Molecular and Cellular Endocrinology 581 (2024) 112110

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

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

# A MUFA/carotenoid-rich oil ameliorated insulin resistance by improving inflammation and oxidative stress in obese rats

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ARTICLE INFO

Handling Editor: Carolyn M. Klinge

*Keywords:*
Obesity
*Caryocar brasiliense*
Oxidative stress
Inflammation
Insulin resistance

ABSTRACT

Obesity is associated with low-grade inflammation and oxidative stress, leading to insulin resistance and type II diabetes. *Caryocar brasiliense* pulp oil (pequi oil – PO) is rich in oleic acid and carotenoids and positively implicated in regulating inflammation and oxidative stress. This study investigated PO's antioxidant and anti-inflammatory effects in a diet-induced obesity model. Male Wistar rats were allocated into three experimental groups: Control (CD), Western Diet (WD), and Western Diet, with 27% of lard switched by PO (WDP). Metabolic, inflammatory, and oxidative stress biomarkers were evaluated after 12 weeks of diet protocols in liver and adipose tissue. WDP rats gained less body mass and epididymal fat, had less hepatic fat infiltration, and were more glucose-tolerant and insulin-sensitive than WD ($p < 0.05$). In the liver, the WDP group had the highest non-enzymatic antioxidant capacity, SOD and GPx activities, CAT, SOD II, and HSP72 expression compared to WD ($p < 0.05$). Adipose tissue IL-6 and TNF were reduced, and IL-10 was increased in WDP compared to WD ($p < 0.05$). Our data suggest that the partial replacement of lard by PO in a Western diet prevented visceral fat accumulation and contributed to reducing inflammation in adipose tissue and liver oxidative stress, improving obesity-related insulin resistance.

## 1. Introduction

Obesity is characterized by excessive fat accumulation in adipose tissue, and its origin is multifactorial. The excessive intake of carbohydrates and lipids is a determinant for fat accumulation in the adipose tissue, which disrupts the adipocyte function. It has been shown that the overload of lipids in the adipocytes generates proinflammatory and oxidative responses, which, systemically, lead to insulin resistance and other disturbances (Echeverría et al., 2019).

Excessive consumption of high-fat foods, mainly saturated fats and trans fats, significantly contributes to the increase in global obesity rates (Krishnan and Cooper, 2014). Changes in dietary habits, such as reducing fat intake and improving fat quality, are proposed to prevent obesity and metabolic complications (Tutunchi et al., 2020a). In this sense, monounsaturated fatty acids (MUFA) have been pointed out as

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https://doi.org/10.1016/j.mce.2023.112110
Received 21 June 2023; Received in revised form 6 November 2023; Accepted 13 November 2023
Available online 20 November 2023

**Abbreviations**

| | |
|---|---|
| **AMP** | adenosine monophosphate |
| **AOAC** | Association of Official Analytical Chemists |
| **AOCS** | American Oil Chemist Society |
| **AUC** | area under the glucose curve |
| **CAT** | catalase |
| **CD** | Control diet group |
| **DPPH** | 2,2-diphenyl-1-picryl-hydrazyl-hydrate |
| **FFAs** | free fatty acids |
| **FRAP** | Ferric reducing antioxidant power |
| **GAPDH** | glyceraldehyde 3-phosphate dehydrogenase |
| **GPx** | glutathione peroxidase |
| **HSP72** | Heat shock protein |
| **IKK** | *Ikappa kinases* |
| **IL** | Interleukin |
| **ipGTT** | intraperitoneal glucose tolerance test |
| **MUFA** | monounsaturated fatty acids |
| **Nrf-2** | *nuclear factor erythroid 2-related factor 2* |
| **OA** | Oleic acid |
| **PO** | *pequi* oil |
| **ROS** | reactive oxygen species (ROS) |
| **SOD** | Superoxide dismutase |
| **TAG** | Triglycerides |
| **TBARS** | thiobarbituric acid reactive substances |
| **TNF** | tumoral necrosis factor |
| **WD** | Western diet group |
| **WDP** | Western diet *pequi* group |

beneficial for managing and preventing obesity (Yang et al., 2017; Tutunchi et al., 2020b). Oleic acid (OA), which is the most common MUFA in diet, is found in several vegetable oils (olive oil, high-oleic varieties of soybean and canola oil), nuts, and animal products (lard, ground beef, pork, and eggs), being olive oil one of the most OA rich-food (Raatz et al., 2018). OA-rich diets can be involved in regulating food intake, body mass, and energy expenditure by stimulating AMP-activated protein kinase (AMPK) signaling, preventing the nucleotide-binding oligomerization domain-like receptor 3 (NLRP3)/caspase-1 inflammasome pathway, inducing oleoyl ethanolamide (OEA) synthesis, and possibly downregulating stearoyl-CoA desaturase-1 activity (Tutunchi et al., 2020c).

In addition, bioactive components in fruits and vegetables, such as carotenoids, could also contribute to the prevention and treatment of obesity (Coronel et al., 2019). Carotenoids are compounds responsible for the yellow, orange, and red colors in fruits and vegetables (Rodriguez-Amaya, 2019). The anti-obesity action of carotenoids and carotenoid conversion products has been observed in several tissues. In adipose tissue, it seems they affect adipogenesis, metabolic capacity for energy storage and release, oxidation, secretory function, and modulate oxidative stress and inflammatory pathways (Takayanagi et al., 2011; Fenni et al., 2017; Liu et al., 2017; Bonet et al., 2015; Mounien et al., 2019). In the liver, some carotenoids have been shown to reduce oxidative stress, inhibit inflammation, and promote M2 macrophage polarization. They also improve mitochondrial oxidative respiration, and insulin sensitivity and suppress fibrosis (Clugston, 2020; Gao et al., 2021; Lim, 2019).

From a dietary perspective, including MUFA/carotenoid-rich foods in the diet could be an aid in preventing obesity or at least delaying its complications. In this context, *pequi oil* (PO), which is extracted from *Caryocar brasiliense* fruit, has high amounts of oleic acid (57.42 g.100g-1) and carotenoids (32.18 mg $g^{-1}$) (Oliveira et al., 2017), especially β-carotene and β-cryptoxanthin (Azevedo-Meleiro and Rodriguez-Amaya, 2004). Because of that, in the last decade, PO antioxidant (Vale et al., 2019), anti-inflammatory (Junior et al., 2020), antitumor (Miranda-Vilela et al., 2014), antigenotoxic (Colombo et al., 2015), and hypolipidemic (Oliveira et al., 2017; Teixeira et al., 2013) effects have been observed in *vitro* and *in vivo* studies. However, to our knowledge, investigation of PO anti-inflammatory and antioxidant effects in diet-induced obesity models is still lacking. Therefore, in this study, we investigated whether PO would have any anti-inflammatory and antioxidant effect on adipose tissue and liver in a diet-induced obesity model. We also wanted to know if those effects would impact insulin resistance and other metabolic disturbances in obese animals. We hypothesized that the partial replacement of lard by PO in a high fat/high sugar diet would improve anti-inflammatory and antioxidant responses in adipose tissue and liver and, accordingly, metabolic disturbances, especially insulin resistance.

