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A comparative study between olive oil and corn oil on oxidative metabolism

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Fats are an important part of diet, but not all lipids have the same structure and chemical properties. Unsaturated fatty acids have one or more double bonds in their structure and can be monounsaturated or polyunsaturated, respectively. Most vegetable oils, such as olive oil and corn oil, contain significant amounts of these fatty acids. The presence of double bonds in the molecule of a fatty acid constitutes vulnerable sites for oxidation reactions generating lipid peroxides, potentially toxic compounds that can cause cellular damage. In response to this oxidative damage, aerobic organisms have intracellular enzymatic antioxidant defense mechanisms. The aim of the present investigation was to study comparatively the effects of control liquid diets, of a defined composition, containing olive oil or corn oil as a lipid source respectively of monounsaturated and polyunsaturated fatty acids, on the oxidative metabolism of rats. Rats were divided into three groups which received a control animal feed diet (A.F.), olive oil liquid diet (O.O) and corn oil liquid diet (C.O) for 30 days. It was observed that the activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), increased in the liver and white fat tissue of rats fed with olive oil when compared to the corn oil group. However, in brown fat tissue and blood cells, the enzyme activities showed a tendency to decrease in the olive oil group. In addition, the effect of olive oil and corn oil on several glucose metabolism parameters (pyruvate, lactate, LDH, acetoacetate and beta-hydroxybutyrate) showed that corn oil impairs to a greater extent the cellular metabolism. All these results helped in concluding that some body tissues are more adversely affected than others by the administration of corn oil or olive oil, and their antioxidant defenses and cellular metabolism respond differently too.

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1. Introduction

Dietary fat is considered an important macronutrient, essential for proper growth and body development.[1–8] Triglycerides (triacylglycerols) are esters of glycerol with fatty acids and constitute the main lipid component of the diet.[9,10] Their excessive consumption has been associated with the development of obesity.[11–13] With reference to fatty acids, they can have double bonds in their chain, which makes them saturated, monounsaturated or polyunsaturated. The presence of unsaturation in the structure of fatty acids makes them vulnerable to oxidation reactions,[14,15] since oxygen is necessary for aerobic life and is involved in the majority of the reactions responsible for ATP production (oxidative metabolism).[16,17] This oxidative metabolism contributes to the production of reactive oxygen species (ROS) responsible for oxidative stress and the development of diseases.[18–23] In order to prevent oxidative damage, aerobic organisms have several intracellular enzymatic defense mechanisms that involve superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx).[24,25] In the absence of oxidative stress, the generated ROS are held to very low levels due to the coordinated action of these enzymes. If there is a reduction in the antioxidant capacity or a unilateral deficiency of one of these enzymes, it will increase the cell vulnerability against ROS.[24,25]

The dietary intake of seed oil, which contains polyunsaturated fatty acids, has increased in recent years.[26–32] Polyunsaturated fatty acids are very sensitive to oxidation and therefore, they require in their composition the presence of natural antioxidants to prevent it.[33–35] Olive oil is one of the most important components of the Mediterranean diet. It contains monounsaturated fatty acids with high levels of oleic

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acid (56–84%) and a content of linoleic acid of 3–21%.[36] It is the most consumed oil due to its composition of polyphenols, hydroxytyrosol, squalene and phytosterols, which have positive effects on oxygenic metabolism, lipid metabolism, defense against free radicals, decreased cardiovascular risk and inflammation.[37–41] Olive oil is characterised by its good stability at high cooking temperatures and high resistance to oxidative deterioration, which is related to the fatty acid composition and the phenol content, two factors necessary to prevent fat oxidation.[42] On the other hand, corn oil is composed of 99% triacylglycerols with polyunsaturated fatty acids (59%), monounsaturated fatty acids (24%) and saturated fatty acids (13%).[43] This type of oil contains vitamin E (tocopherol) which presents an antioxidant effect, but scientific evidence suggested greater oxidative damage by the effect of polyunsaturated fatty acid rich diet.[44]

The aim of the present study is to analyze the effects of liquid diets containing olive oil or corn oil as a source of monounsaturated or polyunsaturated fatty acids, respectively, on the oxidative metabolism of Wistar rats.

2. Materials and methods

2.1. Materials

The reagents used in the present study were analytical grade and were purchased from Panreac (Spain), Boehringer Ingelheim (Germany) and Merck (Germany).

2.2. Animals

Male Wistar rats (150–180 g) (Charles River, Barcelona, Spain), one or two animals housed per cage (Panlab, Barcelona, Spain) under a 12 h light/dark cycle at 22 °C and 60% humidity and maintained on a standard chow diet and water *ad libitum*, were used. This study was carried out in accordance with the guidelines established in the Spanish legislation (Royal Decree RD 53/2013). The protocol was approved by the Experimental Animal Ethics Committee of the Universidad Católica de Valencia "San Vicente Mártir" (Spain). Rats were divided into three groups which received a control animal feed diet (A.F.), olive oil liquid diet (O.O) and corn oil liquid diet (C.O) for 30 days:

Group 1: Control animal feed (A.F)

Group 2: Olive oil (O.O)

Group 3: Corn oil (C.O)

The dietary intake of the animals was recorded daily and the body weight was measured weekly. After 30 days, rats were anaesthetized with an overdose of isoflurane and sacrificed by exsanguination with cardiac puncture as indicated by the Federation of European Laboratory Animal Science Association. All the samples (liver, blood, white and brown fat) were taken for analytical determination.

