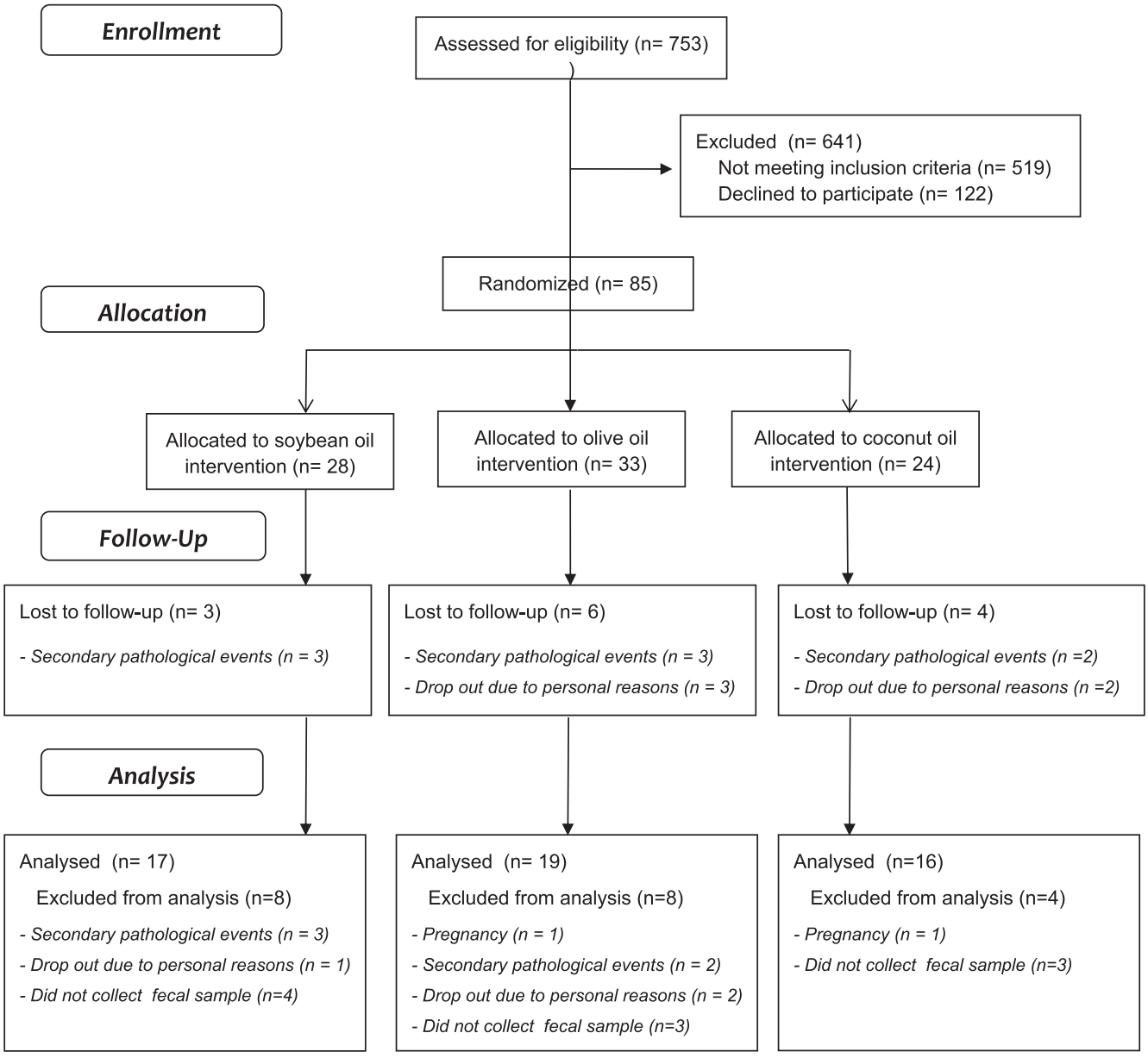

Fig. 1. CONSORT diagram showing the flow of participants through each stage of the trial. CONSORT Consolidated Standards of Reporting Trials.

assessed. The volunteers were instructed to maintain the level of physical activity and not to drink alcohol during the entire intervention.

2.3. Test drinks

Test drinks had seven different flavors (mango, guava, passion fruit, strawberry, grape, chocolate and cappuccino), had similar macronutrients and energy contents, and differing only in fatty acids quality (Table 1). Such drinks were prepared using skimmed milk powder and 25 mL of one of the following oils: soybean oil (Corcovado, Archer Daniels Midland, Uberlândia, Brazil), extra virgin olive oil (Andorinha®, Sovena S.A., Algés, Portugal) or coconut oil (Copra, Copra Indústria Alimentícia Ltda., Alagoas, Brazil). The amount of oil added was based on the range used in previous studies (Assunção, Ferreira, Dos Santos, Cabral, & Florêncio, 2009; Fung et al., 2009), without exceeding the daily dietary fat recommendation (IOM, 2005).

2.4. Prescribed diets

Prescribed diets were calculated considering the mean nutritional composition of the daily breakfast provided and the energy requirements, according to the Estimated Energy Requirement (EER) (IOM, 2002) for overweight and obese women. The level of physical activity (Hagstromer et al., 2006) was based on physical activity coefficients (1.00 for sedentary or 1.16 for active individuals) (IOM, 2005). Then, the energy restriction was applied (−500 kcal/day). No other fat source food having a content (saturated, mono and polyunsaturated) greater than 25 mL was prescribed. The diets prescribed to each experimental group presented similar energy density and macronutrients content (Supplementary table 1). Throughout the intervention period, the types of foods prescribed and the distribution of macronutrients were maintained to reduce the influence of the prescribed diet on the variables of interest.

Table 1
Mean ± SD test drink nutritional composition, according to experimental groups.

Test drink (mean ± SD of 7 menus)	Soybean oil	Extra virgin olive oil	Coconut oil
Energy (kcal)	346.63 ± 25.47	346.63 ± 25.47	346.63 ± 25.47
Carbohydrate (g)	24.23 ± 6.23	24.23 ± 6.23	24.23 ± 6.23
Protein (g)	2.94 ± 0.61	2.94 ± 0.61	2.94 ± 0.61
Dietary fiber (g)	1.29 ± 2.27	1.29 ± 2.27	1.29 ± 2.27
Sodium (mg)	58.58 ± 3.91	58.58 ± 3.91	58.58 ± 3.91
Total fat (g)	26.40 ± 0.11	26.40 ± 0.11	26.40 ± 0.11
Fatty acids (%)			
Lauric acid (C12:0)	–	–	10.59 ± 0.33
Tridecanoic acid (C13:0)	–	–	0.01 ± 0.00
Myristic acid (C14:0)	–	–	5.78 ± 0.13
Pentadecanoic acid (C15:0)	0.002 ± 0.00	–	0.002 ± 0.00
Palmitic acid (C16:0)	2.78 ± 0.00	2.47 ± 0.04	3.60 ± 0.12
Margaric acid (C17:0)	0.05 ± 0.03	0.07 ± 0.00	0.007 ± 0.01
Estearic acid (C18:0)	0.81 ± 0.02	0.53 ± 0.07	0.54 ± 0.03
Arachidic acid (C20:0)	0.09 ± 0.005	0.09 ± 0.005	0.02 ± 0.00
Total SFA	**3.74 ± 0.07**	**3.16 ± 0.12**	**20.56 ± 0.64**
Miristoleic acid (C14:1)	0.02 ± 0.00	0.002 ± 0.00	–
10-pentadecanoic acid (C15:1)	0.005 ± 0.00	–	–
Palmitoleic acid cis (C16:1)	0.02 ± 0.01	0.16 ± 0.00	–
Oleic acid (C18:1)	5.87 ± 0.01	20.20 ± 0.23	3.28 ± 0.07
Gadoleic acid (C20:1 n9)	0.05 ± 0.00	0.07 ± 0.00	0.02 ± 0.00
Total MUFA	**5.97 ± 0.04**	**20.44 ± 0.25**	**3.30 ± 0.07**
Linoleic acid (C18:2)	13.57 ± 0.06	1.22 ± 0.23	1.09 ± 0.02
α-linolenic acid (C18:3)	1.56 ± 0.01	0.14 ± 0.23	0.04 ± 0.04
8.11-eicosadienoic acid (C20:2)	0.11 ± 0.00	0.03 ± 0.00	0.002 ± 0.00
Total PUFA	**15.24 ± 0.08**	**1.39 ± 0.25**	**1.13 ± 0.06**

