The supplementations with 2-hydroxyoleic acid and n-3 polyunsaturated fatty acids revert oxidative stress in various organs of diet-induced obese mice

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ABSTRACT

Obesity and its related diseases have been associated with oxidative stress. Thus, the search for nutritional strategies to ameliorate oxidative stress in obese individuals seems important. We hypothesized that the supplementation with monounsaturated (2-hydroxyoleic acid (2-OHOA)) and with combined n-3 polyunsaturated (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) fatty acids would ameliorate oxidative stress in different organs, including brain, liver, lungs, and kidneys of adult diet-induced obese (DIO) mice. Adult female ICR-CD1 mice were fed a high-fat diet (HFD) for 14 weeks. During the last 6 weeks of HFD feeding, one group of DIO mice received the same HFD, supplemented with 1500 mg of 2-OHOA per kg of HFD and another group with 1500 mg of EPA and 1500 mg of DHA per kg of HFD. At the end of the experiment, several parameters of oxidative stress were assessed. The supplementation with 2-OHOA or with EPA and DHA in DIO mice was able to revert oxidative stress, enhancing the activities of catalase and glutathione reductase, as well as diminishing the activity of xanthine oxidase, the concentration of thiobarbituric acid reactive substances (TBARS) and the ratio between oxidized glutathione and reduced glutathione in several organs. These reached similar values to those of control mice, which were fed a standard diet. These data suggest that supplementation with 2-OHOA and with EPA and DHA could be an effective nutritional intervention to restore an appropriate redox state in DIO mice.

Keywords: Diet-induced obese mice ; 2-OHOA ; EPA and DHA ; oxidative stress ; organs

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Introduction

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The high intake of fat and calories has been associated with the development and maintenance of obesity in both humans and rodents [11,181]. Obesity has become a global health problem and represents an important contributor to the current burden of chronic diseases [2].

Increasing evidence suggests that oxidative stress plays a critical role in the pathogenesis of obesity and its related diseases, such as type-2 diabetes, cardiovascular and neurodegenerative diseases [34]. The possible causes of oxidative stress in obesity include hyperglycemia [45undefined5], hyperleptinemia [56], inadequate antioxidant defenses, increased free radical formation rates [67], elevated tissue lipid amounts [undefined], impaired mitochondria and endothelial function [810,9undefined89], chronic inflammation [10]. Oxidative stress, when maintained for a long period of time, can damage cellular structures and trigger an inflammatory response, closing a detrimental feedback loop [1011,11]. At low/moderate concentrations, ROS are essential to regulate biological and physiological processes, such as several signaling pathways, gene expression, and apoptosis [1212]. However, at high concentrations, ROS lead to oxidative damage. Recent studies have shown that a lower capacity of antioxidant enzymes, such as catalase (CAT), glutathione peroxidase (Gpx), and reductase activities, as well as a higher production of oxidants (xanthine oxidase (XO) activity, glutathione redox ratio, and lipid peroxidation) could be considered markers of oxidative stress [1013,1314,14]. Similar to obesity, the process of aging has also been associated with oxidative stress [1515]. In fact, according to the oxidative-inflammatory theory of aging, the generation of chronic oxidative stress and inflammatory stress is the basis of the age-related impairment of the functions of the organism 15. We have found that obesity results in premature features of aging in adult mice, such as oxidative stress and immunosenescence (i.e. age-related changes of the immune system). Therefore, obesity has been suggested as a possible model of premature and accelerated aging [161617,17]. In this sense, the search for strategies to ameliorate oxidative stress, and consequently to promote healthy aging in obese individuals seems important.

Previous studies confirmed that the high-fat diet (HFD) intake (60% of energy as fat) is a good model to produce obesity in mice [1322,222321–2320–2318–22,1717–22, 5–22]. These diet-induced obese (DIO) mice display increased adipose tissue, liver steatosis, insulin resistance, and oxidative stress in different tissues. Also, these animals exhibit a pro-inflammatory status [1620,201923,232322–24]. Interestingly, this obesity model depletes the synthesis and levels of n-3 PUFA in different tissues, with an enhancement in the n-6/n-3 PUFA ratio favoring a pro-inflammatory state [25,252624–26]. In this context, the diets rich in monounsaturated fatty acids (MUFA) from olive oil and in n-3 polyunsaturated fatty acids (PUFA), namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), from marine-based fish oil and fish, promote beneficial effects for obesity and related diseases [22626]. One of the possible mechanisms involved in these effects could be the incorporation of unsaturated fatty acids into the cell membranes, which occurs in all tissues of the body, following their consumption. In addition, these fatty acids are known to modulate cell functions, by modifying membrane fluidity, lipid peroxide formation, eicosanoid production, and gene regulation [2727,2828]. Emerging evidence indicates that the supplementations with 2-hydroxyoleic acid (2-OHOA), a synthetic derivative of MUFA oleic acid, and with EPA and DHA ameliorate the cardiometabolic risk and revert the altered redox state and functions in immune cells of DIO mice 1329. However, the specific role of these unsaturated fatty acids on the oxidative stress of non-immune organs remains unknown. Therefore, the aim of this study was to evaluate several oxidative stress parameters in the brain, liver, lungs, and kidneys of DIO mice supplemented with 2-OHOA or with the combination of n-3 fatty acids (EPA and DHA).

