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Comparing the Effect of Flaxseed and Fish Oils on Acute Ischemia-Reperfusion Injury in the Rat Kidney

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Abstract

Objectives: Polyunsaturated fatty acids (PUFAs) are useful in reducing the deleterious effects of ischemia-reperfusion (IR). This study was designed to compare the impacts of treatment with flaxseed oil (FLO), rich in linoleic and alpha-linolenic acids, and fish oil containing long PUFAs, as well as eicosapentaenoic and docosahexaenoic acids (DHAs), on IR injury in the rat kidney.

Materials and Methods: In this experimental study, 32 male Wistar rats were randomly divided into 4 groups (8 rats each) including Sham, IR, FLO, and fish oil (FO). Correspondingly, experimental groups were administered saline and flaxseed or FO at doses of 0.4 g/kg by gavage. After 4 weeks, the rats underwent right nephrectomy and 45-minute clamping of the left renal arteries followed by 24 hours reperfusion. Renal function was assessed by measuring the serum creatinine (Cr) and blood urea nitrogen (BUN) levels. The oxidative stress and serum aspartate aminotransferase (AST) activity were measured. Each kidney was sectioned into 2 equal parts. One part was used for pathological evaluations after hematoxylin-eosin staining and the other one was applied in assaying the malondialdehyde (MDA) level.

Results: Serum Cr and BUN levels, AST activity, and tissue MDA content increased in the IR group. Both FO and FLO decreased tissue MDA levels ($P < 0.05$ vs. IR), but could not lead to a significant reduction in the levels of the renal markers. In addition, histological damages which were increased following the IR were markedly reduced by these 2 oils.

Conclusions: Generally, FLO and FO may provide protection against IR-induced renal injury and oxidative stress. However, these effects were not significant between the 2 supplementations.

Keywords: Renal ischemia-reperfusion, Oxidative stress, Fish oil, Flaxseed oil

Introduction

Renal ischemia-reperfusion (IR) injury is the primary cause of acute kidney damage (1). Several mechanisms including disturbances in the cellular Ca^{2+} metabolism, high levels of free radicals, and production of toxic lipid metabolites were proposed to be responsible in this respect (2).

Supplementations with omega-3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are the main (n-3) polyunsaturated fatty acid (PUFA) found in fish were found to have protective effects on IR-induced acute kidney failure (3).

Studies on the renal model of IR demonstrated that 4-week supplementation with EPA improved glomerular filtration rate (GFR) (4) while 8-day DHA supplementation decreased serum creatinine (Cr) level and histological damage (5) In addition, 2-week DHA + EPA treatment led to functional improvement, reduction in oxidative stress, and histological damages (6).

In addition to the above-mentioned long-chain fatty acids, alpha-linolenic acid (ALA) as an essential fatty acid has many useful effects. Zhao et al demonstrated that ALA ameliorates cardiovascular diseases by inhibiting vascular inflammation (7). Further, intake of ALA prevents coronary heart disease (8), and Finally, dietary ALA is associated with fewer coronary events (9). An animal study suggested that ALA has favorable effects on heart failure (10). Furthermore, the coexistence of ALA and lignans in plant oils might exert a better cardioprotective effect on the ALA (11). The cardioprotective activity of flaxseed oil (FLO) is widely accepted (12).

Plants are alternative sources of omega-3 fatty acids. Moreover, the plant flaxseed is the richest source of the essential fatty acids, linoleic acid, omega 6, ALA, omega 3 PUFA, and the most widely available n-3 PUFA for the humans (13). Voss et al reported that long chain nonessential omega-3 PUFAs can be produced from their precursor, namely, ALA, in mammalians (14).

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Additionally, flaxseed contains antioxidants (15) that could decrease the effect of reactive oxygen species (ROS) and prevent lipid oxidation (16).

Although long-chain n-3 fatty acids are beneficial to mammals in preventing IR-induced damages, the evidence is not comparable with the flaxseed. There are many areas in the world that are far from the sea or oceans coasts. The ALA-containing oils such as FLO have advantages over the EPA- and DHA-containing oils mainly fish oil (FO), in that high intake of ALA is possible due to its low cost and high availability. Accordingly, this study aimed to determine the effect of FLO supplementation on renal IR injury and to compare it with FO.

