Journal of Functional Foods 66 (2020) 103780

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Different source of commercial vegetable oils may regulate metabolic, inflammatory and redox status in healthy rats

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ARTICLE INFO

Keywords:
Inflammation
Polyunsaturated fatty acid
Redox process
Saturated fatty acid
Vegetable oils

ABSTRACT

Our goal was to carry out a comparative study to evaluate the metabolic and inflammatory effects and the redox status of commercial vegetable oils supplementation [linseed (LO), coconut (VCO), and sunflower (SO)] in metabolically healthy rats. The results found in this study showed that the LO group decreased the HOMA-IR and hepatic cholesterol, and increased the serum levels of IL-6. Supplementation with VCO increased glucose and HOMA-IR, cholesterol concentration and serum triacylglycerol (TAG). In this group, there was also an increase in TBARS. In the SO group there was a decrease in serum concentrations of cholesterol and TAG and an increase in hepatic concentration of these lipids. In addition, in the SO group there was a decrease in hepatic and serum concentrations of IL-6 and hepatic levels of TNF, as well as a decrease in the GSH/GSSG ratio, suggesting changes in glutathione metabolism and inflammatory mediators.

1. Introduction

It is known that oils usually incorporated into the daily diet, such as those supplemented by formulations, can have significant effects on metabolism (Stawarska, Bialek, & Tokarz, 2018). The type and amount of ingested lipids can regulate hepatic lipid metabolism and gene expression, and the key targets of this control include glycolysis, synthesis, elongation, desaturation and oxidation of fatty acids (Jump et al., 2005). Moreover, excessive lipids intake are also related reactive species generation and redox imbalance (Estadella et al., 2013; Peluso, Morabito, Urban, Ioannone, & Serafini, 2012).

Vegetable fats' composition are mainly by triacylglycerol (TAG), and include different fatty acids types. The imbalance in the fat type may impact differently on health (Harris et al., 2009). Vegetable oils are a complex mixture of various saturated and unsaturated fatty acids, phosphatides, pigments, sterols and tocopherols (Ganesan, Sukalingam, & Xu, 2018). However, each vegetable oil has a specific distribution of fatty acids, depending on its plant source. Thus, the impact of this type of fat on human health can be evaluated according to the individual fatty acids present in each vegetable oil, and thus their different influence on human nutrition (Orsavova, Misurcova, Ambrozova, Vicha, & Mlcek, 2015).

Coconut oil's composition is mainly by lauric, myristic and stearic acid (DebMandal & Mandal, 2011). Although coconut oil is an excellent source of medium chain triglycerides (MCT), its intake should not exceed the daily recommendation (less than 10% of total calorie intake) of the US Department of Agriculture's (USDA's) due to the high percentage of saturated fat (Sacks et al., 2017; Sankararaman & Sferra, 2018). Monounsaturated fatty acids (MUFAs) are mainly present in vegetable oils, especially olive oil (Ganesan et al., 2018). Oleic acid is the main fatty acid found in extra virgin olive oil, which also contain mainly phenolic bioactive compounds (Cicerale, Conlan, Barnett, Sinclair, &

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https://doi.org/10.1016/j.jff.2020.103780
Received 4 September 2019; Received in revised form 18 December 2019; Accepted 4 January 2020
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Keast, 2009). The extra virgin olive oil intake has been associated with protection against cardiovascular diseases and the prevention of neurodegenerative diseases (Toledo et al., 2015).

Regarding polyunsaturated fatty acids (PUFAs), it is known that linoleic acid (LA, n-6) and alpha-linolenic acid (ALA, n-3) are essential dietary components (Misra & Khurana, 2009) and the linseed and sunflower oils are good n-3 (Yashodhara et al., 2009) and n-6 (Misra & Khurana, 2009) sources, respectively. N − 3 and n − 6 PUFAs have opposite effects on metabolic functions in the organism. Diets enriched in n-3 PUFAs exhibit anti-inflammatory properties, whilst n-6 PUFAs favor pro-inflammatory responses (Saini & Keum, 2018). Moreover, dietary modification has led to an imbalance in the n-6/n-3 PUFA ratio, resulting in an increase in this ratio (Patterson, Wall, Fitzgerald, Ross, & Stanton, 2012; Russo, 2009; Simopoulos, 2006). Dietary lipids containing fats of different proportions and types are able to regulate metabolism and may influence both pathogenesis and the prevention of chronic diseases (Jump, 2011; Manio, Matsumura, & Inoue, 2018).

The metabolic effects resulting from these vegetable fats supplementation are few explored in healthy individuals. Most of the studies evaluate the effects of these oils on pathological conditions, such as coronary heart disease (Sayon-Orea, Carlos, & Martinez-Gonzalez, 2015) and metabolic syndrome (Misra & Khurana, 2009). In the last decades, an increase in the vegetable fat intake was observed, and more studies are necessary to understand the effect of these vegetable fats in the metabolic homeostasis. Thus, the objective of this study was to evaluate the effect of chronic intake of different commercial vegetable oils (linseed, coconut and sunflower oils) with different fatty acid compositions on metabolic mediators, redox and inflammatory state in healthy rats.

2. Methods

2.1. Purchase of vegetable oils

As priority, the study has used commercial vegetable oils available for population intake. In our study we used linseed oils (LO), virgin coconut oil (VCO) and sunflower oil (SO). Linseed and sunflower oils were acquired from Duom® (Colombo, Paraná, Brazil). The coconut oil was acquired from UNILIFE VITAMINS C.R. Vertuan Ind. of natural and Nutraceuticals products® (Maringá, Paraná, Brazil). The nutritional information of each oil was based on quantity per serving (13 mL serving - 1 tablespoon) and are presented below:

Flaxseed oil: Quantity per serving (13 mL serving − 1 tablespoon): total fats 12 g; saturated fats 1.3 g, monounsaturated fats 2.5 g, n-9: 2.4 g, polyunsaturated fats 7.5 g, n-6: 1.6 g, n-3: 5.9 g, vitamin E 2.7 g. **Coconut oil:** total fats 12 g; saturated fats 11 g, monounsaturated fats 0.7 g, polyunsaturated fats 0.2 g. **Sunflower oil:** total fats 13 g, saturated fats 1.2 g, monounsaturated fats 3.0 g, n-9: 2.6 g, polyunsaturated fats 8.8 g, n-6: 8.5 g and n-3: 0.1 g. It does not contain significant amounts of carbohydrates, proteins, trans fats, dietary fiber and sodium.

