Effects of Omega-3 Fatty Acid Supplementation on Inflammatory Cytokines and Advanced Glycation End Products in Patients With Diabetic Nephropathy

A Randomized Controlled Trial

Seyyed Mehdi Mirhashemi,[1,2] Faeze Rahimi,[1] Alireza Soleimani,[3] Zatollah Asemi[1]

[1]Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, Iran
[2]Department of Clinical Biochemistry and Genetics, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran
[3]Department of Internal Medicine, School of Medicine, Kashan University of Medical Sciences, Kashan, Iran

Keywords. omega-3 fatty acids, diabetic nephropathy, inflammatory markers, advanced glycation end products

Introduction. This study was performed to evaluate the effects of omega-3 fatty acid supplementation on inflammatory cytokines and advanced glycation end products (AGEs) in patients with diabetic nephropathy (DN).

Materials and Methods. This randomized double-blind placebo-controlled trial was done on 60 patients with DN who were randomly divided into 2 groups to receive either 1000 mg/d of omega-3 fatty acid from flaxseed oil (n = 30) or placebo (n = 30) for 12 weeks. The primary outcome variables were tumor necrosis factor-α, receptor tumor necrosis factor-α and growth differentiation factor 15. Fasting blood samples were taken at the onset and the end of the study to quantify the related markers.

Results. Compared with the placebo, omega-3 fatty acid supplementation resulted in a significant decrease in serum AGEs (-2.3 ± 2.8 AU versus 0.2 ± 2.5 AU, P = .001). Despite a significant reduction in serum level of receptor for AGEs (-0.1 ± 0.3 AU, P = .02) in the omega-3 fatty acid group, no significant difference was found between the two groups in terms of their effects on the receptor for AGEs. Supplementation with omega-3 fatty acid had no significant effect on the inflammatory cytokines as compared with the placebo.

Conclusions. Our study demonstrated that omega-3 fatty acid supplementation among DN patients had favorable effects on AGEs and the receptor for AGEs.

IJKD 2016;10:197-204
www.ijkd.org

Original Paper

INTRODUCTION

Diabetic nephropathy (DN) is one of the most devastating complications of diabetes mellitus and accounts for a significant increase in morbidity and mortality.[1] Previous studies have demonstrated that inflammatory cytokines, including tumor necrosis factor-α (TNF-α) is directly involved in the pathogenesis of DN by tissue damage and albuminuria related to a enhanced stimuli for overexpression of TNF-α.[2] In addition, the accumulation of advanced glycation end products (AGEs) and increased inflammatory cytokines play an important role in diabetes complications.[3,4] Extracellular AGEs, through their interaction with receptor for AGEs (RAGEs), and intracellular AGEs can result in increased oxidative stress and generation of reactive oxygen species and activation of protein kinase C.[5]

Despite recent advancements in improving inflammatory cytokines and AGEs of DN patients, no single medication has thus far been able to correct

inflammatory state and AGEs. Several published studies have recently exhibited the beneficial effects of omega-3 fatty acids supplementation in DN patients, with most focusing on metabolic profiles.[6,7] However, some studies have evaluated the effects of omega-3 fatty acids supplementation on inflammatory cytokines in patients without DN, the results of which are conflicting. It is well established that omega-3 fatty acids supplementation can improve inflammatory cytokines and RAGEs expression.[8,9] However, some researchers did not observe the beneficial effects of omega-3 fatty acids administration on inflammatory markers.[10,11] These discrepancies in various studies may be explained by the different population studies, the origin of omega-3 fatty acids, dosage of used omega-3 fatty acids, and duration of treatment.

Omega-3 fatty acids intake may reduce inflammatory cytokines and AGEs through nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) activation by increasing peroxisome proliferator-activated receptor-γ (PPAR-γ) gene expression in a chain length-dependent manner.[12-14] We hypothesized that omega-3 fatty acids supplementation may have a beneficial effect on inflammatory cytokines, AGEs, and RAGEs in patients with DN. This clinical trial was to assay the effects of omega-3 fatty acid supplementation on inflammatory cytokines, AGEs and RAGEs among DN patients.