Materials and Methods

Animals

Male Wistar rats (250-300 g) were obtained from Gonabad University of Medical Sciences (Gonabad, Iran) and were maintained in an air-conditioned animal room on a light/dark (12/12 hours) cycle at 21-23°C having free access to food and tap water.

Experimental Design

A total of 32 rats were randomly assigned into 4 groups of 8 rats: Sham, IR, FLO, and FO. The rats in the experimental, FLO, FO, and IR groups received 0.4 g/kg of FLO (Barij essence, Iran), FO (Omega Flex, International Agencies, Davie, FL, USA) containing EPA 180 mg and DHA 120 mg/g and normal saline through gavage for 4 weeks, respectively. Then, the rats were anesthetized using ketamine (100 mg/kg) and xylazine (10 mg/kg) and after right nephrectomy, the left renal pedicle was occluded for 45 minutes using a vascular clamp. The clamp was then released and reperfusion was established subsequently. The same protocol was followed for the sham group, but the kidneys remained intact. After 24 hours, all the rats of the groups were anesthetized again. After an incision on abdominal skin, blood samples were obtained from their left ventricle and centrifuged at 4000 RPM for 10 minutes, and their serum was kept at -80°C until assay. Further, the left kidneys were removed, cut longitudinally, and divided into 2 sections. One part was fixed in 10% formalin (Sigma-Aldrich) and used for histological evaluation and the other part was snap frozen, powdered under liquid nitrogen, and stored at -80°C for further investigations including assay of malondialdehyde (MDA).

Measurement of Serum Parameters

Serum Cr and blood urea nitrogen (BUN) levels and aspartate aminotransferase (AST) activity were measured by commercial kits (Pars Azmoon, Iran).

Assay of MDA Levels in Renal Tissue

All the chemicals were prepared from the Sigma-Aldrich (Sigma, St. Louis, MO, USA). The MDA levels as an index of lipid peroxidation level in renal tissue were measured according to Ohkawa et al (17) in order to evaluate the oxidative stress status. Briefly, the powdered tissues were homogenized in 1.15 % KCl in a ratio of 10 % w/v and then the homogenates were centrifuged at 10 000 RPM for 10 minutes. The supernatant of each sample was then used for MDA evaluation. The reaction mixture containing 100 µL sample, 100 µL 8.1% sodium dodecyl sulfate (SDS), and 750 µL 20% acetic acid solution with a pH of 3.5 was adjusted by adding NaOH, 750 µL 0.8% solution of TBA, and finally 300 µL distilled water (DW) was heated at 95°C for 60 minutes. After cooling with tap and addition of 500 µL DW, an equal volume of n-butanol and pyridine mixture (15: 1, v/v) was added and the mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 minutes, the absorbance of the upper layer was measured at 532 nm. The levels of MDA in tissues were calculated using its extinction coefficient of the MDA adduct (1.56×10^5 $cm^{-1}M^{-1}$).

Measurement of Superoxide Dismutase and Glutathione Peroxidase Activity

Superoxide dismutase (SOD) and glutathione peroxidase activity (GPX) in serum were assayed in accordance with the protocols of the kits which was used (Randox, UK).

Histological Assessment

Three sections were prepared from each sample and stained with hematoxylin and eosin (H & E). Histological evaluation and grading of kidney damage included tubular cell necrosis, number of erythrocytes in glomerular capillaries, intracellular vacuolization, vascular congestion, and casts formation.

Sections were graded as follows: (1) <25% of tubules (0), (2) 25%–50% of tubules (minimal), (3) > 50% of tubules (no lesions) were involved. The total histopathological score, namely, the sum of all the scores regarding different damages were calculated.

Data Analysis

Kolmogorov–Smirnov test was implemented to determine if the data were normally distributed. Furthermore, the one-way ANOVA test was performed followed by Tukey post hoc test in order to compare the kidney function parameters and oxidative stress values. Moreover, the nonparametric tests including Kruskal-Wallis and Mann-Whitney tests were used to compare the total histopathological scores. In all comparisons, statistical significance levels were considered $P < 0.05$ and all data were presented as mean and standard error (M ± SE).

