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## Original Article

# Metabolic Effects of Coconut Oil on Fatty Liver and Oxidative Stress Induced by a High-fat Diet in Rats

Laura Perreira Barreto[1], Raissa Yolanda de Oliveira Silva[2], Bianca Bellizzi de Almeida[1], Paula Payão Ovídio[2] and Alceu Afonso Jordao[2*]

[1]*Department of Internal Medicine at Ribeirão Preto Medical School, University of São Paulo, São Paulo, Brazil;* [2]*Department of Health Sciences at Ribeirão Preto Medical School, University of São Paulo, São Paulo, Brazil*

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## Abstract

**Background and objectives:** This study aimed to evaluate the effect of coconut oil (CO) on steatosis and oxidative stress in rats fed a high-fat diet.

**Methods:** Three groups of male Wistar rats were used: the control group (CG, *n* = 10) received a standard diet for 50 days, the hyperlipidic group (HL, *n* = 10) received a high-fat diet with 50% lard for 50 days, and the hyperlipidic-CO group (HL+CO, *n* = 10) received a high-fat diet with 50% lard for 30 days followed by 25% lard and 25% CO for 20 days. Then, the animals were euthanized, and their blood, liver, and adipose tissue were collected for biochemical analyses.

**Results:** The groups that received a high-fat diet had pronounced liver steatosis. Compared to the CG and HL groups, the HL+CO group had less weight gain, but liver fat and triglycerides were increased, with a significant reduction in liver cholesterol. Glutathione increased significantly and vitamin E decreased in the livers of the experimental groups compared to the control. Lipid peroxidation in the serum and liver was less in the HL+CO group compared to that in the HL group, but it was higher than that in the control group. CO caused significant accumulation of hepatic fat, triglycerides, and fat content, despite decreasing the hepatic cholesterol levels. There was a better hepatic antioxidant response in the CO group, especially compared with the HL group.

**Conclusions:** CO was not able to prevent or improve liver fat levels, but the HL+CO group had a better antioxidant profile. Additional clinical studies are necessary to verify the efficacy and safety of different CO doses on both hepatic and lipid metabolism.

## Introduction

Acute and chronic liver diseases can be caused by chemicals, viruses, pharmacological agents, or other toxic components, which alter the morphological structure and functional capacity of hepatocytes. Among the liver diseases, nonalcoholic fatty liver disease (NAFLD) is characterized by the hepatic accumulation of lipids, mainly in the form of triglycerides, which, due to progressive inflammatory activity, can evolve into a more severe form, nonalcoholic steatohepatitis.[1,2]

NAFLD and nonalcoholic steatohepatitis are associated with insulin resistance, type 2 diabetes mellitus, obesity, hyperlipidemia, hypertension, and metabolic syndrome as well as extrahepatic manifestations, such as sleep apnea, chronic kidney disease, and cardiovascular disease.[1,3] Also, it is believed that the first event necessary for fat accumulation originates from insulin resistance, resulting in altering lipid and apolipoprotein metabolisms.[4] Hormonal characteristics of fatty liver disease are hyperglucagonemia, hyperinsulinemia, hypercortisolemia, high sympathetic tone, and deficient growth hormone levels. These characteristics influence lipid metabolism.[5]

**Keywords:** Steatosis; Oxidative stress; Coconut oil; Animal fat; Hyperlipidic diet.

**Abbreviations:** ALT, alanine aminotransferase; AST, aspartate aminotransferase; CO, coconut oil; DTNB, dithionitrobenzoic acid; EDTA, ethylenediamine tetraacetic acid; GSH, reduced glutathione; HDL, high-density lipoprotein; HL, hyperlipidic diet; HL+CO, hyperlipidic group with coconut oil; MDA, malondialdehyde; NAFLD, nonalcoholic fatty liver disease.

*Correspondence to: Alceu Afonso Jordão, Department of Health Sciences at Ribeirão Preto Medical School. University of São Paulo, Ribeirão Preto-SP, ZIP Code 14049-900, Brazil. ORCID: https://orcid.org/0000-0003-1288-0802. Tel: 551633154564, Fax: 551633154582, E-mail: alceu@fmrp.usp.br

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Free fatty acids and their metabolic factors influence the development of NAFLD, since their levels increase significantly during the development of the disease.[6] Elevated "*de novo*" lipogenesis generates an indirect response, hampering the beta-oxidative flow, which can strongly induce steatosis.[5]

