Nutrition 66 (2019) 173–179

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

Effects of lipoic acid and ω-3 long-chain polyunsaturated fatty acids on the kidney in the ovariectomized rat model of menopause

Jordana S. Putti M.Sc., Vanessa K. Engers M.Sc., Fernanda M. Heemann M.Sc., Tiago B. Salomon Ph.D., Mara S. Benfato Ph.D., Fernanda S. Hackenhaar Ph.D. *

Department of Biophysics, Postgraduate Program in Cellular and Molecular Biology, Federal University of Rio Grande do Sul, Porto Alegre, Brazil

ARTICLE INFO

Article History:
Received 4 February 2019
Received in revised form 26 April 2019
Accepted 21 May 2019

Keywords:
Antioxidants
Kidney
Lipoic acid
Menopause
ω-3
Oxidative stress

ABSTRACT

Objectives: The loss of antioxidant protection from estrogen during menopause may lead to oxidative stress in the kidneys. Thus, antioxidant supplementation may potentially decrease the menopause-derived oxidative stress. The aim of this study was to evaluate the effect of α-lipoic acid (LA) and ω-3 long-chain polyunsaturated fatty acids on the redox profile of the kidneys in the ovariectomized rat model of menopause.
Methods: We assessed oxidative damage markers and antioxidant defenses in the kidneys of ovariectomized rats supplemented with LA, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). Animals received 3 mo of dietary supplementation.
Results: Ovariectomy did not increase the levels of the damage markers carbonyl and malondialdehyde. EPA supplementation increased carbonyl and malondialdehyde levels. Ovariectomy increased fumarase activity but did not affect the levels of vitamin C, glutathione, and glutathione S-transferase activity. LA, DHA, and EPA supplementation decreased fumarase activity, but increased the levels of vitamin C, glutathione, and glutathione S-transferase activity. Vitamin E, superoxide dismutase, glutathione peroxidase, and peroxide consumption were not affected by ovariectomy or supplementation.
Conclusions: The results suggest that ovariectomy did not affect the redox profile in the kidneys. LA, DHA, and EPA supplementation increased certain endogenous antioxidants; however, EPA may have a prooxidant effect on the kidneys.

Introduction

Menopause can be defined as a phase of reproductive senescence in women, a natural process characterized by the permanent cessation of ovarian follicular activity and the menstrual cycle [1]. In addition, there is a marked decrease in estrogen levels that contributes to the development of menopause-related disorders [2,3]. The risk for renal disease and cardiovascular diseases such as hypertension and atherosclerosis, increase after menopause [3,4]. Estrogen is an antioxidant hormone that is well known for its cardioprotective properties. Accordingly, studies have demonstrated that a decrease in estrogen levels at menopause is associated with oxidative stress. Generation of reactive oxygen species (ROS) above physiologic levels is often the mechanism underlying vascular alterations and vascular damage [3–5].

The uncomfortable symptoms and diseases associated with menopause have become a worldwide health concern. Many therapies have targeted hormone replacement therapy, which is not free of risk [6]. Expanded therapy including lifestyle modifications such as physical exercise and diet may greatly contribute to women's health [5]. Food rich in antioxidants have shown evident benefits. Dietary supplementation with antioxidants could help the body to cope with the loss of estrogen protection against oxidative stress [7,8].

An alternative for hormone replacement therapy is the use of ω-3 long-chain polyunsaturated fatty acids (LCPUFAs) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). DHA and EPA are cardioprotective and neuroprotective antioxidants absorbed from the diet and are found in seafood, especially oily

This research was supported by PPGBCM-Postgraduate Programme in Cellular and Molecular Biology of UFRGS -Universidade Federal do Rio Grande do Sul and CAPES - Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Brazilian Federal Agency for Support and Evaluation of Graduate Education) - Finance Code 001. All animal studies were approved by the Ethics Committee of the Universidade Federal do Rio Grande do Sul (project 20137).

JSP contributed to manuscript preparation, oxidative stress assays, animal feed, and statistical tests. VKE, FMH, TBS, and FSH contributed to the oxidative stress assays, animal feed, and surgical procedures. MSB and FSH contributed to manuscript preparation and orientation.

The authors have no conflicts of interest to declare.

* Corresponding author: Tel.: +55 51 330 87372; Fax: +55 51 330 87003.
E-mail address: Fernanda.hackenhaar@ufrgs.br (F.S. Hackenhaar).

https://doi.org/10.1016/j.nut.2019.05.003
0899-9007/

fish, and in fish oil–type supplements [9,10]. α-Lipoic acid (LA) is also an antioxidant supplement used worldwide. The biological effects of LA are primarily associated with its antioxidant role, showing antimutagenic and anticarcinogenic activity in experimental and clinical studies. LA is used as a therapeutic agent in diverse pathologic conditions, including diabetes, atherosclerosis, insulin resistance, neuropathy, neurodegenerative diseases, and ischemia-reperfusion injury [11–13]. LA is an endogenous lipid; however, it is also obtained in the diet from both animal and plant sources. LA is found at high concentrations in animal-derived food products, such as red meat, liver, heart, and kidney. The most abundant plant sources of LA are spinach and broccoli [13,14].

The kidneys are the central organs of the urinary system and play several essential regulatory roles in vertebrates, including removal of excess molecules and excretion of by-products of metabolism in the blood [14,15]. Thus, the kidneys are targets of systemic toxicity and oxidative stress. For these reasons, renal disease may be a consequence of menopause-related oxidative damage.