The oils have been kept away from light and heat, which are essential conditions for them not to produce, develop or aggregate physical, chemical or biological substances that pose a risk to animal health. In addition, the researchers evaluated daily the scent, colour and texture of oils.

2.2. Experimental design

All of the experimental procedures were approved by the Ethics Committee on Animal Use of the Federal University of Ouro Preto (Protocol 079/2016) and were carried out in accordance with the regulations described in the Guiding Principles Manual of the Committee.

The animals used in the study belong to the Experimental Nutrition Laboratory (LABNEX) of the Federal University of Ouro Preto (UFOP), Minas Gerais, Brazil. In LABNEX there is a control/sorting of the Fischer 344 (F344) lineage that was kept in the laboratory for several generations. When saying “metabolically healthy” we refer to the fact that the animals do not present any pre-existing pathology before the experimental design. Thirty-five female Fischer healthy rats (~90 days, 216.9 g ± 11.55), were randomly divided into the following four groups: control group (C) (N = 8); linseed oil group (LO) (N = 9); extra virgin coconut oil group (VCO) (N = 9) and sunflower oil group (SO) (N = 9), during 90 days. The experimental groups have received daily vegetable oil per gavage, at a dose of 3.6 g/kg/day (equivalent to 1 mL/250 g) (Ortiz-Avila et al., 2015).

The use of females is justified by the fact that the research group of the Experimental Nutrition Laboratory generated a database with the average values of biochemical parameters obtained from control rats of the Fischer lineage over the years. These data provided subsidies to name the rats used in this study as metabolically healthy rats.

The control group has received 1 mL/250 g/day of water per gavage. All groups were fed *ad libitum* a commercial chow diet (Nuvital®, São Paulo, Brazil) and water during the experimental period. At the end of the 90 days, the animals were euthanized after 8 h fasted by isoflurane (Isoforine®, São Paulo, Brazil).

The blood samples were collected and the liver was immediately removed, weighed, snap-frozen in liquid nitrogen and stored at −80 °C until further analysis.

2.3. Analysis of the fatty acid composition of vegetable oils

Quantitative oil analyses were performed by gas chromatography (Agilent 7890B) equipped with a mass spectrometry detection system (Agilent 5977A-MSD) with a quadrupole mass analyzer. The column used was a capillary type CP - WAX 52 CB (Polyethylene glycol, 30 m × 0.25 mm × 0.25 μm internal diameter). The oil was injected automatically into the chromatograph using an injection volume of 1.0 μL in split mode at 1:10 injection ratio. Data acquisition took place in SCAM mode, using a mass to charge ratio (m/z) of 14–500. The mass analyzer was a simple quadrupole type operated at 150 °C. The mass and fragmentation profile of the peaks found were compared with standard and the National Institute of Standards and Technology (NIST) library spectra database. The analyzes were performed in triplicate and the results were expressed by the mean of the percentage in normalized area relative to the chromatographic peaks.

2.4. Serum parameters

Serum total cholesterol, triacylglycerol (TAG) and plasma glucose (N = 25) levels were measured using a Labtest® kits (Lagoa Santa, Minas Gerais, Brazil), following the manufacturer's intructions. Serum insulin (N = 25) was determined using a commercial Rat/Mouse Insulin enzyme-linked immunoabsorbent assay *ELISA* (*Enzyme Linked Immuno Sorbent Assay*) *Kit for Insulin (INS), Rattus norvegicus (Rat)* (Cloud-Clone Corp, Katy, TX), according to the manufacturer's recommendations. The homeostasis model assessment (HOMA) was computed as follows: fasting insulin (mU/L) × fasting glucose (mmol/L)/ 22.5 (Matthews et al., 1985).

2.5. Liver lipids determination

Hepatic lipids were extracted from liver samples (N = 35) using a chloroform/methanol method (2:1, v/v), as described previously (Folch, Lees, & Sloane Stanley, 1957). The total lipids content was gravimetrically quantified by evaporation and dried lipids were resuspended in 1 mL of isopropanol. Total cholesterol and TAG were measured using Labtest® kits, previously described.

2.6. Quantitative reverse transcription polymerase chain reaction (RT-qPCR) assay

Total RNA was obtained from the liver (N = 24) using a combination of Trizol™ reagent (Invitrogen, Carlsbad, CA) and chloroform (Sigma-Aldrich, St. Louis, MO) and were purified using the SV Total RNA *Isolation System* kit (Promega, Madison, WI), according to the manufacturer's protocol. Total RNA was quantified using the NanoVue® system (GE Healthcare, Little Chalfont, United Kingdom) and RNA integrity was analyzed by electrophoresis on a 1.2% agarose formamide-TBE gel. Total RNA was treated with RNase-free DNase I (Promega) for 30 min, and the optical density of the solution was measured at 230, 260, and 280 nm. Ratios greater than 1.8 (260/ 280 and 260/230) were considered to be acceptable for gene expression quantification (Becker, Hammerle-Fickinger, Riedmaier, & Pfaffl, 2010).

Total RNA (1000 ng) were reverse transcribed into cDNA using the High Capacity cDNA RT kit (Thermo Fisher, Waltham, MA), following the manufacturer's instructions. Then, mRNA expression was quantified by RT-qPCR, using the SYBR® Green PCR Master Mix kit (Thermo Fisher) and the ABI 7300 Real-Time PCR System to detect the targets. Rat-specific primers were used to detect *Srebf1* [NM_001276707.1; F: 5′ GTGAGTGGAGGGACCATCCTG 3′ and R: 5 CCAGCTGCTAGTCGGTGG ATC 3′], *Acaca* [NM_022193; F: 5′ TGTAGAAACCCGAACCGTGG 3′ and R: 5′ CTGGAAACCAAACTTGGCCG 3′]; and *Fasn* [NM_017332.1; F: 5′ GCTTGGTGAACTGTCTCCGA 3′ and R: 5′ GTGAGATGTGCTGCTGAGGT 3′]. Quantification cycle (Cq) (Bustin et al., 2009) were determined based on the SYBR® Green emission intensity during the exponential phase. Cq data were normalized using *Ppia* [NM_017101.1; F: 5′ GCA AGCATGTGGTCTTTGGG 3′ and R: 5′ GTCCACAGTCGGAGATGGTG 3′], which was stably expressed in all experimental groups. The relative gene expression was calculated using the $2^{-\Delta Cq}$ method (Livak & Schmittgen, 2001), and the data were expressed as Log of relative expression.