MATERIALS AND METHODS

Participants

In a randomized double-blind placebo-controlled trial, we selected 60 patients with DN referred to Akhavan Clinic in Kashan, Iran, from April 2015 to July 2015. We defined DN as diabetic renal disease with a proteinuria level greater than 0.3 g/24 h, with or without elevation of serum creatinine levels.[15] To determine the sample size, we used the standard formula suggested for clinical trials considering a type 1 error of 0.05 and a type 2 error of 0.20 (power = 80%). Based on a previous randomized controlled trial,[8] we used 0.20 ng/mL as the standard deviation and 0.16 ng/mL as the change in the mean value of TNF-α as the key variable. Based on these, we planned to reach 25 patients in each group. Assuming 5 dropouts in each group, the final sample size was determined to be 30 participants per group.

Inclusion and Exclusion Criteria

Eligible participants were patients with DN aged 45 to 85 years old. The exclusion criteria were the intake of omega-3 fatty acid supplements within the past 3 months, uncontrolled diabetes mellitus (hemoglobin A1c [HbA1c], > 7.5%; fasting plasma glucose, > 126 mg/dL; and blood glucose 2-hour postprandial, > 180 mg/dL), current use of warfarin, malignancy, liver cirrhosis, and smoking.

Ethics Statements

This study was conducted in accordance with the Declaration of Helsinki. Participants were informed about the nature and purpose of the study and signed an informed consent form. The study protocol was approved by the ethics committee of Kashan University of Medical Sciences and was registered in the Iranian Registry of Clinical Trials (IRCT201504095623N41).

Study Design

The participants were randomly divided into 2 groups to receive either 1000 mg/d of omega-3 fatty acid from flaxseed oil or placebo for 12 weeks. The appearance of the placebo was indistinguishable in color, shape, size, packaging, smell, and taste from the omega-3 fatty acid capsule. Omega-3 fatty acid supplements and placebos were produced by Barij Pharmaceutical Company (Kashan, Iran). Randomization assignment was performed by the use of computer-generated random numbers. Randomization and allocation were concealed from the researchers and participants until the main analyses were completed. The randomized allocation sequence, enrolling, and allocating participants to the interventions were conducted by a trained nutritionist at the clinic. At the onset of the study, the participants were requested not to change their routine physical activity or usual dietary intakes throughout the study and not to consume any supplements other than the one provided to them by the investigators as well as not to take any medications that might affect the study results during the 12-week intervention. All of the patients completed 3-day food records and 3 physical activity records at the study baseline, during the study (weeks 3, 6, and 9), and at the end of the study. Physical activity was defined as metabolic equivalents in hours per day. To quantify the metabolic equivalents for each patient, we

multiplied the times (in hour per day) reported for each physical activity by its related metabolic equivalents coefficient by standard tables.[16] Daily macro- and micro-nutrient intakes were analyzed by nutritionist IV software (First Databank, San Bruno, CA).

Treatment Adherence

Every 4 weeks, the patients were given enough supplements to last 3 days after their next scheduled visit and were instructed to return all unused supplements at each visit. The remaining supplements were counted and subtracted from the number provided to determine the number taken. To increase the compliance, all of the patients were receiving short messages on their cell phones to take the supplements every day.

Assessment of Anthropometric Measures

Weight and height of the patients were recorded in an overnight fasting status using a standard scale (Seca, Hamburg, Germany) at study baseline and after a 12-week treatment period. All anthropometric measures were conducted by a trained nutritionist who was blinded to the randomization assignments.