Materials and methods

Animals

Female ICR/CD1 mice, 8 weeks of age, were purchased from Harlan Interfauna Iberica (Barcelona, Spain). For the first five days of acclimatization to a new environment, all mice were fed a standard maintenance diet (Teklad Global 14%). The animals were housed in polyurethane cages (two animals per cage) and maintained at a constant temperature of 22 ± 2 °C, with adequate ventilation and relative humidity of 50–60% on a 12/12 h reversed light/dark cycle (lights on at 8:00 pm). The experiments procedures and handling of animals were performed with approval of the Committee for Animal Experimentation of the Complutense University of Madrid (ref. CEA-UCM 06/2012) and were conducted in accordance with the guidelines and protocols of the Royal Decree 53/2013 regarding the care and use of laboratory animals.

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Experimental groups

The animals, at 9 weeks of age, were separated into four groups (*n* = 8, per group). (1) The control mice group (C) were fed a standard maintenance diet until the end of the study. (2) The DIO mice group were fed an HFD (60% of calories from fat, reference TD. 06414, Harlan Interfauna Iberica) for 14 weeks. (3) The 2-OHOA-supplemented DIO (2-OHOA-DIO) mice group were fed a HFD for 14 weeks, but received during the last 6 weeks of HFD, the supplementation with 2-OHOA (1500 mg of 2-OHOA per kg of HFD, BTSA-Applied Biotechnologies S.L., Bedford, MA). (4) The EPA and DHA-supplemented DIO mice group (EPA and DHA-DIO) were fed a HFD for 14 weeks, this being supplemented during the last 6 weeks with the combination of EPA and DHA (1500 mg of EPA and 1500 mg DHA per kg of HFD, BTSA-Applied Biotechnologies S.L., Bedford, MA). In order to progressively increase the amount of fat in the diet of groups 2, 3, and 4, the animals were fed with an intermediate-fat diet (IFD, 22% of calories from fat, Teklad Global 2019, Harlan Interfauna Iberica) for 4 weeks prior to the ingestion of HFD. The 2-OHOA is a synthetic derivative of oleic acid, and it is also known as 2-hydroxy-D9-cis-octadecenoic acid. The n-3 PUFA were extracted from fish (anchovy). The supplements 2-OHOA as well as EPA and DHA were provided in powdered form and in oil form, respectively, and were mixed into the chow, which was of malleable consistency. After the chow was uniformly mixed, it was made into pellets. The 2-OHOA and the combined EPA and DHA supplementation doses were chosen according to a previous study, in which the same supplemented doses were associated with changes in the gut microbiota of DIO mice [2929]. Throughout the entire study, all mice were given *ad libitum* access to water and diet. The nutritional composition of all the diets used are shown in Table 1. Weight and food intake were measured every week from the start to the end of the study.

Table 1. Composition of the diets.

Components	Standard maintenance diet	Intermediate-fat diet	High-fat diet	High-fat diet + 2-OHOA supplementation	High-fat diet + EPA and DHA supplementation
Energy (kcal/g)	2.9	3.3	5.1	5.1	5.1
Protein (%)	14.3	19.0	23.5	23.5	23.5
Carbohydrate (%)	48.0	44.9	27.3	27.3	27.3
Fat (%)	4.0	9.0	34.3	34.4	34.6
Saturated (%)	0.6	1.2	12.5	12.5	12.5
Monounsaturated (%)	0.7	1.7	16.1	16.2	16.1
Polyunsaturated (%)	2.1	4.4	5.4	5.4	5.7
Protein (% of energy)	20.0	23.0	18.4	18.4	18.3
Carbohydrate (% of energy)	67.0	55.0	21.3	21.3	21.2
Fat (% of energy)	13.0	22.0	60.3	60.4	60.5
Fatty acid composition (%)					
16:0 palmitic	0.5	0.9	8.2	8.2	8.2
18:0 stearic	0.1	0.2	3.9	3.9	3.9
18:1n-9 oleic	0.7	1.7	14.7	14.8	14.7
18:2n-6 linoleic	2.0	3.9	4.7	4.7	4.7
18:3n-3 linolenic	0.1	0.4	0.5	0.5	0.8
n-6:n-3 ratio	20:1	10:1	9:1	9:1	6:1

Collection of organs

Animals were euthanized at 8:00 am by decapitation, and no anesthetic was used to avoid the effect of anesthesia on the results. Organs were extracted *post-mortem* immediately and washed carefully with phosphate buffer, pH 7.4. The brain, liver, lungs, and kidneys were frozen in liquid nitrogen and stored at −80 °C until performing the oxidative stress assays.

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Catalase activity assay

The activity of CAT was determined following the method previously described [3032]. The assays were performed using aliquots of the homogenized tissue samples (50 mg/ml, for kidneys and lungs, and 25 mg/ml, for liver) in phosphate buffer (66 mM, pH 8.4) and centrifuged at 3200×*g* for 20 min at 4 °C. The supernatant extract (30 µl) was mixed with 670 µl of hydrogen peroxide (H_2O_2) (14 mM of H_2O_2 in phosphate buffer) (Merck, Darmstadt, Germany). The enzymatic assay was followed using spectrophotometry for 80 s at 240 nm through the decomposition of H_2O_2 into $H_2O + O_2$. Protein content of the samples was assessed following the bicinchoninic acid (BCA) protein assay kit protocol (Sigma-Aldrich, Madrid, Spain). The results were expressed as units (U) of enzymatic activity per milligram of protein (U CAT/mg protein).