Results

Biochemical Assays

In this study, renal IR significantly increased ($P < 0.05$) serum Cr (1.54 ± 0.124 mg/dL) and BUN levels (53.307 ± 6.43 mg/dL) compared to the sham group (1.06 ± 0.32 & 23.07 ± 0.81, respectively). Although the 4-week treatment

with FLO and FO decreased serum Cr and BUN levels, these differences were not significant compared to the IR group, (Figure 1A-1B).

Additionally, AST activity in the serum of IR group was higher than that of the sham group ($P < 0.05$). There were no significant differences among the other groups in serum AST activities (Figure 2). As Figure 3 illustrates, IR in its related group significantly increased renal MDA contents compared to the Sham group (690 ± 189.868 nmol/g of wet tissue, vs. 265 ± 33.78 nmol/g, $P < 0.05$). However, FLO and FO significantly reduced the MDA values in the corresponding groups compared to the IR group (FLO group, 315 ± 65.357 nmol/g & FO group, 270 ± 23.944 nmol/g vs. IR group, 690 ± 135.97 nmol/g, both $P < 0.05$). The decreasing effect of FLO on renal MDA content was not the same as that of the FO. However, the MDA value of the kidney tissue in the FLO group was not significantly different from that of the FO group. In addition, the level of SOD decreased but not significantly in all the other groups as compared to the sham group (Figure 4). There was no significant difference between the FLO and FO groups as compared to the IR group in this regard. Finally, there was no significant difference among the groups in the level of GPX (Figure 5).

Evaluation of Renal Histology

The results of histological studies are summarized in Table 1 and Figure 6. Sham group demonstrated no histological damages (Figure 6A). The number of erythrocytes decreased in the glomerular capillaries located in the cortex of the IR group. Further, epithelial cells in the proximal tubules were vacuolated and exfoliated into the lumens. FLO and FO reduced these changes except for vacuolization in the FLO and FO groups. In the medulla of the IR group (Figure 6B), vascular congestion and intra-tubular cast formation had higher scores compared to the FLO and FO groups (Figure 6C-6D, respectively). Furthermore, the total histopathological score significantly increased in the IR group compared to the sham group ($P < 0.05$). Although this score reduced in FLO and FO groups compared to the IR group (both $P < 0.05$), its amount was still significantly different from that of the sham group ($P < 0.05$), the obtained data are represented in Table 1.

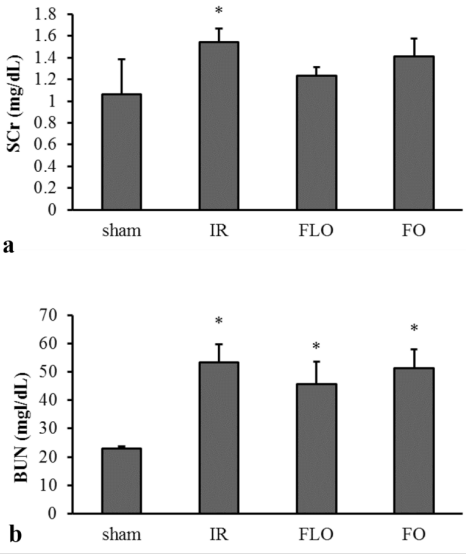

Figure 1. Renal Functional Assessment at the End of the Reperfusion Period in Different Investigated Rat Groups.
Abbreviations: SCr, serum creatinine; BUN, blood urea nitrogen.
Data are expressed as M ± SEM. Groups (n = 8): Sham; IR: Ischemia-reperfusion; FLO: Flaxseed oil; FO: Fish oil (gavage, 0.4 g/kg/d for 4 weeks before the ischemia).* $P < 0.05$ vs. sham.