2.3. Diets

The solid non-purified IPM R-20 diet commercialized by Letica (Barcelona, Spain) and commonly used in our university animal facility was taken as the reference for preparing the composition of our experimental liquid diets.

The reagents used in the liquid diet production were obtained from Sigma-Aldrich (St Louis, Missouri, USA) (DL-methionine, choline, olive oil, corn oil, dextrin, cellulose and xantham gum) and ICN-Biomedicals (Santa Ana, California, USA) (casein, vitamins and minerals). The liquid diets were produced following the directions of the American Institute of Nutrition.[45]

The model of the olive oil liquid diet was obtained with the addition of olive oil as the lipid component (8.5 g/1000 mL) in the liquid diet. The corn oil liquid diet model was formulated with the same dietary characteristics as the previous one but with corn oil as the lipid component (8.5 g/1000 mL). Table 1 shows the final conditions of the dietary liquid treatment which the rats received during the present study. The two models of liquid diets administered have the composition of macronutrients and energy as shown in Table 2.

All the diets given to the rats were stable not only during the administration to the animals, but also during the storage period. Since the diets did not receive any conservative agent, they were kept at 4 °C in a closed environment protected from light and for a maximum period of 4 days.

The composition of fatty acids from the olive oil and corn oil supply of the diets was determined by gas chromatography at the "Instituto de la grasa", Sevilla, Spain. In the olive oil diet

Table 1 Composition of liquid diets

Composition	Olive oil diet (O.O.)	Corn oil diet (C.O.)
Casein	52.0 g	52.0 g
DL-Methionine	0.8 g	0.8 g
Choline, hydrochloride	0.3 g	0.3 g
Olive oil	**8.5 g**	
Corn oil		**8.5 g**
Dextrin	162.7 g	162.7 g
Cellulose	10.0 g	10.0 g
Xantham gum	2.0 g	2.0 g
Vitamins AIN-76A	2.6 g	2.6 g
Minerals AIN-76	9.1 g	9.1 g
Water	1000 mL	1000 mL

O.O.: olive oil diet; C.O.: corn oil diet.

Table 2 Final composition of the liquid diets

Composition	O.O. diet	C.O. diet
Proteins	52.8 g	52.8 g
Fats	**8.5 g**	**8.5 g**
Carbohydrates	166.3 g	166.3 g
Energy	**960 kcal**	**960 kcal**
Intake	Isoenergetically limited compared to animals fed with the laboratory diet	Isoenergetically limited compared to animals fed with the laboratory diet
Treatment period	30 days	30 days

O.O.: olive oil diet; C.O.: corn oil diet.

model, the content of oleic acid was 81.3% and the content of antioxidants was namely alfa-tocoferol 20.9 ppm, gamma-tocoferol 2 ppm and delta-tocoferol 4 ppm. While in the corn oil diet model, the content of oleic acid was 25.2% and the content of antioxidants was namely alfa-tocoferol 71.3 ppm, gamma-tocoferol 10 ppm and delta-tocoferol 240 ppm.

The ingested quantity was obtained taking into account the administered quantity and the consumed amount of diet.

2.4. Sample processing

Immediately after the sacrifice of the rats, 2 mL of blood samples were collected and 0.2 mL of heparine at 5% (Rovi, Madrid, Spain) were added and the liver was extracted and white adipose tissue from the abdominal area and all the brown fat from the dorsal part of the thorax were extracted as well.

Each aliquot of the liver sample was homogenized in a Tris-HCl buffer (10 mM) with 0.25 M sucrose, 1 mM EDTA, pH 7.4, 1 : 4 (w : v). The homogenized mixture was filtered through a thin gauze and then centrifuged at 1900*g* for 20 min. After centrifugation, a 1 mL sample of supernatant fluid was kept for the determination of proteins of total liver, and the remaining supernatant was once more centrifuged at 18 000*g* for 60 min in order to obtain the cytosolic fraction for the determination of antioxidant enzymes.

The brown and white fat were homogenized and centrifuged in the same way as the liver sample. The supernatant was used also for the determination of antioxidant enzymes.