There are still few studies investigating the effect of menopause on the kidneys. Moreover, no previous studies address the effects of supplementation with ω-3 LCPUFA or LA on the kidney redox system. Thus, in the present study we assessed oxidative damage biomarkers and antioxidant response of the kidneys in ovariectomized rats treated with the antioxidants DHA, EPA, and LA.

Materials and methods

Animals

Fifty 3-mo-old female Wistar rats (*Rattus norvegicus*) were used in the present study. They were divided in five groups containing 10 animals each. Five rats per cage were maintained in a room under controlled conditions (24 ± 1° C, 12- h light/dark cycle) with free access to water. The surgical procedure was performed under general anesthesia. Immediately after surgery, while still under anesthesia, the rats received a combination of antibiotics and anti-inflammatory drugs (Pencivet PPU Plus, Intervet/Schering-Plough Animal Health, 0.1 mL/100 g, i.m.; containing [per 100 mL] procaine benzylpenicillin G, 10 000 IU, benzathine benzylpenicillin G, 10 000 IU dihydrostreptomycin, 10.5 mg, piroxicam 1 mg). After surgery, the animals were maintained under a heat lamp until recovered from anesthesia. The details of the animal preparation were previously published [16].

All animal studies followed the rules from the EU Directive for animal experiments 2010/63/EU and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (DHEW Publication No. (NIH) 85-23, revised in 1996, Office of Science and Health Reports, Division of Research Resources/NIH, Bethesda, MD, USA) and were approved by the university ethics committee.

Diets

One week after surgery, the animals were randomly assigned to the five groups. Two groups received a standard diet: the sham-operated control group (Sham) and one ovariectomized group (OVX); the other three groups where formed only by ovariectomized animals and were grouped according to the received supplementation: DHA (predominance of docosahexaenoic acid ethyl ester: 1 g · kg body weight [BW] · d^{-1} DHA plus 0.2 g · kg BW · d^{-1} EPA; capsule 1 g fish oil with 500 mg DHA and 100 mg EPA [PO9037701 Naturalis, SP, Brazil]), EPA (predominance of EPA ethyl ester: 1 g/kg g · kg BW · d^{-1} EPA plus 0.2 g g · kg BW · d^{-1} DHA; capsule 1 g fish oil with 540 mg EPA and 100 mg DHA [PO9037701 Naturalis, SP, Brazil]), and LA (supplementation of LA: 180 mg · kg BW · d^{-1} LA [Pharmanostra, SP, Brazil]). All five groups received the diets for a period of 16 wk. The experimental diets were formulated with fish oil and LA and were blended daily into the standard diet to avoid fatty acid oxidation and loss of antioxidants before their use. The food intake was recorded daily, and all animals were weighed weekly. All diets contained 4% fat and ≥2% corn, soybean, and wheat oils.

Kidney obtaining and processing

All animals were sacrificed according to an experimental protocol by intraperitoneal injection of a mixture of ketamine (60 mg/kg) and xylazine (10 mg/kg) [17]. After perfusion using a saline infusion, the kidneys were removed and immediately frozen in liquid nitrogen. For the analysis, the kidneys of each animal were processed with manual maceration while still frozen. The macerated tissues were mixed in 1 mL with 30 mmol/L phosphate buffer, 120 mmol/L KCl, and 100 mmol/L PMSF (pH 7.4) after sonication three times for 10s each and centrifuged for 10 min at 1700*g* for cell membrane lysis. The supernatant of each tube was transferred to a second tube and centrifuged again for 10 min at 1700*g*. The supernatant from the second centrifugation was aliquoted and frozen at −80°C for later analysis and assays.

Estrogen measurement

Levels of 17 β-estradiol in serum were estimated by solid phase radioimmunoassay using the Estrogen Coat-a-Count DPC kit (Diagnostic Products Corporation, Los Angeles, CA, USA).

Assays for oxidative damage

As an index of protein damage, carbonyl levels were marked with 2,4-dinitrophenyl hydrazine and measured at 370 nm [18]. Malondialdehyde (MDA), an index of lipid peroxidation, was measured by high-perfomance liquid chromatography (HPLC) simultaneously with vitamin C as described later [19].

Assay for fumarase activity

The fumarase activity was assessed by the conversion of fumarate to malate and measured at 240 nm [20].

Assays for enzymatic antioxidants

Superoxide dismutase (SOD) activity was assessed with the Ransod Kit (Randox Laboratories Ltd, Crumlin, UK) and glutathione peroxidase (GPx) activity was assessed with the Ransel Kit (Randox Laboratories Ltd, Crumlin, UK). The activities were expressed as U/mg of protein. The hydrogen peroxide consumption was evaluated by measuring the rate of hydrogen peroxide decrease using absorbance at 240 nm [21]. The activity was expressed as U/mg of protein. Glutathione S-transferase (GST) activity was measured by the GST-catalyzed reaction of 1-chloro-2,4-dinitrobenzene with reduced glutathione (GSH) using absorbance at 340 nm [22]. GST activity was expressed as U/mg of protein.

Assays for non-enzymatic antioxidants

Vitamin C levels were assayed by HPLC employing a reverse-phase Supercosil LC-18- DB HPLC column (15 cm × 4.6 mm) and a mobile phase (30 mmol/L monobasic potassium phosphate (pH 3.6) and methanol in flow rate of 1 mL/min. The absorbance of the column effluent was monitored at 250 nm [19]. The amount of vitamin E was measured by HPLC using a 15 cm × 4.6 mm column (Nucleosil 120 C-18) with a continuous flow of 2 mL/min methanol-to-water ratio. Detection was carried out by fluorescence (295 nm excitation and 350 nm emission) [23]. The assay method for total GSH involves oxidation of GSH by the sulfhydryl reagent 5,5′-dithiobis(2-nitrobenzoic acid) to form the yellow derivative 5′-thio-2-nitrobenzoic acid, measurable at 412 nm. The glutathione disulfide formed can be recycled to GSH by glutathione reductase in the presence of NADPH [24]. Color development was read at 412 nm, and the level was expressed as μmol of GSH/mg of protein.