The nutritional composition of the test drinks was obtained from the Brazilian Food Composition Table (NEPA, 2011). The fatty acids profile of the test oils was determined according to Hartman and Lago (1973) SFA: saturated fatty acids, PUFA: polyunsaturated fatty acids, MUFA: monounsaturated fatty acids.

2.5. Food intake

Food intake was assessed by applying 3 non-consecutive days food records (2 days of the week and 1 weekday). Participants filled out the food records one week before the beginning of the study (baseline), during the study (4th week of intervention) and in the last week (9th week) to verify adherence to the prescribed diet. Macronutrients, fibers and energy intake was analysed and conducted by a single nutritionist, using the DietPro software (version 5.2i, Agromedia, MG, Brazil), based on food composition tables (NEPA, 2011; Philippi, 2016; USDA, 2017).

2.6. Anthropometrics and body composition

Anthropometrics evaluations were performed by a single investigator. Body weight was measured on a 0.5 kg platform scale (Toledo®, Model 2096PP/2, São Paulo, Brazil). Height was measured with an accuracy scale of 0.1 cm (Wiso, Chapecó, SC, Brazil). BMI (Body Mass Index) was calculated from the weight/height^2 ratio (kg/m^2) (World Health Organization, 2000). Waist circumference and abdominal sagittal diameter were measured at the midpoint between the last rib and iliac crest (Vasques et al., 2010). Body composition was evaluated using dual X-ray densitometry equipment (DEXA, model H8610FE ProdigyAdvance, General Electric Medical Systems).

2.7. Metabolic biomarkers

When blood samples were collected, they were centrifuged (3500 rpm, 4 °C, 15 min) and stored at −80 °C until analyses. The quantification of glucose, uric acid, total cholesterol, high density lipoprotein (HDL-c) and low density lipoprotein (LDL-c), triglycerides, aspartate amino transferase (TGO) and alanine amino transferase (TGP) was performed by the bs-200 automatic analytical system (Mindray Medical International Ldt., Shenzen, China) using commercial kits (Bioclin®, Minas Gerais, Brazil). Insulinemia was determined by electrochemilumluminescence method (Elecsys-Modular E-170, Roche Diagnostics Systems) and HOMA-IR (Homeostasis Model Assessment Index) assessed as proposed by Grisham, Johnson, and Lancaster-Junior (1996).

2.8. Lipopolysaccharide (LPS)

LPS concentrations were analysed by the Limulus Amebocyte Lysate (LAL) method, using a commercial kit (Hycult Biotech, Noord-Brabant, The Netherlands), according to the manufacturer's guidelines. For the analysis, plasma samples were manipulated in pyrogenic containers and heated to 75 °C for 5 min to neutralize endotoxin inhibitors. Plasma aliquots (5 mL) and patterns were diluted in pyrogenic water, added 30 mL of the LAL reagent and incubated for 30 min. Absorbance was read at 405 nm (Multiskan Go, Thermo Scientific, USA). Acetic acid solution was added to stop the reaction when necessary and rereading the absorbance was performed. The standard curve used to calculate LPS concentrations was adjusted by logistic regression and the LPS values corrected by the dilution factor (1:6). LPS concentration data are presented as EU/ml.

2.9. Intestinal microbiota, fecal short-chain fatty acids (SCFAs) and pH

Participants were instructed to collect the stool samples as close as possible to their delivery to the laboratory, and to keep it refrigerated (4 °C) until the time of delivery.

2.9.1. Intestinal microbiota

Collected stool samples were kept at −80 °C until analyses. DNA extraction from stool samples was performed by mechanical disruption (bead-beating) (Stevenson & Weimer, 2007). The quality and quantity of the extracted DNA were verified using μDropTM Plate (Thermo Fisher Scientific, Finland), the integrity and size were evaluated by electrophoresis in agarose gels, and subsequently the DNA samples were stored at −20 °C until sequencing analyses.

The sequencing of the V3-V4 hypervariable regions of the 16S rRNA gene of members of the Bacteria domain was performed at the Argonne National Laboratory® (Ilinois, USA), using the MiSeq platform (Illumina, San Diego, California, USA). Data processing and analysis were performed in Mothur v.1.40.0 program (Schloss et al., 2009). Paired-end sequences were superimposed from predefined parameters in the "make.contigs" command. Very short and very long sequences with ambiguous characters and homopolymers were removed. The sequences were aligned using the SILVA (v.132) 16S rRNA gene reference database (Quast et al., 2013). Chimeric sequences were detected and removed using UCHIME (Edgar, Haas, Clemente, Quince, & Knight, 2011). The taxonomic classification was performed using the SILVA database (v.132). The operational taxonomic units (OTU) were grouped with a cutoff point of 97% sequence similarity and the sequencing coverage for all samples was evaluated by "Good's Coverage". For the calculation of alpha diversity indices, the Chao1 (community richness), Shannon and Simpson indices (estimate diversity) were used. Beta diversity was evaluated by Principal Coordinate Analysis (PCoA) based on Bray-Curtis dissimilarity index. The raw sequences were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database under the access number PRJNA 542277.

2.9.2. Fecal short-chain fatty acids (SCFAs) and pH

For analysis, stool samples (~500 mg) were homogenized in 1 mL of Milli-Q water with the aid of vortex and centrifuged at 12,000*g* for 10 min. The supernatant was removed and the other steps were performed as described by Siegfried, Ruckemann, and Stumpf (1984).

Subsequently, the samples were analyzed by high performance liquid chromatography (HPLC), using a Dionex Ultimate 3000 Dual chromatograph coupled to a Shodex RI-101 refractive index (IR) detector maintained at 40 °C, and Phenomenex Rezex ROA ion exclusion column, 300 × 7.8 mm maintained at 40 °C. The mobile phase used was sulfuric acid (H_2SO_4) 5 mM with flow of 0.7 mL/min. Acetic, propionic, butyric, isobutyric, valeric and isovaleric acids were used as standards in the calibration curve.

For fecal pH analysis, about 1 g of feces was homogenized in 10 mL of distilled water, with the aid of vortex glass spheres. Subsequently, the glass electrode of the pHmeter was inserted. The measurements were performed in duplicate.