The blood samples (2 mL) were centrifuged at 1100*g*. The pellet was resuspended in the same amount of bidistilled water as the discarded supernatant and after vortexing the solution, it was left for 2 h at 4 °C to achieve complete hemolysis. 0.4 mL of sample aliquots were kept for the determination of glutathione peroxidase (GPx) and hemoglobin. In order to obtain the precipitation of hemoglobin, chloroform : ethanol (3 : 5, v/v) and 0.3 mL of bidistilled water were added. The samples were gently shaken and then centrifuged at 1100*g* for 10 min, and the supernatant fluid was kept in an Eppendorf tube to measure the activity of superoxide dismutase (SOD) and catalase (CAT).

2.5. Antioxidant enzymatic systems and hemoglobin quantification

Superoxide dismutase activity was determined at 450 nm by the inhibition of the xanthine/xanthine oxidase-mediated oxidation of cytochrome c using an assay kit manufactured by Biovision (reference K335-100). The assay kit uses WST-1 that produces a water-soluble formazan dye upon reduction with superoxide anions. The rate of reduction with a superoxide anion is linearly related to the xanthine oxidase activity and is inhibited by SOD. Therefore, the inhibition activity of SOD is assessed by a colorimetric method. Catalase activity was determined spectrophotometrically at 240 nm by the reduction of H_2O_2 at 25 °C. CAT catalyzes the breakdown of hydrogen peroxide into oxygen and water. The reduction of hydrogen peroxide per unit of time is proportional to the amount of catalase in the sample. Glutathione peroxidase activity was measured at 340 nm from the NADPH decrease at 37 °C.[46] The quantification is based on the coupled reaction in which glutathione peroxidase catalyzes the oxidation reaction of glutathione (GSH) to glutathione disulfide (GSSG). Then, the enzyme glutathione reductase catalyzes the reduction of the disulfide glutathione (GSSG) in the presence of NADPH by forming glutathione (GSH).

Total hemoglobin was measured using a colorimetric commercial kit supplied by Boehringer Mannheim (reference 124729). The spectrophotometric determination was done at 546 nm. This determination consists of the reaction of hemoglobin with a reagent that contains cyanide and ferrocyanide, which oxidizes hemoglobin to methaemoglobin which is converted into cyanomethaemoglobin. The color intensity of this compound is measured photocolorimetrically.

2.6. Hepatocytes

2.6.1. Isolation of rat hepatocytes. Hepatocytes were obtained from male Wistar rats (2–3 months old) fed with the control animal feed, olive oil liquid diet or corn oil liquid diet. The isolation procedure was performed by following the perfusion method described by Seglen.[47]

Cells were centrifuged at 200*g* for 3 min, the supernatant was discarded and the pellet was resuspended again with Krebs buffer supplemented with calcium at 37 °C. The samples were centrifuged once more at the same speed. The supernatant was discarded and the pellet was weighed and resuspended at the proportion of 10 mL per g of cells.

The metabolic studies used 2 mL of cell suspension and 2 mL of Krebs–Henseleit buffer was added to the samples. The hepatic suspension was distributed into 25 mL Erlenmeyer flasks.

At t = 0, 0.4 ml of 20% perchloric acid was added to the samples to stop cellular metabolic processes. Samples at t = 60 min were immediately gassed with an O_2/CO_2 mixture (95/5, v/v) for 30 seconds. Then, the flask was hermetically sealed and placed in a thermostatic bath (37 °C) with constant agitation for 60 minutes. After incubation, the reaction was stopped with perchloric acid at 20%. All samples were centrifuged at 1100*g* for 10 min, and the supernatant fluid was neutralized with KOH (20%).

A special treatment of the samples was required for the determination of LDH. After incubation and before cellular destruction with PCA at 20%, the samples were centrifuged at a low speed (200*g*) for 5 min to avoid the breaking of the cell membranes in order to get the cytosolic lactate dehydrogenase (LDH) release. The supernatant fluid was kept for analysis.

2.6.2. Analysis of metabolites. Pyruvate and L-lactate were determined according to Valero and García-Carmona.[48] Lactate dehydrogenase was measured using the commercial kit from Spinreact (reference: 1001200.01). Acetoacetate and β-hydroxybutyrate were determined spectrophotometrically using the enzymatic procedure described by Williamson and Mellanby.[49]

2.7. Statistical analysis

Data are shown as the mean ± SEM of n experiments obtained from different animals. Statistical analysis was performed with SPSS software (19 version). Statistically significant differences in mean values were tested by the analysis of variance. Then, the Scheffé test was carried out. The groups studied fitted into the normality that was analyzed by the Kolmogorov–Smirnov test and there was homogeneity of variances that was analyzed by the Levene test. Statistical differences were set at $p < 0.05$.

3. Results

3.1. Body and tissue development

After the animals were sacrificed, the variation in the body weight, white fat, brown fat and liver was evaluated (Table 3).

The increase in white fat in the animals of the corn oil group (C.O.) with respect to the olive oil group (O.O.) was 12.3%. The results obtained show that the animals of the corn oil group experienced a higher liver development, according to their higher body weight, with respect to the animals of the olive oil group, with a variation in the liver weight of 14.7%.