For diagnosis, noninvasive measures have a practical advantage to assess the disease,[7] such as measuring serum aminotransferase levels and performing imaging tests like ultrasound, computed tomography, and magnetic resonance; however, they do not reliably reflect the spectrum of liver histology in patients with NAFLD. Therefore, there has been significant interest in developing clinical prediction rules and noninvasive biomarkers for identifying steatohepatitis in patients with NAFLD.[8] The degree of infiltration can be classified as mild (grade 1), affecting 10–30% of hepatocytes; moderate (grade 2), affecting 30–70% of hepatocytes; or severe (grade 3), affecting ≥70% of hepatocytes.[9]

However, even with these changes, disease progression is more frequent. Approximately one-third of patients develop fibrosis or cirrhosis, averaging 5–10 years after the diagnosis; but cirrhosis can be observed in a smaller time period, ranging from one to two years.[10]

As a subtype of NAFLD, steatohepatitis is strongly progressive and can lead to cirrhosis, hepatocellular carcinoma, liver transplantation, and death.[3] Factors such as obesity, hypertension, diabetes, and even genetic polymorphisms have influenced the severity of the disease, especially regarding the probability of developing cirrhosis and hepatocarcinoma. Therefore, a greater understanding of the factors that may modify the natural course of the disease is needed so that more specific therapies can be developed.[11] Nevertheless, it is acknowledged that physical exercise and changes in diet are important for all NAFLD patients.[12]

The prevalence of NAFLD is increasing worldwide, and 25% of the adult population is affected by the disease. Its increasing prevalence is related to an unhealthy lifestyle, especially diet, with a high intake of glucose, fructose, and saturated fat, among other deleterious nutrients.[13] There has been an increase in fructose consumption, which is associated with obesity, insulin resistance, and fatty liver disease. Even in acute animal models, it has been demonstrated that fructose induces lipogenesis, and the mechanisms indicate that it can cause the progression of disease.[14] Although NAFLD is more associated with nutrition and a Western diet, cases such as side effects to drugs, endocrine disorders, and viral infections can also induce this condition.[5] As diet plays a role in the induction or prevention of NAFLD, some nutrients such as vitamins D and E as well as some types of fatty acids can act positively, reducing steatosis, inflammation, and ballooning in nonalcoholic liver steatosis, although the results of some studies are conflicting.[15]

The use of coconut oil (CO) has shown an improvement in the antioxidant status in addition to preventing oxidative damage of lipids and proteins, and this effect may be associated with its composition of phenolics and tocopherols.[16] When comparing extra virgin CO with other oils, like peanut oil, it has both a lower amount of aldehydes and peroxides and a greater amount of polyphenols, which may then improve the antioxidant capacity.[17] When the coconut is cold pressed, the product is called virgin CO, which is rich in phytosterols and antioxidants.[18] CO contains an average of approximately 91% medium-chain-saturated fatty acids, with lauric and meristic acids predominating. While 12-carbon lauric acid is rapidly oxidized in the cell, 14-carbon myristic acid has an intermediate rate of oxidation compared with longer chain saturated fatty acids, such as 18-carbon stearic acid, which are oxidized at a slower rate.[18] The medium-chain triglycerides are transported in the blood by albumin, reaching the liver through the portal vein, unlike long-chain triglycerides, which have their metabolism prolonged by a process of esterification and chylomicron formation and then are absorbed by the lymphatic route. Their fast absorption is the reason that they are widely included in Parenteral Nutritional Therapy and in the syndrome of bad intestinal absorption.[18] Virgin CO has been recognized as a multipurpose nutritional supplement due to the nutritional and medicinal benefits of its medium-chain fatty acids, vitamins, amino acids, antioxidants, antimicrobials, and antiviral compounds.[19]

In view of the above, the present study aimed to evaluate the possible positive effects of dietary CO on the liver steatosis, lipid profile, and oxidative stress in animals fed with a high-fat diet or control diet.

## Methods

### *Diet*

The standard diet (Nuvilab) consisting of 56% carbohydrates, 19% protein, and 3.5% lipids was used, in addition to 4.5% cellulose and 5% vitamins and minerals, with 3.78 kcal/g, which is the commercial ration for rats.

For the control group, the diet was offered in a ground form. The hyperlipidic diet (HL) that was used to induce steatosis in animals was composed of 50% of the ground diet and 50% of lipids from thermolyzed animal fat (lard), adapted from Leonardi *et al.*,[20] in which it was submitted to heating at 130° for 30 min, adapted from De Assis *et al.*[21] The hyperlipidic group with coconut oil (HL+CO) received a diet composed of 50% of the ground feed, 25% of the same thermolyzed animal fat (lard) that was carried out in the HL group, and 25% of virgin CO, purchased in local stores. The CO information is reported by the manufacturer at https://www.copra.com.br/en/nossosprodutos/extra-virgin-coconut-oil/.