Glutathione peroxidase activity assay

The Gpx activity was determined according to the method previously described 32. The assays were performed with aliquots of the homogenate tissue samples (50 mg/ml, for brain, kidneys and lungs, and 25 mg/ml for liver) in phosphate buffer (50 mM, pH 7.4) and centrifuged at 3200×*g* for 20 min at 4 °C. The total activity was determined using cumene hydroperoxide (Sigma-Aldrich, Madrid, Spain), which carried out the oxidation of β-nicotinamide adenine dinucleotide phosphate, in its reduced form (β-NADPH, Sigma-Aldrich, Madrid, Spain), in the presence of glutathione reductase (GR) (Sigma-Aldrich, Madrid, Spain). The reaction was measured spectrophotometrically by the decrease of the absorbance at 340 nm. The protein contents were evaluated following the previously mentioned protocol. The results were expressed as milliunits of enzymatic activity per milligram of proteins (mU GPx/mg protein).

Glutathione reductase activity assay

The GR activity was measured by the technique previously described 32. The assays were performed with aliquots of the homogenate tissue samples (50 mg/ml, for kidneys and lungs, and 25 mg/ml for liver) in phosphate buffer 50 mM, pH 7.4 with 6.3 mM ethylenediaminetetraacetic acid (EDTA), and centrifuged at 3200×*g* for 20 min at 4 °C. The total activity was measured through the oxidation of NADPH spectrophotometrically at 340 nm. The protein contents of samples were evaluated following the previously described protocol. The results were expressed as milliunits of enzymatic activity per milligram of proteins (mU GR/mg protein).

Glutathione concentrations assay

Both reduced (GSH) and oxidized (GSSG) concentrations of glutathione were measured using a fluorometric method. This method is based on the reaction of a fluorescence probe, o-phthaldialdehyde (OPT; Sigma-Aldrich, Madrid, Spain), with GSH at pH 8 and with GSSG at pH 12, which generates a fluorescence derivative. The tissue samples were homogenized (50 mg/ml, for kidneys and lungs, and 25 mg/ml for liver) in sodium phosphate–EDTA buffer (0.1 M, pH 8) and proteins were precipitated by adding 5 µl of 60% perchloric acid ($HClO_4$) (60%, Sigma-Aldrich, Madrid, Spain). The homogenate tissue samples were centrifuged at 9500×*g* for 10 min at 4 °C and supernatants were maintained in ice for the measurement of GSH and GSSG concentrations. For GSH content determination, 10 µl of the supernatant, 190 µl of phosphate–EDTA buffer, and 20 µl of OPT solution (1 mg/ml in methanol) were added to a 96-well black plate (Nunc, Roskilde, Denmark), and incubated at room temperature for 15 min. Fluorescence was determined in a plate reader (Fluostar Optima, BMG Labtech, Barcelona, Spain) using excitation at 350 nm and emission detection at 420 nm. For the measurement of GSSG contents, 8 µl of N-ethylmaleimide (NEM, 0.04 M, Sigma-Aldrich, Madrid, Spain) were added to each well and incubated at room temperature for 30 min. Then, 182 µl of sodium hydroxide (NaOH) (0.1 N, Panreac Quimica SA, Barcelona, Spain) with 20 µl of OPT solution were added to a 96-well black plate. After incubation (room temperature, 15 min), fluorescence was measured as previously described for GSH determination. Protein concentration of the samples was measured following the BCA protein assay kit protocol (Sigma-Aldrich, Madrid, Spain). The results were analyzed with GSH and GSSG standard curves at different concentrations and expressed as nmol/mg protein. The GSSG/GSH ratios were then calculated for each sample.

Xanthine oxidase activity assay

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Xanthine oxidase activity was measured, by a fluorescence assay, in homogenates of tissues, using a commercial kit (Amplex Red Xanthine/Xanthine Oxidase Assay Kit, Molecular Probes, Paisley, UK). The H_2O_2 produced by XO reacts with the horseradish peroxidase (HRP) present in the reaction mixture and generates a fluorescent oxidation compound resorufin whose fluorescence is measured in a plate reader (Fluorestar Optima, BMG Labtech Biomedal, Seville, Spain). Tissue samples were homogenized (50 mg/ml, for kidneys and lungs, and 25 mg/ml for liver) in phosphate buffer (50 mM, pH 7.4) containing 1 mM EDTA. The homogenate was centrifuged (5000×*g*), and the supernatant (50 µl) was collected and incubated with 50 µl working solution of Amplex Red reagent (100 µM) containing HRP (0.4 U/ml) and xanthine (200 µM). After 30 min of incubation at 37 °C, measurement of fluorescence was performed in the microplater reader, using excitation at 530 nm and emission detection at 595 nm. XO supplied in the kit was used as the standard. Protein content of the samples was assessed using the BCA protein assay (Sigma-Aldrich, Madrid, Spain). The results were expressed as milliunits of enzymatic activity per milligram of protein (mU XO/mg protein).