3.2. Oxidative stress

The activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) was evaluated in the following: liver tissue, white adipose tissue, brown fat tissue and blood cells, in the groups of rats fed with olive oil and corn oil, with respect to the animal feed group.

3.2.1. Liver tissue. Fig. 1 shows the activity of SOD, CAT and GPx in liver tissue for the different treatment groups.

The results obtained show a significant increase of the enzymatic SOD activity in the group treated with olive oil with respect to the animal feed. In addition, a significant decrease of 47% in the activity of this enzyme was observed in the group treated with corn oil with respect to the group treated with olive oil.

The catalase activity observed in the liver tissue decreased in the groups treated with olive oil and with corn oil, compared to the control diet. It is important to highlight the 23% decrease in the enzymatic activity in the corn oil group with respect to the animal feed group.

The values corresponding to the group whose lipid source was corn oil showed a significantly lower GPx enzymatic activity with respect to the groups fed with olive oil and a solid laboratory diet.

3.2.2. White fat. Fig. 2 shows the activity of SOD, CAT and GPx in white adipose tissue for the different treatment groups.

The activity of the SOD enzyme in the groups treated with olive oil and corn oil shows significantly higher values when compared to the group treated only with the solid laboratory diet.

The catalase activity observed in the white adipose tissue shows a significant decrease in the group treated with corn oil, in relation to the untreated group.

The enzymatic activity of GPx was significantly decreased in the corn oil-treated group compared to both animal feed and olive oil. Furthermore, in the case of the olive oil-treated

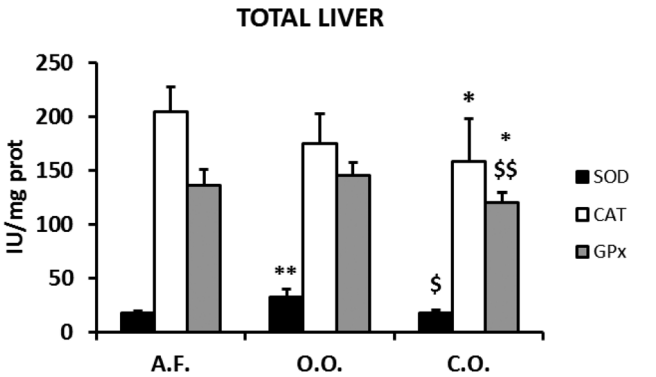

Fig. 1 SOD, CAT and GPx activity in the liver. A.F.: animal feed (n = 6); O.O.: olive oil diet (n = 8); C.O.: corn oil diet (n = 9). Values are expressed as mean ± SEM. The statistical difference is indicated as follows: *$p < 0.05$, **$p < 0.005$ A.F. *vs.* O.O. and A.F. *vs.* C.O.; $^{\$}p < 0.05$, $^{\$}$ $^{\$}p < 0.005$ O.O. *vs.* C.O.

Table 3 Body and tissue development

Body development	A.F. (n = 6)	O.O. (n = 8)	C.O. (n = 9)	%
Initial body weight (g)	180.00 ± 0.00	181.00 ± 2.90	184.00 ± 17.00	0.00
Final body weight (g)	259.99 ± 3.34	241.00 ± 4.70	255.00 ± 34.00	+5.80
White fat (g)	1.38 ± 0.30	1.87 ± 0.43	2.10 ± 0.76	+12.30
Brown fat (g)	0.37 ± 0.06	0.38 ± 0.05	0.35 ± 0.05	−7.90
Liver (g)	9.20 ± 1.44	8.37 ± 0.59	9.60 ± 0.74	+14.70

O.O.: olive oil diet; C.O.: corn oil diet; %: C.O. *vs.* O.O. Values are expressed as mean ± SEM.

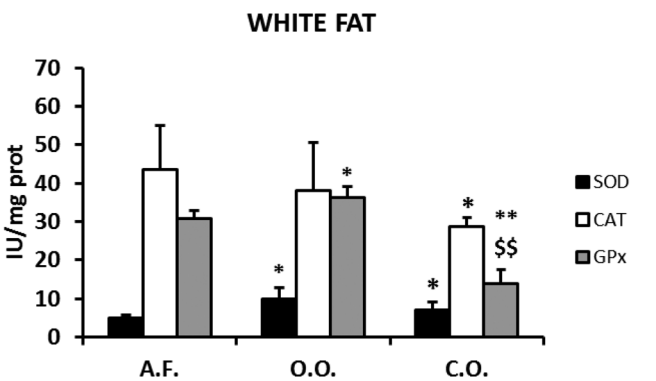

Fig. 2 SOD, CAT and GPx activity in white fat tissue. A.F.: animal feed (n = 6); O.O.: olive oil diet (n = 8); C.O.: corn oil diet (n = 9). Values are expressed as mean ± SEM. The statistical difference is indicated as follows: *$p < 0.05$, **$p < 0.005$ A.F. *vs.* O.O. and A.F. *vs.* C.O.; $^{\$}p < 0.05$, $^{\$}$ $^{\$}p < 0.005$ O.O. *vs.* C.O.