Data normalization and statistical analysis

All results were normalized to protein content with bovine serum albumin as the standard [25]. All assays were independently performed in triplicate.

Data were expressed as mean ± SEM. Statistical analysis of different variables was performed with one-way analysis of variance, and multiple comparisons for variables that showed significant differences were performed using post hoc Tukey test. Differences were considered significant at $P \leq 0.05$. Statistical analysis was accomplished with the support of the Statistical Nucleus of the university (NAE).

Results

Kidneys and body weights

Table 1 shows the weight of the kidneys as well as the initial and final body weights of the rats during the study.

Oxidative damage and fumarase activity in kidney tissue

Ovariectomy did not cause significant changes in carbonyl levels, a marker used to evaluate protein oxidation. The LA and EPA groups exhibited an increase in protein carbonylation compared with the OVX group, and the EPA group also had significantly higher levels than the Sham group (Fig. 1A). Similarly, ovariectomy did not increase MDA, a marker used to evaluate lipid peroxidation.

Table 1
Kidney and initial and final animal weights (g)

	Sham	OVX	LA	DHA	EPA
Kidneys weight	2.05 ± 0.072	1.83 ± 0.047	2.14 ± 0.066	2.35 ± 0.048	1.94 ± 0.07
Kidneys/body ratio	0.008	0.006	0.007	0.008	0.007
Initial body weight	218.8 ± 19.2	224.5 ± 15.3	217 ± 19.7	223 ± 19.6	190.2 ±12.1
Final body weight	262.8 ± 22.3	297.2 ± 28.4	284.9 ± 19.3	311 ± 44.7*	299.4 ± 21.1*
Δ body weight	51.0	72.7	67.9	88	109.2

ANOVA, analysis of variance; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, α-lipoic acid; OVX, ovariectomized.
Results are expressed as mean ± SEM. Significance was determined using factorial ANOVA of repeated measures.
All groups 10 per group.
*$P \leq 0.05$ vs Sham group final weight.

However, MDA levels were higher in the EPA group than in the Sham group (Fig. 1B).

The activity of the enzyme fumarase increased in the OVX group compared with the Sham group and returned to levels similar to those of the Sham group after LA and EPA supplementation. In the case of DHA supplementation, fumarase activity was significantly lowered compared with the Sham group (Fig. 1C).

Non-enzymatic antioxidant in kidney tissue

Ovariectomy did not change vitamin C levels. All supplemented groups showed higher vitamin C levels than the Sham group but did not differ when compared with the OVX group (Fig. 2A). After ovariectomy, GSH levels were increased compared with the Sham group; supplementation with LA, DHA, and EPA significantly

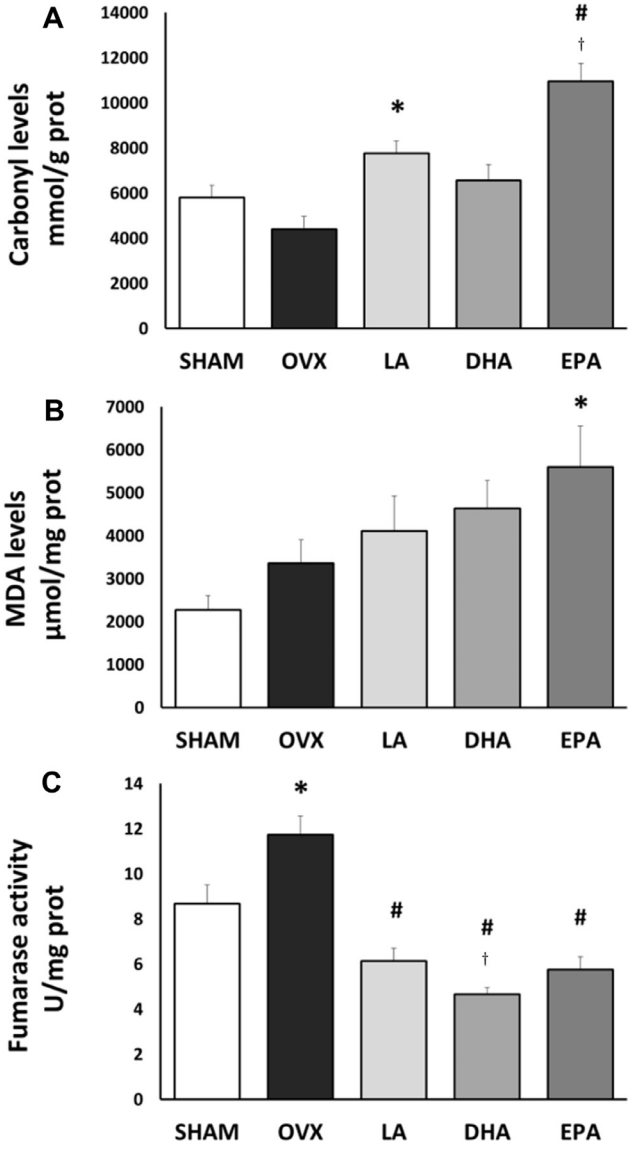

Fig. 1. Levels of oxidative damage biomarkers (**A**) carbonyl and (**B**) malondialdehyde, and (**C**) fumarase activity in the kidney of OVX rats treated with DHA, EPA, or LA. *$P \leq 0.05$ vs OVX. †$P \leq 0.05$ vs Sham. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, α-lipoic acid; OVX, ovariectomized.