Lipid peroxidation (thiobarbituric acid reactive substances (TBARS) assay)

Lipid peroxidation was evaluated using a colorimetric assay kit (BioVision, Mountain View, CA), which measures the reaction of MDA with thiobarbituric acid (TBA) and the MDA-TBA adduct formation. The tissue samples were homogenized (50 mg/ml, for brain, kidneys, and lungs, and 25 mg/ml for liver) in 300 µl of MDA lysis buffer with 3 µl butylhydroxytoluene (BHT) (×100) and then centrifuged (13,000×*g*, 10 min, 4 °C) to remove insoluble material. An aliquot (200 µl) of each supernatant was added to 600 µl of TBA and incubated at 95 °C for 60 min. The samples were then maintained in an ice bath for 10 min and 200 µl from each 800 µl reaction mixture were placed into a 96-well microplate for spectrophotometric measurement at 532 nm. Protein concentration was measured following the BCA protein assay kit (Sigma-Aldrich, Madrid, Spain). The results were obtained using a TBARS standard curve at different concentrations and expressed as nmol TBARS/mg protein.

Statistical analysis

The statistical analysis of results was performed in SPSS IBM, version 25.0 (SPSS, Inc., Chicago, IL). The data were expressed as mean ± standard error of the mean (SEM). Each value is the mean of the data from an assay performed in duplicate or triplicate. The normality of the samples was checked by the Kolmogorov–Smirnov test and homogeneity of variances with the Levene test. The data were statistically evaluated by one-way ANOVA followed by Tukey's post hoc test for homogenous variances. Games-Howell's post hoc test was used for unequal variances. $p < .05$ was considered statistically significant.

Results

Body weight and food intake

As shown in Table 2, body weight and food intake at the beginning of the study (9 weeks of age) were similar among the experimental groups. At the end of the study (27 weeks of age), body weight and food intake were significantly increased in DIO mice in comparison with controls ($p = .02$ and $p = .004$, respectively; Table 2). In addition, DIO mice supplemented with 2-OHOA significantly decreased their body weight when compared with DIO mice ($p = .006$; Table 2).

Table 2. Average body weight and food intake.

	Control	DIO mice	2-OHOA-DIO mice	EPA and DHA-DIO mice
Average initial weight (g) (9 weeks)	27 ± 0.5	28 ± 0.5	26 ± 0.7	26 ± 0.5
Average final weight (g) (27 weeks)	34 ± 1.2	51 ± 4.2*	28 ± 1.3##	48 ± 4.4
Average initial food intake (kcal/mouse/day) (9 weeks)	11 ± 0.03	11 ± 0.3	11 ± 0.2	11 ± 0.2
Average final food intake (kcal/mouse/day) (27 weeks)	13 ± 0.5	17 ± 0.8**	15 ± 1	17 ± 1

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DIO: diet-induced obese; 2-OHOA-DIO: 2-hydroxyoleic acid supplemented DIO; EPA and DHA-DIO: eicosapentaenoic acid and docosahexaenoic acid supplemented DIO.

Each value represents the mean ± SEM of the data weekly for the period indicated corresponding to 8 animals.

*$p < .05$ with respect to the values in non-obese control mice.

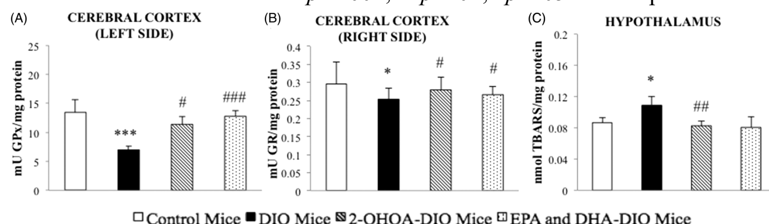
**$p < .01$ with respect to the values in non-obese control mice.

##$p < .01$ with respect to the values in DIO mice.

Left and right cerebral cortex and hypothalamus

The GPx and GR activities, two antioxidant enzymes of the glutathione system, were significantly lower in the left ($p < .001$; Figure 1(A)) and right cerebral cortex ($p = .026$; Figure 1(B)), respectively, of DIO mice as compared with non-DIO controls. However, DIO mice that were supplemented with 2-OHOA or with EPA and DHA showed significantly higher activity of these enzymes in comparison with DIO mice ($p = .03$ and $p < .001$ for GPx activity, respectively; Figure 1(A)) and ($p = .04$ and $p = .01$ for GR activity, respectively; Figure 1(B)).

Figure 1. Oxidative stress parameters in the brain. (A) Left cerebral cortex glutathione peroxidase (GPx) activity (mU/mg protein). (B) Right cerebral cortex glutathione reductase (GR) activity (mU/mg protein). (C) Hypothalamic thiobarbituric acid reactive substances (TBARS) concentration (nmol/mg protein). Each column represents the mean ± SEM of 8 values corresponding to that number of animals and each value being the mean of duplicate or triplicate assays. ***$p < .001$; *$p < .05$ with respect to the values in non-obese control mice. ###$p < .001$; ##$p < .01$; #$p < .05$ with respect to the values in DIO mice.