2.10. Intestinal permeability

Initially, participants eliminated all residual urine. They then ingested an isosmolar solution (200 mL) containing 6.25 g of lactulose and 2.0 g of mannitol, and remained for 6 h in the laboratory. During this period, participants ingested about 600 mL of water at regular intervals and all urine produced was collected and transferred to vials containing 12 mg of thimerosal to prevent bacterial growth. Samples were stored in a freezer at −20 °C until analyses. For the analyses, two milliliters of urine were incubated in a water bath for 10 min at 56 °C and centrifuged at 10,000 rpm for 7 min. They were filtered through a polyetersulfonic microporous membrane (PES) (0.22 μm × 13 mm) and about 600 μL transferred to vials. Urinary excretions of lactulose and mannitol were evaluated by high- performance liquid chromatography (HPLC) using a Dionex Ultimate 3000 Dual coupled to a refractive index (IR) detector (Shodex RI-101) maintained at 40 °C. Analytes were separated in a Phenomenex Rezex ROA ion exclusion column (300 × 7.8 mm) maintained at 40 °C. The mobile phase used was sulfuric acid 5 mM with flow of 0.7 mL/min. For the normalization curve, lactulose and mannitol patterns were used. The total volume of urine collected was multiplied by the determined concentration of each sugar, thus obtaining the amount excreted in the urine. From the dose of mannitol and lactulose administered, the percentage of lactulose (%L) and mannitol (%M) was calculated. Next, the ratio Lactulose/Mannitol (L/M) was calculated (Teixeira et al., 2012; Vilela et al., 2008).

2.11. Statistical analyses

The present study presented a statistical power of 99% (Mera, Thompson, & Prasad, 1998) to detect a difference of 50% in the LPS concentrations (Boutagy, Mcmillan, Frisard, & Hulver, 2017), considering the mean and standard deviation (0.56 ± 0.26 EU/ml) of theat variable presented by our participants at baseline. LPS concentrations were adopted as the main variable, since this is an indicator of metabolic endotoxemia that is associated with changes in metabolism and intestinal microbiota. Anthropometric variables, food intake, biochemical markers, intestinal permeability, fecal pH data, besides LPS and SCFAs concentrations statistical analyses were performed in SPSS, version 22.0 for Windows (SPSS, Inc., Chicago, IL, USA). Outlier values for these variables were excluded (lower = lower quartile – (1.5 × interquartile interval) and upper = upper quartile (1.5 × interquartile interval). Intragroup differences (9 weeks vs.baseline) were evaluated by paired *t*-test or Wilcoxon. Differences between groups were evaluated by the ANOVA or Kruskal-Wallis test, followed by Bonferroni's post hoc test. The α level of 5% was considered significant. Normality was tested by the Shapiro-Wilk test for all variables analysed.

Alpha diversity indices were calculated using Mothur (Schloss et al., 2009), intra and group differences were analysed by SPSS version 22.0 for Windows (SPSS, Inc., Chicago, IL, USA), using the Wilcoxon and Kruskal-Wallis test, respectively. Beta diversity analyses to compare the microbial composition at baseline and after 9 weeks of intervention in each group and between groups, were assessed at the level of OTUs, phyllo and gender, the Principal Coordinate Analysis (PCoA) was used based on the Bray-Curtis paired distance and nonparametric similarity analysis (ANOSIM) with permutation number 10,000 and the aid of the PAST software (Hammer, Haper, & Ryan, 2001). Differences in the relative abundance of OTUs after the consumption of each oil was evaluated by the White's non-parametric *t*-test and differences between groups evaluated by the Kruskal-Wallis test, using the software STAMP v 2.1.3 (Statistical Analysis of Taxonomic and Functional Profiles Statistical Analysis of Taxonomic and Functional Profiles) (Parks, Tyson, Hugenholtz, & Beiko, 2014).

For analyses of relative abundance at phylum and gender level, the Wilcoxon test was used to detect intra-group differences after the intervention, differences between groups were analyzed by the Kruskal-Wallis test. To view the differences, the "fold change" (ratio between two quantities measured at different times, final/baseline) was calculated. The p-values were adjusted using Benjamini-Hochberg's False Discovery Rate (FDR). Values of $P < 0.05$ and $P\_FDR < 0.05$ were considered significant in all analyzes. The analyses were performed in the R program (version 3.5.0), using the packages phyloseq and DESeq2 (Love, Huber, & Anders, 2014; McMurdie & Holmes, 2013). Correlation analyzes were also performed in the R program (version 3.5.0).

3. Results

3.1. Characteristics of study participants

Fifty-two women completed the study protocol and were included in the analyses. Anthropometric measurements, body composition and biochemical variables were similar between groups at baseline (Table 2). Participants mean of body fat percentage at baseline was equivalent to 46.83 ± 0.58%. According to the BMI, 53.85% (n = 28) of the participants were overweight and 46.15% were obese (n = 24). Women were 26.81 ± 0.74 years old and they had a mean waist circumference of 96.88 ± 1.04 cm. According to the cutoff points

Table 2
Baseline characteristics of study subjects.

	Soybean oil	Extra virgin olive oil	Coconut oil
Subjects (n)	17	19	16
Age (years)	26.18 ± 1.21	26.79 ± 1.10	27.50 ± 1.62
Body weight (kg)	80.90 (16.92)	77.55 (11.95)	75.00 (15.95)
Body mass index (kg/m^2)	30.05 (5.78)	29.70 (5.31)	29.98 (5.99)
Total body fat percentage (%)	47.20 ± 0.74	46.93 ± 1.01	46.32 ± 1.25
Total lean mass percentage (%)	38.73 (2.63)	38.68 (5.77)	36.28 (7.25)
Waist circumference (cm)	96.23 ± 1.65	97.57 ± 1.64	96.75 ± 2.21
Sagittal abdominal diameter (cm)	19.70 ± 0.49	19.53 ± 0.43	19.50 ± 0.57
Insulin (pmol/L)	55.98 ± 3.82	63.68 ± 4.51	54.03 ± 5.97
Glucose (mmol/L)	4.74 ± 0.11	4.94 ± 0.09	4.86 ± 0.12
HOMA -IR	1.58 (0.79)	1.96 (1.18)	1.56 (1.04)
Total cholesterol (mmol/L)	4.11 ± 0.20	4.49 ± 0.21	4.50 ± 0.14
HDL-c (mmol/L)	1.19 ± 0.07	1.28 ± 0.08	1.17 ± 0.05
LDL-c (mmol/L)	2.29 ± 0.15	2.58 ± 0.15	2.80 ± 0.11
Triglycerides (mmol/L)	1.01 ± 0.10	1.22 ± 0.12	1.01 ± 0.06
Uric Acid (mg/dL)	3.52 ± 0.14	3.36 ± 0.19	3.69 ± 0.24
TGO (U/L)	34.00 (12.75)	29.00 (13.00)	33.00 (13.50)
TGP (U/L)	16.00 (6.50)	14.00 (8.00)	14.00 (9.00)

Values are mean ± SE or median (interquartile range). Different letters indicate statistical significance (ANOVA or Kruskal-Wallis, $P > 0.05$, followed by Bonferroni's post hoc). HOMA-IR homeostasis model assessment of insulin resistance , HDL-c: high-density lipoprotein cholesterol, LDL-c: low-density lipoprotein cholesterol , TGO:aspartate amino transferase; TGP: alanine amino transferase.

established by the Brazilian Society of Cardiology (Faludi et al., 2017), these participants had a high concentration of cholesterol (23.07%), triglycerides (9.61%) and LDL (7.69%). None of them had diabetes (World Health Organization, 2016), symptoms of infection and/or inflammation, changes in the functioning of the gastrointestinal tract or menstrual changes.