2.7. Inflammatory mediators: IL-6 and TNF

IL-6 and TNF levels were measured both in serum (N = 21) and liver (N = 23) using a Rat IL-6 and Rat TNF ELISA kits (Peprotech, Rocky Hill, NJ), according to the manufacturer's instructions. Briefly, the hepatic tissue was fractionated in 30 mg and homogenized with 500 μL of PBS (Phosphate Buffered Saline) (1 ×, pH 7.4). After homogenization, the samples were centrifuged at 3500 rpm for 10 min, at 4 °C. The supernatant was collected and used as the biological sample.

Assays were performed on 96-well plates, which was sensitized with 100 μL of the capture antibody diluted for each cytokine at 1 μg/mL and incubated overnight at room temperature. After the incubation period, the blockade was carried out with a solution containing PBS and 1% fetal bovine serum (FBS) for two hours. Samples (serum and supernatant) and the standard cytokines were added in a volume of 100 μL per well (the initial concentration of the curve was 5 ng/ml for IL-6 and 3 ng/ml for TNF). Subsequently, secondary antibodies diluted in PBS and 1% FBS were added. The absorbance readings were run in a plate reader at 405 nm with wavelength correction set at 630 nm.

2.8. Redox status analyses

2.8.1. Antioxidant defense: SOD, catalase, total glutathione and GSSG

The activity of the total antioxidant enzyme superoxide dismutase (SOD) was measured in an indirect way, according to the method proposed by (Marklund, Holme, & Hellner, 1982). Briefly, 100 mg of liver samples (N = 35) were homogenized with PBS (0.1 M, pH 7.2) and, subsequently, centrifuged at 10.000 rpm for 10 min. The assay was based on SOD competition with superoxide radical, which is generated by pyrogallol self-oxidation, and is responsible for MTT reduction, resulting in formazan crystals that can be detected in the spectrophotometer at 570 nm (Marklund et al., 1982). The SOD activity was expressed as U SOD/mg of protein.

The catalase activity was measured in liver samples (N = 35) according to (Aebi, 1984). Reading was performed in a spectrophotometer at 240 nm and the catalase activity was expressed as U CAT/per mg of protein.

Total glutathione was determined in liver samples (N = 26) by a kinetic assay using an adapted protocol from the Glutathione Assay Kit (Catalog #CS0260; Sigma-Aldrich, St. Louis, MO). Briefly, the DTNB [5,5′-Dithiobis (2-nitrobenzoic acid)] was reduced to TNB (2-nitro-5-thiobenzoate) and this reduction was directly proportional to the tripeptide concentration in the assessed tissue, once reduced glutathione (GSH) is the reaction's cofactor. In order to determine oxidized glutathione (GSSG) concentration, homogenate derivatization with 2,2′,2″-nitrilotriethanol, tris (2-hydroxyethyl) amine (TEA), and vinylpyridine was performed. The concentrations of total glutathione (GSHt) and GSSG were obtained by a standard curve performed for each assays. The GSH concentration was obtained by subtracting the oxidized glutathione value from the total glutathione concentration. We performed a homogenate derivatization with TEA and 2-vinylpyridine (Sigma-Aldrich) to access oxidized glutathione (GSSG). The GSH concentration was obtained as follows: GSHt - GSSG. The total glutathione was expressed as nmol/mL and GSH and GSSG was expressed as the GSH/GSSG ratio.

2.8.2. Oxidative stress markers: TBARs and carbonylated proteins

Proteins sensitive methods were used in order to evaluate the oxidative damage to thiobarbituric acid reactive substances (TBARS) and the formation of carbonyl derivatives. The TBARS concentration was determined according to thiobarbituric acid (TBA) binding to oxidized lipids, previously described (Buege & Aust, 1978). The reading was performed in ELISA plate reader at 535 nm. The TBARS concentration was determined based on the line equation, according to the Lambert Beer law, which was used 2,2,6,6-Tetramethylpiperidine (TMP) as a standard. The values were normalized with the total protein determined using the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951) and the results were expressed as $nmol{\cdot}mL^{-1}$/per mg of protein.

Measurements of carbonylated protein were performed according to (Levine, Williams, Stadtman, & Shacter, 1994). The supernatant absorbance was 370 nm. The total protein was determined using the Lowry method (Lowry et al., 1951). The results were expressed as nmol/mL per mg of protein.

2.9. Statistical analysis

The statistical analyses were performed using the Graph Pad Prism (version 6.01) software (GraphPad Software Inc., Irvine, CA). The normal data distribution was verified by Kolmogorov-Smirnov test. The results were expressed as mean ± standard deviation (SD) (parametric) or median and interquartile range (IQR) (non-parametric). Differences among groups were evaluated using a one-way ANOVA, followed by the Tukey post-hoc test for parametric data. The non-parametric data was evaluated using Kruskal-Wallis, followed by the Dunn's post-hoc test. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Fatty acid profile of vegetable oils

The chromatographic analysis of the vegetable oils used in this study was performed and the results show that the percentage distributions of every fatty acid are different, allowing the identification of the type and content of fatty acid present in every oil. In Table 1, when analyzing the levels of fatty acids present in the VCO, it was observed that there is a predominance of saturated medium chain fatty acids in its constitution, wherein lauric acid (C 12:0) (38.78%); myristic acid (C

Table 1
Comparative data on the relative percentage (%) of fatty acids in vegetable oils.

Compound	Coconut	Sunflower	Linseed
Caproic acid (C 6:0)	0.6801	–	–
Caprylic acid (C 8:0)	7.3364	0.2312	0.11281
Capric acid (C 10:0)	7.1457	–	–
Lauric acid (C 12:0)	38.7812	–	–
Myristic acid (C 14:0)	24.5877	0.0761	0.1231
Palmitic acid (C 16:0)	10.2921	13.9676	8.1450
Palmitoleic acid (C16:1)	–	–	–
Oleic acid (C 18:1 n-9)	10.2303	34.9225	30.6873
Linoleic acid (C 18:2 n-9,12)	–	45.6326	15.9283
Linolenic acid (C 18:3 n-3,6,9)	–	5.1696	45.0033

(–) not detected.