## 2. Materials and methods

### 2.1. Preliminary analysis of pequi oil

Samples of PO were acquired from the local market. The fatty acid profile was determined by gas chromatography (CGC Agilent 6850 Series GC System) according to the AOCS Ce 1–62 method (AOCS, 2020). Total carotenoids were determined according to the AOAC Official Methods of Analysis (Latimer and Latimer, 2023) using a spectrophotometer (Specord 210, model Analytikjena) at 450 nm. All analyses were performed in three samples of pequi oil. *In vitro,* PO antioxidant activity was performed in 60% ethanolic and 50% methanolic/acetone extracts employing the DPPH Radical Scavenging (Brand-Williams et al., 1995) and the ferric reducing antioxidant power (FRAP) (Benzie and Strain, 1996) methods.

### 2.2. Experimental protocol

Thirty-six male Wistar rats (Universidade Federal de Viçosa, Viçosa-Brazil), aged four weeks, were individually housed in stainless steel cages and maintained in a humidity and temperature (22 ± 2 °C) controlled room, with a 12:12 h light/dark cycle and free access to water and food during the experimental period. The study protocol was conducted as the National Research Council's Guide for the Care and Use of Laboratory Animals and was approved by the Ethics Commission on Animal Use from Universidade Federal dos Vales do Jequitinhonha e Mucuri, Brazil. After a one-week acclimation period, rats were randomly allocated into three groups (n = 12 in each group). CD: fed a control diet - AIN93G (Reeves et al., 1993); WD: fed a western diet – high-fat (mostly lard), high-sugar (sucrose); and WDP: fed western diet with 27% of lard switched by PO (Table 1). The experiment lasted 12 weeks. The amount

**Table 1**
The composition of experimental diets. CD: control diet. WD: western diet; WDP: western diet with 27% of lard switched by PO.

| Ingredients ($g.kg^{-1}$) | Experimental diets* | | |
|---|---|---|---|
| | CD | WD | WDP |
| Casein | 200.0 | 200.0 | 200.0 |
| Dextrinized starch | 132.0 | 100.0 | 100.0 |
| Sucrose | 100.0 | 341.0 | 341.0 |
| Starch | 397.5 | 48.5 | 48.5 |
| Soybean oil | 70.0 | 10.0 | 10.0 |
| Lard | – | 200.0 | 146.0 |
| *Pequi* oil | – | – | 54.0 |
| Cellulose | 50.0 | 50.0 | 50.0 |
| Mineral mix | 35.0 | 35.0 | 35.0 |
| Vitamin mix | 10.0 | 10.0 | 10.0 |
| L-cystine | 3.0 | 3.0 | 3.0 |
| Choline bitartrate | 2.5 | 2.5 | 2.5 |

of *pequi* oil added to the WDP diet was based on the daily intake of 10% from lipid calories, based on a 1800-kcal diet (for humans = ~20 g/day). This amount meant replacing 27% of lard with pequi oil (w/w), and is suitable for human consumption. CD had 3.9 kcal $g^{-1}$; WD and WDP, 4.7 kcal $g^{-1}$. CD had 63.8, 15.9, and 20.0 kcal% from carbohydrates, lipids, and protein, respectively. WD and WDP had 42.0, 40.7, and 17.2 kcal% for carbohydrates, lipids, and protein, respectively. Body mass and food intake were recorded throughout the experiment. One week before euthanasia, the intraperitoneal glucose tolerance test (ipGTT) was performed. On the day of euthanasia, overnight fasted rats were anesthetized (ketamine/xylazine at 50 mg $kg^{-1}$/10 mg $kg^{-1}$ respectively) and euthanized by decapitation for blood and tissue harvesting.

### 2.3. Intraperitoneal glucose tolerance test (ipGTT)

After 6-h fasting, rats were intraperitoneally injected with a dextrose solution (1 g.$kg^{-1}$ of body mass). Blood samples were collected from the tail vein at 0, 15, 30, 60, and 120 min to measure glucose levels using a portable blood glucose meter (Roche, Accu-Chek Performa Nano, Rio de Janeiro, Brazil), according to Villas-Boas et al. (Villas Boas et al., 2020). The area under the glucose curve (AUC) was calculated using the trapezoidal rule (Prisma 8.0 software).

### 2.4. Hormone and cytokine analysis

Serum insulin (Sigma-Aldrich EZRMI-13 K), leptin (EZRL-83 K Sigma-Aldrich), and adiponectin (R&D System RRP300) concentrations were determined using commercial ELISA kits. Following the manufacturer's recommendations, fasting serum glucose was measured using a commercial kit (Bioclin®, Brazil). Insulin resistance was determined by the homeostatic model assessment of insulin resistance (HOMA-IR) from fasting blood glucose and insulin concentrations, according to Antunes et al. (2016). From leptin and adiponectin values, the adiponectin/leptin ratio was calculated, according to Frühbeck et al. (2018).

Interleukin 10 (IL-10 – R&D System R1000), 6 (IL-6 - R&D System R6000B), and tumoral necrosis factor (TNF - R&D System RTA00) concentrations were determined in samples of epididymal adipose tissue, using ELISA commercial kits (R&D Systems) according to the manufacturer's recommendations. Cytokine concentrations were expressed in pg/mg of total protein content.

### 2.5. Histological analyzes

After euthanasia, the liver and epididymal adipose tissue were carefully removed and weighed on an analytical scale. Liver relative mass (liver mass/body mass × 100) and epididymal fat relative mass (epididymal fat mass/body mass × 100) were calculated. Results were presented as % of body mass.