After the 9-week intervention period, all three groups showed a reduction in body weight, waist circumference and abdominal sagittal diameter. While only those who consumed soy oil and extra virgin olive oil showed a reduction in BMI (Supplementary Table 2). Similarly, a reduction in glucose concentration was observed in the OS and OOEV groups after the intervention. However, changes (9 weeks - baseline) in metabolic biomarkers did not differ significantly between groups after the 9 weeks of experiment (Supplementary Table 3).

The consumption of calories, carbohydrate, protein, total fat and dietary fiber did not differ significantly between groups during the intervention period. As expected, due to differences in the ingested test drinks fatty acids quality, the consumption of saturated, monounsaturated and polyunsaturated fatty acids was significantly different between groups (Supplementary Fig. 1).

3.2. Fecal short-chain fatty acids concentration and pH

The consumption of soybean oil for 9 weeks reduced the concentration of isovaleric acid. The other short chain fatty acids and fecal pH did not differ at the end of each intervention and between groups (Table 3). Correlation between changes of isovaleric acid concentration and of intestinal bacteria, and between changes of isovaleric acid concentration and intake of PUFAs not was verified.

3.3. Intestinal permeability and LPS concentrations

There was an increase in urinary lactulose excretion in response to the consumption of extra virgin olive oil and coconut oil, while the consumption of coconut oil resulted in an increase in the excretion of mannitol at the end of the experiment. The consumption of extra virgin olive oil also increased the lactulose/mannitol ratio after 9 weeks of intervention. There were no significant changes in the LPS concentrations between groups (Table 4). Correlation between changes of LPS concentration and intestinal permeability level not was observed.

3.4. Intestinal microbiota

In total, 3,540,737 bacterial sequences were generated, with a maximum length of 302 bp, an mean length of 253 bp and a minimum length of 151 bp in all samples. After trimming, filtering and removing chimeras, 2,541,747 bacterial sequences were obtained. The mean number of sequences and OTUs that passed through the filtration, cleaning and normalization steps are shown in supplementary Table 4. The "Good's coverage" of the bacterial community was 0.99 for all treatments, indicating that the sequencing efforts were sufficient to capture the community diversity in the samples under investigation.

Bacterial communities did not vary in terms of the alpha diversity estimated by the Shannon and Simpson indices. However, the microbial richness, estimated by the Chao 1 index, increased after the soybean oil consumption, while in the other groups it remained unchanged (Fig. 2). According to the beta-diversity analyses, no significant changes between bacterial communities were observed in response to the consumption of the three types of oils, at the level of OTU, phyla and genera, as indicated by PCoA the analysis and analysis of similarities (ANOSIM) (Fig. 3A–C). We did not observe significant differences, in terms of OTU, phyla and genera, when comparing the three experimental groups at baseline and at the end of the experiment (Supplementary Fig. 2).

According to the beta diversity analyses, no significant changes between bacterial communities were observed in response to the consumption of the three types of oils, at the level of OTU, phyla and genera, indicated by the PCoA analysis and ANOSIM (Fig. 3A–C). There were no significant differences, in terms of OTU, phyla and genera, when comparing the three experimental groups at baseline and at the end of the experiment (Supplementary Fig. 2).

Table 3
Fatty acids and pH values at baseline, after 9 weeks of intervention, and delta values (9 weeks - baseline), according to the experimental group.

		Soybean oil (n = 17)	P_{Intra}	Extra virgin olive oil (n = 19)	P_{Intra}	Coconut oil (n = 16)	P_{Intra}	P_{Inter}
Acetic (g/L)	B	5.90 (2.98)	0.084	5.35 (2.24)	0.968	4.25 (1.68)	0.569	0.142
	E	5.57 (3.92)		5.62 (3.84)		4.51 (2.64)		0.455
	Δ	0.82 ± 0.41		−0.03 ± 0.44		0.16 ± 0.55		0.406
Propionic (g/L)	B	2.90 (2.24)	0.246	3.38 (1.82)	0.948	2.16 (1.88)	0.955	0.437
	E	3.27 (2.86)		3.42 (2.56)		2.71 (1.90)		0.264
	Δ	0.69 ± 0.50		0.10 ± 0.42		0.04 ± 0.46		0.552
Butyric (g/L)	B	4.53 (3.82)	0.535	4.86 (3.44)	0.058	2.56 (2.40)	0.878	0.240
	E	4.89 (4.99)		3.59 (4.01)		2.97 (4.48)		0.757
	Δ	0.44 ± 0.55		−1.01 ± 0.57		0.67 ± 0.82		0.085
Isobutyric (g/L)	B	0.69 (0.58)	0.925	0.58 (0.49)	0.215	0.94 (0.90)	0.638	0.668
	E	0.69 (0.46)		0.56 (0.66)		0.71 (0.78)		0.421
	Δ	−0.01 ± 0.13		−0.11 ± 0.11		−0.05 ± 0.22		0.890
Valeric (g/L)	B	1.04 (1.01)	0.379	0.91 (0.62)	0.420	0.80 (0.85)	0.374	0.733
	E	0.94 (1.11)		0.94 (0.77)		0.90 (1.09)		0.873
	Δ	−0.09 ± 0.18		−0.11 ± 0.13		0.17 ± 0.27		
Isovaleric (g/L)	B	0.58 ± 0.08	**0.026**	0.48 ± 0.07	0.416	0.48 ± 0.07	0.687	0.562
	E	0.39 ± 0.04		0.45 ± 0.06		0.49 ± 0.06		0.353
	Δ	−0.20 ± 0.07		0.10 ± 0.11		0.05 ± 0.13		0.114
Total Fatty acids (g/L)	B	14.25 ± 1.36	0.302	16.39 ± 1.05	0.464	12.59 ± 1.37	0.610	0.106
	E	15.84 ± 1.73		15.23 ± 1.58		13.56 ± 2.06		0.661
	Δ	1.59 ± 1.50		−1.16 ± 1.56		0.97 ± 1.87		0.448
pH	B	7.22 (0.59)	0.510	7.08 (0.62)	0.093	7.32 (0.25)	0.629	0.170
	E	7.23 (0.64)		7.24 (0.27)		7.20 (0.52)		0.942
	Δ	−0.23 ± 0.15		0.13 ± 0.13		−0.14 ± 0.13		0.143

Values are mean ± SE or median (interquartile range). B: Baseline; E: End (after 9 week). **Δ** values (9 week s – baseline). $\mathbf{P_{Intra}}$ – differences within groups (paired *t*-test or Wilcoxon signed-rank test, $P < 0.05$). $\mathbf{P_{Inter}}$ _ differences between groups (ANOVA or Kruskal Wallis, $P < 0.05$). Bold numbers indicate significant differences ($P < 0.05$).

Table 4
Variables of intestinal permeability and LPS concentration at baseline, after 9 weeks of intervention and delta values (9 weeks - baseline), according to the experimental group.