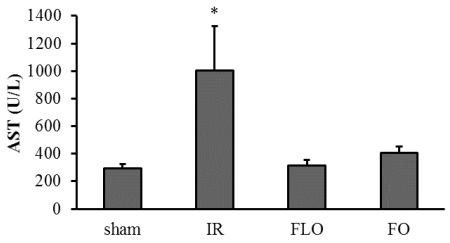

Figure 2. Serum AST Level at the End of the Reperfusion Period in Different Investigated Rat Groups.
Data are expressed as M ± SEM. Groups (n = 8): Sham; IR: Ischemia-reperfusion; FLO: Flaxseed oil; FO: Fish oil (gavage, 0.4 g/kg/d for 4 weeks before the ischemia).* $P < 0.05$ vs. Sham.

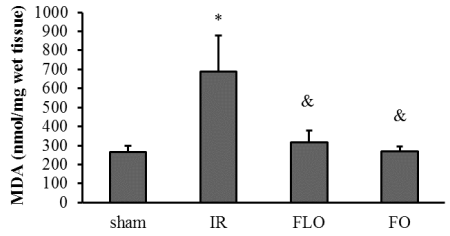

Figure 3. Tissue MDA Content at the End of the Reperfusion Period in Different Investigated Rat Groups.
Data are expressed as M ± SEM. Groups (n = 8): Sham; IR: Ischemia-reperfusion; FLO: Flaxseed oil; FO: Fish oil (gavage, 0.4 g/kg/d for 4 weeks before the ischemia).* $P < 0.05$ vs. sham and & $P < 0.05$ compared to the IR group.

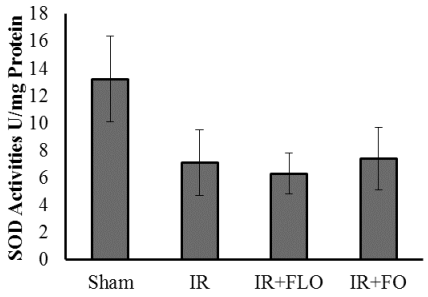

Figure 4. Tissue SOD Activity at the End of the Reperfusion Period in Different Investigated Rat Groups.
Data are expressed as M ± SEM. A comparison of the SOD in Sham; IR: Ischemia-reperfusion; FLO: Flaxseed oil; FO: Fish oil (gavage, 0.4 mg/kg/d for 4 weeks before the ischemia). *$P < 0.05$ vs. sham.

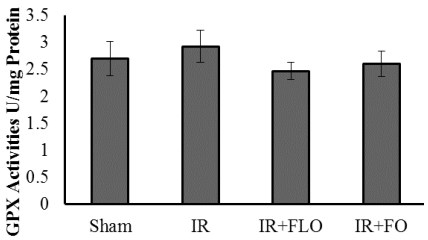

Figure 5. Tissue GPX Activity at the End of the Reperfusion Period in Different Investigated Rat Groups.
Data are expressed as M ± SEM. A comparison of the GPX in Sham; IR: Ischemia-reperfusion; FLO: Flaxseed oil; FO: Fish oil (gavage, 0.4 mg/kg/d for 4 weeks before the ischemia). *$P < 0.05$ vs. sham.

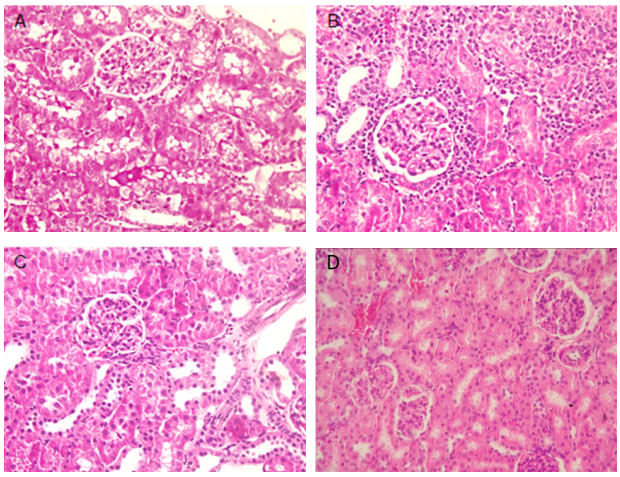

Figure 6. Renal histological changes. Renal histological changes including intracellular vacuolization, tubular necrosis, tubular cast, and vascular congestion in the (A) Sham, (B) Ischaemia–reperfusion (IR), (C) FLO, and (D) FO groups (hematoxylin-eosin, ×400).