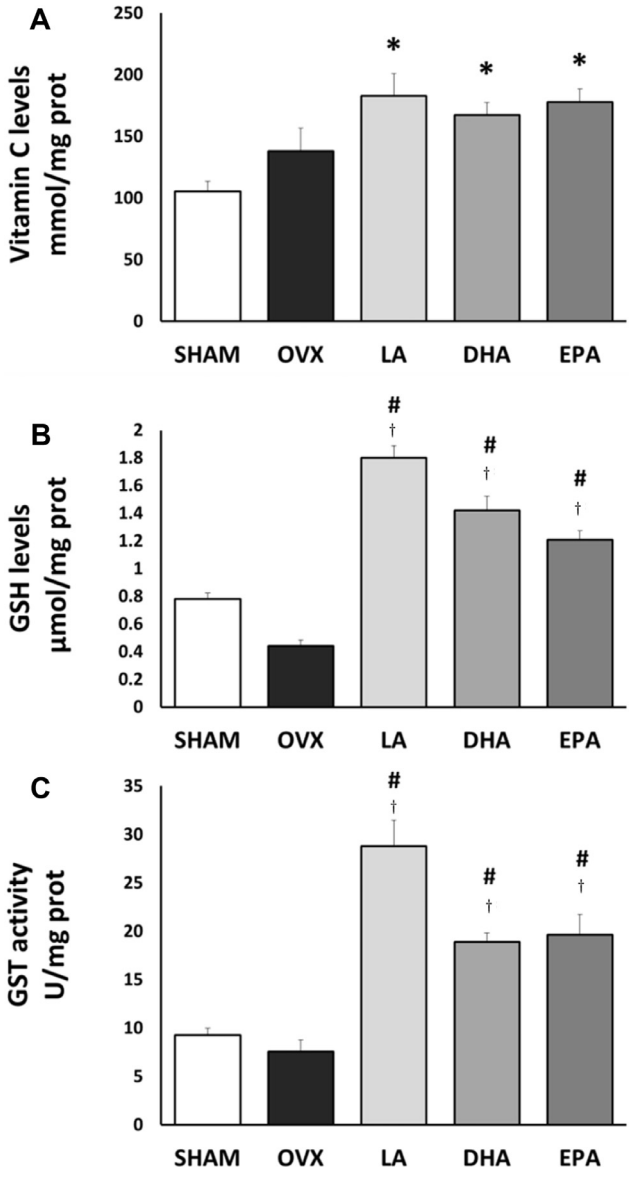

Fig. 2. Levels of antioxidant (**A**), vitamin C, and (**B**) total GSH and of (**C**) GST activity in the kidney of OVX rats treated with DHA, EPA, or LA. $^{*}P \leq 0.05$ vs Sham. $^{\dagger}P \leq 0.05$ vs OVX. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GSH, glutathione GST, glutathione-S-transferase; LA, α-lipoic acid; OVX, ovariectomized.

increased GSH levels compared with both OVX and Sham groups. There were no differences between GSH levels in the OVX and Sham groups (Fig. 2B). No significant changes were found in vitamin E levels among the analyzed groups (Table 1).

Enzymatic antioxidants in kidney tissue

After ovariectomy, GST activity did not change compared with the Sham group; supplementation with LA, DHA, and EPA significantly increased GST activity compared with both OVX and Sham groups (Fig. 2C). No significant changes were found in the activities of SOD, GPx, and hydrogen peroxide consumption (Table 2).

Discussion

Ovariectomy has been described as a valid model for induction of menopause, reproducing the numerous physiologic changes that occur as a consequence of estrogen deprivation in different organs [26]. The estrogen levels in all ovariectomized groups were significantly decreased compared with the Sham group. This result demonstrates the successful induction of the surgical menopause model.

Despite not being significant, there was a tendency of the weight of the kidneys of ovariectomized animals to decrease. Ovariectomy and LA did not alter BW; however animals receiving ω-3 LCPUFA supplementation with both EPA and DHA had greater BW than other groups. Although ω-3 LCPUFA are high-calorie supplements, previous studies have shown that they are beneficial for blood cholesterol and do not alter blood triacylglycerol (TG) and glucose levels. For example, ω-3 LCPUFA supplementation normalizes plasma total cholesterol and non–high-density lipoprotein cholesterol increased by ovariectomy, whereas blood TGs are not altered by ovariectomy or ω-3 LCPUFA supplementation [27]. Moreover, a meta-analysis examining plasma glucose control and patients with diabetes did not observe changes after ω-3 LCPUFA supplementation [28]. Regarding LA, a meta-analysis supports that LA supplementation decreases serum TGs and cholesterol [29]. Furthermore, LA intake reduces hyperglycemia and insulin resistance

Table 2
Estrogen levels, enzymatic antioxidants activity, and vitamin E levels in the kidney of ovariectomized rats