For histological analysis, the liver's right lobe and epididymal adipose tissue fragments were fixed in buffered-formalin, paraffin-embedded, sliced, and stained with hematoxylin and eosin. The degree of hepatic steatosis was classified from I to IV, based on the percentage of hepatocytes with accumulated fat: grade 0 indicated no steatosis and normal liver; grade I indicated ≤25% of steatosis affected hepatocytes; grade II indicated 26–50% of steatosis affected hepatocytes; grade III indicated 51–75% of steatosis affected hepatocytes; and grade IV, >76% of steatosis affected hepatocytes (Tzeng et al., 2013). We determined the percentual of animals that showed liver steatosis in each experimental group in each classification grade. We also characterized the hepatic fatty infiltration in micro or macrovesicular and evaluated cellular ballooning characteristics, such as size greater than its neighboring cells, flocculent cytoplasm, and hyperchromatic nucleus (Brunt et al., 2022).

In epididymal adipose tissue, adipocyte sizes were determined by measuring the cell area. Fields were photographed at 400× magnification, and 50 adipocytes per animal were measured to assess hypertrophy. Histological analysis of the microscopic liver and adipose tissue images was performed using the ImageJ 1.51 software.

### 2.6. Redox status analysis

Liver samples (300 mg) were macerated in phosphate-buffered saline (PBS, 0.015 M and pH7.4), and the assays were performed as described (Costa et al., 2022). The tissue homogenates were centrifuged, and the supernatants harvested for analysis. It was determined the thiobarbituric acid reactive substances (TBARS) (Ohkawa et al., 1979) and the Ferric Reducing Antioxidant Power (FRAP) (Benzie and Strain, 1996). According to the manufacturer's specifications, the glutathione peroxidase (GPx) activity was evaluated through a commercially available kit (Cayman Chemical Company, Ann Arbor, MI, USA, kit no. 703102). The superoxide dismutase (SOD) enzymatic activity assay was carried out as proposed by Marklund and Marklund (1974). The catalase (CAT) activity was determined according to Nelson and Kiesov (Nelson and Kiesow, 1972). Protein concentration was determined using the Bradford method (Bradford, 1976), and results were normalized according to hepatic protein content.

### 2.7. Western blotting

Protein (60 μg) from liver samples was loaded onto a 15% polyacrylamide gel for electrophoresis. After electrophoresis, proteins were transferred to a polyvinylidene difluoride membrane, blocked with a 5% non-fat milk solution, and washed in PBS containing 0.1% Tween 20. Membranes were incubated overnight at 4 °C with the following primary monoclonal antibodies: glyceraldehyde 3-phosphate dehydrogenase – β-actin (1:4000 dilution; Sigma-Aldrich, St. Louis, Missouri, USA); SOD1 (1:2000 dilution; Sigma-Aldrich) and SOD2 (1:3000 dilution; Sigma-Aldrich), GPx (1:1000 dilution; Santa Cruz Biotechnology, Dallas, Texas, USA), CAT (1:1000; Cell Signaling Technology, Danvers, MA, USA), Heat shock protein - Hsp72 (1:5000 dilution; Sigma-Aldrich). Thereafter, it was used monoclonal anti-sheep (1:3000, Santa Cruz Biotechnology), anti-mouse (1:3000, Cell Signaling), anti-goat (1:5000, Santa Cruz Biotechnology), or anti-rabbit (1:5000, Cell Signaling) secondary antibodies conjugated with peroxidase. Detection was carried out using enhanced chemiluminescence (Amersham Biosciences, Little Chalfont, UK), and images were obtained by a scanner (Gel Logic 200 imaging system, Kodak, Rochester, NY, USA). Protein levels were corrected to β-actin and expressed as a ratio of optical densities.

### 2.8. Statistical analyses

All values were expressed as means ± standard error (SE). After verifying the data normality by the Shapiro-Wilk test, the comparisons between groups were conducted using one-way ANOVA and the Tukey test, as necessary. The Prisma 10.0 software was used for all analyses, and the p-value $<0.05$ was considered significant.

## 3. Results

PO had expressive amounts of monounsaturated fatty acids (MUFA), with oleic acid as its primary component. Among saturated fatty acids, palmitic was the most abundant (Table 2). The total unsaturated fatty acid content was almost twice the saturated one. Also, PO presented 30.14 ± 4 mg. $g^{-1}$ of total carotenoids. In the *in vitro* antioxidant activity, it was observed a higher ferric reducing ability (1319.55 ± 202.58 μmolTE.$g^{-1}$) and a higher capacity of free radical scavenging (358.01 ± 135.61 μmolTE.$g^{-1}$) in the methanolic extract compared to the ethanolic (445.01 ± 63.29 and 89.03 ± 22.25 μmolTE.$g^{-1}$, respectively).

**Table 2**
Fatty acid composition of *pequi* oil (g.100 $g^{-1}$). * Mean of 3 samples. SD: standard deviation.

| Fatty acid | Carbon num. | Mean*±SD |
|---|---|---|
| Lauric | C12:0 | 0.03 ± 0.002 |
| Myristic | C14:0 | 0.07 ± 0.000 |
| Palmitic | C16:0 | 35.60 ± 0.035 |
| Margaric | C17:0 | 0.07 ± 0.001 |
| Stearic | C18:0 | 2.81 ± 0.002 |
| Arachidonic | C20:0 | 0.19 ± 0.000 |
| Behenic | C22:0 | 0.06 ± 0.007 |
| Lignoceric | C24:0 | 0.06 ± 0.010 |
| Palmitoleic | C16:1 | 0,94 ± 0.002 |
| Cis-10-heptadecenoic | C17:1 | 0.08 ± 0.000 |
| Oleic | C18:1 | 57.54 ± 0.030 |
| α-Linoleic | C18:2 | 1.47 ± 0.003 |
| α Linolenic | C18:3 | 0.94 ± 0.001 |
| Eicosenoic | C20:1 | 0.14 ± 0.001 |
| Total saturated | – | 38.89 ± 0.031 |
| Total unsaturated | – | 61.11 ± 0.031 |

### 3.1. The lard switch by PO oil in a western diet reduced the body mass gain

Food intake was lower in WD and WDP groups compared to CD (Fig. 1A, $p < 0.01$), but there was no difference among groups for caloric intake (Fig. 1B). All rats started the protocol with similar body masses (CD = 63.91 ± 1.20; WD = 64.61 ± 1.34; WDP = 64.84 ± 1.30 g). In the 12th week, WD and WDP groups weighted more than CD (Fig. 1C, $p < 0.05$). Body mass gain was higher for WD, followed by WDP and, at last, CD (Fig. 1D, $p < 0.01$).