The TBARS concentration, which is an indicator of lipid oxidation and oxidative damage in cells, was significantly higher in the hypothalamus of DIO mice with respect to non-DIO mice ($p = .04$; Figure 1(C)). In turn, the supplementation with 2-OHOA was able to lower the concentration of TBARS in comparison with non-supplemented DIO mice ($p = .009$; Figure 1(C)). No significant differences were observed between EPA and DHA-DIO supplemented and non-supplemented mice regarding the concentration of TBARS in the hypothalamus ($p = .4$; Figure 1(C)).

Liver

The antioxidant CAT and GR activities were significantly lower in the liver of DIO mice as compared with that in non-DIO controls ($p = .008$ and $p = .012$, respectively; Figure 2(A,C)). By contrast, the GPx activity was significantly higher in DIO mice than in their respective non-DIO controls ($p = .015$; Figure 2(B)). The supplementation with 2-OHOA or with EPA and DHA resulted in significantly higher CAT activity in the liver ($p = .015$ and $p < .001$, respectively; Figure 2(A)) in comparison with non-supplemented DIO mice. In addition, the supplementation with EPA and DHA resulted in lower activity of GPx and higher activity of GR in the liver of DIO mice ($p < .001$ and $p = .038$, respectively; Figure 2(B,C)) than in non-supplemented DIO mice. No statistically significant differences were observed in the activities of GPx and GR in the liver of 2-OHOA-DIO mice and non-supplemented DIO mice ($p = .073$ and $p = .108$, respectively; Figure 2(B,C)).

Figure 2. Oxidative stress parameters in the liver. (A) Hepatic catalase (CAT) activity (U/mg protein). (B) Hepatic glutathione peroxidase (GPx) activity (mU/mg protein). (C) Hepatic glutathione reductase (GR) activity (mU/mg protein). (D) Hepatic xanthine oxidase (XO) activity (mU/mg protein). (E) Hepatic oxidized glutathione (GSSG)/reduced glutathione (GSH) ratio ($\times 10^{-2}$). (F) Hepatic thiobarbituric acid reactive substances (TBARS) concentration. Each column represents the mean ± SEM of 8 values corresponding to that number of animals and each value being the mean of duplicate or triplicate assays. ***$p < .001$; **$p < .01$; *$p < .05$ with respect to the values in non-obese control mice. ###$p < .001$; ##$p < .01$; #$p < .05$ with respect to the values in DIO mice.

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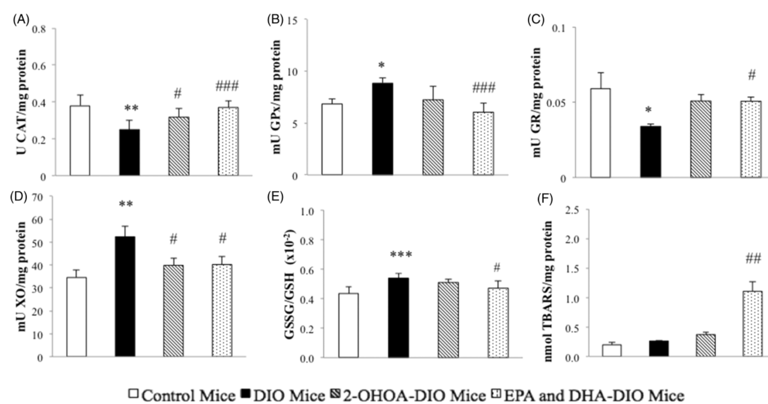

The activity of XO, which is associated with the production of free radicals, as well as the GSSG/GSH ratio, an indicator of oxidative stress, was significantly higher in DIO mice in comparison with those in non-DIO controls ($p = .001$ and $p < .001$, respectively; Figure 2(D,E)). No statistically significant differences were observed in the concentration of TBARS in the liver of DIO mice and non-DIO mice ($p = .564$; Figure 2(F)). The supplementation with 2-OHOA or with EPA and DHA resulted in significantly lower XO activity in the liver ($p = .028$ and $p = .032$, respectively; Figure 2(D)) in comparison with non-supplemented DIO mice. In addition, the supplementation with EPA and DHA resulted in lower GSSG/GSH ratios and higher TBARS concentration in the liver of DIO mice ($p = .017$ and $p = .006$, respectively; Figure 2(E,F)) than in non-supplemented DIO mice. No statistically significant differences were observed in the GSSG/GSH ratios and TBARS concentration in the liver of 2-OHOA-DIO mice and non-supplemented DIO mice ($p = .510$ and $p = .079$, respectively; Figure 2(E,F)).

Lungs

The activities of CAT and GR were significantly lower in the lungs of DIO mice than in non-DIO controls ($p < .001$ and $p < .001$, respectively; Figure 3(A,C)). However, the GPx activity was significantly higher in DIO mice than in their respective non-DIO controls ($p < .001$; Figure 3(B)). The supplementation with 2-OHOA or with EPA and DHA in DIO mice resulted in significantly lower activity of GPx ($p < .001$ and $p < .001$, respectively; Figure 3(B)) and higher activity of GR ($p = .001$ and $p = .001$, respectively; Figure 3(C)) with respect to non-supplemented DIO mice. In addition, the supplementation with EPA and DHA in DIO mice resulted in significantly higher CAT activity ($p < .001$; Figure 3(A)) than in non-supplemented DIO mice. No statistically significant differences were observed in the CAT activity in the lungs of 2-OHOA-DIO mice and non-supplemented DIO mice ($p = .062$; Figure 3(A)).