### *Animals*

Thirty male Wistar rats, with an average weight of 60 g, from the vivarium of the Faculty of Medicine of Ribeirão Preto - University of São Paulo were used in this study. The rats were housed in cages (3 animals/cage) and kept at a temperature of 25 °C under a 12-h light-dark cycle. Weighing the animals and cleaning the cages were carried out weekly, since the diets were weighed and replaced three times a week. The animals were separated into three groups and received water and diet *ad libitum* for 60 days. All animals were handled according to the Brazilian College of Animal Experimentation recommendations, and all procedures, which were based on the Animal Research: Reporting of *in-vivo* experiments (ARRIVE), were approved by the CEUA – Ethics Committee of Animals Use of FMRP/USP (protocol no. 012/2009).

The control group ($n$ = 10) received the standard diet throughout the experiment. The adaptation of the diet occurred during the first three days. The HL ($n$ = 10) and HL+CO ($n$ = 10) groups received the high-fat diet with animal fat for 30 days. The animal adaptation to the diet occurred during the first five days; initially, the animals were supplied with the experimental diets added to the control diet in a proportion of 2:1 (control: experimental), and later, in a proportion of 1:1, with the high-fat diet consisting of 50%. The HL+CO group started to receive a diet with animal fat added with CO. It was added progressively, with the first three days being at a 2:1 ratio (animal fat: CO), and then 1:1, reaching the total diet ratio of 25% animal fat, 25% CO, and 50% ground feed), similar to that reported by Leonardi.[22] Each group received their specific diet for an additional 20 days.

At the end of the experiment, the animals were euthanized by beheading. The blood, liver, and adipose tissue of the rats were collected for biochemical analyses. The blood was promptly centrifuged at 3,500 rpm at 4 °C for 15 min to obtain the serum. The liver and the epididymal and retroperitoneal adipose tissue were removed, weighed, and promptly frozen in liquid nitrogen. The samples were stored at −80 °C for further biochemical analysis.

### *Biochemical analysis*

#### Determination of total liver fat

The determination of total liver fat was performed according to the method proposed by Bligh and Dyer.[23]

#### Determination of total cholesterol and hepatic and serum triglycerides

The lipid fractions from the liver were measured after the extraction of total fat by the method of Bligh and Dyer[23] and later determined by commercial kits used for analysis in serum, as described below.

#### Determination of total cholesterol

The total cholesterol content was determined by the enzymatic colorimetric method using commercial kits from Labtest (Labtest Diagnóstica SA, Brazil).

#### Determination of triglycerides

The determination of hepatic triglycerides was also carried out by enzymatic colorimetric methodology using commercial kits from Labtest (Labtest Diagnóstica SA, Brazil).

#### Determination of serum and hepatic protein

The determination of serum and liver protein was carried out using commercial kits, using the Biuret method (Labtest Diagnóstica, Lagoa Santa, MG, Brazil).

#### Determination of blood glucose

Glycemia in the animals was determined from the serum, using a LiquiformLabtest ® Glucose PAP dosing kit.

#### Determination of high-density lipoprotein (HDL) cholesterol

For the determination of HDL blood cholesterol through the selective precipitation of low- and very-low-density lipoproteins, a combination of two kits was used: the HDL Cholesterol Labtest and Cholesterol LiquiformLabtest.

#### Determination of serum concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT)

Serum aminotransferase concentrations were assessed using commercial Labtest kits.

#### Determination of serum and liver lipid peroxidation

This analysis was performed according to the reaction of 1-methyl-2-phenylindole with malondialdehyde (MDA) and 4-hydroxyalkenals,[24,25] with some adaptations. For the measurement of MDA in serum, 200 µL of sample was used. The determination of MDA in the liver was performed with a 200-µL aliquot taken from the liver homogenate (200 mg of tissue in 1 L of phosphate buffer), in which both 650 µL of a 10 mM solution of 1-methyl-phenylindole in acetonitrile and methanol (2:1, v/v) and 150 µL of 37% hydrochloric acid were added. Soon after, the samples were vortexed and incubated for 40 min in a water bath at 45 °C. Next, the samples were cooled on ice, and then the Eppendorf tubes were centrifuged at 4,000 rpm for 10 min. The absorbance of the supernatant was read at a wavelength of 586 nm. The MDA concentration was calculated using a standard curve.