Outcomes

The primary outcome variables were inflammatory cytokines, and the secondary outcome variables were AGEs and RAGEs concentrations. Before the onset and after 12 weeks of intervention, 5-mL blood samples were taken in a fasting status from each participant at the Kashan University of Medical Sciences Reference Laboratory. To separate the serum, blood samples were immediately centrifuged (Hettich D-78532, Tuttlingen, Germany) at 3500 rpm for 10 minutes. Then, we stored the samples at -70°C before the final analysis. Serum TNF-α and TNF-α receptor levels were quantified using an enzyme-linked immunosorbent assay kit (Bioassy Technology Laboratory, Shanghai, China) with inter- and intra-assay coefficient variances of 6.5% and 8.5% for TNF-α and 6.7% and 8.9% for TNF-α receptor, respectively. Serum growth differentiation factor 15 concentrations were determined by the use of an enzyme-linked immunosorbent assay kit (Bioassy Technology Laboratory, Shanghai, China) with inter- and intra-assay coefficient variances of 7.1% and 9.3%, respectively. Serum AGEs were assessed by the fluorometeric method with inter- and intra-assay coefficient variances of 3.9% and 4.8%. Serum RAGEs concentrations were quantified using an enzyme-linked immunosorbent assay kit (Bioassy Technology Laboratory, Shanghai, China) with inter- and intra-assay coefficient variances of 7.3% and 9.4%, respectively. The HbA1c levels in the whole blood were determined by a Glycomat kit (BiocodeHycel, Massy, France) using the method of exchange chromatography. However, effects of omega-3 fatty acid supplementation on HbA1c levels were not evaluated. Data on HbA1c at study baseline were taken from the records of the patients available at the clinic. Enzymatic kits (Pars Azmun, Tehran, Iran) were used to determine fasting plasma glucose and serum creatinine concentrations (Jaffe method) with intra- and inter-assay coefficient variances less than 5%.

Statistical Analyses

We conducted the Kolmogrov-Smirnov test to assess the normality of distribution of variables. The analyses were performed based on the intention-to-treat principle. To detect differences in anthropometric measures as well as in daily dietary intakes between the two groups, we used independent samples Student *t* test. Data on AGEs, RAGEs, and inflammatory cytokines are presented as mean ± standard deviation. To determine the effects of omega-3 fatty acid administration on AGEs, RAGEs, and inflammatory cytokines, we used the 1-way repeated measures analysis of variance. In this analysis, the treatment (omega-3 fatty acid versus placebo group) was regarded as the between-subject factor and time, with 2 time-points (the beginning of the study and after 12-week intervention), was considered as the within-subject factor. To examine if age, baseline body mass index and baseline values of the biochemical parameters influenced our findings, we applied the analysis of covariance controlling for these variables. *P* values less than .05 were considered significant. All statistical analyses were done by the use of the Statistical Package for Social Science, version 18 (SPSS Inc, Chicago, Illinois, USA).

RESULTS

At the screening visit, 205 diabetic patients were screened, of whom 70 had DN. Ten patients did not meet the criteria and 60 patients were enrolled and assigned to the two groups. Each group included 30

patients, but 2 from each group were lost to follow up (Figure). Finally, 56 participants completed the study (28 in the omega-3 and 28 in the placebo group). In accordance with the intention-to-treat principle, all of the 60 patients were included in the final analysis.

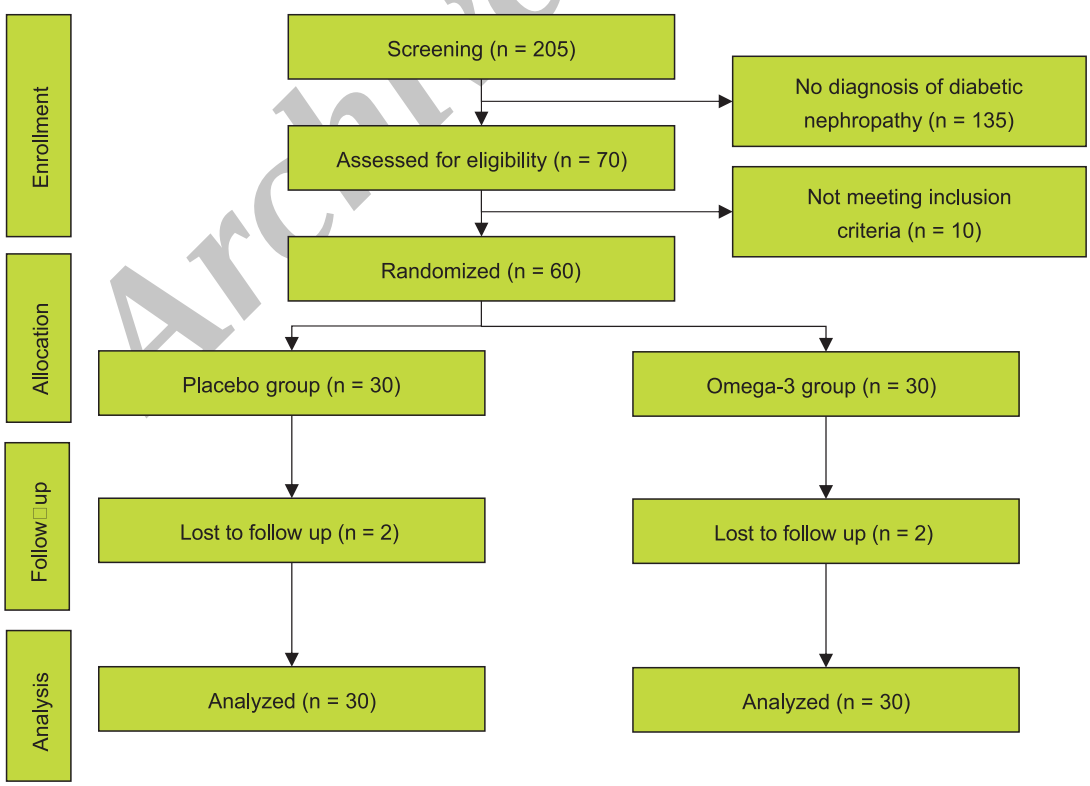
The two groups were comparable in terms of age, height, weight at study baseline and end of the study, weight change, duration of diabetes mellitus, and administration of antidiabetic and antilipidemic medications, angiontensin-converting enzyme inhibitors, and aldosterone receptor blockers (Table 1). Based on the 3-day dietary records obtained at the study baseline and on weeks 3, 6, 9, and 12, no significant changes were observed in the daily dietary macro- and micro-nutrient intakes between the two groups (Table 2).