Group	Estrogen (pg/mL)	H_2O_2 consumption (U/mg prot)	Glutathione peroxidase (U/mg prot)	Superoxide dismutase (U/mg prot)	Vitamin E (nmol/mL)
Sham	26.52 ± 10.03	1469.73 ± 134.03	3539.27 ± 259.36	162.58 ± 13.56	336.89 ± 33.72
OVX	8.32 ± 2.48*	1820.74 ± 209.99	3794.47 ± 293.53	149.71 ± 18.24	254.26 ± 23.01
LA	6.63 ± 2.89*	1495.63 ± 172.89	4897.16 ± 1093.76	143.28 ± 10.20	491.58 ± 84.38
DHA	9.88 ± 6.77*	1198.60 ± 77.98	3207.26 ± 294.98	141.57 ± 6.95	346.51 ± 60.03
EPA	10.39 ± 3.11*	1453.66 ± 138.25	5434.67 ± 1176.36	160.39 ± 25.09	374.07 ± 68.52

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; H_2O_2, hydrogen peroxide; LA, α-lipoic acid; OVX, ovariectomized.
Data expressed as mean ± SEM.
*Lower than Sham ($P \leq 0.05$).

in an animal model of high-fat diet [30]. Whether LA supplementation is beneficial for lipid and glucose parameters in menopausal women needs to be further investigated.

Physiologic changes and symptoms affecting menopausal and postmenopausal women are linked not only to the decrease of estrogen, but also to redox misbalance, and may lead to oxidative damage to the kidneys. In the present experimental model, ovariectomy did not increase carbonyl and MDA levels. These results suggest that at least 3 mo postovariectomy, the kidneys are not subjected to free radical damage from proteins and lipids.

Supplementation with ω-3 LCPUFA can decrease protein carbonylation in the liver, blood, kidney, and skeletal muscle of healthy female rats supplied with vegetable oils from soybean and linseed as controls. However, the antioxidant effect turned out to be dependent on the ratio of EPA to DHA. The most protective ratio was 1:1 when compared with 2:1 and 1:2 ratios [31]. In the present study, the groups supplemented with DHA and EPA did not also receive this pure ω-3 LCPUFA. The commercially available ω-3 capsules for human supplementation are a mixture of the two types of ω-3 LCPUFA. Therefore, the DHA group received 83.3% DHA and 16.67% EPA, and the EPA group received 83.3% EPA and 16.67% DHA.

Moreover, it is necessary to point out that high doses (1 g/kg) of ω-3 LCPUFA were used, as recommended for human adults by the UN's Food and Agriculture Organization (FAO) [8, 32]. We based the ω-3 LCPUFA dose on a study by Cappellari et al. that was able to reverse endothelial dysfunction after 2 mo of daily supplementation (0.8 g/kg) but using esophageal gavage for administration [27]. For this reason we used 1 g/kg daily to compensate the possible oxidation of the ω-3 LCPUFA during the preparation of the chow pellets. Because high doses of ω-3 LCPUFA were used, we choose comparable high daily doses of LA (180 mg/kg). These doses may be elevated for rats, with this being an important limitation of the study. In the present case, EPA supplementation increased both protein and lipid damage biomarkers. It is possible that the high administered dose of these antioxidants was, at least in part, prooxidant to the kidneys. Previous data support the hypothesis of a prooxidant effect of EPA because EPA induced ROS production and lipid damage in liver cells treated with ethanol, which has been reported in the liver of rats fed with fish oil as well [33]. Furthermore, F3-isoprostanes and F4-neuroprostanes are formed by the oxidation of EPA and DHA, respectively [34]. Whereas F3-isoprostane is considered an oxidative stress marker [35], the biological effect of F4-neuroprostane is still under investigation, but it may be considered a protective molecule in the cardiovascular system [36]. F3-isoprostanes are excreted by the kidneys into urine [35]. It is possible that the F3-isoprostanes generated from EPA have a prooxidant effect on the kidneys compared with DHA by-products. Excretion of secondary metabolites may in part explain the kidney-specific response to ω-3 LCPUFA by-products.

Our carbonyl results also point to a difference between the kidney cell response to EPA versus DHA. Importantly, EPA and DHA supplementation affects membrane lipid content. In renal cell culture, ω-3 LCPUFA supplementation increased its incorporation into the cell membrane, but also increased saturated fatty acid incorporation to preserve the ω-6 to ω-3 ratio [37]. According to a study performed with rat kidney cells, incorporation of EPA, but not DHA, into cells decreased membrane fluidity by decreasing membrane arachidonic acid [38]. Whether alterations in membrane lipids are affecting membrane proteins or protein oxidation needs to be further investigated.

LA is considered a unique molecule owing to its antioxidant properties in both oxidized and reduced forms and for its presence in both lipid and aqueous environments [11,39]. LA scavenges ROS, chelates metals, and regenerates endogenous antioxidants. It is possible that LA contributes to maintaining the reduced forms of GSH, vitamin C, and vitamin E [40], which are the forms evaluated by the methods applied in the present study. Vitamin C acts as a reducing agent against peroxyl radicals, including oxidized vitamin E [41]. Dietary ω-3 LCPUFAs are inserted into membranes [27], which also may happen with LA owing to its nonpolar portion. Vitamin E is inserted into the membranes as well, being the main barrier against membrane lipidic peroxidation. Diets rich in lipids such as ω-3 LCPUFA impair vitamin E absorption in the liver [42,43], and for this reason vitamin E supplementation is recommended proportionally to the increase of unsaturated fatty acid intake. In the present study, vitamin E was not altered by ω-3 LCPUFA or LA supplementation in the kidneys, showing that this impairment is tissue specific. Moreover, despite the known synergy between vitamins C and E, vitamin C is a water-soluble antioxidant, and lipid supplements influence vitamin C differently; in contrast to vitamin E, LA, DHA, and EPA increased vitamin C levels in the present study. It is important to emphasize that rats can synthesize vitamin C in the liver, whereas humans absorb it from the diet [44]; thus, in the present study, supplementation may have induced the synthesis of vitamin C. To our knowledge, there are no previous studies on the influence of LA or ω-3 LCPUFAs on vitamin C synthesis, which needs to be investigated by future studies.