#### Determination of hepatic and serum reduced glutathione (GSH)

The measurement of GSH was carried out in the liver tissue according to the method described by Sedlak and Lindsay.[26] Approximately 100 mg of the tissue sample was homogenized with 4.0 mL of ethylenediamine tetraacetic acid (EDTA) buffer (0.02 M), on ice. A 2.5-mL aliquot of this homogenate was removed and mixed with 2.0 mL of deionized water and 0.5 mL of 50% trichloroacetic acid. The reaction lasted about 15 min, and then the sample was centrifuged at 4,000 rpm and room temperature for 15 min. After centrifugation, 1.0 mL of the supernatant was removed, and 2.0 mL of TRIS buffer (0.4 M, pH 8.9) and 0.05 mL of dithionitrobenzoic acid (DTNB; 0.01 M in methanol) were added. At 5 min after the addition of DTNB, the absorbance was read at a wavelength of 412 nm, against a blank with EDTA (0.02 M) in place of the supernatant.

The absorbance of GSH was measured using an aliquot of 25 µL of serum with the addition of 1 mL of the Tris-EDTA buffer to perform the first reading at a wavelength of 412 nm, obtaining A1. Subsequently, 25 µL of DTNB was added to this solution, and after 15 min of reacting at room temperature, the second reading was performed, obtaining A2, against a DTNB blank. After subtracting the values (A2–A1), the concentration in the sample was calculated using a standard curve of GSH, expressed in mmol/L, according to a method described previously.[27]

#### Determination of serum retinol and serum vitamin E and oils and fats from experimental diets

Liver analyses of vitamin E (a-tocopherol) and vitamin A retinol were performed by high-performance liquid chromatography, according to an adapted method.[28,29] For the analyses, a Shimadzu chromatograph model LC-20AT was used, with column type C-18 (150 × 4.6 mm – 5 µm), UV-visible detector model SPD-20A, a mobile phase composed of 7:2:1 acetonitrile: dichloromethane: methanol, a flow rate of 1.0 mL/min, and detection at 292 nm and 352 nm for α-tocopherol and retinol, respectively. The concentrations were determined by using an external standard, and the results were expressed in µmol/L of serum/plasma.

About 200 mg of oil/fat or 200 µL of serum were mixed with 400 µL of absolute ethanol. Subsequently, 400 µL of n-hexane was added for extraction. The samples were vortexed for 1 min and subsequently centrifuged at 3,000 rpm for 10 min. The supernatant (about 200 µL) was removed and dried under a flow of nitrogen. The dry residue was resuspended in 200 µL of the mobile phase, and a 20-µL aliquot was injected into the chromatograph.

### *Calculations*

#### Calculation of the ratio between serum triglycerides and HDL cholesterol

The triglyceride/HDL cholesterol ratio was calculated as a predictor of insulin resistance.[30,31]

#### Calculation of the ratio between vitamin E and cholesterol

The vitamin E/cholesterol ratio was calculated as described by Ford *et al.*[32]

**Table 1. Changes in body weight on a weekly basis (g)**

| | Control | HL | HL+CO |
|---|---|---|---|
| Start | 69 ± 6.83 | 67.5 ± 5.32 | 66.4 ± 3.06 |
| Week 1 | 126.7 ± 20.00 | 126.7 ± 10.77 | 128.1 ± 10.03 |
| Week 2 | 192.6 ± 19.13 | 166.7 ± 24.32[a] | 162.4 ± 17.84[b] |
| Week 3 | 251.1 ± 15.77 | 201.2 ± 39.35[b] | 197.7 ± 35.82[b] |
| Week 4 | 312.6 ± 21.77 | 240.1 ± 34.96[c] | 223.1 ± 42.23[c] |
| Week 5 | 363.5 ± 40.50 | 281.11 ± 42.61[b] | 282 ± 61[b] |
| Week 6 | 386.88 ± 62.44 | 314.33 ± 41.24[a] | 310.3 ± 57.59[a] |
| Week 7 | 416.29 ± 62.32 | 346.33 ± 42.12[a] | 299 ± 49.54[c] |
| Week 8 | 439 ± 83.01 | 379.22 ± 48.10 | 345.33 ± 34.81[b] |
| Week 9 | 402.57 ± 74.47 | 378.56 ± 43.46 | 348.22 ± 31.46 |

The results are expressed as the mean ± standard deviation. Statistical analysis: a indicates $p < 0.05$ vs. control; b indicates $p < 0.01$ vs. control; c indicates $p < 0.001$ vs. control. HL, hyperlipidic group; HL+CO, hyperlipidic group with coconut oil.

### *Statistical analysis*

The comparison between the experimental groups was performed by one-way analysis of variance with Tukey's post-test, considering $p < 0.05$, $p < 0.01$, and $p < 0.001$ as the levels of significance. The variables are presented as the mean ± standard deviation.