After 12 weeks of treatment, omega-3 fatty acid supplements compared with the placebo resulted in a significant decrease in serum AGEs levels (-2.3 ± 2.8 AU versus 0.2 ± 2.5 AU, P = .001; Table 3). Despite a significant reduction of serum RAGEs levels (-0.1 ± 0.3 AU, P = .02) in the omega-3 group, no significant difference was found between the two groups in terms of their effects on RAGEs. Supplementation with omega-3 fatty acid had no significant effect on the inflammatory cytokines compared with the placebo. Intake of omega-3 fatty acid did not significantly affect fasting blood glucose levels compared with the placebo (-3.1 ± 50.5 mg/dL versus 4.8 ± 46.2 mg/dL, P = .52). Adjustments for baseline values of biochemical parameters did not affect the findings, except for serum RAGEs (P = .002; Table 4). Further adjustments for age and baseline body mass index did not influence the findings except for serum RAGEs (P= .002).

DISCUSSION

In the current study, we evaluated the effects of omega-3 fatty acid supplementation on AGEs and inflammatory cytokines in patients with DN. However we have previously reported the beneficial effects of omega-3 fatty acid supplementation on markers of insulin resistance and triglycerides levels in patients with DN,[6] to our knowledge, this randomized clinical trial is the first evaluating the effects of omega-3 fatty acid supplementation on AGEs and inflammatory cytokines these patients.

Patients with DN are susceptible to increased inflammatory cytokines and oxidative stress.[2,17]



Summary of patient flow diagram.

Table 1. Characteristics of Study Participants

Characteristic	Placebo Group (n = 30)	Omega-3 Group (n = 30)	*P*
Sex, %			
Male	10 (33.3)	11 (36.7)	
Female	20 (66.7)	19 (63.3)	.78
Duration of diabetes mellitus, y	15.2 ± 3.2	15.8 ± 3.0	.48
Age, y	63.1 ± 8.7	63.5 ± 10.1	.86
Height, cm	162.5 ± 7.4	165.0 ± 7.4	.20
Weight at study baseline, kg	79.5 ± 9.2	80.2 ± 13.5	.35
Weight change, kg	0.1 ± 0.5	0.1 ± 0.4	.89
Antidiabetic medication, %			
Metformin	3 (10.0)	3 (10.0)	
Metformin and gliclazide	14 (46.7)	13 (43.3)	
Metformin, gliclazide, and repaglinide	13 (43.3)	14 (46.7)	.96
Antilipidemic therapy, %	25 (82.5)	25 (82.5)	> .99
Antilipidemic medication, %			
Statins	19 (76.0)	17 (68.0)	
Fibrates	2 (8.0)	3 (12.0)	
Statins and fibrates	4 (16.0)	5 (20.0)	.81
Angiotensin-converting enzyme inhibitors, %	26 (86.7)	26 (86.7)	> .99
Angiotensin receptor blockers, %	4 (13.3)	5 (16.7)	.78
Hemoglobin A1c ,%	6.8 ± 0.4	6.9 ± 0.4	.77
Urinary albumin excretion, mg/d	496.0 ± 68.0	512.3 ± 63.9	.34
Serum creatinine, mg/d	1.3 ± 0.5	1.4 ± 0.6	.79