14:0) (24.58%), palmitic acid (C 16:0) (10.29%), oleic acid (C 18:1 n-6) (10.23%), caprylic acid (C 10:0) (7.33%), capric acid (7.14%) and caproic acid (C 6:0) (0.68%) are present.

When analyzing the fatty acid contents present in the LO, we observed that in this oil there are more polyunsaturated fatty acids (n-3 family) and monounsaturated fatty acids (n-9 family), represented by linolenic acid (C 18:3 n-3) (45%) and oleic acid (C 18:1 n-9) (30.68%), respectively. In addition, linoleic acid (C18:2 n-6) (15.92%), palmitic acid (C 16:0) (8.14%), myristic acid (C14:0) (0.12%) and caprylic acid (C 8:0) (0.11%) are also present.

Finally, the chromatographic analysis showed that SO presents in its composition higher contents of polyunsaturated fatty acids (n-6 family) and monounsaturated fatty acid represented by linoleic acid (C 18:3 n-9) (45.63%) and oleic acid oleic acid (C 18:1 n-9) (34.92%), respectively. It is also present in SO palmitic acid (C16:1) (13.96%), linolenic acid (C18:3 n-3) (5.16%), caprylic acid (0.23%) and myristic acid (C14:0) (0.076%).

3.2. VCO changes in the glycometabolic parameters

In order to evaluate whether the chronic intake of the different vegetable oils resulted in alterations in biochemistry parameters, glucose, insulin, and HOMA-IR were determined. The results showed an increase in glycaemia in the VCO group compared to the C group (41%, $P < 0.05$). No change was observed in insulin levels; however, we observed an increase in HOMA-IR in the animals of the VCO (± 42% $P < 0.01$, VCO *versus* LO) and SO groups (33%, $P < 0.01$, SO *versus* LO) (Table 2).

3.3. VCO increases serum cholesterol and TAG, while SO alters liver lipid profile

Total cholesterol (Fig. 1, Panel D) and TAG levels (Fig. 1 Panel E) were increased in the serum of VCO group, when compared to LO (± 25%, $P < 0.001$), SO (± 29%, $P < 0.05$) and C (± 128%, $P < 0.05$) groups, respectively. Interestingly, a decrease in liver fat (Fig. 1 Panel A) and hepatic cholesterol (Fig. 1 Panel B) was observed in the VCO group comparing to LO (± 26%, $P < 0.05$) and C (± 26%, $P < 0.05$) groups, respectively.

Regard to the SO group, a decrease in serum cholesterol (± 23%, $P < 0.05$) (Fig. 1 Panel D) and TAG (± 176%, $P < 0.001$) (Fig. 1 Panel E) was observed when compared to the VCO group. There was also an increase in total liver fat (± 47%, $P < 0.001$, SO *versus* VCO) and hepatic TAG (± 17%, $P < 0.001$; SO *versus* LO group).

Regarding hepatic cholesterol (Fig. 1 Panel B), there was an increase in their levels in the SO group, when compared to the VCO (± 14%, $P < 0.05$) and LO groups (± 22%, $P < 0.01$). The LO group also showed a decrease in hepatic cholesterol levels (± 14%, $P < 0.05$, C *versus* LO).

3.4. The commercial vegetable oils supplementation does not alter the lipogenic genes expression

After the observation that the chronic intake of the different vegetable oils had an effect on the serum and hepatic lipids profile, the next goal was to verify if the intake of these oils could act on the gene expression levels of the regulatory enzymes of the lipogenic pathway, acetyl Coa carboxylase (*Acaca*), fatty acid synthase (*Fasn*) as well as the transcription factor related to lipid metabolism, sterol regulatory element-binding protein 1 (*Srebf1*). However, no significant changes in gene expression were observed in any experimental groups (Fig. 2).

3.5. The chronic intake of VCO cause an increase in the serum and hepatic concentration of IL-6 and hepatic TNF

Considering that fatty acids may play an important role in the regulation of immune and inflammatory response, the effect of chronic intake of vegetable oils on liver and serum concentrations of inflammatory cytokines interleukin 6 (IL-6) and tumor necrosis factor (TNF) were evaluated (Fig. 3).

Regarding hepatic levels, a decrease of IL-6 (± 88%, $P < 0.001$, SO *versus* C; ± 89%, $P < 0.001$, SO *versus* LO; ± 89%, $P < 0.001$, SO *versus* VCO) (Fig. 3 Panel A) and TNF (± 75%, $P < 0.001$, SO *versus* C; ± 81%, $P < 0.001$, SO *versus* LO; ± 80%, $P < 0.001$, SO *versus* VCO) (Fig. 3 Panel C) was observed in the SO group when compared to the other experimental groups. Also, an increase of hepatic TNF was observed in LO groups (± 32%, $P < 0.05$, LO *versus* C).

Regarding serum levels, a decrease of IL-6 in the SO group was observed when compared to the LO group (± 77%, $P < 0.05$) and an increase of IL-6 in the LO group when compared to the control (± 343%, $P < 0.001$) (Fig. 3 Panel B). No significant changes were observed in serum levels of TNF in any experimental group (Fig. 3, Panel D).

3.6. Chronic intake of SO increases SOD activity and alters the glutathione cycle

The antioxidant status was evaluated by the activity of the enzymes superoxide dismutase (SOD) and catalase, in addition to the total glutathione concentration and the GSH/GSSG ratio (Fig. 4 Panel A-D). In the Fig. 4 Panel E-F, biomarkers were evaluated as indicators of lipid

Table 2
Effect of different vegetable oils chronic intake on body weight gain and glucometabolic parameters of healthy Fischer rats.