## Results

As for the weekly weight gain, a lower weight gain was observed in the groups that were fed high-fat diets after the second **week**, compared to the control group. In the sixth week, when CO was included in the diet of the HL+CO group, it was possible to observe a lower weight gain in the HL group, but without statistical significance, as shown in Table 1.

The animal diet intake results are shown on a weekly basis due to the fact that it took an average of 3 days to consume their food. The results are shown in Table 2. After the first week of the experiment, some reduction in food intake by the animals that received the high-fat diets was observed. From the first week until the end of the experiment, the HL and HL+CO groups presented a similar consumption, despite the introduction of CO in the sixth week. However, compared to the control group, both the HL and HL+CO groups showed a significant difference in consumption, with the control group consuming more than the other two groups.

As shown in Table 3, we observed that the hepatic weight did not present a statistically significant difference among the groups, but the liver/body weight ratio revealed an increase in liver weight in relation to the body weight in the HL and HL+CO groups, compared to the control group. The HL group revealed an increase in epididymal adipose tissue and the ratio of epididymal/retroperitoneal adipose tissue compared to the control and HL+CO groups. The HL and HL+CO groups showed a significant increase in retroperitoneal adipose tissue compared to the control group.

As shown in Table 4, total liver fat, percentage liver fat, and hepatic triglycerides were significantly greater in the groups that received a high-fat diet, with the triglyceride level in the HL+CO group also being significantly greater than that in the HL group. The total hepatic cholesterol level showed a significant difference between the HL group and the control and HL+CO groups. The total liver protein level was significantly less in the HL and HL+CO groups compared to that in the control group.

The serum changes did not match the liver changes found. Only

**Table 2. Food intake (g) of animals throughout the experimental period on a weekly basis**

| | Control | HL | HL+CO |
|---|---|---|---|
| Start | 14.77 ± 4.78 | 18.2 ± 3.05 | 17.7 ± 4.6 |
| Week 1 | 24.35 ± 6.91 | 17.3 ± 3.29[a] | 19.08 ± 2.76[b] |
| Week 2 | 25.55 ± 3.22 | 15.83 ± 2.62[c] | 14.45 ± 2.97[c] |
| Week 3 | 25.85 ± 3.67 | 15.10 ± 3.19[c] | 14.33 ± 4.02[c] |
| Week 4 | 31.78 ± 5.53 | 15.25 ± 4.70[c] | 15.6 ±6.32[c] |
| Week 5 | 35.35 ± 9.84 | 15.63 ±3.51[c] | 15.35 ± 4.91[c] |
| Week 6 | 41.5 ± 6.85 | 15.22 ± 2.53[c] | 15.22 ± 3.54[c] |
| Week 7 | 38.31 ± 6.85 | 14.67 ± 3.21[c] | 12.58 ± 4.23[c] |
| Week 8 | 29.53 ± 17.10 | 15.42 ± 3.75[b] | 17.22 ± 4.37[b] |
| Week 9 | 24.42 ± 13.97 | 16.12 ± 2.11 | 16.59 ± 2.25 |

The results are expressed as the mean ± standard deviation. Statistical analysis: a indicates $p < 0.05$ vs. control; b indicates $p < 0.01$ vs. control; c indicates $p < 0.001$ vs. control; d indicates $p < 0.05$ vs. lard, e indicates $p < 0.5$ vs. lard. HL, hyperlipidic group; HL+CO, hyperlipidic group with coconut oil.

**Table 3. Liver weight (g), liver/body weight ratio (%), epididymal adipose tissue (g), retroperitoneal adipose tissue (g), and ratio of epididymal adipose tissue weight to body weight (%)**

| | Control | HL | HL+CO |
|---|---|---|---|
| Liver weight (g) | 9.41 ± 1.99 | 14.05 ± 3.22 | 15.81 ± 3.54 |
| Liver/body weight ratio (%) | 2.33 ± 0.11 | 3.76 ± 0.5[a] | 4.47 ± 1.04[b] |
| Epididymal adipose tissue (g) | 4.47 ± 1.63 | 7.9 ±2.46[a] | 5.51 ± 1.27 |
| Retroperitoneal adipose tissue (g) | 3.82 ± 1.88 | 9.91 ± 2.69[b] | 9.76 ± 1.18[b] |
| Weight ratio epididymal adipose tissue/body (%) | 1.12 ± 0.44 | 2.09 ±0.45[a] | 1.54 ± 0.28 |

The results are expressed as the mean ± standard deviation. Statistical analysis: a indicates $p < 0.05$ vs. control; b indicates $p < 0.01$ vs. control. HL, hyperlipidic group; HL+CO, hyperlipidic group with coconut oil.