GSH is the most abundant intracellular antioxidant and is used as substrate for GPx and GST [41]. It can reduce peroxides, xenobiotics, and disulfide bonds of cysteines in cell proteins, serving as an electron donor. The present results showed that all ovariectomized-supplemented groups had increased GSH levels, but menopause had no effect on GSH synthesis. GST conjugates the reduced GSH to detoxify harmful endogenous compounds and xenobiotic substrates and can be reversibly activated by nitrosylation and irreversibly activated by nitration [41]. GST activity is similarly enhanced in all ovariectomized-supplemented groups, although it was not altered by ovariectomy. It is possible that GST was activated to help kidney cells cope with the high dose of the antioxidant supplementation, as explained previously. There is evidence that the analyzed supplements are able to increase GSH and GST. LA is known for activating the nuclear factor erythroid-2-related factor 2 [45], a transcription factor that induces GSH synthesis and

GST expression in the kidneys [46]. Treatment with DHA increases GSH and GST in fibroblast cells [47] and EPA supplementation also increased GSH levels in a rat model of kidney damage, but in contrast to the present results, SOD activity was increased [48]. Unfortunately, reports in kidney tissue are scarce and the previous studies did not provide data for menopausal women.

In contrast to GSH and GST results, neither ovariectomy nor supplementation had an effect on the activity of SOD, GPx, and hydrogen peroxide consumption. The hydrogen peroxide consumption assay is able to evaluate the ability of all hydrogen peroxide detoxifying enzymes together, including GPx, peroxiredoxins, and catalase. It is known that estrogen induces the expression of SOD, GPx, GST, and catalase through the antioxidant response element signaling pathway [41]. Although nuclear factor erythroid-2-related factor 2 regulates SOD, GPx, and GST mRNA expression, their enzymatic activity may be differentially regulated after transcription [41]. Therefore, we would suggest that kidney enzymatic defenses do not directly respond to an estrogen decrease or to the supplementation analyzed in the present study.

Fumarase catalyzes the conversion of L-malic acid to fumaric acid. In eukaryotes, fumarase is known to participate in the tricarboxylic acid cycle in the mitochondrial matrix, but is also required in the cytoplasm during the cellular response to DNA damage [49]. Mutation of the gene that encodes fumarase impairs its activity and leads to renal, skin, and uterus cancers [50]. We hypothesized that a decrease in fumarase activity may be linked with an increase in the risk for cancer after menopause. Contrary to our expectations, however, ovariectomy resulted in an increase of fumarase activity. To our knowledge, there are no previous studies relating menopause to fumarase activity in the kidney; however, in brain tissue and brain cells, fumarase is not altered by ovariectomy or estrogen [51,52]. For these reasons, we hypothesize that this increase is related to the cytoplasmic response of fumarase to DNA damage. Future studies will be necessary to verify this hypothesis. LA and ω-3 LCPUFA supplementation restored fumarase activity to Sham levels, which may reinforce our DNA damage hypothesis. Vitamin C, GSH, and GST are important cytoplasmic antioxidants; therefore, their increase after LA and ω-3 LCPUFA supplementation may be related to the response of fumarase to DNA damage.

The benefits of antioxidant supplementation are controversial. Our research group is analyzing the effect of LA and ω-3 LCPUFA on the redox system in dry eye disease [39], and in different tissues of the brain [16], liver [43], and heart (unpublished data). There is a clear tissue-dependent redox response in our experimental model of menopause and antioxidant supplementation. Although the same animals and the same dosage of ω-3 LCPUFAs and LA were used, dietary supplementation seems to be protective to some tissues but may be harmful to others. In our dry eye disease study, DHA, EPA, and LA supplementation improved the impaired tear production after ovariectomy, and supplementation with LA was able to fully restore tear production [39]. In the brain, dietary supplementation with LA showed a protective effect, leading to decreased protein carbonyls and MDA, whereas in contrast, DHA showed a prooxidant effect [16]. In the heart, ovariectomy increased vitamin C, GSH, and hydrogen peroxide consumption, and supplementation with DHA, EPA, and LA reduced the increased levels of these antioxidants, with LA also decreasing protein and lipid damage (unpublished data). In contrast to the present study, where LA had a prooxidant effect, in dry eye disease and brain and heart tissue, LA supplementation had an evident antioxidant effect. In contrast to the other analyzed organs, in the liver all antioxidants decreased protein damage with DHA presenting the most evident antioxidant effect, decreasing protein and lipid damage [43].

To our knowledge, there are no previous studies on the kidneys focusing on antioxidant supplementation in the rat ovariectomy model of menopause. The present data provides evidence that ovariectomy has no effect on the redox profile in the kidneys; however, LA and ω-3 LCPUFA supplementation increased a portion of the antioxidant defenses. Unexpectedly, at the doses applied in the present study, EPA supplementation may have a prooxidant effect on the kidneys.

Acknowledgments

The authors acknowledge Z. Almeida for technical assistance.