		Soybean oil (n = 17)	P_{Intra}	Extra virgin olive oil (n = 19)	P_{Intra}	Coconut oil (n = 16)	P_{Intra}	P_{Inter}
LPS (EU/ml)	B	0.52 (0.36)	0.087	0.60 (0.56)	0.053	0.39 (0.40)	0.875	0.112
	E	0.44 (0.27)		0.34 (0.54)		0.43 (0.18)		0.836
	Δ	−0.05 (0.24)		−0.15 (0.41)		0.03 (0.24)		0.246
Lactulose (%)	B	0.37 ± 0.10	0.167	0.52 ± 0.08	**0.005**	0.45 ± 0.07	**0.001**	0.672
	E	0.88 ± 0.27		1.33 ± 0.20		1.83 ± 0.29		0.305
	Δ	0.51 ± 0.33		0.81 ± 0.24		1.38 ± 0.31		0.137
Mannitol (%)	B	4.09 ± 0.50	0.357	4.71 ± 0.38	0.289	4.06 ± 0.37	**0.004**	0.679
	E	5.39 ± 1.06		5.59 ± 0.66		7.82 ± 1.16		0.098
	Δ	1.31 ± 1.21		0.88 ± 0.80		3.76 ± 1.05		0.112
L/M	B	0.10 ± 0.04	0.287	0.15 ± 0.06	**0.022**	0.17 ± 0.08	0.158	0.830
	E	0.26 ± 0.06		0.23 ± 0.04		0.28 ± 0.08		0.731
	Δ	0.12 ± 0.10		0.17 ± 0.03		0.12 ± 0.05		0.713

Values are mean ± SE or median (interquartile range). B: Baseline; E: End (after 9 week). **Δ** values (9 weeks – baseline). $\mathbf{P_{Intra}}$ – differences within groups (paired *t*-test or Wilcoxon signed-rank test, $P < 0.05$). $\mathbf{P_{Inter}}$ – differences between groups (ANOVA or Kruskal Wallis, $P < 0.05$). Bold numbers indicate significant differences ($P < 0.05$).

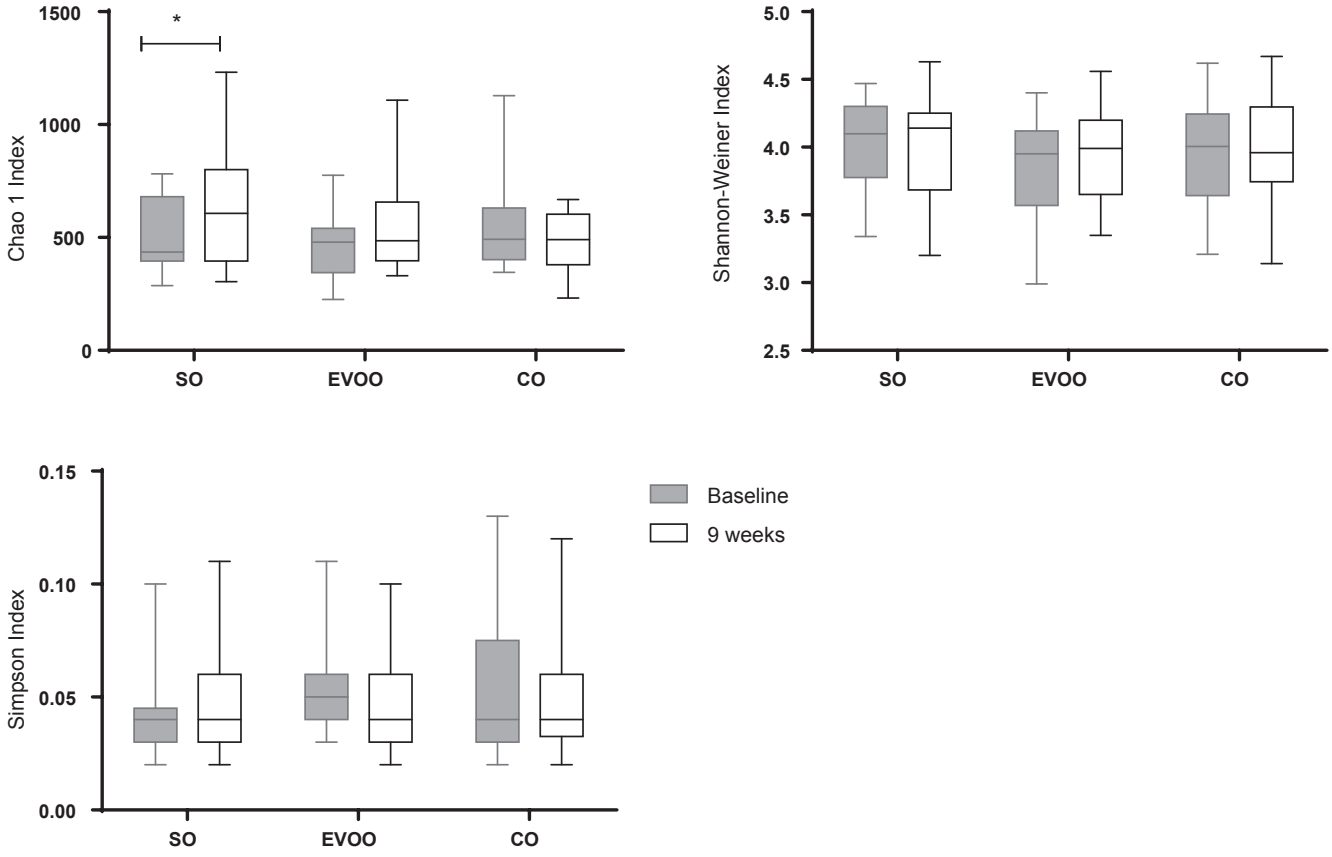

Fig. 2. Alpha-diversity estimated by Chao 1, Shannon-Weiner and Simpson indices at baseline and after 9 weeks of ingestion of 25 mL of soybean oil (SO) (n = 17), extra virgin olive oil (EVOO) (n = 19) and coconut oil (CO) (n = 16). * Differences within groups (paired *t*-test or Wilcoxon test, $P < 0.05$). ** Differences between groups (ANOVA or Kruskal Wallis, $P < 0.050$).

Taxonomic analyses of the bacterial community in response to soybean oil consumption revealed the existence of 1927 OTUs that were classified into 15 phyla, 20 classes, 35 orders, 66 families, and 226 genera. We observed 1865 OTUs, 16 phyla, 23 classes, 38 orders, 71 families and 243 genera in the group that consumed extra virgin olive oil, while in the group that consumed coconut oil, 1645 OTUs, 15 phyla, 22 classes, 38 orders, 70 families, and 225 genera were identified. OTUs that showed relative abundance > 0.5% after the consumption of each oil are shown in Supplementary Table 5. However, no significant changes in OTU composition was observed between the experimental groups.