Parameters	C (n = 5)	LO (n = 6)	VCO (n = 6)	SO (n = 6)
Body weight gain (g)	21.70 ± 5,86	24.76 ± 2,54	17.04 ± 6,36§	18.28 ± 4,16
Glucose (mmol/ L)	6.67 ± 1,84	7.32 ± 1.59	9.41 ± 1.48*	8.64 ± 0.86
Insulin(mU/ L)	24.44 (22.6–27.4)	27.06 (26–27.4)	25.95 (22.7–27.3)	25.33 (21.6–27.3)
HOMA-IR	8.29 ± 1.33	7.08 ± 1.15	10.03 ± 1.04§	9.42 ± 1.29§

Data were expressed as mean ± standard deviation (SD) (parametric data) or median and IQR (non-parametric data). The effect of vegetable oil chronic intake was evaluated by one-way ANOVA, followed by the Tukey post-test (parametric data) or by Kruskal-Wallis test, followed by Dunns post-test (non-parametric data). Significant differences were considered when P < 0.05. *when compared to C group; § when compared to the LO group; & when compared to the VCO group. C: Control; LO: Linseed oil; VCO: Virgin coconut oil; SO: Sunflower oil.

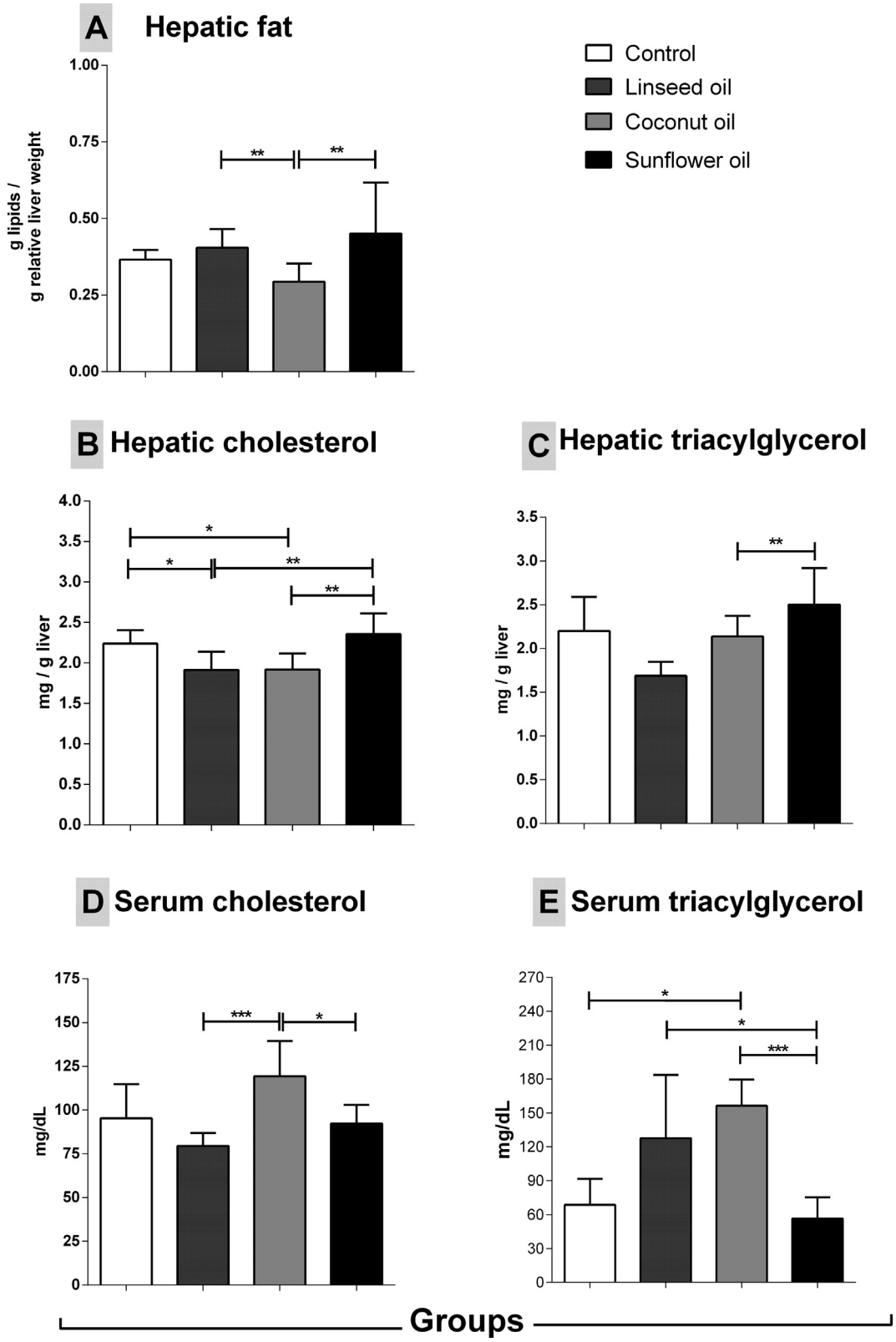

Fig. 1. Effect of different vegetable oils chronic intake on serum and hepatic lipid profile of healthy Fischer rats. Data were expressed as means ± SD (parametric data) and median ± IQR (non-parametric data). The effect of vegetable oil chronic intake was evaluated by one-way ANOVA followed by Tukey's post hoc analyses (parametric data) or Kruskal-Wallis test, followed by Dunn's post hoc analyses (non-parametric data). Significant differences were considered $P < 0.05$; *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

peroxidation and protein oxidation, TBARS and protein carbonyl groups, respectively. In panel A, an increase in SOD activity in the animals of the SO group (± 29%, $P < 0.05$, SO *versus* VCO) was observed. Regarding catalase activity (Fig. 4 Panel B), there was no significant change observed among the experimental groups. Regarding total glutathione levels, a decrease was observed in the SO group