Discussion

The results indicated that flaxseed and FOs could be beneficial in reducing IR-induced renal injury. It was found that the 4-week treatment with FLO and FO reduced histological and oxidative stress damages. However, these effects were not accompanied by significant attenuation in plasma levels of Cr and BUN and AST activity.

In this study, increases in Cr and BUN levels and histological damages after IR is probably related to a significant decrease in renal function. This effect is assumed to be due to an increase in oxidative stress found in the present study in which MDA content significantly increased as demonstrated by the other researchers (18-20). Moreover, SOD level, as an antioxidant, decreased in IR groups compared to the sham group. However, such a reduction was not significant.

Additionally, although the rats which received the FLO and FO supplements demonstrated reduced Cr and BUN levels following the renal IR, this decline was not statistically significant, which is probably related to the reperfusion period since in the study of Tucci Junior et al, Cr and BUN levels decreased seven days after the reperfusion (21). In addition, it was found that dietary supplementation with n-3 PUFAs, DHA, and EPA decreased IR-induced functional and histological injury in the hippocampus after 10 days (22). Another possible explanation for such an effect is the renal compensatory impact by which Cr and BUN levels could not rise to such a level in the IR group so that their decreases in oil-treated groups became significant (23).

Further, based on the results, FLO and FO in treated rats decreased the levels of MDA content. This is in agreement with the findings of the other studies (18,19). However, in line with our previous study, SOD and GPX levels in treated groups indicated no differences (24). This would be due to the histological improvement of the kidney as demonstrated by other studies in which omega-3 restored the antioxidant systems of the tissues (25,26). Several studies found that DHA had anti-inflammatory and immunosuppressive effects (27,28) and could decrease damages induced by tissue reperfusion in rats and dogs (5,18,29).

Histological evaluation revealed that IR-mediated renal injury resulted in significant tubular necrosis, cell vacuolization, decreased number of erythrocytes in the cortex glomerular capillaries, intra-tubular cast formation,

Table 1. Histopathological Scores

Kidney Areas	Damage Marker	Histopathological Score			
		Sham	IR	FLO	FO
Cortex	Reduced number of erythrocytes in glomerular capillaries	0	2	1	1
	Tubular cells necrosis	0	3	1	1
	Intracellular vacuolization	0	2	2	2
Medulla	Intra-tubular proteinaceous casts	0	2	1	1
	Vascular congestion	0	2	1	1
Total score		0	11*	6*&	6*&

Groups (n = 8): Sham; IR: Ischemia-reperfusion; FLO: Flaxseed oil; FO: Fish oil.
Sections were graded as follows: 0, minimal, or no lesions; (1) < 25% of tubules; (2) 25–50 % of tubules; (3) > 50 % of tubules were involved. Both Sham (*P) and IR (&P) were significant at < 0.05.

and medullar congestion in the IR group, which is in line with the findings of the other studies (18,19). Based on the results of the other studies, pathological changes including the release of ROS (30) and apoptosis, as well as necrosis (31,32) are considered as the main results of IR-mediated injury of the kidney. Cellular ATP depletion, disruption in mitochondria function, inflammatory processes activation, and decreased sodium/calcium exchange were proposed as probable mechanisms of IR-induced injury.

A less histological injury which was observed in the FLO and FO-treated rats compared to the IR received rats, are possibly due to the decreased oxidative stress, functional improvement of mitochondria in maintaining the myocardial ATP levels, and therefore preventing the apoptosis (33-35).

The FLO in the FLO group could decrease the MDA level to the level similar to that of FO in the FO group but to a lesser extent. In this case, flax lignans in FLO could contribute to attenuated oxidative stress (36).