References

[1] Koebele SV, Bimonte-Nelson HA. Modleing menopause: the utility of rodents in translational behavioral endocrinology research. Maturitas 2016;87:5–17.
[2] Akcay T, Dincer Y, Kayali R, Colgar U, Oral E, Cakatay U. Effects of hormone replacement therapy on lipid peroxides and oxidation system in postmenopausal women. J Toxicol Environ Health A 2000;59:1–5.
[3] Mercantepe T, Unal D, Selli J, Mercantepe F, Unal B, Karabiyik TN. Protective effects of estrogen and bortezomib in kidney tissue of post-menopausal rats: an ultrastructural study. Ren Fail 2016;38:1129–35.
[4] Bednarek-Tupikowska G, Tupikowski K, Bidzinska B, Bohdanowicz-Pawlak A, Antonowicz-Juchniewicz J, Kosowska B, et al. Serum lipid peroxides and total antioxidant status in postmenopausal women on hormone replacement rtherapy. Gynecol Endocrinol 2004;19:57–63.
[5] Moiety FMS, Salem HA, Mehanna RA, Abdel-Ghany BS. Comparative study on induction and effects of surgical menopause in a female rat model: a prospective case control study. Int J Clin Exp Med 2015;8:9403–11.
[6] Yang XP, Reckelhoff JF. Estrogen, hormonal replacement therapy and cardiovascular disease. Curr Opin Nephrol Hypertens 2011;20:133–8.
[7] Miquel J, Ramirez-Bosca A, Ramirez-Bosca JV, Alperi JD. Menopause: a review on the role of oxygen stress and favorable effects of dietary antioxidants. Arch Gerontol Geriatr 2006;42:289–306.
[8] Gebauer SK, Psota TL, Harris WS, Kris-Etherton PM. N-3 fattyacid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. Am J Clin Nutr 2006;83:1526–35.
[9] Vericel E, Polette A, Bacot S, Calzada C, Lagarde M. Pro- and antioxidant activities of docosahexaenoic acid on human blood platelets. J Thromb Haemost 2003;1:566–72.
[10] Richard D, Kefi K, Barbe U, Bausero P, Visioli F. Polyunsaturated fatty acids as antioxidants. Pharmacol Res 2008;57:451–5.
[11] Bilska A, Wlodek L. Lipoic acid - the drug of the future? Pharmacol Rep 2005;57:570–7.
[12] Demir U, Demir T, Ilhan N. The protective effect of alpha-lipoic acid against oxidative damage in rabbit conjunctiva and cornea exposed to ultraviolet radiation. Ophthalmologica 2005;219:49–53.
[13] Goraca A, Huk-Kolega H, Piechota A, Kleniewska P, Ciejka E, Skibska B. Lipoic acid - biological activity and therapeutic potential. Pharmacol Rep 2011;63:849.
[14] Cakir T, Polat C, Basturk A, Gul M, Aslaner A, Durgut H, et al. The effect of alpha lipoic acid on rat kidneys in methotrexate induced oxidative injury. Eur Rev Med Pharmacol Sci 2015;19:2132–9.
[15] Cheung KL, Stefanick ML, Allison MA, LeBlanc ES, Vitolins MZ, Shara N. et al. Menopausal symptoms in women with chronic kidney disease. Menopause 2015;22:1006–11.
[16] Behling CS, Andrade AS, Putti JS, Mahl CD, Hackenhaar FS, da Silva AC, et al. Treatment of oxidative stress in brain of ovariectomized rats with omega-3 and lipoic acid. Mol Nutr Food Res 2015;59:2547–55.
[17] Hackenhaar FS, Salomon TB, Alabarse PVG, Ehrenbrink G, Benfato MS. Pulmonary antioxidant defences and protein damage during the ageing process of both sexes. Cell Biochem Funct 2009;27:378–82.
[18] Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, et al. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 1990;186:464–78.
[19] Karatepe M. Simultaneous determination of ascorbic acid and free malondialdehyde in human serum by HPLC-UV. Lc Gc N Am 2004;22:362–5.
[20] Mescam M, Vinnakota KC, Beard DA. Identification of the catalytic mechanism and estimation of kinetic parameters for fumarase. J Biol Chem 2011;286:21100–9.
[21] Aebi H. Catalase in vitro. Methods Enzymol 1984;105:121–6.
[22] Tsuchida S. Regulation of glutathione transferase expression and function in stress response. Seikagaku 2001;73:89–92.
[23] Barbas C, Castro M, Bonet B, Viana M, Herrera EJ. Simultaneous determination of vitamins A and E in rat tissues by high-performance liquid chromatography. Chromatogr A 1997;778:415–20.
[24] Rahman I, Kode A, Biswas SK. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. Nat Protoc 2006;1:3159–65.