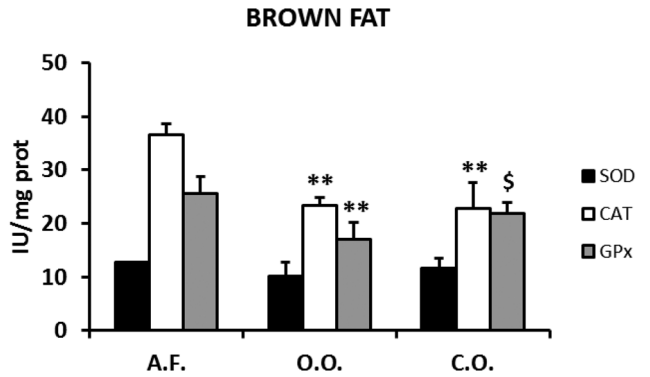

Fig. 3 SOD, CAT and GPx activity in brown fat tissue. A.F.: animal feed (n = 6); O.O.: olive oil diet (n = 8); C.O.: corn oil diet (n = 9). Values are expressed as mean ± SEM. The statistical difference is indicated as follows: *p < 0.05, **p < 0.005 A.F. *vs.* O.O. and A.F. *vs.* C.O.; $^{\$}p$ < 0.05, $^{\$}$ $^{\$}p$ < 0.005 O.O. *vs.* C.O.

group, a significant increase in GPx enzyme activity was observed compared to the other two groups.

3.2.3. Brown fat. Fig. 3 shows the activity of SOD, CAT and GPx in brown fat tissue for the different treatment groups.

The results obtained in the study of SOD activity in brown fat tissue showed a decrease in this enzymatic activity in the group treated with olive oil, compared to the groups treated with corn oil and animal feed.

The CAT enzyme activity was significantly lower in the groups fed with olive oil and corn oil than in the untreated group.

The administration of olive oil produced a significant decrease in GPx. When comparing the groups treated with olive oil and corn oil, a significant increase in GPx activity was observed in the group treated with corn oil with respect to olive oil.

3.2.4. Blood cells. Fig. 4 shows the activity of SOD, CAT and GPx in blood cells for the different treatment groups.

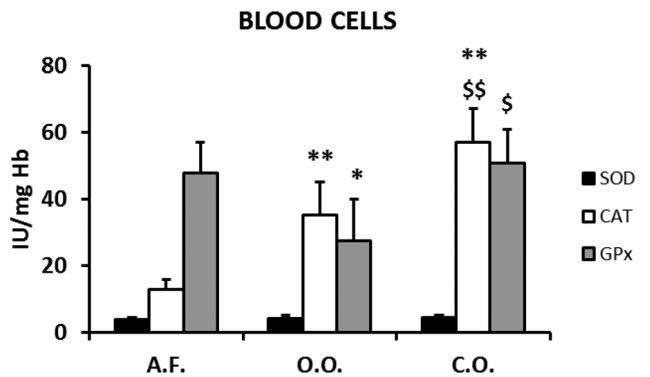

Fig. 4 SOD, CAT and GPx activity in blood cells. A.F.: animal feed (n = 6); O.O.: olive oil diet (n = 8); C.O.: corn oil diet (n = 9). Values are expressed as mean ± SEM. The statistical difference is indicated as follows: *p < 0.05, **p < 0.005 A.F. *vs.* O.O. and A.F. *vs.* C.O.; $^{\$}p$ < 0.05, $^{\$}$ $^{\$}p$ < 0.005 O.O. *vs.* C.O.

When evaluating SOD enzyme activity in rat blood cells, no significant differences were observed in the groups treated with olive oil and corn oil.

In contrast, the CAT enzyme activity was significantly higher in the groups treated with olive oil and corn oil, with respect to the group treated with animal feed. Moreover, it was observed that the increase was greater in the group of rats treated with corn oil than in the group of rats treated with olive oil.

The GPx enzyme activity in blood cells was different for the olive oil and corn oil treated groups. Thus, in the olive oil treated group, a significant decrease was observed with respect to the animal feed, whereas in the corn oil treated group, a significant increase was observed with respect to the group of rats fed with olive oil.

3.3. Metabolites

The next step in this study was to determine the metabolic interactions in hepatocytes isolated from rats subjected to a diet whose lipid component was olive oil or corn oil. The metabolites studied were LDH, β-OH-butyrate, acetoacetate, lactate, and pyruvate.

LDH concentrations in the two treatment groups, olive oil and corn oil, before and after the incubation period, were higher than those in the untreated group (Fig. 5A). When relating the LDH concentration between the groups treated with olive oil and corn oil, a significant increase was observed in the group treated with corn oil after 60 minutes of incubation (Fig. 5A). This result indicates that the protective effect induced by olive oil is significantly higher than that of corn oil.