Table 2. Dietary Intakes of Study Participants Throughout the Study

Dietary Intake	Placebo Group (n = 30)	Omega-3 Group (n = 30)	*P*
Energy, kcal/d	2175 ± 226	2256 ± 225	.17
Fat, g/d	74.4 ± 11.3	77.3 ± 14.9	.40
Saturated fatty acids, g/d	23.5 ± 4.6	24.5 ± 4.9	.44
Polyssaturated fatty acids, g/d	22.0 ± 5.2	23.5 ± 6.2	.34
Monossaturated fatty acids, g/d	20.5 ± 5.1	21.1 ± 5.8	.65
Cholesterol, mg/d	192.9 ± 88.7	189.3 ± 94.3	.88
Omega-3 fatty acids, g/d	0.2 ± 0.1	0.2 ± 0.1	.23
Omega-6 fatty acids, g/d	20.6 ± 4.3	21.7 ± 5.1	.38
Selenium, µg/d	53.8 ± 5.7	53.6 ± 5.0	.86
Magnesium, mg/d	263.2 ± 44.5	279.7 ± 52.1	.19

Although limited data are available evaluating effects of omega-3 fatty acid supplementation in human models. Wang and colleagues[18] demonstrated that docosahexaenoic acid inhibited AGEs-induced microglia activation in in vitro microglia culture system. In addition, 2 individual studies have indicated that the transcription of downstream genes such as RAGE and TNF-α were inhibited with docosahexaenoic acid treatment.[19,20] These findings indicated that docosahexaenoic acid and eicosapentaenoic acid intake may efficiently inhibit NF-kB activity. These results are consistent with other studies in skeletal muscle and cardiomyocytes,[19] showing eicosapentaenoic acid and docosahexaenoic acid inhibited NF-kB activation by preventing I kappa B alpha phosphorylation and further inhibited NF-kB activation by reducing degradation of the inhibitory I kappa B alpha protein. Furthermore, it was noteworthy that omega-3 fatty acids were the known natural ligands of PPAR-γ,[21] and few studies have demonstrated that omega-3 fatty acids decreased NF-kB activation by increasing PPAR-γ gene expression in a chain length-dependent manner.[12,13] Being a member of the immunoglobulin superfamily with a high affinity for AGEs,[20] RAGE

Table 3. Advanced glycation end products and inflammatory cytokines at study baseline and after 3-month intervention in patients with diabetic nephropathy that received either omega-3 fatty acid supplements or placebo

	Placebo Group (n = 30)				Omega-3 fatty acids group (n=30)				P		
	Baseline	End of Study	Change	P	Baseline	End of Study	Change	P1	Time	Group	Time x Group
TNF-α, ng/L	19.0 ± 2.5	20.3 ± 5.8	1.3 ± 8.6	.42	19.1 ± 4.1	20.5 ± 4.5	1.4 ± 4.5	.11	.14	.88	.96
TNF-α receptor, ng/mL	21.0 ± 4.1	21.9 ± 3.4	0.9 ± 3.2	.13	19.7 ± 3.4	20.8 ± 4.1	1.1 ± 4.7	.24	.07	.14	.89
GDF15, ng/L	73.9 ± 8.8	73.6 ± 6.6	-0.3 ± 13.5	.89	77.1 ± 7.7	75.2 ± 9.3	-1.8 ± 10.9	.35	.49	.08	.63
AGEs, AU	25.8 ± 3.3	26.0 ± 2.0	0.2 ± 2.5	.67	27.0 ± 3.6	24.7 ± 2.7	-2.3 ± 2.8	< .001	.004	.90	.001
RAGEs, ng/mL	2.0 ± 0.2	2.1 ± 0.2	0.1 ± 0.3	.53	2.0 ± 0.4	1.9 ± 0.3	-0.1 ± 0.3	.02	.33	.03	.05

*TNF-α indicates tumor necrosis factor-α; AGEs, advanced glycation end products; GDF15, growth differentiation factor 15; and RAGEs, receptor advanced glycation end products.