The most abundant phyla (>0.1%) in all experimental groups before and after the intervention were: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Tenericutes, Verrucomicrobia and Lentisphaerae. We did not observe significant variations in the relative abundance of phyla after 9 weeks of consumption of any of the tested oils or between the oils at baseline and at the end of the experiment (Fig. 4). When considering the two predominant phyla, Firmicutes and

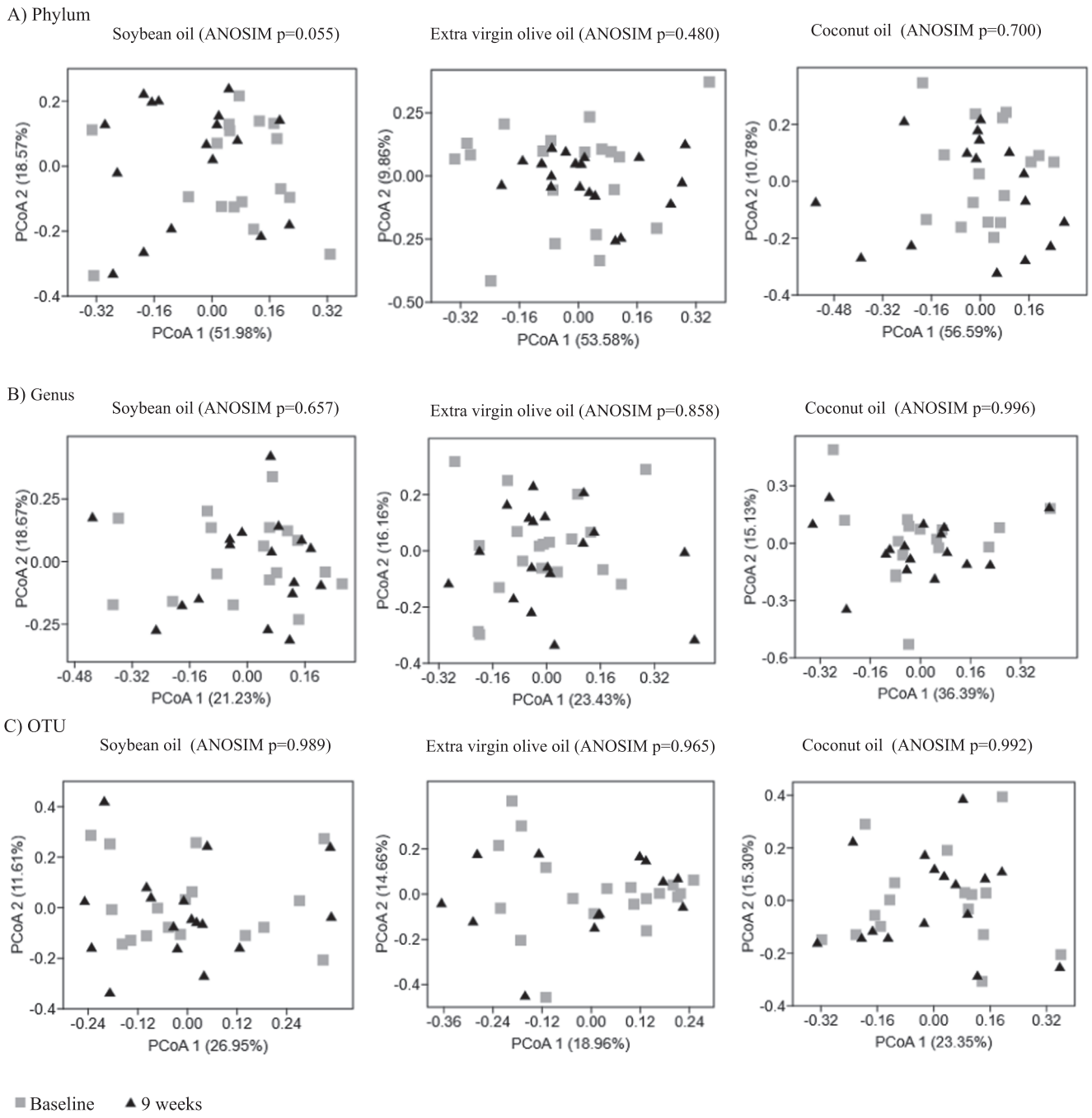

Fig. 3. Changes in β-diversity after the intervention. A) Principal Coordinate Analysis (PCoA) based on the Bray-Curtis distance at the Phylum level. B) Principal Coordinate Analysis (PCoA) based on Bray-Curtis distance at Genus level. C) Principal Coordinate Analysis (PCoA) based on Bray-Curtis distance at OTU level. ■ Baseline ▲ 9 Weeks.

Bacteroidetes, we did not observe significant changes between and after oil consumption. We also did not observe significant changes in the Firmicutes/Bacteroidetes ratio within and between experimental groups (Fig. 4).

At the gender level, some significant changes were observed after the consumption of the three different types of oils. However, after removing false positives, using the Benjamini-Hochberg ratio of false discoveries (FDR), these differences did not remain significant at the α level of 5%. In addition, there were no differences between groups (Supplementary Table 6).

4. Discussion

To our knowledge, this is the first randomized, parallel and double-blind clinical trial to investigate the effect of consuming different oil types, associated with an energy-restricted normofat diet, on gut microbiota and intestinal permeability in overweight women. Although the consumption of 25 mL/day of soybean oil (rich in PUFAs), extra virgin olive oil (rich in MUFAs) or coconut oil (rich in SFAs), for 9 weeks, did not induce significant changes in microbial diversity intestinal, we observed an increase in bacterial richness in response to soybean oil consumption. In addition, although the integrity and absorptive function of the intestinal mucosa were altered after extra virgin olive oil and coconut oil ingestions, such changes did not lead to metabolic