In the groups of rats treated with olive oil and corn oil, at the initial time of incubation (t = 0) and after 60 minutes, the values of β-OH-butyrate were higher than those obtained in the hepatocytes of the untreated group (animal feed) (Fig. 5B). This increase was significantly higher in the group of rats fed with corn oil.

The levels of acetoacetate in the hepatocytes of the olive oil and corn oil treated groups were higher than those in the hepatocytes of the untreated group (Fig. 5C). In addition, it is important to note that hepatocytes from rats fed with corn oil had significantly higher levels of acetoacetate when compared to those from rats fed with olive oil (Fig. 5C). This difference was observed in both freshly isolated hepatocytes (t = 0) and those incubated at 60 minutes.

Contrary to what has been observed in the previous metabolites, the lactate concentrations found in the isolated hepatocytes of the rats treated with olive oil and corn oil were lower than those observed in the hepatocytes of the untreated rats, although this decrease was not significant before incubation (t = 0) in the group of rats treated with olive oil (Fig. 5D). On the other hand, when comparing the lactate concentration between the two treated groups, a significant decrease was observed in the group treated with corn oil, both at time 0 and at 60 minutes of incubation (Fig. 5D).

Pyruvate levels were lower in the groups treated with olive oil and corn oil when compared to the untreated group

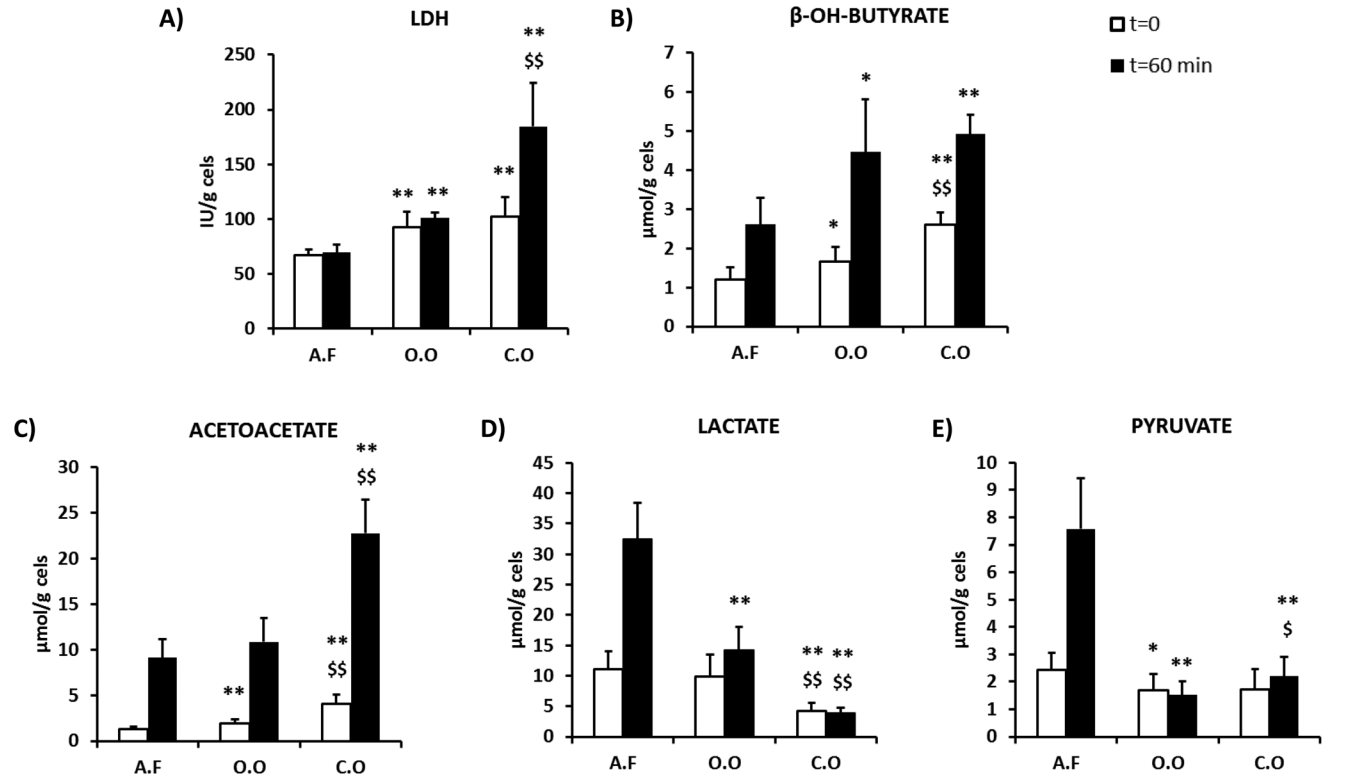

Fig. 5 Effects of metabolic interactions on LDH release levels (A), β-OH-butyrate (B), acetoacetate (C), lactate (D) and pyruvate (E) in rat isolated hepatocytes. A.F.: animal feed; O.O.: olive oil diet; C.O.: corn oil diet. Values are expressed as mean ± SEM. The number of rats per group was 5–8. The statistical difference is indicated as follows: *$p < 0.05$, **$p < 0.005$ A.F. *vs.* O.O. and A.F. *vs.* C.O.; $^{\$}p < 0.05$, $^{\$\$}p < 0.005$ O.O. *vs.* C.O.