Furthermore, several studies found that high intake of ALA, which is the main fatty acid in FLO, prevents coronary heart disease (8,9,37,38), and may have favorable effects on heart failure (10). Moreover, in the current study, the corresponding rats were supplemented with 0.4 g/kg of both oils whereas based on molecular weights of these 2 fatty acids (ALA in FLO & EPA & DHA in FO), this concentration makes different molar levels of fatty acids in the corresponding groups, which partially explains the different protection effects. It is reasonable that the higher is the level of n-3 PUFAs in the body, the further would be the production of MDA as an oxidized product of n-3 PUFAs. Higher content of n-3 PUFAs in FLO may justify a higher level of MDA in the FLO group compared to the FO group.

Previous studies demonstrated that a considerable amount of ALA, about 0.7% in humans, in comparison with the only 0.1% of DHA, incorporates into adipose tissue. Additionally, partitioning the dietary ALA into the β-oxidation pathway of mitochondria in men is about 30%. These findings reveal that availability of ALA to the site of IR-induced injury might be less compared to the DHA received by the rats. Similar results were observed in women by increased availability of ALA for conversion to longer chain PUFA compared to men, due to their lower muscle mass up-taking ALA for β-oxidation (39).

There is a positive linear relationship between ALA ingestion and DHA cell content. Due to the common pathway for the conversion of linoleic acid (LA) and ALA into long-chain PUFAs (arachidonic acid & EPA/DHA, respectively), increases in LA content of diet may decrease the content of DHA. Since there are additional reactions involved in the synthesis of DHA from EPA, increases in synthesis of EPA from ALA would not normally result in sufficient DHA synthesis. In addition, based on previous studies, the overall efficiencies of conversion of ALA into EPA and DHA are only 0.2 and 0.05%, respectively. Due to the feedback inhibition regarding conversing ALA into EPA and DHA by these 2 fatty acids, higher intake of EPA and DHA could inhibit the mentioned pathway. Totally, due to the restriction of ALA conversion into EPA, especially DHA, a diet lacking EPA and DHA would be sufficient to maintain the cellular requirement only when the demand for these 2 long PUFAs are not high (39).

Conclusions

Generally speaking, the results of the present study revealed that both FLO and FO provided protection against IR-induced renal injury and oxidative stress. However, these effects were not significant between the 2 supplementations.

Conflict of Interests

Authors have no conflict of interests.

Ethical Issues

All the experiments were performed in accordance with the institutional guidelines of the Research Ethics Committee of Gonabad University of Medical Sciences with the allocated code of 92.1145.9-2013.06.06. In addition, the guidelines of the National Ethics Committee of the Ministry of Health and Medical Education for the care and use of laboratory animals were observed.

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References

1. Yamamoto S, Hagiwara S, Hidaka S, et al. The antioxidant EPC-K1 attenuates renal ischemia-reperfusion injury in a rat model. Am J Nephrol. 2011;33(6):485-490. doi:10.1159/000327820
2. Gezici A, Ozturk H, Ozturk H. The effects of mibefradil, a T-type Ca2+ channels blocker, on the renal dysfunction and injury caused by ischemia-reperfusion of the rat kidney. Ren Fail. 2005;27(6):775-781.
3. Donadio JV Jr. Omega-3 polyunsaturated fatty acids: a potential new treatment of immune renal disease. Mayo Clin Proc. 1991;66(10):1018-1028.
4. Torras J, Soto K, Riera M, et al. Changes in renal hemodynamics and physiology after normothermic ischemia in animals supplemented with eicosapentaenoic acid. Transpl Int. 1996;9 Suppl 1:S455-459.
5. Kielar ML, Jeyarajah DR, Zhou XJ, Lu CY. Docosahexaenoic acid ameliorates murine ischemic acute renal failure and prevents increases in mRNA abundance for both TNF-alpha and inducible nitric oxide synthase. J Am Soc Nephrol. 2003;14(2):389-396.
6. Ajami M, Eghtesadi S, Habibey R, et al. Effect of short and long-term treatment with omega-3 Fatty acids on scopolamine-induced amnesia. Iran J Pharm Res. 2012;11(2):533-540.