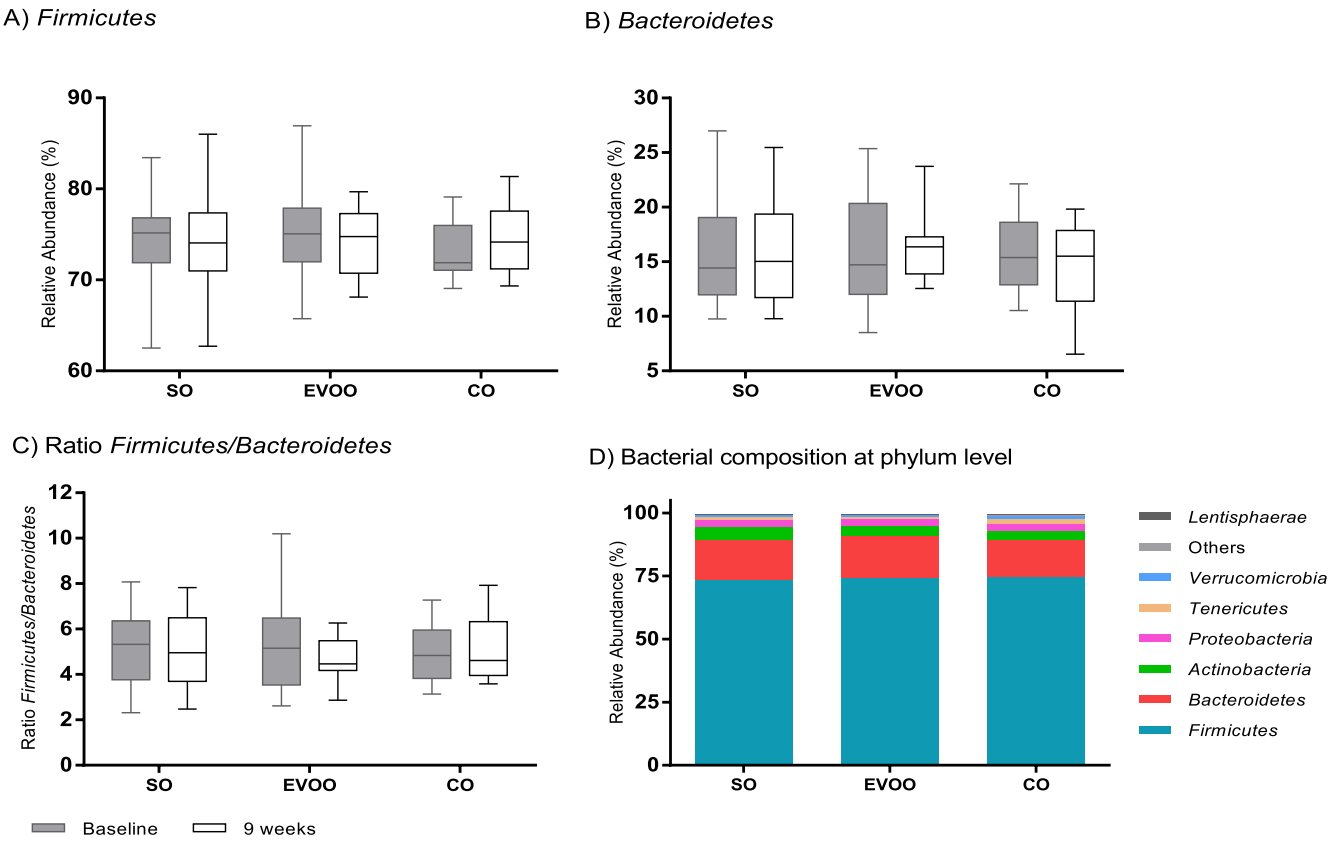

Fig. 4. Bacterial composition at phylum level. A) Relative abundance of the Firmicutes before and after 9 weeks according to experimental groups. B) Relative abundance of the Bacteroidetes before and after 9 weeks according to experimental groups. C) Firmicutes/Bacteroidetes ratio before and after 9 weeks according to experimental groups. D) Bacterial composition at phylum level at the end of 9 weeks of intervention, according to the experimental group. SO: soybean oil; EVOO: extra virgin olive oil; CO: coconut oil; The category "Others" corresponds to phyla with relative abundance < 0.1%, they are: Fusobacteria, Spirochaetes, Elusimicrobia, Epsilonbacteraeota, Synergistetes, Kiritimatiellaeota, Acidobacteria, Planctomycetes, Cloacimonetes, Deferribacteres, Patescibacteria.

endotoxemia.

Our results suggest that the consumption of different types of oils can lead to modest changes in the intestinal microbiota and permeability in overweight women. The results obtained in other human intervention studies also suggest that the effect of dietary fat on the intestinal microbiota composition may be modest and even null (Blaedel et al., 2016; Lang et al., 2018; Morales et al., 2016; Rajkumar et al., 2014). On the other hand, authors of observational studies suggest a strong association of the microbiota and dietary fat versus cardiometabolic factors (Org et al., 2017; Röytiö, Mokkala, Vahlberg, & Laitinen, 2017; Simoes et al., 2013).

Dietary fat digestion begins with the action of lingual and gastric lipases, and is completed by pancreatic lipase. After hydrolysis, free fatty acids can be absorbed in the small intestine (Wang, Liu, Portincasa, & Wang, 2013). It is estimated that 7% of the ingested fat are not absorbed and reach the large intestine (Gabert et al., 2011). Thus, in our study, about 1.75 mL must have reached the colon. We believe that this amount was insufficient to promote significant changes in intestinal bacterial diversity and relative abundance in our study. However, we observed an increase in species richness, estimated by the Chao 1 index, after soybean oil ingestion, rich in PUFAs.

Long-chain fatty acids (>12 carbon atoms) are digested and absorbed more slowly than short- and medium-chain ones (Ye et al., 2019). In addition, such fatty acids are incorporated into chylomicrons and transported by the lymphatic system, while short and medium chain fatty acids are absorbed directly into the systemic circulation (Ye et al., 2019). Thus, it is possible that soybean oil, in which long-chain fatty acids predominate, had a lower rate of digestion and absorption and a longer time of intestinal transit, allowing them to reach the colon, interacting with the intestinal microbiota and increasing the bacterial richness.

In contrast to our results, the consumption of a diet containing soybean oil as the main fat source and with moderate fat content (30% fat in relation to energy) by healthy individuals did not affect Chao1 and Ace richness, after 6 months of experiment (Wan et al., 2019). The intake of oils rich in MUFAs and PUFAs in diets containing 35% fat, for 30 days by individuals at risk of metabolic syndrome also had no impact on the alpha-diversity indexes (Pu et al., 2016). Changes in the energy content of the diet can also modulate changes in the microbiota (Heinsen et al., 2016). Thus, we believe that the association of energy-restriction with the consumption of PUFAs is responsible for the increase in the number of bacterial species, thus explaining the divergence of the results in the current study compared to those obtained by Pu et al. (2016) and Wan et al. (2019).

Apparently, a lower microbial richness is one of the factors associated with greater adiposity, insulin resistance, dyslipidemia and inflammatory factors (Chatelier et al., 2013). Although alpha diversity alone is not a determinant of the microbiota's benefits on the host, increased species richness can have a positive impact on host health (Chatelier et al., 2013), in addition to being associated with lower intestinal permeability (Mokkala, Ro, & Ekblad, 2016). In the present study, we observed a reduction in glucose concentration in the OS and OOEV groups after the intervention. Despite of that, intestinal permeability remained unchanged after soybean oil ingestion, suggesting that the bacterial composition may have a greater impact on the barrier function than the number of species (richness) itself.

The results of previous studies suggest that the ingestion of high fat diets may increase intestinal permeability, facilitating the passage of

endotoxins, activating the immune system and leading to low-grade inflammation, associated with the pathogenesis of several metabolic diseases (Moreira, Teixeira, & Peluzio, 2012; Rohr, Narasimhulu, Rudeski-rohr, & Parthasarathy, 2019; Wisniewski, Dowden, & Campbell, 2019). However, in our study, we did not observe changes in LPS concentrations after consuming a normofat diet containing the three different types of fatty acids. Thus, we believe that losses in intestinal permeability were not a sufficient condition to generate metabolic endotoxemia, since the overweight and obese subjects essentially have increased intestinal permeability.

After ingesting extra virgin olive oil, we observed an increase in the excretion of lactulose and the lactose/mannitol ratio, reflecting the intestinal mucosa integrity impairment, with losses in the multiprotein complex that form the firm junctions, responsible for paracellular pathway of transport (Wells et al., 2017). In addition to the increase in lactulose excretion, we observed a greater excretion of mannitol after coconut oil ingestion, indicating an increase in absorptive function with decreased resistance of intestinal epithelial cells via the transcellular route (Wells et al., 2017). These results suggest that the consumption of different types of fatty acids for 9 weeks led to different responses in intestinal permeability in overweight women.

In an in vitro study, PUFAs were able to improve the epithelial barrier integrity, while palmitic saturated fatty acid (C16: 0) showed little impact on this function (Willemsen et al., 2008). These effects can be attributed to the ability of PUFAs to increase the formation of firm junctions and to stimulate the differentiation and maturation of intestinal cells (Teixeira, Moreira, Souza, Frias, & Peluzio, 2014). In animals, increased permeability in response to ingestion of SFAs was associated with a greater abundance of sulfidogenic bacteria, such as Biophilia (Lam et al., 2015). While n-3 PUFAs consumption was related to the presence of bacteria that preserve the barrier function, such as bifidobacteria (Griffiths et al., 2004; Patterson et al., 2017).