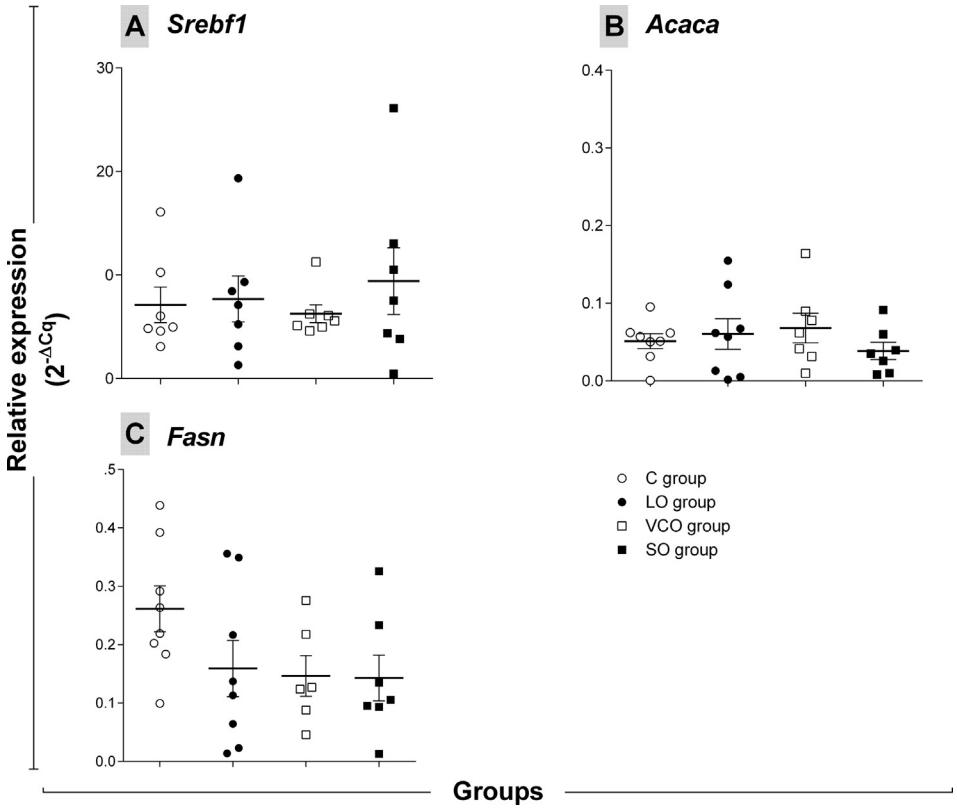

Fig. 2. Effect of different vegetable oils chronic intake on gene expression of regulatory enzymes of the lipogenic pathway Acc (*Acaca*) and Fas (*Fasn*) and the transcription factor Srebp1 (*Srebf1*) in the liver of healthy rats. Data were expressed as means ± SD (parametric data) and median ± IQR (non-parametric data). Statistically significant differences were determined using a one-way ANOVA to examine the effects of vegetable oil intake on the gene expression of regulatory enzymes of the lipogenic pathway, followed by Tukey's post hoc analyses (parametric data) and Kruskal-Wallis test, followed by Dunn's post hoc analyses for (non-parametric data). It was considered statistically significant $P < 0.05$; *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

(± 38%, $P < 0.05$, SO *versus* C and VCO), and the same profile was observed when the GSH/ GSSG ratio was compared to the C (43%, $P < 0.01$) and VCO (41%, $P < 0.05$) groups. No significant changes were observed in carbonylated protein levels (Fig. 4 Panel F). Regarding TBARS levels, an increase in the VCO group was observed (± 63%, $P < 0.05$, VCO *versus* C) (± 61%, $P < 0.05$, VCO *versus* LO) (Fig. 4 Panel E).

4. Discussion

The liver plays a central role in the whole body lipid metabolism and adapts rapidly to changes in dietary fat composition (Jump, 2008). Dietary fat is an essential macronutrient for growth and development of all organisms, being a substrate for energy metabolism, membranes, signaling molecules and regulation of gene expression (Jump et al., 2005). The chronic intake of coconut, linseed or sunflower oil, which differ only in the fat type (saturated, n-3 or n-6, respectively) may change the homeostasis in metabolically healthy rats. Due to the dietary pattern that predominates nowadays, known as the Western diet, even metabolically healthy individuals usually eat high amounts of vegetable fat. However, it is known that excessive vegetable fat intake can lead to changes in metabolic homeostasis and generate long-term damage (Mori, 2018).

Our results showed that virgin coconut oil intake promoted a decreased body weight gain, and increase in plasma glucose and HOMA-IR when compared to the linseed oil group, as well as an increase in serum cholesterol and TAG. The literature reports many articles that associate the consumption of coconut oil and weight loss (Gunasekaran et al., 2017; Liau, Lee, Chen, & Rasool, 2011; Nosaka et al., 2003; St-Onge & Bosarge, 2008; Tsuji et al., 2001), as well as health benefits similar to those of middle chain triglycerides (MCT) (Clegg, 2017). The effects of coconut oil on weight loss are related to the combination of two factors: energy expenditure increases and satiety induced by MCT. Therefore, MCTs are a readily available energy source that can be oxidized faster (Hirsch, Stahl, & Lodish, 1998). Virgin coconut oil, consist, mainly, in saturated fatty acids (~91%), namely lauric acid (12:0) and myristic acid (14:0) (Katragadda, Fullana, Sidhu, & Carbonell-Barrachina, 2010). Some studies have shown that diets rich in saturated fat, mostly lauric, myristic and palmitic acids, are associated with an atherogenic blood lipid profile and insulin resistance (Horowitz et al., 2018; Zong et al., 2016). Plasma cholesterol levels are closely related with the liver function (Habib et al., 2005), and our results suggest that the increase in serum cholesterol levels in the coconut oil group may be related to its reduction in the liver. This suggested that saturated fatty acids contribute to the increase of plasma cholesterol through reduction of B/E receptors, which causes inhibition of removal LDL cholesterol particles of blood (Pereira et al., 2012). It was also observed that sunflower oil intake resulted in an increase in liver fat content, which may be related to the increase in cholesterol and TAG in the organ. These results are corroborated by (Go et al., 2015). It was observed that sunflower oil intake by rats for 22 days caused a decrease on serum TAG and lipids in the liver.

Our next goal was to assess the hepatic genes expression related to lipid metabolism. It is noteworthy that the fat type and the amount ingested may regulate hepatic lipid composition and gene expression. Three genes involved in the lipogenic pathway, *Srebf11, Acaca and Fasn*, which encode the Esterol Regulatory Element Binding Protein-1c (SREBP-1c), Acetyl-CoA Carboxylase (ACC) and Acid-Fatty Syntase (FAS), respectively, were evaluated. Our results showed that there was no difference in gene expression levels among the groups. Lipogenic enzymes can be regulated by multiple mechanisms, such as allosteric control and transcriptional and post-translational modification (Wang, Viscarra, Kim, & Sul, 2015). Thus, in our model, we suggested that coconut and sunflower oils modulate hepatic lipid metabolism regardless of transcriptional regulation, suggesting the participation of post-translational or allosteric mechanisms, such as phosphorylation or dephosphorylation.