**Table 3**
Effects of switching lard by *pequi* oil in a Western diet for twelve weeks in serum metabolic markers of rats. HOMA-IR: Homeostatic model assessment of insulin resistance. Data are expressed as mean ± SE (n = 12). Different superscript letters indicate a significant difference ($p < 0.05$) by One-way ANOVA and Tukey test. CD: control diet; WD: western diet; and WDP: western diet with 27% of lard switched by PO.

| Variables | Experimental diets/groups | | | *p*-value |
|---|---|---|---|---|
| | CD | WD | WDP | |
| Glucose (mg.$dL^{-1}$) | 117.4 ± 4.06$^{c}$ | 139.0 ± 5.22$^{a}$ | 122.0 ± 2.70$^{b}$ | 0,002 |
| Insulin (ng.$mL^{-1}$) | 2.34 ± 0.23$^{b}$ | 3.55 ± 0.21$^{a}$ | 3.50 ± 0.36$^{a}$ | 0,005 |
| HOMA-IR | 15.75 ± 1.14$^{c}$ | 30.58 ± 2.66$^{a}$ | 26.36 ± 3.01$^{b}$ | 0,0004 |
| Leptin (ng.$mL^{-1}$) | 11.61 ± 0.78$^{b}$ | 15.14 ± 1.14$^{a}$ | 14.36 ± 1.03$^{a}$ | 0,043 |
| Adiponectin (ng.$mL^{-1}$) | 28.07 ± 1.04$^{b}$ | 23.67 ± 1.03$^{b}$ | 24.39 ± 1.92$^{b}$ | 0,07 |
| Adiponectin/Leptin ratio (ng.$ng^{-1}$) | 2.61 ± 0.90$^{a}$ | 1.69 ± 0.61$^{b}$ | 1.80 ± 0.58$^{b}$ | 0,02 |

### 3.2. The lard switch by PO oil in a western diet ameliorated insulin resistance and glucose metabolism disturbance

WD rats had increased plasma glucose, insulin, leptin, and HOMA-IR and reduced adiponectin/leptin ratio compared to CD ($p < 0.05$, Table 3). WDP had plasma insulin, leptin, adiponectin, and adiponectin/leptin ratio values similar to WD. Plasma glucose and HOMA-IR values were in between CD and WD values in the WDP group ($p < 0.05$; Table 3). In addition, in the ipGTT, the WDP group had fasting glucose values between WD and CD ($p < 0.05$; Fig. 2A). At 15 min, glucose

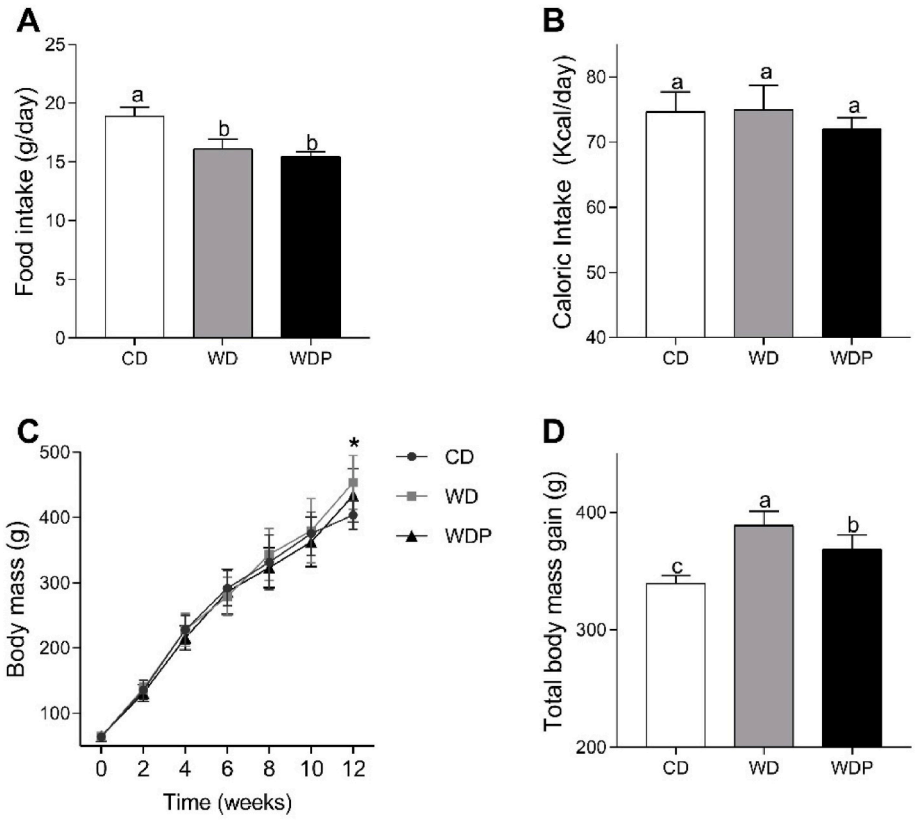


**Fig. 1.** Effects of switching lard by *pequi* oil in a Western diet for twelve weeks on food **(A)** and caloric intake **(B)**, body mass **(C),** and total body mass gain **(D)**. Data are mean ± SE (n = 12 animals/group). Bars with different letters indicate a significant difference ($p < 0.05$). * Indicates significant difference of CD vs WD or WDP ($p < 0.05$) by One-way ANOVA and Tukey test. CD: control diet. WD: western diet; WDP: western diet with 27% of lard switched by PO. The figure is a 2-column fitting image.

values were similar among all groups (Fig. 2A). At 30 min, the WD group showed higher blood glucose compared to CD and WDP. At the end of the test, the WDP and CD groups had similar glucose values lower than WD ($p < 0.05$, Fig. 2A). Indeed, the glucose AUC and HOMA-IR in the WDP was lower than WD and higher than CD ($p < 0.05$, Fig. 2B and Table 3), indicating that PO improved glucose tolerance and insulin resistance in WDP rats.

### 3.3. The lard switch by PO in a western diet ameliorated liver fat accumulation and antioxidant capacity

Liver photomicrographs showed that the WD group had extensive fat infiltration in hepatocytes, predominantly macrovesicular with size greater than its neighboring cells, flocculent cytoplasm, and hyperchromatic nucleus, suggesting ballooning changes (Fig. 3A). WDP, otherwise, had almost no fat infiltration (only microvesicular, when found) (Fig. 3A). CD had no fat infiltration or other alterations (Fig. 3A). In addition, WD rats showed accumulated fat in hepatocytes, 66% of the samples in grade III and 33% in grade IV (Fig. 3B and C). Conversely, WDP rats showed grade I (63%) or II (36%) liver steatosis (Fig. 3B and C). All CD rats showed grade I (100%) steatosis. The relative liver weight was higher in the WD group compared to the other two groups, CD and WDP, which had similar weights (Fig. 3D).