7. Zhao G, Etherton TD, Martin KR, West SG, Gillies PJ, Kris-Etherton PM. Dietary alpha-linolenic acid reduces inflammatory and lipid cardiovascular risk factors in hypercholesterolemic men and women. J Nutr. 2004;134(11):2991-2997. doi:10.1093/jn/134.11.2991
8. Campos H, Baylin A, Willett WC. Alpha-linolenic acid and risk of nonfatal acute myocardial infarction. Circulation. 2008;118(4):339-345. doi:10.1161/circulationaha.107.762419
9. Baylin A, Kabagambe EK, Ascherio A, Spiegelman D, Campos H. Adipose tissue alpha-linolenic acid and nonfatal acute myocardial infarction in Costa Rica. Circulation. 2003;107(12):1586-1591. doi:10.1161/01.cir.0000058165.81208.c6
10. Fiaccavento R, Carotenuto F, Minieri M, et al. Alpha-linolenic acid-enriched diet prevents myocardial damage and expands longevity in cardiomyopathic hamsters. Am J Pathol. 2006;169(6):1913-1924.
11. Abdel-Moneim AE, Dkhil MA, Al-Quraishy S. The redox status in rats treated with flaxseed oil and lead-induced hepatotoxicity. Biol Trace Elem Res. 2011;143(1):457-467. doi:10.1007/s12011-010-8882-z
12. Rodriguez-Leyva D, Dupasquier CM, McCullough R, Pierce GN. The cardiovascular effects of flaxseed and its omega-3 fatty acid, alpha-linolenic acid. Can J Cardiol. 2010;26(9):489-496.
13. Connor WE. Alpha-linolenic acid in health and disease. Am J Clin Nutr. 1999;69(5):827-828. doi:10.1093/ajcn/69.5.827
14. Voss A, Reinhart M, Sankarappa S, Sprecher H. The metabolism of 7,10,13,16,19-docosapentaenoic acid to 4,7,10,13,16,19-docosahexaenoic acid in rat liver is independent of a 4-desaturase. J Biol Chem. 1991;266(30):19995-20000.
15. Moran L, Andres S, Bodas R, et al. Antioxidants included in the diet of fattening lambs: Effects on immune response, stress, welfare and distal gut microbiota. Anim Feed Sci Technol. 2012;173(3-4):177-185. doi:10.1016/j.anifeedsci.2012.01.010
16. Cortinas L, Barroeta A, Villaverde C, Galobart J, Guardiola F, Baucells MD. Influence of the dietary polyunsaturation level on chicken meat quality: lipid oxidation. Poult Sci. 2005;84(1):48-55. doi:10.1093/ps/84.1.48
17. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-358.
18. Ajami M, Davoodi SH, Habibey R, Namazi N, Soleimani M, Pazoki-Toroudi H. Effect of DHA+EPA on oxidative stress and apoptosis induced by ischemia-reperfusion in rat kidneys. Fundam Clin Pharmacol. 2013;27(6):593-602. doi:10.1111/j.1472-8206.2012.01066.x
19. Ashtiyani SC, Najafi H, Kabirinia K, Vahedi E, Jamebozorky L. Oral omega-3 fatty acid for reduction of kidney dysfunction induced by reperfusion injury in rats. Iran J Kidney Dis. 2012;6(4):275-283.
20. Abtahi-Evari SH, Shokoohi M, Abbasi A, Rajabzadeh A, Shoorei H, Kalarestaghi H. Protective effect of Galega officinalis extract on Streptozotocin-induced kidney damage and biochemical factor in diabetic rats. Crescent J Med Biol Sci. 2017;4(3):108-114.
21. Tucci Junior S, Carvalho RM, Celini FM, et al. Renal ischemia and reperfusion injury: influence of chorpromazine on renal function and lipid peroxidation. Acta Cir Bras. 2008;23 Suppl 1:42-46; discussion 46.
22. Ajami M, Eghtesadi S, Razaz JM, et al. Expression of Bcl-2 and Bax after hippocampal ischemia in DHA + EPA treated rats. Neurol Sci. 2011;32(5):811-818. doi:10.1007/s10072-011-0621-5