We believe in the possibility of a link between changes in the composition of the microbiota induced by the diet and intestinal permeability in our study. However, the question is how the composition of the colon microbiota interferes with the permeability of the small intestine or vice versa. We believe that the increase in small intestine permeability observed in our study may be related to changes in the composition of the microbiota at the site, which were not identified by the stool analysis performed. Fecal samples analysis, as conducted in our study, most effectively reflects the composition of bacterial communities in the distal portions of the intestine (Kastl, Terry, Albenberg, & Wu, 2019; Zoetendal et al., 2012). Interestingly, supporting our idea, the reduction in the abundance of the Clostridia class, commonly present in the small intestine, was associated with reduced barrier function and increased permeability in this portion of the intestine (Araújo, Tomas, Brenner, & Sansonetti, 2017).

Although the population of bacteria in the small intestine is less diverse and abundant (duodenum 10^3, jejunum 10^4, ileo 10^7, colon 10^{12} cells/g) (Sommer & Beackhed, 2016), the interaction between these bacteria and the host seems to be essential to regulate fat digestion and absorption processes in animals (Martinez-guryn et al., 2018). In humans, the role of bacteria present in the small intestine has been neglected, and studies on this topic are scarce (Chang & Martinez-guryn, 2019). Future research investigate the microbiota-host mechanisms and interactions in the small intestine and their relationship with fat metabolism and excess weight is needed (Kastl et al., 2019).

In our study, we observed that the consumption of soybean oil reduced the concentration of isovaleric fatty acid in fecal samples. Probably, the bacterial species were less efficient on colonic fermentation, leading to less SCFAs production. Possible changes in abundance of proteolytic bacteria, such as Bacteroides and Clostridium, may be associated with the production of short chain branched fatty acids, such as isovaleric (Granado-serrano, Martín-garí, Sánchez, Solans, & Berdún, 2019). Isovaleric acid is often the predominant fatty acid end product of microbial origin produced from the metabolism of leucine. The branched short-chain fatty acids can have effects on adipocyte lipid and glucose metabolism that can contribute to improved insulin sensitivity in individuals with disturbed metabolism (Heimanna, Nyman, Lbrink, Lindkvist-Petersson, & Degerman, 2016). In addition, isovaleric acid may be related to other health symptoms such as depression and cortisol levels. Additionally bacteria that previously have been identified as being correlated with depression were also correlated with isovaleric acid (Szczesniak, Hestad, Hanssen, & Rudi, 2016).

Apparently, the influence of the microbiota on metabolism may reside in the presence of less abundant bacteria (Cuevas-sierra et al., 2019). In addition, some authors report that changes in the intestinal microbiota can be hidden by high inter-individual variations, especially in dietary interventions consuming moderate amounts of nutrients (Lang et al., 2018; Wan et al., 2019). Another possible explanation for modest changes in the intestinal microbiota observed in the present study is the antimicrobial and anti-inflammatory action exerted by fatty acids (Coelho et al., 2018). In an in vitro study carried out by our research group, we observed that coconut oil had great antibacterial activity, even at very low concentrations. Exposure to fatty acids present in coconut oil, mainly lauric, was able to inhibit the growth of bacteria related to excess body weight (data not yet published). Long-chain fatty acids, MUFAs and PUFAs, including alpha-linolenic (C-18: 3), linoleic (C-18: 2) and oleic (C-18: 1) can also affect bacterial survival (Coelho et al., 2018; Jackman, Yoon, Li, & Cho, 2016).

Significant effects of dietary fat on the intestinal microbiota have been reported in response to higher than recommended fat intake (>35% of energy from fat) associated with lower carbohydrate intake (Fava et al., 2012; Wan et al., 2019), or in response to unsustainable dietary treatments (69.5% lipids compared to energy) applied for a short period of time (10 days) (David et al., 2014). However, in our study we applied a nutritional strategy commonly used to treat excess weight. The experimental groups received isocaloric, normolipidic, energy-restricted diets, presenting similar macronutrients and dietary fiber contents, varying only in the type of fatty acid ingested. Therefore, the results were obtained without altering the proportion of the intake of other nutrients, especially those that are metabolized primarily by the intestinal bacteria, such as carbohydrate.

Another strong point of our study was using amplicon-based target sequencing based on the 16S rRNA gene, to allow a more complete and comprehensive assessment of the intestinal microbiota. In addition, we conducted a randomized, double-blind clinical trial, with rigorous participants selection criteria and double data entry. The consumption of the type of oil tested, adherence to the prescribed diet and the level of physical activity exercised by the participants were well controlled.

On the other hand, the analyses of fecal samples that was assessed may not reflect the intestinal microbiota prevalent in all parts of the intestine, being associated mainly with colonic communities. Despite being more invasive and difficult to access, the collection of material from the small intestine would have been challenging and interesting to obtain an analysis of the microbiota in the entire intestine. Fecal determination of saturated, mono and polyunsaturated fatty acids concentrations in the test oils would be an important indicator of their level of absorption. Furthermore, as the method used for taxonomic classification did not allow the assignment of sequences at the species level, small taxonomic changes may not have been identified. It is also possible that the 9 weeks of intervention were not enough to observe the effects of the test oils on the intestinal microbiota. On the other hand, in an animal study it was clear the effect of the type of oil consumed on the microbiota after 5 weeks of intervention (Abulizi et al., 2019).

5. Conclusion

Daily consumption of 25 mL of oils presenting different types of fatty acids associated with the normofat and energy-restricted diet did not affect the intestinal bacteria diversity and relative abundance. However, the consumption of soybean oil, a source of PUFAs, increased the

richness and reduced the concentration of isovaleric short chain fatty acid. Paracellular and transcellular permeability increased after ingesting extra virgin olive oil and coconut oil. However, LPS concentrations remained unchanged, configuring the absence of metabolic endotoxemia.

Thus, the intake of a normolipidemic diet, containing different types of fatty acids, associated with an energy-restricted diet modestly affected the intestinal microbiota and permeability in overweight women. Knowledge about the effects of oils, sources of different types of fatty acids (SFAs. MUFAs and PUFAs) on the composition of the microbiome and on the intestinal permeability in overweight individuals may have potential therapeutic implications for the maintenance of human health.

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CRediT authorship contribution statement

Thalita Lin Netto Cândido: Conceptualization, Methodology, Validation, Formal analysis, Writing - original draft. **Laís Emilia da Silva:** Conceptualization, Methodology, Validation, Formal analysis. **Flávia Galvão Cândido:** Methodology, Investigation. **Flávia Xavier Valente:** Methodology, Investigation. **Juliana Soares da Silva:** Software, Validation, Formal analysis, Data curation. **Déborah Romaskevis Gomes Lopes:** Software, Validation, Formal analysis, Data curation. **Maria do Carmo Gouveia Peluzio:** Supervision. **Hilário Cuquetto Mantovani:** Conceptualization, Resources, Supervision, Project administration. **Rita de Cássia Gonçalves Alfenas:** Conceptualization, Resources, Visualization, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2020.109951.

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