[25] Bradford MM. Rapid and sensitive method for quantitation of microgram quantities of protein utilizing principle of protein-dye binding. Anal Biochem 1976;72:248–54.
[26] Baeza I, De Castro NM, Gimenez-Llort L, De la Fuente M. Ovariectomy, a model of menopause in rodents, causes a premature aging of the nervous and immune systems. J Neuroimmunol 2010;219:90–9.
[27] Cappellari GG, Losurdo P, Mazzucco S, Panizon E, Jevnicar M, Macaluso L, et al. Treatment with n-3 polyunsaturated fatty acids reverses endothelial dysfunction and oxidative stress in experimental menopause. J Nutr Biochem 2013;24:371–9.
[28] Chen C, Yu XF, Shao SY. Effects of omega-3 fatty acid supplementation on glucose control and lipid levels in type 2 diabetes: a meta-analysis. PLoS One 2015;10:e0139565.
[29] Mousavi SM, Shab-Bidar S, Kord-Varkaneh H, Khorshidi M, Djafarian K. Effect of alpha-lipoic acid supplementation on lipid profile: a systematic review and meta-analysis of controlled clinical trials. Nutrition 2019;59:121–30.
[30] Yang Y, Li W, Liu Y, Li Y, Gao L, Zhao JJ. Alpha-lipoic acid attenuates insulin resistance and improves glucose metabolism in high fat diet-fed mice. Acta Pharmacol Sin 2014;35:1285–92.
[31] Mendez L, Pazos M, Gallardo JM, Torres JL, Pérez-Jiménez J, Nogués R, et al. Reduced protein oxidation in Wistar rats supplemented with marine omega 3 PUFAs. Free Radic Biol Med 2013;55:8–20.
[32] Kris-Etherton PM, Grieger JA, Etherton TD. Dietary reference intakes for DHA and EPA. Prostaglandins Leukot Essent Fatty Acids 2009;81:99–104.
[33] Aliche-Djoudi F, Podechard N, Chevanne M, Nourissat P, Catheline D, Legrand P, et al. Physical and chemical modulation of lipid rafts by a dietary n-3 polyunsaturated fatty acid increases ethanol-induced oxidative stress. Free Radic Biol Med 2011;51:2018–30.
[34] Albert BB, Cameron-Smith D, Hofman PL, Cutfield WS. Oxidation of marine omega-3 supplements and human health. Biomed Res Int 2013;2013:464921.
[35] Song WL, Paschos G, Fries S, Reilly MP, Yu Y, Rokach J. et al. Novel eicosapentaenoic acid-derived F-3-isoprostanes as biomarkers of lipid peroxidation. J Biol Chem 2009;284:23636–43.
[36] Roy J, Fauconnier J, Oger C, Farah C, Angebault-Prouteau C, Thireau J, et al. Non-enzymatic oxidized metabolite of DHA, 4(RS)-4-F-4t-neuroprostane protects the heart against reperfusion injury. Free Rad Biol Med 2017;102:229–39.
[37] Fares M, Armand M, Francois C, Maixent JM. ω6/ω3 polyunsaturated fatty acid supplementations in renal cell model lead to a particular regulation through lipidome for preserved omega 6/omega 3 ratios. Cell Mol Biol 2012;58 (suppl):1715–9.
[38] Hagve TA, Woldseth B, Brox J, Narce M, Poisson JP. Membrane fluidity and fatty acid metabolism in kidney cells from rats fed purified eicosapentaenoic acid or purified docosahexaenoic acid. Scand J Clin Lab Invest 1998;58:187–94.
[39] Andrade AS, Salomon TB, Behling CS, Mahl CD, Hackenhaar FS, Putti J, et al. Alpha-lipoic acid restores tear production in an animal model of dry eye. Exp Eye Res 2014;120:1–9.
[40] Petronilho F, Florentino D, Danielski LG, Vieira LC, Martins MM, Vieira A. et al. Alpha-lipoic acid attenuates oxidative damage in organs after sepsis. Inflammation 2016;39:357–65.
[41] Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford, UK: Oxford University Press; 2015.
[42] Koo SI, Noh SK. Phosphatidylcholine inhibits and lysophosphatidylcholine enhances the lymphatic absorption of alpha-tocopherol in adult rats. J Nutr 2001;131:717–22.
[43] Schuller AK, Canata DAM, Hackenhaar FS, Engers VK, Heemann FM, Putti JS, et al. Effects of lipoic acid and n-3 long-chain polyunsaturated fatty acid on the liver ovariectomized rat model of menopause. Pharmacol Rep 2018;70:263–9.
[44] Gupta SD, Choudhury PK, Chatterjee IB. Synthesis of L-ascorbic-acid from D-glucurono-1,4-lactone conjugates by different species of animals. Int J Biochem 1973;4:309–14.
[45] Steele ML, Fuller S, Patel M, Kersaitis C, Ooi L, Münch G. Effect of Nrf2 activators on release of glutathione, cysteinylglycine and homocysteine by human U373 astroglial cells. Redox Biol 2013;1:441–5.
[46] Shelton LM, Park BK, Copple IM. Role of Nrf2 in protection against acute kidney injury. Kidney Int 2013;84:1090–5.
[47] Arab K, Rossary A, Flourie F, Tourneur Y, Steghens JP. Docosahexaenoic acid enhances the antioxidant response of human fibroblasts by upregulating gamma-glutamyl-cysteinyl ligase and glutathione reductase. Br J Nutr 2006;95:18–26.
[48] Fayez AM, Zaafan MA. Eicosapentaenoic acid and vitamin E against doxorubicin-induced cardiac and renal damages: role of cytochrome C and iNOS. Arch Iran Med 2018;21:502–8.
[49] Yogev O, Naamati A, Pines O. Fumarase: a paradigm of dual targeting and dual localized functions. Febs J 2011;278:4230–42.
[50] Sudarshan S, Linehan WM, Neckers L. HIF and fumarate hydratase in renal cancer. Br J Cancer 2007;96:403–7.
[51] Ramara A, Duckles SP, Krause DN, Procaccio V. Estrogen suppresses brain mitochondrial oxidative stress in female and male rats. Brain Res 2007;1176:71–81.
[52] Razmara A, Sunday L, Stirone C, Wang XB, Krause DN, Duckles SP, et al. Mitochondrial effects of estrogen are mediated by estrogen receptor alpha in brain endothelial cells. J Pharmacol Exp Ther 2008;325:782–90.