(Fig. 5E). Under basal conditions, no differences were observed in the pyruvate levels released by isolated hepatocytes from rats treated with corn oil. In contrast, at 60 minutes of incubation, a significant increase of this metabolite was observed in the group of rats treated with corn oil when compared to the group treated with olive oil (Fig. 5E). Importantly, these values were three times higher at 60 minutes of incubation in the untreated group.

4. Discussion

Studies that relate changes in biochemical parameters with nutritional alterations require a strict control of all the factors involved in the feeding of experimental animals. In our study, we focused on an experimental diet model, in which the experimental animals received an isoenergetically limited liquid diet with respect to the group of animals fed with a solid laboratory diet, varying only the type of lipid and keeping the same proportions of minerals with the same nutritional quality. In this way, the possible modifications of the oxygenic metabolism that we studied would be exclusively due to the modification of the type of lipid and the nature of the fatty acids provided to the experimental animals, without the introduction of other nutritional variables, such as differences in the proportion of micronutrients, quality of proteins, and variation in the quantity and nature of carbohydrates, all factors that could directly or indirectly affect the oxygenic metabolism.

It has been well demonstrated that there is a relationship between the quantity and quality of dietary fat and certain chronic pathologies such as cancer and cardiovascular diseases.[50–53] Dietary habits have always been a sociocultural reference and the scientific evidence generated in recent years relating diet and health status has been decisive to consider eating habits as a social and health indicator.[54,55]

4.1. Body and tissue development

One of the objectives of our study was to determine the relationship between the type of oil administered in the diet and the development of white adipose, brown adipose and liver tissues. Our results showed that animals treated with corn oil experienced an increase in weight that was accompanied by a considerable increase in white adipose tissue. Several studies indicated that not only the amount of dietary fat, but also the type of fat ingested are the cause of the production of different effects on body weight and metabolism.[56] On the other hand, the development of the liver followed the same trend as observed in the evolution of the total weight of the animals.

4.2. Oxidative stress

Dietary lipids are used as energy substrates (on entering the mitochondria they are used for ATP generation) and for their

incorporation into cell membranes, which depends on the amount and type of fatty acid ingested.[57] However, these lipids can be damaged by a situation of oxidative stress. The main targets for lipid oxidation are polyunsaturated fatty acids (PUFAs) and cholesterol.[58,59] As a protective response to this oxidative damage, living organisms have developed an antioxidant enzyme system consisting of three enzymes SOD, CAT and GPx.[60] Therefore, we studied if these systems are able to act effectively against the possible generation of reactive oxygen species (ROS) formed in our experimental model. If the antioxidant defense is lower than the production of reactive species, ROS could react with each other or with other molecules enhancing the production of other radicals responsible for damage at the cell membrane, in addition to other molecular alterations. Lipid oxidation is commonly observed in many diseases, especially those involving inflammatory processes.[61]

Our results seem to indicate that when the type of lipid in the diet is varied, in this case by a polyunsaturated fatty acid such as corn oil, there is damage caused by oxygenic species, when compared to olive oil (monounsaturated), since a significant decrease in SOD activity is observed in the following compartments studied, the liver (Fig. 1) and white fat tissue (Fig. 2), while in brown fat tissue (Fig. 3) and blood cells (Fig. 4), the SOD activity shows a slight tendency to induce this enzyme.

Regarding CAT, its function is doubly important since, on the one hand, it removes from the cellular medium the product of the enzymatic action of SOD, thus decreasing the concentration of an oxidizing agent such as hydrogen peroxide, and also prevents the interaction of this with other reactive species such as transition metals and the subsequent formation of hydroxyl radicals through the Haber–Weiss and Fenton reaction.[62] This oxygenic species are excellent factors for the induction of oxidative modification of DNA and as a consequence for its mutagenic potential.[63] Catalase is distributed across a large and diverse family of species, while in others it is absent or with very little activity. Changes in the enzyme's activities have been found to be associated with the development of various organisms. In mammals, these changes appear to be not as widespread and more dependent on the type of tissue under study compared to what is observed with SOD.[64]

In our experimental system, we observed that in the liver and white fat tissue, the catalase activity is lower in the groups treated with corn oil when compared to the group treated with olive oil, showing no differences in brown fat tissue in both groups studied. In contrast, catalase suffered an increase in blood cells in both treated groups, although this increase was more significant in the group treated with corn oil.