Liver peroxidation was equally increased in WD and WDP, compared to CD group ($p < 0.05$; Fig. 4A). Total non-enzymatic antioxidant capacity was lower in WD ($p < 0.05$), compared to WDP and CD groups, and they did not differ (Fig. 4B). The western diet did not change SOD activity. However, PO in the western diet raised its activity compared to WD and CD ($p < 0.05$, Fig. 4C). CAT activity was not altered among experimental groups (Fig. 4D). GPx activity was increased in the liver of WDP rats, followed by WD and CD ($p < 0.05$; Fig. 4E). The western diet reduced SOD1, SOD 2, CAT and HSP72 (Fig. 4D and **J**) expression compared to the CD. SOD-II expression levels were higher in WDP compared to WD and equal to the CD ($p < 0.05$, Fig. 4D and **H**). WDP showed similar values of WD and CD for SOD I, catalase and HSP72 expression (Fig. 4D and **J**).

### 3.4. The lard switch by PO in a western diet reduced adipocyte sizes and improved cytokine profile in epididymal fat

The epididymal fat photomicrographs in Fig. 5A revealed that WD adipocyte sizes were extensively bigger than CD and WDP. Epididymal fat relative weight and adipocyte areas were higher in WD (Fig. 5B and **C**, $p < 0.05$). Despite the Western diet, WDP adipocyte size and area were preserved and similar to CD (Fig. 5A and **C**, $p < 0.05$). WDP epididymal fat relative weight was similar to CD (Fig. 5B, $p < 0.05$). About the cytokines, WD rats had the lowest IL-10 concentration, followed by WDP and then CD ($p < 0.05$; Fig. 5D). WDP showed the lowest liver content of IL-6, followed by CD and WD ($p < 0.05$; Fig. 5E). TNF content was increased in WD, and WDP reduced it, but it did not reach the CD value ($p < 0.05$; Fig. 5F).

## 4. Discussion

PO is a food usually consumed in Brazil, especially in its geographical areas of occurrence. This oil has a peculiar chemical composition, with a high content of MUFA, especially oleic acid, and several antioxidant compounds, mostly carotenoids (Oliveira et al., 2017). Because of that and the growing scientific interest in understanding PO biological effects, our research group has investigated the beneficial properties of *pequi* pulp or PO in healthy (Oliveira et al., 2017; César et al., 2017; Moreno et al., 2016) and animal diseases models, such as obesity (Rodrigues et al., 2020; Evangelista-Silva et al., 2021) and inflammatory bowel disease (Moreno et al., 2021). In the present study, we have demonstrated improved metabolic, anti-inflammatory, and antioxidant effects of PO in male rats fed a high-fat/high-sugar diet. We believe that PO oleic acid and carotenoid abundance were determinants, at least in part, of our observations.

The chemical composition of the PO found in our study agreed with previously published data. PO has, indeed, high oleic acid and total carotenoid content, the latter higher than in usually consumed fruits, such as acerola (29.6 μg $g^{-1}$), papaya (28.7 μg $g^{-1}$), mango (25.0 μg $g^{-1}$) and pumpkin (22.1 μg $g^{-1}$) (Rodríguez-Amaya et al., 2008). Although the specific carotenoids present in our PO samples were not determined, other authors have already demonstrated specific carotenoids in PO samples from the same geographical region, such as beta-carotene, anteroxanthin, zeaxanthin and beta-cryptoxanthin. Phenolic compounds, vitamin A, and selenium were also found (Carneiro et al., 2023). It is described in the literature the antioxidant activity of these compounds *in vitro* and *in vivo* studies (Goulas and Georgiou, 2020; Du et al., 2021). Therefore, we believe such compounds may have contributed to the higher antioxidant activity observed in the PO methanolic extract. Previous data from our lab also found a high antioxidant activity in a methanolic extract from *pequi* pulp (Morais et al., 2013).

As expected, in the bioassay, the high-fat/high-sugar diet promoted higher body mass gain and visceral and hepatic fat accumulation, even though the same caloric intake was observed between WD and WDP. It is possible that, in the WD group, the high glycemic load, induced hormonal disturbances related to visceral fat accumulation (Ludwig and Ebbeling, 2018), and the long-term high fat intake were able to disrupt the energy balance, favoring the increase in body mass (Woods et al., 2003). In WD rats, such changes were followed by hyperinsulinemia, hyperglycemia, low glucose tolerance, and insulin resistance, extensively described as effects of excessive fat and sugar intake (Kothari

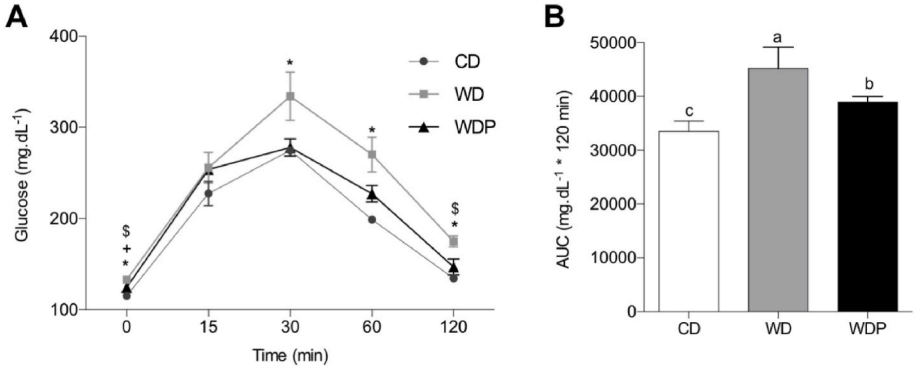


**Fig. 2.** Effects of switching lard by *pequi* oil in a Western diet for twelve weeks on glucose homeostasis. (**A**): Intraperitoneal glucose tolerance test. (**B**): Area under the curve. Data are expressed as mean ± SE ($n = 12$). Bars with different letters indicate a significant difference ($p < 0.05$) by One-way ANOVA and Tukey test. * = CD vs WD ($p < 0.05$), $ = WD vs WDP ($P < 0.05$), + = CD vs WDP ($p < 0.05$). CD: control diet. WD: western diet; WDP: western diet with 27% of lard switched by PO. The figure is a 2-column fitting image.