23. Tucci S Jr, Molina CA, Cologna AJ, et al. Compensatory renal growth and mitochondrial function: the influence of warm ischemia and reperfusion. Acta Cir Bras. 2008;23 Suppl 1:31-35; discussion 35.
24. Moghimian M, Soltani M, Abtahi H, Adabi J, Jajarmy N. Protective effect of tunica albuginea incision with tunica vaginalis flap coverage on tissue damage and oxidative stress following testicular torsion: Role of duration of ischemia. J Pediatr Urol. 2016;12(6):390.e391-390.e396. doi:10.1016/j.jpurol.2016.06.002
25. Xi S, Chen LH. Effects of dietary fish oil on tissue glutathione and antioxidant defense enzymes in mice with murine aids. Nutr Res. 2000;20(9):1287-1299. doi:10.1016/S0271-5317(00)00214-1
26. An WS, Kim HJ, Cho KH, Vaziri ND. Omega-3 fatty acid supplementation attenuates oxidative stress, inflammation, and tubulointerstitial fibrosis in the remnant kidney. Am J Physiol Renal Physiol. 2009;297(4):F895-903. doi:10.1152/ajprenal.00217.2009
27. Tomobe YI, Morizawa K, Tsuchida M, Hibino H, Nakano Y, Tanaka Y. Dietary docosahexaenoic acid suppresses inflammation and immunoresponses in contact hypersensitivity reaction in mice. Lipids. 2000;35(1):61-69.
28. De Caterina R, Liao JK, Libby P. Fatty acid modulation of endothelial activation. Am J Clin Nutr. 2000;71(1 Suppl):213s-223s. doi:10.1093/ajcn/71.1.213S
29. Neumayer HH, Heinrich M, Schmissas M, Haller H, Wagner K, Luft FC. Amelioration of ischemic acute renal failure by dietary fish oil administration in conscious dogs. J Am Soc Nephrol. 1992;3(6):1312-1320.
30. Maenpaa CJ, Shames BD, Van Why SK, Johnson CP, Nilakantan V. Oxidant-mediated apoptosis in proximal tubular epithelial cells following ATP depletion and recovery. Free Radic Biol Med. 2008;44(4):518-526. doi:10.1016/j.freeradbiomed.2007.10.040
31. Havasi A, Borkan SC. Apoptosis and acute kidney injury. Kidney Int. 2011;80(1):29-40. doi:10.1038/ki.2011.120
32. Bonegio R, Lieberthal W. Role of apoptosis in the pathogenesis of acute renal failure. Curr Opin Nephrol Hypertens. 2002;11(3):301-308.
33. Stanley WC, Recchia FA, Lopaschuk GD. Myocardial substrate metabolism in the normal and failing heart. Physiol Rev. 2005;85(3):1093-1129. doi:10.1152/physrev.00006.2004
34. Neubauer S. The failing heart--an engine out of fuel. N Engl J Med.2007;356(11):1140-1151.doi:10.1056/NEJMra063052
35. Abdel Moneim AE. Flaxseed oil as a neuroprotective agent on lead acetate-induced monoamineric alterations and neurotoxicity in rats. Biol Trace Elem Res. 2012;148(3):363-370. doi:10.1007/s12011-012-9370-4
36. Prasad K. Hypocholesterolemic and antiatherosclerotic effect of flax lignan complex isolated from flaxseed. Atherosclerosis. 2005;179(2):269-275. doi:10.1016/j.

atherosclerosis.2004.11.012
37. Gebauer SK, Psota TL, Harris WS, Kris-Etherton PM. n-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. Am J Clin Nutr. 2006;83(6 Suppl):1526s-1535s. doi:10.1093/ajcn/83.6.1526S
38. Djousse L, Arnett DK, Carr JJ, et al. Dietary linolenic acid is inversely associated with calcified atherosclerotic plaque in the coronary arteries: the National Heart, Lung, and Blood Institute Family Heart Study. Circulation. 2005;111(22):2921-2926. doi:10.1161/circulationaha.104.489534
39. Burdge GC, Calder PC. Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. Reprod Nutr Dev. 2005;45(5):581-597. doi:10.1051/rnd:2005047