Figure 3. Oxidative stress parameters in the lungs. (A) Lungs catalase (CAT) activity (U/mg protein). (B) Lungs glutathione peroxidase (GPx) activity (mU/mg protein). (C) Lungs glutathione reductase (GR) activity (mU/mg protein). (D) Lungs xanthine oxidase (XO) activity (mU/mg protein). (E) Lungs oxidized glutathione (GSSG)/reduced glutathione (GSH) ratio ($\times 10^{-2}$). (F) Lungs thiobarbituric acid reactive substances (TBARS) concentration. Each column represents the mean ± SEM of 8 values corresponding to that number of animals and each value being the mean of duplicate or triplicate assays. ***$p < .001$ with respect to the values in non-obese control mice. ###$p < .001$; ##$p < .01$ with respect to the values in DIO mice.

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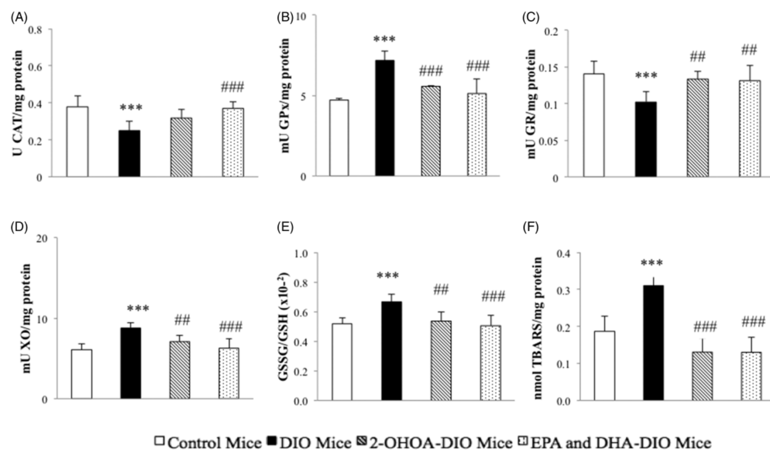
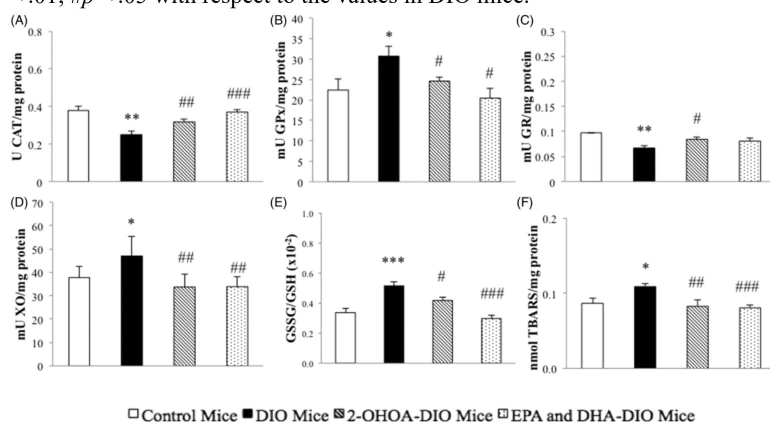


The XO activity, GSSG/GSH ratios, and TBARS concentration were significantly higher in DIO mice than in non-DIO controls ($p < .001$, $p < .001$, and $p < .001$, respectively; Figure 3(D–F)). The supplementation with 2-OHOA or with EPA and DHA resulted in significantly lower XO activity ($p = .005$ and $p < .001$, respectively; Figure 3(D)), GSSG/GSH ratios ($p = .002$ and $p < .001$, respectively; Figure 3(E)), and TBARS concentration ($p < .001$ and $p < .001$, respectively; Figure 3(F)) in the lungs of DIO mice in comparison with non-supplemented DIO mice.

Kidneys: renal medulla and cortex

The activities of CAT and GR were significantly lower in the renal medulla of DIO mice in comparison with non-DIO controls ($p = .001$ and $p = .006$, respectively; Figure 4(A,C)). By contrast, the GPx activity was significantly higher in DIO mice than in their respective non-DIO controls ($p = .025$; Figure 4(B)). The supplementation with 2-OHOA or with EPA and DHA resulted in significantly higher activity of CAT ($p = .001$ and $p < .001$, respectively; Figure 4(A)) and lower activity of GPx ($p = .024$ and $p = .015$, respectively; Figure 4(B)) in the renal medulla of DIO mice in comparison with non-supplemented animals. In addition, the supplementation with 2-OHOA resulted in significantly higher GR activity ($p = .022$; Figure 4(C)) in the renal medulla of DIO mice in comparison with non-supplemented DIO mice. No statistically significant differences were observed in the GR activity in the renal medulla of EPA and DHA-supplemented DIO mice and non-supplemented DIO mice ($p = .995$; Figure 4(C)).