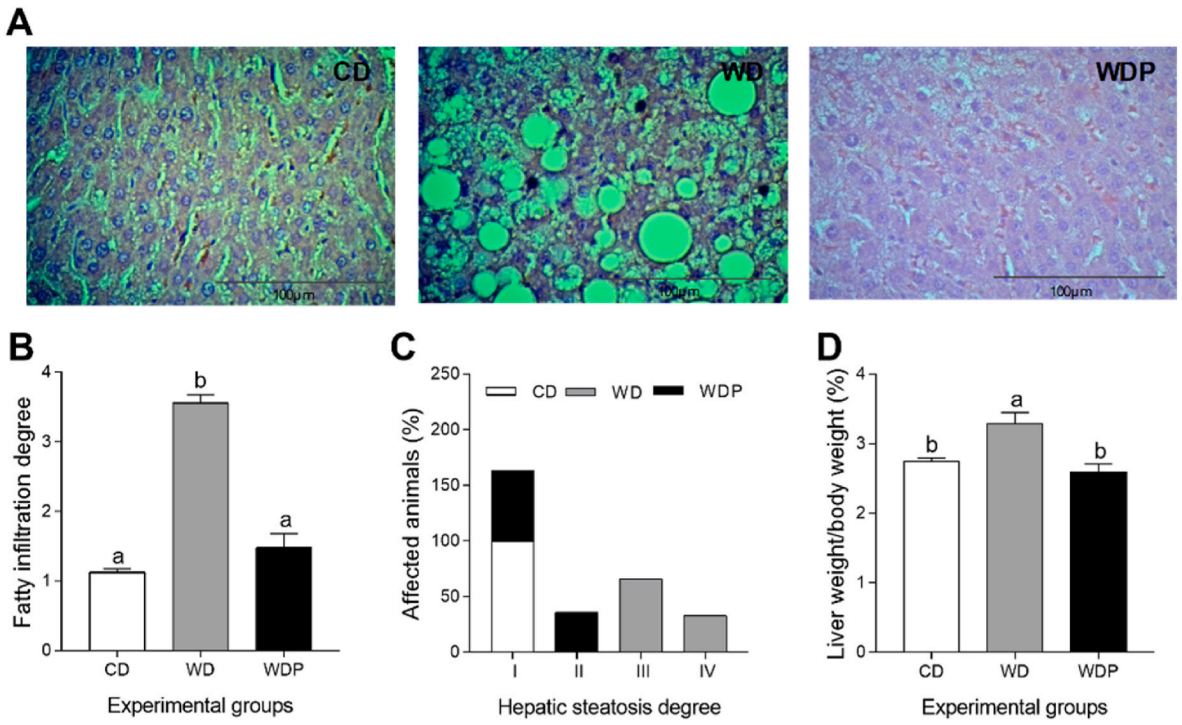


**Fig. 3.** Effects of switching lard by *pequi* oil in a Western diet for twelve weeks on liver fat infiltration in rats. (**A**): Representative photomicrographs of liver fatty infiltration. (**B**): Fatty infiltration degree. (**C**): Affected animals (%). (**D**) Liver relative weight. Data are expressed as mean ± SE (n = 12). Bars with different letters indicate a significant difference ($p < 0.05$) by One-way ANOVA and Tukey test. (**A**) Magnification: 10 (ocular) × 40 (object lens). CD: control diet. WD: western diet; WDP: western diet with 27% of lard switched by PO. The figure is a 2-column fitting image.

et al., 2017; Panchal et al., 2011).

Conversely, when pequi oil was ingested in the Western diet, the animals gained less body mass and less epididymal fat, indicating lower visceral fat accumulation. This fact possibly ameliorated the local inflammation since TNF and IL-6 were reduced, and IL-10 was increased. Indeed, it has been shown that lowering fat in adipose tissue ameliorates the immune metabolic disruption in adipocytes, characterized by a reduction of proinflammatory biomarkers such as IL-6 and TNF and an increase in anti-inflammatory ones, such as IL-10 (Escalante-Aburto et al., 2023; Wang et al., 2021).

Nevertheless, despite the lower epididymal fat accumulation in the WDP group, adiponectin and leptin levels did not change compared to WD, and the adiponectin/leptin ratio remained lower than in control lean rats. The lower adiponectin/leptin ratio is a marker of the early stages of insulin resistance in animals and humans (Diwan et al., 2018). Leptin and adiponectin are hormones produced mainly by adipocytes, with circulating concentrations of leptin being positively correlated (Jean-Philippe et al., 2006) and adiponectin negatively correlated with the degree of adiposity (Diwan et al., 2018). Under physiological conditions, appetite control is a primary function of leptin (Diwan et al., 2018); it reduces food intake in response to triacylglycerol deposition in adipose tissue (Crujeiras et al., 2015). Adiponectin, in turn, exerts protective effects against inflammation and modulates the endocrine system, as it stimulates fatty acid oxidation and glucose uptake by skeletal muscle and inhibits hepatic glucose production (Li et al., 2020). Although we did not evaluate leptin and adiponectin tissue levels, we believe that even though they were changed in epididymal adipose tissue because of PO replacement in the Western diet, it was insufficient to impact their circulating concentrations.

In our study, the Western diet treatment resulted in liver macrovesicular fat accumulation. The liver is the most sensitive organ to insulin signaling impairment in obesity, which occurs faster than in other organs, and hepatic insulin resistance is the primary event that leads to the subsequent development of peripheral tissue insulin resistance (Ahmed et al., 2021). The excess of FFA and dietary lipids from obese adipose tissue leads to increased ectopic fat content, especially triacylglycerols. Ectopic fat diverges the lipid metabolism to the production of ceramides and diacylglycerols, which activate many molecules involved in the dysregulation of insulin action, including *protein kinase-C*, *c-Jun N-terminal*, and *Ikappa kinases* (IKK) activation, oxidative and endoplasmic reticulum stress (Vázquez-Jiménez et al., 2018). When PO replaced lard, fat liver accumulation was reduced, with the predominance of microvesicular fat, indicating that PO reduced the hepatic impairment caused by the Western diet. Therefore, the improvements in fasting glucose, glucose tolerance, and insulin resistance could be partly due to the lower lipid accumulation.

PO also improved the liver's antioxidant capacity. The exogenous supply of antioxidants in WDP was higher (carotenoids), resulting in higher total antioxidant capacity, similar to the one observed in lean animals. In addition, the higher expression and activity of SOD were followed by an increase in the GPx activity and CAT expression in the WDP group. This finding may imply a better ability to stop oxidoreduction reactions and minimize oxidative damage, which could have been a determinant for maintaining cellular integrity (Ribas et al., 2014; Trachootham et al., 2008). In addition, the improvement in the hepatic redox state may be a consequence of the lower hepatic fat deposition, and, accordingly, it may contribute to ameliorating glucose and insulin disturbances (Henstridge et al., 2014). However, the Western diet increased liver lipid peroxidation, and PO did not modify it, which indicates that the adverse effects of saturated fat and sucrose overload may have overcome the PO antioxidant effects (Aleksandrova et al., 2021).