Dietary fat also influences the susceptibility to oxidative damages due to redox imbalance (El-Sayed, Elsanhoty, & Ramadan, 2014). Deleterious effects of reactive species are counteracted by antioxidant

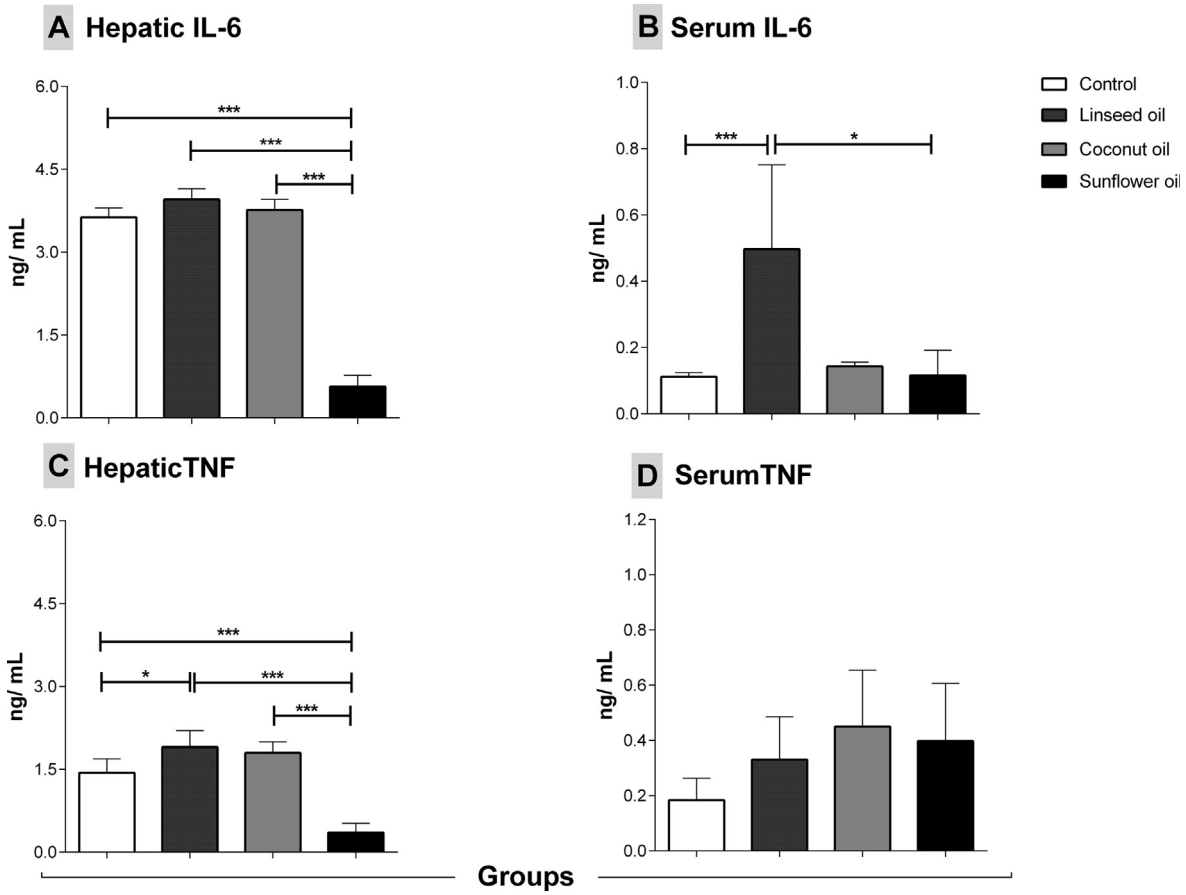

Fig. 3. Effect of different vegetable oils chronic intake on levels of inflammatory cytokines in the liver and serum of healthy Fischer rats. Data were expressed as means ± SD (parametric data) and median ± IQR (non-parametric data). Statistically significant differences were determined using a one-way ANOVA to examine the effects of vegetable oil intake on levels of inflammatory cytokines in the liver and serum of healthy, followed by Tukey's post hoc analyses (parametric data) and Kruskal-Wallis test, followed by Dunn's post hoc analyses for (non-parametric data). It was considered statistically significant $P < 0.05$; *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

defense mechanisms. The antioxidant system involves enzymatic and non-enzymatic pathways. The enzymatic system is superoxide dismutase (SOD), which catalyzes the dismutation of superoxide anion to hydrogen peroxide, glutathione peroxidase (GPx) or catalase, responsible for the water conversion to hydrogen peroxide (Gebhardt, 2002; Hefnawy & Ramadan, 2013). The coconut oil intake did not change the antioxidant status; however, it was responsible for increasing the production of TBARS. The increase of TBARS after the coconut oil intake was also observed in other studies (Lima et al., 2017; Oliveros, Videla, Ramirez, & Gimenez, 2003). The sunflower oil intake also altered the redox status. Studies in animal models showed that the intake of vegetable fat rich in n-6 increases redox imbalance (de Catalfo, de Alaniz, & Marra, 2013; El-Sayed et al., 2014). There was an increase in SOD activity which could be explained as an adaptive response to hold the oxidative damage observed by the increase of TBARS, since the redox imbalance induces physiological and pathological responses in the cells (Yoshiike et al., 2012). Moreover, a decrease in total glutathione levels and GSH/GSSG ratio was observed. Glutathione is predominantly found in its reduced form and the GSH/GSSG ratio is an important measure to evaluate the cellular redox state, and a decrease in this ratio is indicative of oxidative stress and reduction of antioxidant defenses (Franco, Schoneveld, Pappa, & Panayiotidis, 2007). Alteration in homeostasis of liver GSH contributes to an increase in reactive species, compromising signaling pathways that may affect intermediate metabolism and survival, contributing to the pathogenesis of different liver diseases (Yuan & Kaplowitz, 2009). Several nutrients, including dietary fatty acids, are able to influence the immune response, through suppression or activation of this response (Harrison, Balan, & Babu, 2013). TNF and IL-6 are two pro-inflammatory and immunoregulatory cytokines with pleiotropic function (Drutskaya, Efimov, Kruglov, & Nedospasov, 2017). In our study, it was observed that sunflower oil chronic intake was responsible for decreasing the serum and hepatic IL-6 levels, as well as the liver TNF levels. However, the linseed oil chronic intake increased serum IL-6 and hepatic TNF levels. There are few studies assessing the effect of commercial vegetable oils on the inflammatory profile modulation and none of them were carried out in healthy individuals. Most studies have evaluated the isolated effect of fatty acids, such as linoleic acid and alpha-linolenic, not the blend included in vegetable oils. In this line of thinking, it is possible to infer that the commercial oils effects do not, necessarily, correlate with the effects of isolated fatty acids supplementation, since in commercial vegetable oils there may be synergistic or antagonistic interactions of the different fatty acids. This hypothesis could explain the conflicting results with the literature, which show that n-3 fatty acids have an anti-inflammatory effect (Labrousse et al., 2018; Thota, Ferguson, Abbott, Dias, & Garg, 2018) and n-6 a more inflammatory profile (Labrousse et al., 2018).