**Table 4. Total liver fat, percentage of liver fat, total hepatic cholesterol, hepatic triglycerides, and total hepatic protein**

| | Control | HL | HL+CO |
|---|---|---|---|
| Total liver fat (mg/gT) | 0.05 ± 0.01 | 0.21 ± 0.06[a] | 0.24 ± 0.06[a] |
| Percentage of liver fat (%) | 9.9 ± 1.71 | 40.85 ± 14.19[a] | 45.85 ± 13.31[a] |
| Total hepatic cholesterol (Ug/gT) | 36.69 ± 3.47 | 69.86 ± 23.31[b] | 40.20 ± 0.97[d] |
| Hepatic triglycerides (Ug/gT) | 3.36 ± 1.65 | 74.42± 29.93[c] | 132.87 ± 71.19[a,d] |
| Total hepatic protein (g/mL) | 0.023 ± 0.005 | 0.019 ±0.002[c] | 0.017 ± 0.003[a] |

The results are expressed as the mean ± standard deviation. Statistical analysis: a indicates $p < 0.05$ vs. control; b indicates $p < 0.01$ vs. control; c indicates $p < 0.001$ vs. control; d indicates $p < 0.05$ vs. lard, e indicates $p < 0.5$ vs. lard. HL, hyperlipidic group; HL+CO, hyperlipidic group with coconut oil.

serum triglycerides showed a significant difference between the HL+CO group and the HL group, with the latter group having higher values. The values of glucose, total protein, total cholesterol, HDL cholesterol, and the triglyceride/HDL cholesterol ratio did not show significant differences, as shown in Table 5.

We observed that the serum concentrations of ALT, AST, and the AST/ALT ratio were not significantly different, despite the fact that all of these parameters were greater in the HL+CO group in relation to the other groups (Table 6).

The parameters of the serum and liver antioxidant system are shown in Table 7. Although the serum GSH concentrations of the HL group did not present significant differences, there was a significant decrease between the HL+CO group in relation to the control group, and a significant increase in hepatic GSH in the groups that received a high-fat diet compared to the control group. The serum vitamin E and retinol levels as well as the vitamin E/cholesterol ratio were significantly different between the groups receiving a high-fat diet and the control group, with higher retinol

**Table 5. Serum values of glucose, total protein, triglyceride, total cholesterol, HDL cholesterol, and triglyceride/HDL cholesterol ratio**

| | Control | HL | HL+CO |
|---|---|---|---|
| Blood glucose (mg/dL) | 75.78 ± 8.25 | 89.31 ± 17.97 | 82.14 ± 12.83 |
| Total protein (g/mL) | 0.08 ± 0.01 | 0.07 ± 0.01 | 0.07 ± 0.008 |
| Triglycerides (mg/dL) | 61.03 ± 11 | 80.26 ± 19.31 | 57.33 ± 13.33[a] |
| Total cholesterol (mg/dL) | 67.35 ±18.81 | 81.26 ± 11.2 | 90.09 ± 22.2 |
| HDL cholesterol (mg/dL) | 45.44 ± 8.77 | 51.46 ± 9.06 | 46.85 ± 11.34 |
| Triglyceride/HDL cholesterol ratio | 1.56 ± 0.36 | 1.56 ± 0.29 | 1.27 ± 0.36 |

The results are expressed as the mean ± standard deviation. Statistical analysis: a indicates $p < 0.05$ vs. control. HDL, high-density lipoprotein; HL, hyperlipidic group; HL+CO, hyperlipidic group with coconut oil.

**Table 6. Serum concentrations of AST and ALT and the AST/ALT ratio**

| | Control | HL | HL+CO |
|---|---|---|---|
| AST (U/L) | 77.8 ± 18.88 | 66 ± 8.72 | 82.6 ± 13.32 |
| ALT (U/L) | 30.57 ± 18.17 | 24.5 ± 6.09 | 32.69 ± 6.09 |
| AST/ALT | 2.42 ± 1.18 | 2.84 ± 0.99 | 2.61 ± 0.62 |

The results are expressed as the mean ± standard deviation. Data are not significantly different among the experimental groups. ALT, alanine aminotransferase; AST, aspartate aminotransferase; HL, hyperlipidic group; HL+CO, hyperlipidic group with coconut oil.