An important outcome of our study was the increased protein expression of HSP72 upon PO intake. This protein acts as a molecular chaperone and is crucial in inhibiting signaling pathways that induce stress and protect cell integrity (Henstridge et al., 2014). It is essential for hepatic mitochondrial integrity and fatty acid oxidation (Archer et al., 2018a) and is involved in other metabolic pathways (Henstridge et al., 2014; Archer et al., 2018a; Chung et al., 2008). Whereas in human

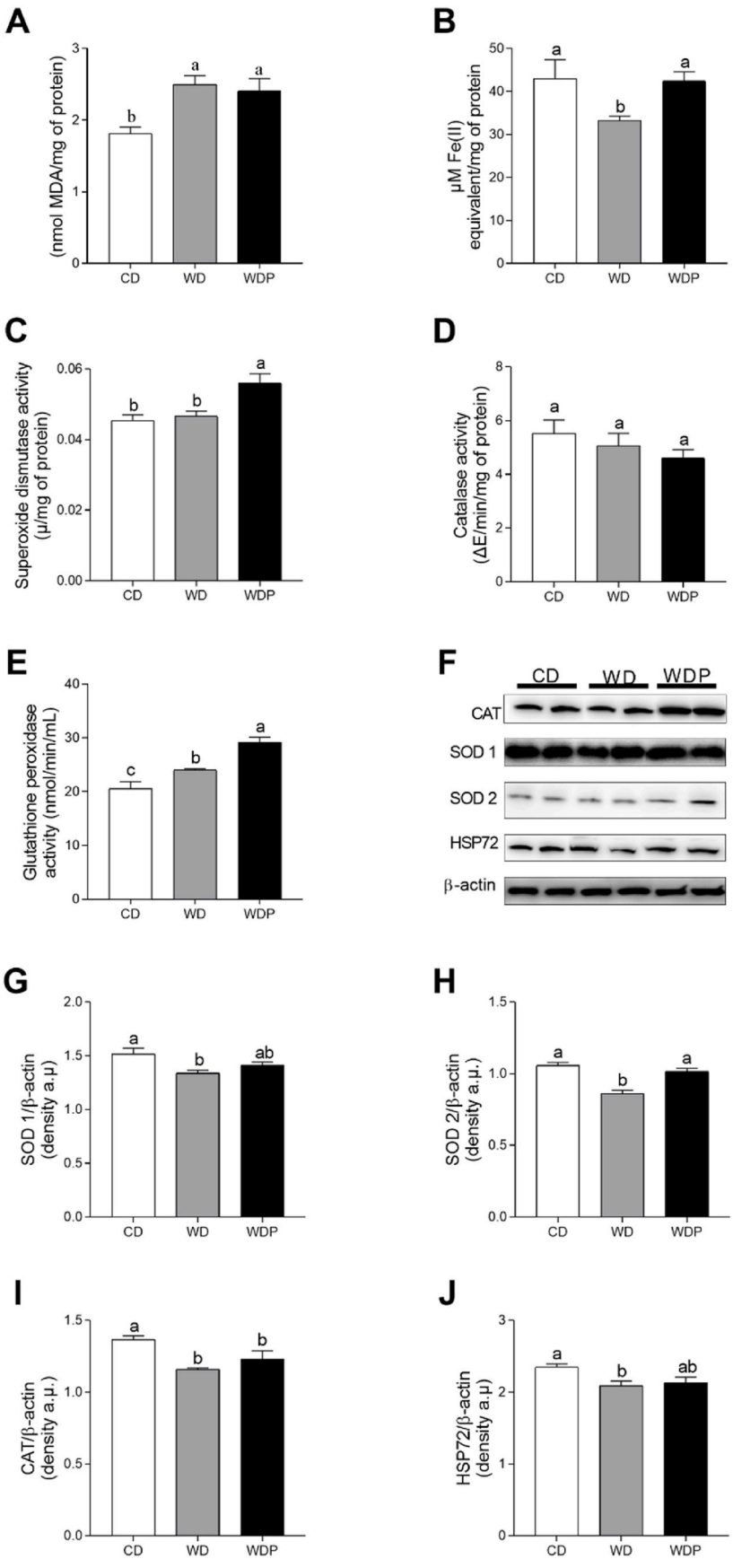


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**Fig. 4.** Effects of switching lard by *pequi* oil in a western diet for twelve weeks on liver redox status of rats. (**A**): Lipid peroxidation (Thiobarbituric acid reactive substances – TBARS). (**B**): Total antioxidant capacity (Ferric Reducing Antioxidant Power – FRAP. (**C**): Superoxide dismutase activity. (**D**): Catalase activity. (**E**): Glutathione peroxidase activity. (**F**): Cropped images represent Western blot analysis showing antioxidant enzymes and HSP72 expression in the liver of rats receiving different dietary treatments. (**G**): Superoxide dismutase I (SOD-I) expression. (**H**): Superoxide dismutase II expression (SOD-II). (**I**): Catalase expression (CAT) (**J**): Heat-shock protein-72 expression (HSP72). Data are expressed as mean ± SE (n = 12). Bars with different letters indicate a significant difference ($p < 0.05$) by One-way ANOVA and Tukey test. CD: control diet. WD: western diet; WDP: western diet with 27% of lard switched by PO. The figure is a 2-column fitting image.