Figure 4. Oxidative stress parameters in the renal medulla. (A) Renal medulla catalase (CAT) activity (U/mg protein). (B) Renal medulla glutathione peroxidase (GPx) activity (mU/mg protein). (C) Renal medulla glutathione reductase (GR) activity (mU/mg protein). (D) Renal medulla xanthine oxidase (XO) activity (mU/mg protein). (E) Renal medulla oxidized glutathione (GSSG)/ reduced glutathione (GSH) ratio ($\times 10^{-2}$). (F) Renal medulla thiobarbituric acid reactive substances (TBARS) concentration. Each column represents the mean ± SEM of 8 values corresponding to that number of animals and each value being the mean of duplicate or triplicate assays. ***$p < .001$; **$p < .01$; *$p < .05$ with respect to the values in non-obese control mice. ###$p < .001$; ##$p < .01$; #$p < .05$ with respect to the values in DIO mice.



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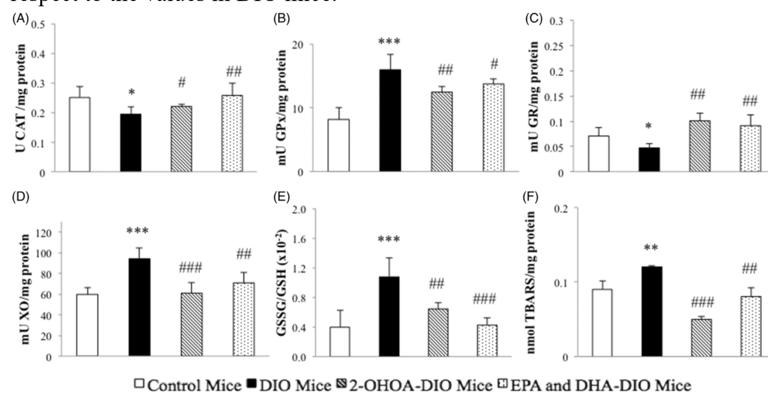
The XO activity, GSSG/GSH ratios, and TBARS concentration were significantly higher in DIO mice than in non-DIO controls ($p = .032$, $p < .001$, and $p = .034$, respectively; Figure 4(D–F)). The supplementation with 2-OHOA or with EPA and DHA resulted in significantly lower XO activity ($p = .003$ and $p = .003$, respectively; Figure 4(D)), GSSG/GSH ratios ($p = .015$ and $p < .001$, respectively; Figure 4(E)), and TBARS concentration ($p = .001$ and $p < .001$, respectively; Figure 4(F)) in the renal medulla of DIO mice than in non-supplemented animals.

The activities of CAT and GR were significantly lower in the renal cortex of DIO mice than in non-DIO controls ($p = .020$ and $p < .001$, respectively; Figure 5(A,C)). However, the GPx activity was significantly higher in DIO mice than in their respective non-DIO controls ($p < .001$; Figure 5(B)). The supplementation with 2-OHOA or with EPA and DHA resulted in significantly higher activities of CAT ($p = .039$ and $p = .011$, respectively; Figure 5(A)) and GR ($p = .003$ and $p = .007$, respectively; Figure 5(C)) in the renal cortex of DIO mice in comparison with non-supplemented animals. The activity of GPx was significantly lower after the supplementation with 2-OHOA or with EPA and DHA ($p = .006$ and $p = .039$, respectively; Figure 5(B)) in the renal cortex of DIO animals in comparison with non-supplemented DIO mice.

Figure 5. Oxidative stress parameters in the renal cortex. (A) Renal cortex catalase (CAT) activity (U/mg protein). (B) Renal cortex glutathione peroxidase (GPx) activity (mU/mg protein). (C) Renal cortex glutathione reductase (GR) activity (mU/mg protein). (D) Renal cortex xanthine oxidase (XO) activity (mU/mg protein). (E) Renal cortex oxidized glutathione (GSSG)/reduced glutathione (GSH) ratio ($\times 10^{-2}$). (F) Renal cortex thiobarbituric acid reactive substances (TBARS) concentration. Each column represents the mean ± SEM of 8 values corresponding to that number of animals and each value being the mean of duplicate or triplicate assays. ***$p < .001$; **$p < .01$; *$p < .05$ with respect to the values in non-obese control mice. ###$p < .001$; ##$p < .01$; #$p < .05$ with respect to the values in DIO mice.



The XO activity, GSSG/GSH ratios, and TBARS concentration were significantly higher in DIO mice than in non-DIO controls ($p < .001$, $p = .001$, and $p = .002$, respectively; Figure 5(D–F)). The supplementation with 2-OHOA or with EPA and DHA resulted in significantly lower XO activity ($p < .001$ and $p = .001$, respectively; Figure 5(D)), GSSG/GSH ratios ($p = .013$ and $p = .001$, respectively; Figure 5(E)), and TBARS concentration ($p < .001$ and $p = .002$, respectively; Figure 5(F)) in the renal cortex of DIO mice in comparison with non-supplemented DIO animals.

Discussion

Although we previously demonstrated that the supplementations with 2-OHOA as well as with DHA and EPA improved the functions and redox state of immune cells [illegible], the effect of these supplementations on the oxidative stress of the brain, liver, lungs, and kidneys from DIO mice, has not been studied.