**Table 7. Serum and hepatic GSH, serum vitamin E and retinol, vitamin E/choleterol ratio, serum and hepatic MDA**

| | Control | HL | HL+CO |
|---|---|---|---|
| Serum GSH (µmol/gP) | 4.03 ± 1.44 | 5.7 ± 1.69 | 3.71 ± 1.01[e] |
| Hepatic GSH (µmol/gP) | 395.32 ± 90.42 | 533.59 ± 81.42[a] | 560.24 ± 98.15[a] |
| Serum vitamin E (µmol/L) | 5.24 ± 2.04 | 1.77 ± 0.7[b] | 1.21 ± 0.84[b] |
| Vitamin E/cholesterol ratio | 0.79 ± 0.16 | 0.17 ± 0.13[b] | 0.1 ± 0.08[b] |
| Serum retinol (µmol/L) | 0.36 ± 0.08 | 0.96 ± 0.41[a] | 0.85 ± 0.43[c] |
| Serum MDA (nmol/gP) | 85.80 ± 24.16 | 104.23 ± 29.19 | 62.03 ± 24.81[e] |
| Hepatic MDA (µmol/gP) | 11.46 ± 1.83 | 50.92 ± 20.12[b] | 31.13 ± 6,74[c,d] |

The results are expressed as the mean ± standard deviation. Statistical analysis: a indicates $p < 0.05$ vs. control; b indicates $p < 0.01$ vs. control; c indicates $p < 0.001$ vs. control; d indicates $p < 0.05$ vs. lard, e indicates $p < 0.5$ vs. lard. HL, hyperlipidic group; HL+CO, hyperlipidic group with coconut oil; MDA, malondialdehyde; GSH, reduced glutathione.

values, lower vitamin E values, and a lower vitamin E/cholesterol ratio in the high-fat groups compared to the control group. The serum MDA values were significant only in the HL+CO group.

## Discussion

The data from the present experiment confirmed the induction of steatosis through the high-fat diet, compared to the control diet, which is a traditional model of steatosis induction.[33] The use of CO as a partial substitute for lard was not able to prevent or modulate hepatic steatosis; these data were corroborated with the increase in liver fat levels, 45% of fat. One recent study has described that in overweight males, a saturated-fat-enriched diet was more harmful for elevating the intrahepatic triglyceride content than a diet enriched in free sugars.[34]

In the second week of the experiment, we observed that the groups that received a high-fat diet showed less weight gain compared to the control group, and, in the sixth week, the HL+CO group had a lower weight gain than the HL group. In a comparison of different oils, when a diet with 40% CO was offered, it showed less weight gain than the group receiving 36% CO and 4% soy oil.[35] In a study of male Wistar rats fed high-fat diets containing triacylglycerols composed of medium- or long-chain fatty acids for 4 weeks on isocaloric diets, the long-chain fatty acids worsened insulin sensitivity and lipid metabolism, without influencing the body weight. However, medium-chain fatty acids appeared to protect the rats from lipotoxicity and subsequent insulin resistance.[36]

When analyzing liver weight, in the present study, it was possible to observe that although there was no statistically significant difference among groups, when we analyzed the liver/body weight ratio, there was an increase in liver weight in relation to body weight in the HL and HL+CO groups compared to the control group. Likewise, in a study by Wang *et al.*,[37] a significant increase was observed between animal weight and liver weight as well as in the liver/body weight ratio in the group that received a high-fat diet.

A greater weight of retroperitoneal fat in the groups that received a high-fat diet was observed, compared with the control, and only the HL group presented a greater weight of epididymal fat compared to the control group. In a study by De Castro *et al.*,[38] which used a hyperlipidic diet with 40% fat including lard, young rats showed some increase in the weight of retroperitoneal and epididymal fat compared to the control rats.

We observed that the hepatic cholesterol level was significantly higher in the HL group than in the control group. In relation to the triglyceride levels, the HL+CO group showed a significant difference compared to the other groups. In another study, the animals that received high or low doses of CO did not show a significant difference in liver cholesterol among groups, whereas the triglyceride levels were significantly different compared to the control group.[39] In addition, rats fed virgin CO on a diet for 5 weeks had a beneficial effect on the lipid profile, kidney status, hepatic antioxidant defense system, and cardiovascular risk indexes, in contrast with the diet without CO.[40]

In a study by Narayanankutty *et al.*,[39] the groups that received a low or high dose of CO showed higher GSH levels compared to the control group. Analysis of virgin CO polyphenols has documented the presence of gallic acid, ferulic acid, quercetin, and other phenols, which protect cells from pro-oxidant insults and modulate the cellular antioxidant status, making it a potential functional food.[41] In this scenario, CO act as an antioxidant oil.