GPx plays a very important role as a mechanism for the removal of oxidation products from the cell and is responsible for the degradation of lipid peroxides and H_2O_2. As in the case of catalase, GPx is widely distributed, although in some types of cells or organelles it is found in very low concentrations.[65] The results obtained indicate that the activity of this enzyme in the liver and white fat tissue of rats fed with corn oil is lower than that of rats fed with olive oil. A similar situation was observed with the antioxidant enzymes SOD and CAT, as previously mentioned. However, the results at the brown fat level reveal a significant increase in GPx activity in animals fed with corn oil.

Therefore, all these findings demonstrate a clear relationship in terms of oxidative damage between the 3 enzymes studied. In addition, it seems that there is a pattern of behavior in the 4 tissues studied that shows that antioxidant defenses vary from one tissue to another. The liver and white fat tissue have a similar way of responding to oxidative stress, whereas brown fat tissue and blood cells respond differently, which could be due to the physiological function that these tissues perform in the body. Brown fat has a thermogenic function and has a higher number of mitochondria,[66] and therefore, a higher oxygen metabolism. As for the blood cells, they carry out the oxygen transporting function. These two functions of brown fat and blood cells would make these two tissues better prepared for oxidative stress. Moreover, as indicated above, PUFA are more sensitive to oxidation[58,59] and that is why, in its natural composition, corn oil has a higher amount of antioxidant compounds (see the Materials and methods section). Our results seem to show that this antioxidant defense does not seem to be completely effective against the oxidative stress generated in living beings, since the activity of SOD, CAT and GPX usually decreases in the groups treated with corn oil.

4.3. Metabolites

Metabolic pathways are remarkably responsive to changing biological conditions, as metabolic adaptations to exercise, diet, and environmental exposure are well established.[67] For example, during aging, glucose utilization decreases in the specific regions of the brain,[68] and is most severe in brain regions where disease initiates through a number of neurodegenerative disorders, including Parkinson's[69] and Alzheimer's diseases.[70] Therefore, to complete our study and to know the effect of oxidative stress on cellular metabolism, we characterized the release of lactate dehydrogenase (LDH) and the production of beta-hydroxybutyrate, acetoacetate, lactate and pyruvate.

Glucose metabolism is integrated with redox signaling, and dysfunction of these pathways has been linked to disease pathogenesis. Increased cellular peroxides are formed as a consequence of decreased glucose metabolism.[67] Thus, variations in the cellular levels of pyruvate and lactate are an index of the cytosolic redox state.[71,72] In our study, pyruvate and lactate levels decreased after the administration of olive oil and corn oil (Fig. 5D and E) and, in the last case, the decrease is more significant. This shows that corn oil generates a more oxidative environment that impairs glucose metabolism and thus energy production in the cell. Energy generation and conservation are essential for cell survival and normal function, which is essential for normal physiological processes such as cell growth and differentiation. Glucose metabolism dysfunction

contributes to a variety of pathologies, including cancer, cardiovascular diseases, diabetes, stroke and neurodegeneration.[73–78] Interestingly, it is now becoming clear that the surveillance of these pathways is at the interface between pathology and physiology. Under these conditions, acetoacetate and beta-hydroxybutyrate serve as fuels for obtaining energy by the tissues. Thus, as observed in our work, the levels of these ketone bodies are increased in a higher proportion when corn oil is administered compared to olive oil and even more compared to animal feed (Fig. 5B and C). However, this response could be beneficial since a possible protective effect of these ketone bodies against the cell damage caused by this glucose metabolism dysfunction has been recently demonstrated.[79]

Regarding cell membranes, LDH is rapidly released when the plasma membrane is damaged, a key feature of cells undergoing apoptosis, necrosis, and other forms of cellular damage.[80] This would mean that, in the hepatocytes treated with corn oil, the release of LDH is greater (Fig. 5A) than those treated with olive oil, and even more, compared to the animal feed. This could be explained (as has been discussed throughout this work) by the greater oxidative stress generated by corn oil due to the presence of a greater number of unsaturation bonds. Therefore, it could be said that the chemical composition of corn oil would generate a more oxidative metabolic situation compared to olive oil.

5. Conclusions

It is well known that double bonds in the molecule of a fatty acid constitute vulnerable sites for oxidation reactions generating lipid peroxides that can cause cellular damage. Our results showed that the presence of these double bonds and therefore, the different oxidative capacities of two oils such as olive oil and corn oil influence their different response patterns in the body. Thus, some tissues are more adversely affected than others by the administration of corn oil or olive oil, and their antioxidant defenses respond differently. Similarly, cellular metabolism is greatly impaired when taking corn oil with respect to olive oil. Nevertheless, further investigations are required to shed light on the specific action mechanisms that are behind the different physiological behaviors of these two oils.

Author contributions

E. D. and M. L. M.: conceptualization; E. D., M. A. N. and S. C.: investigation; M. L. M.: methodology; E. D., M. A. N. and S. C.: formal analysis; M. L. M.: supervision; M. A. N. and E. D.: writing the original draft; M. L. M.: writing–review and editing.

Conflicts of interest

There are no conflicts to declare.

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