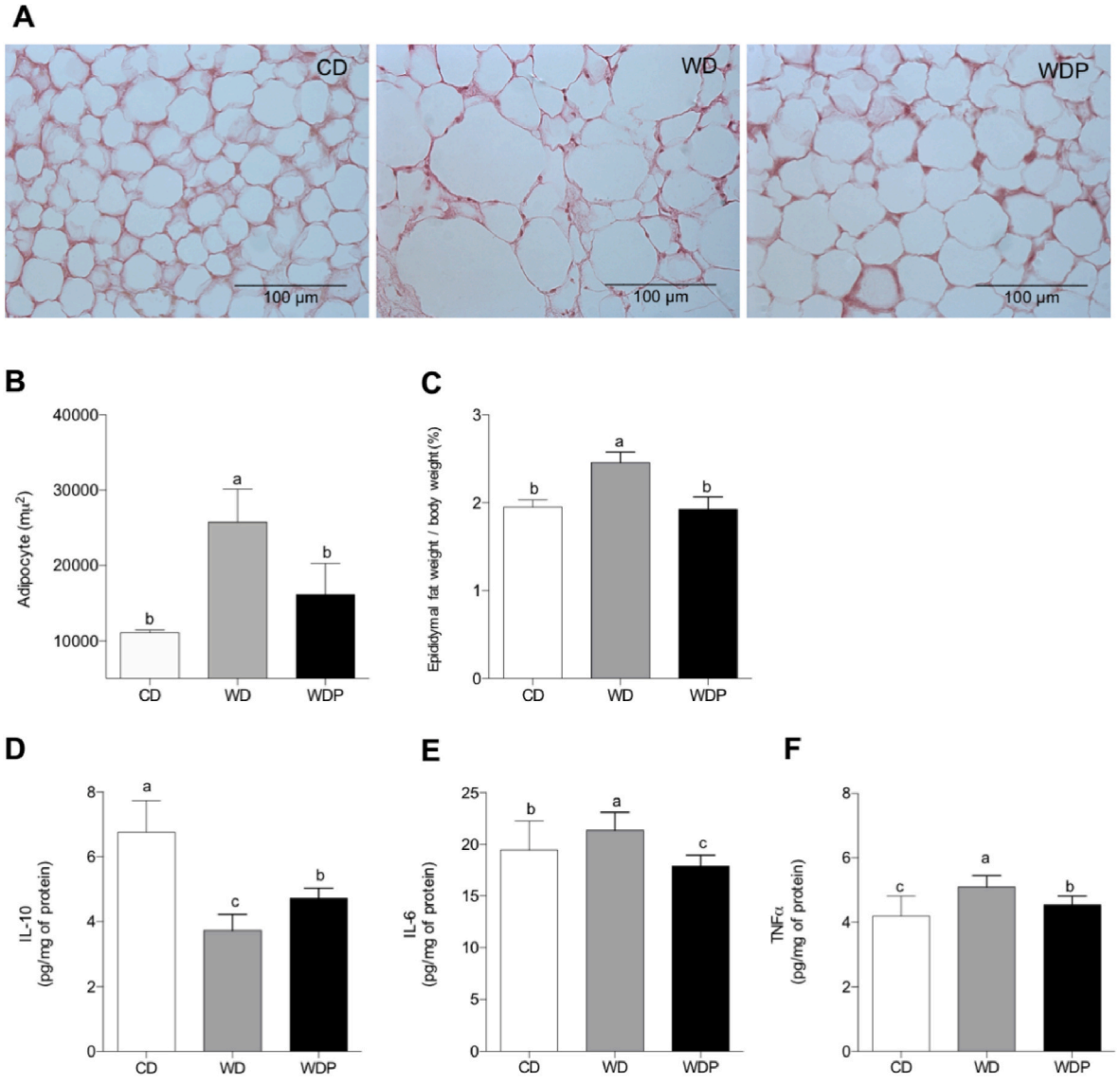


**Fig. 5.** Effects of switching lard by *pequi* oil in a Western diet for twelve weeks on adipose tissue fat deposition and cytokine content. (**A**) Representative photomicrographs of epididymal adipose tissue. (**B**) Epididymal adipocytes area. (**C**) Epididymal tissue relative weight. (**D**) Interleukine-10 – IL-10. (**E**) Interleukine-6 – IL-6. (**F**) Tumor necrosis factor-α – TNF-α. Data are mean ± SE (n = 12 animals/group). Bars with different letters indicate a significant difference ($p < 0.05$) by One-way ANOVA and Tukey test. CD: control diet. WD: western diet; WDP: western diet with 27% of lard switched by PO. The figure is a 2-column fitting image.

and animal obesity, the expression of HSP is reduced (Chung et al., 2008; Archer et al., 2018b; Costa et al., 2021), the maintenance of HSP72 expression in PO-treated animals suggests that this protein contributed to improving glucose metabolism and inflammatory and adiposity markers.

The effects caused by PO could be primarily associated with its oleic acid and carotenoid contents. Oleic acid was shown to protect hepatocytes from lipotoxicity caused by saturated fatty acids (Chen et al., 2018), leading to lower fat accumulation. In adipose tissue, these fatty acids are associated with lower TAG deposition, reducing the inflammatory status (Cruz et al., 2020). We believe the overload of oleic acid from PO could have caused these effects. Carotenoids are antioxidant molecules and may have significantly contributed to the better redox response observed in hepatocytes (Serpeloni et al., 2012). These compounds can interact with nuclear factors, such as the *nuclear factor erythroid 2-related factor 2* (Nrf-2), inducing its translocation to the nucleus and increasing the antioxidant enzymes expression (Linnewiel et al., 2009; Bohn, 2019). Therefore, it is plausible to speculate that these effects ameliorate systemic glucose disturbances in WDP animals.

It is important to note that we chose to study anti-diabetic and anti-obesity PO effects in male rats because, in Brazil, data from extensive population studies pointed out that from 2019 to 2023, there was a

higher increase in obesity rates for men (4,5%) compared to women (3,2%) (Brasil, 2023). However, our results cannot be implied for females because there are several sex-specific differences in adipose tissue metabolism and immune response between males and females. For example, non-oxidative free fatty acids (FFA) clearance is lower in males, catecholamine-induced rate of FFA mobilization from visceral fat to the portal venous system is higher in males; they have the lowest rate of triglycerides synthesis, show higher leptin and adiponectin concentrations (Braga Tibaes et al., 2022; Karastergiou et al., 2017) among others. Studying relationships between food/diet and obesity in female models is a future perspective for our group.

## 5. Conclusion

Taken together, our data point out a potential use of PO in managing obesity health consequences in male biological sex. PO intake in a Western diet (high-fat/high-sugar) reduced epididymal fat accumulation, contributing to reduced adipose tissue inflammation. These events may have promoted improvements in the enzymatic and non-enzymatic antioxidant capacity and fat accumulation in the liver. At last, all these effects contributed, at least in part, to improving glucose tolerance and insulin resistance in the PO animals.

## Funding source

This work was supported by *Fundação de Amparo à Pesquisa do Estado de Minas Gerais* - FAPEMIG, Brazil (#: APQ-01119-12 and APQ-02529-17).

## CRediT authorship contribution statement

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## 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.

## Data availability

Data will be made available on request.

## Acknowledgements

The authors would like to acknowledge Centro Integrado de Pós-Graduação e Pesquisas em Saúde from Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, MG, for granting the necessary structure and equipment.

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