As for the hepatic GSH, the values were higher in the groups that received a high-fat diet, compared to the control; although there was no significant difference, the values of the HL+CO group were higher. The increase in GSH values might have been a physiological adaptation due to lower levels of vitamin E, in accordance with the fact that vitamin E deficiency may be compensated by the increase in hepatic GSH.[42] In another study, our group has demonstrated that a high-fat diet increases oxidative stress, as shown by reduced concentrations of hepatic vitamin E.[20] The low levels of liver tocopherol influence the serum tocopherol levels and other parameters, such as GSH and MDA.

When analyzing serum triglycerides, a significant difference was only observed between the groups that received a high-fat diet, with the HL+CO group having a lower value. In a study by Panchal, Carnahan, and Brow,[43] the rats that received a diet rich in carbohydrates and CO had a higher concentration of plasma triglycerides compared to the control group.

Other strategies can be used for mitigation of oxidative stress and a fatty liver provoked by a high-fat diet, for example, the n-3 polyunsaturated fatty acid docosahexaenoic acid modulates lipid metabolism and the antioxidant hydroxytyrosol diminishes oxidative stress underlying a fatty liver.[44] One recent review discusses various aspects of steatosis, with the roles of glucotoxicity and lipotoxicity, and it was proposed that metabolic (dysfunction)-associated fatty liver disease is a more appropriate term.[45] Moreover, the comparison of high-fat diets enriched with lauric acid, the main fatty acid of CO, with a palmitic acid-supplemented diet demonstrated that both diets increased adipose tissue inflammation, systemic insulin resistance, and liver injury, but with a lesser extent of metabolic derangements caused by lauric acid compared to palmitic acid.[46]

In another experimental study, the consumption of CO modulated the serum lipid profile in a dose-dependent manner as well as the tissue incorporation of saturated fatty acids, inflammation in adipose tissue, and antioxidant effects. The authors did not establish the best dosage of CO because, in higher doses, there was a greater incorporation of saturated fatty acids in the liver and adipose tissues.[47] Furthermore, according to a recent study, rats fed a high-fat diet and supplemented with virgin CO had high levels of plasma lipid peroxidation and liver damage, as evidenced by rising aminotransferase activity, and they also had an increased weight of liver tissue and a higher content of liver cholesterol and triglycerides.[48] In the current study, we found similar results in the hepatic triglyceride levels and percentage of liver fat in the HL+CO group, besides being significantly greater than those in the HL group, but the aminotransferase level did not show any significant difference.

The novel formulation with virgin CO and phosphatidylcholine, which is named as Phoscoliv, has demonstrated hepatoprotective effects in a model using paracetamol in Wistar rats by enhancing the antioxidant status.[49] Vasconcelos has shown that in obese rats that received 3,000 mg/kg of E-virgin CO via gavage, E-virgin CO reduced the body mass and adiposity index as well as improved hormonal parameters compared with those of the nontreated animals.[50] Similar to the current study, another study has demonstrated that virgin CO can be useful for the treatment of a fatty liver by reducing lipid levels and increasing antioxidant levels.[51] Moreover, an interesting study with high-fat diet-induced obesity in mice has revealed that CO enhanced the expression of thermogenesis markers in brown adipose tissue, which was consistent with the increased brown adipose tissue activity.[52] Finally, a recent review by Sanches *et al.*[53] has shown a relationship among excessive CO consumption and consequences on metabolic syndrome and nonalcoholic steatohepatitis. This study agrees with the perspective that new clinical studies are necessary to verify the efficacy and safety of different doses of CO on hepatic and lipid metabolism.

## Conclusion

In conclusion, CO used in the animal model was not able to prevent or improve the status of hepatic steatosis compared to the group that received only animal fat. Furthermore, there was a better hepatic antioxidant response in the group that received CO, especially compared to the group that received only animal fat. These results indicate a possible role of CO in relieving oxidative stress caused by a high-fat diet.

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## Conflict of interest

The authors declare that they have no conflicts of interest.

## Author contributions

Study concept and design (LPB, RYS, BBA, AAJ), database organization (LPB, RYS, PPO), statistical analysis of the data (PPO, AAJ), drafting of the manuscript (LPB, RYS, AAJ, PPO). All authors revised the manuscript critically and approved the version to be published.

## Ethical statement

All animals were handled according to the Brazilian College of Animal Experimentation recommendations, and all procedures, which were based on the Animal Research: Reporting of in-vivo experiments (ARRIVE), were approved by the CEUA – Ethics Committee of Animals Use of FMRP/USP (protocol no. 012/2009).

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