In conclusion, our results suggest that the intake of different vegetable fat may result in distinct alterations in the biochemical parameters, lipid profile and inflammatory mediators, which may compromise metabolic homeostasis in healthy rats. Thus, the intake of vegetable fat should be carried out carefully.

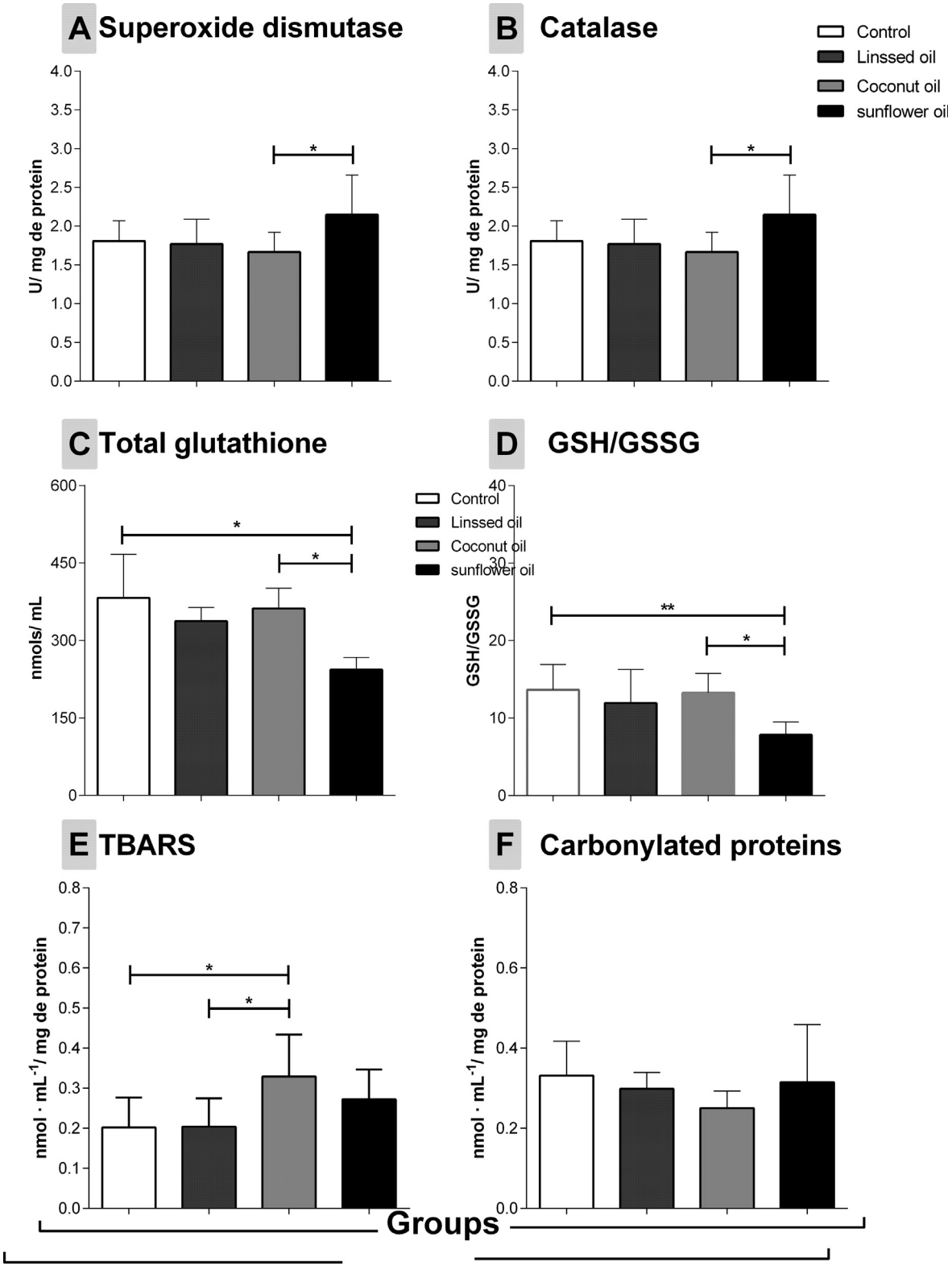

Fig. 4. Effect of different vegetable oils chronic intake on oxidative status in the liver of healthy rats. Data were expressed as means ± SD (parametric data) and median ± IQR (non-parametric data). Statistically significant differences were determined using a one-way ANOVA to examine the effects of vegetable oil intake on antioxidant status in the liver, followed by Tukey's post hoc analyses (parametric data) and Kruskal-Wallis test, followed by Dunn's post hoc analyses for (non-parametric data). It was considered statistically significant $P < 0.05$; *$P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. GSH/GSSG ratio = reduced glutathione (GSH)/ oxidized glutathione (GSSG) ratio; TBARS = Thiobarbituric Acid Reactive Substance.

Ethics statements

This is a research article and include animal experiments. This work was conducted in accordance with the National Council of Animal Experimentation (CONCEA), Brazil. All experiments were approved by the Ethics Committee on Animal Use (CEUA) of the University Federal of Ouro Preto – UFOP, Brazil.

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.

Acknowledgments

This research study was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Universidade Federal de Ouro Preto (UFOP), Brazil.

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