Our results showed that the mice fed an HFD gained significantly more body weight than controls (fed a standard diet). Previous studies from our laboratory confirmed that the HFD intake promotes obesity in ICR-CD1 mice. Additionally, these DIO mice show common features of obesity, such as elevated levels of triglycerides, total cholesterol, and systolic arterial pressure [1316171816–1821]. In turn, we observed that the supplementation with 2-OHOA, but not with EPA and DHA, resulted in significantly lower body weight of high-fat fed mice than that of non-supplemented high-fat fed animals. In this sense, 2-OHOA seems to promote body weight loss through the induction of uncoupling protein-1 (UCP-1) expression in the adipose tissue, a process probably accompanied by enhanced energy expenditure [3131]. In contrast, the supplementation with EPA and DHA seems to not affect the body weight of obese

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individuals [3234]. A recent study from our laboratory showed that the supplementation with 2-OHOA reduces blood pressure, triglycerides, and leptin, and improves adiponectin and resistin secretion, while EPA and DHA only reduce triglyceride levels 21.

Our current results demonstrated increased oxidative stress in the liver, lungs, and kidneys (renal medulla and cortex) of DIO mice in comparison with non-DIO controls. Thus, these DIO animals displayed lower antioxidant defenses, such as CAT and GR activities, as well as a higher production of oxidants, including XO activity, GSSG/GSH ratio, and lipid peroxidation (TBARS concentration) than non-DIO mice. In addition, we found diminished antioxidant defenses (i.e. GPx and GR activities) in the cerebral cortex and elevated TBARS concentration in the hypothalamus of DIO mice. The altered redox state seems to be associated with lipotoxicity in these organs, which is generated when triglycerides are inappropriately stored in non-adipose tissues. The excessive accumulation of intracellular triglycerides is known to reduce the efficacy of the electron transport chain, causing the release of ROS and the generation of oxidative damage 27. greement with our current findings, previous reports also indicate that obesity increases oxidative stress in the bral cortex [3333] er and kidneys [3635,35,363634-37] so, HFD increases mitochondrial H_2O_2 production, causing the abnorma pression of antioxidant enzymes (suc CAT) [37]. These studies suggest that oxidative stress is related to adiposity, lipotoxicity, mitochondrial dysfunction, and endoplasmic reticulum stress in a variety of organs from obese rodents [20373836-3835-38] addition, these alterations are accompanied by the inhibition of the expression and activity of the nuclear factor throid 2-related factor (Nrf2), which is a key transcription factor involved in the regulation of antioxidant defenses 2023. estingly, we found higher values of GPx activity (an antioxidant enzyme) in the liver, lungs, and kidneys of D nice in comparison with non-DIO mice. However, lower values of GPx activity were observed in the brain (right cerebral cortex) of DIO mice in comparison with non-DIO mice. The higher activity of this antioxidant enzyme could possibly be explained as a compensatory mechanism to protect these cells against oxidative damage [7] nilarly, another study from our laboratory also found increased values of GPx in peritoneal leukocytes of DIO 17. s, the activity of this enzyme has been reported to increase or decrease in response to oxidative damage depend on the moment of its evolution and the amount of peroxides generated [3941,4141]

In turn, the dietary supplementations with 2-OHOA or with the combination of EPA and DHA in general were able to restore oxidative stress in the liver, lungs, and kidneys of DIO mice (with higher values of CAT and GR activities and lower values of XO activity, GSSG/GSH ratio, and TBARS concentration) in comparison with non-supplemented DIO mice. In addition, these supplementations led to higher values of GPx and GR activities in the cerebral cortex and lower values of TBARS in the hypothalamus of DIO mice when compared with non-supplemented DIO animals. In all cases, the values showed similar levels to those of non-DIO controls. Similarly, our recent studies in spleen and peritoneal immune cells also ameliorated oxidative stress after the supplementations with 2-OHOA or n-3 PUFA (EPA and DHA) (with increased values of CAT activity and total glutathione concentration and decreased values of XO activity, GSSG/GSH ratio, and TBARS concentration) 918. These results are also in agreement with previous studies showing the antioxidant properties of the supplem tion with n-3 PUFA in the liver, kidneys, and brain of rats [404243] addition, the supplementations with extra virgin olive oil and n-3 PUFA are able to decrease the oxidative str elated parameters in HFD-fed mice [20223744242] this regard, specific n-3 fatty acid oxidation products, particularly isoprostanes, and extra virgin oil polyphenols c ead to the stabilization and activation of the Nrf2 transcription factor. Nrf2 activation provides antioxidant defense against oxidative stress [43-4543-4643-4643-46] contrast to the beneficial effects of MUFA and n-3 PUFA on oxidative stress parameters, conjugated linoleic (CLA), isomers of linoleic acid (n-6 PUFA), have been associated with the enhancement of oxidative stress markers, both non-enzymatic and enzymatic markers [46-5046-5146-5146-5146-5146-51] Furthermore, CLA supplementation fails to show beneficial effects on lipid peroxidation and antioxidant metabolism in overweight/obese individuals [52,5251] wever, other studies showed that the supplementation with CLA ameliorates oxidative stress in atherosclerotic 53 l in non-alcoholic fatty liver disease (NAFLD) patients [53]

In conclusion, diet-induced obesity resulted in increased oxidative stress in the liver, lungs, and kidneys of mice. In turn, both supplementations with 2-OHOA as well as with EPA and DHA were able to restore the redox state in these organs, bringing the values to similar levels to those of non-DIO controls. Thus, these supplementations could be a promising nutritional strategy to reduce the progression of obesity-related diseases as well as to promote a healthy aging, probably through the reduction of oxidative stress.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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