Table 4. Adjusted Changes in Metabolic Variables in Patients With Diabetic Nephropathy Who Received Omega-3 Fatty Acid Supplements or Placebo*

Variables	Placebo Group (n = 30)	Omega-3 Group (n = 30)	P
TNF-α, ng/L			
Model 1	1.2 ± 0.9	1.4 ± 0.9	.90
Model 2	1.2 ± 0.9	1.4 ± 0.9	.91
TNF-α receptor, ng/mL			
Model 1	1.3 ± 0.6	0.7 ± 0.6	.51
Model 2	1.3 ± 0.6	0.7 ± 0.6	.52
GDF15, ng/L			
Model 1	-2.1 ± 1.5	-0.1 ± 1.5	.30
Model 2	-2.1 ± 1.5	-0.1 ± 1.5	.28
AGEs, AU			
Model 1	-0.1 ± 0.3	-2.0 ± 0.3	< .001
Model 2	-0.1 ± 0.3	-2.0 ± 0.3	< .001
RAGEs, ng/mL			
Model 1	0.1 ± 0.1	-0.1 ± 0.1	.002
Model 2	0.1 ± 0.1	-0.1 ± 0.1	.003

*Model 1 is adjusted for baseline values of biochemical parameters, and model 2, for baseline values of biochemical parameters, age and baseline body mass index. TNF-α indicates tumor necrosis factor-α; AGEs, advanced glycation end products; GDF15, growth differentiation factor 15; and RAGEs, receptor advanced glycation end products.

is associated with various diseases including Alzheimer disease, cardiovascular disease, and diabetic vasculopathy.[22]

Supporting with our findings, taking 15 mL/d of either flaxseed oil among healthy young adults for 6 weeks did not decrease inflammatory markers.[23] In another study, low- and high-dose plant and marine (n-3) fatty acids did not influence plasma inflammatory markers in adults with metabolic syndrome after 8 weeks.[24] In addition, no significant effect on inflammatory factors was observed following the consumption of omega-3-enriched supplements of flaxseed and fish oil among firefighters for 12 weeks.[25] Similar findings were seen after omega-3 fatty acids supplementation from flaxseed oil among healthy volunteers for 12 weeks and healthy abdominally obese adults for 8 weeks.[26,27] However, some researchers could observe the beneficial effects of omega-3 fatty acids supplementation on inflammatory cytokines. For instance, Perunicic-Pekovic and colleagues[28] demonstrated a significant decrease in serum TNF-α and interleukin-6 levels of hemodialysis patients treated with 2.4 g/d of omega-3 fatty acids from fish oil for 2 months. Moreover, circulating levels of TNF-α and intterleukin-1β were reduced after

omega-3 fatty acids supplementation from fish oil in obese but not in normal-weight women for 4 weeks.[29] The discrepancies between our findings and those of previous reports might be explained by the origin of omega-3 fatty acids, the dosage of omega-3 fatty acids supplements used, the intervention time, the study participants, the quality of the supplements, their purity and bioavailability, and the time and period of administration.

Our study had some limitations. The main limitation of our study was the lack of measurements of circulating levels of omega-3 fatty acids at study baseline and at the end of the study due to budget limitations. The sample size of our study was low. Future studies with cross-over design, higher dose of omega-3 fatty acid, and bigger sample size are needed to confirm the validity of our findings in DN patients. In addition, we agreed that to determine other inflammatory cytokines (especially interleukin-6) in patients with DN is valuable. Actually, due to limited funding for research projects in developing countries, we did not evaluate these markers. Therefore, measurement of other inflammatory cytokines, biomarkers of oxidative stress, and HbA1c after intervention are warranted in future studies.

CONCLUSIONS

Overall, the current study demonstrated that omega-3 fatty acid supplementation for 12 weeks had favorable effects on serum AGEs and RAGEs levels among DN patients, but did not affect inflammatory cytokines.

CONFLICT OF INTEREST

None declared.

FINANCIAL SUPPORT

The study was supported by a grant (No 5979) from Kashan University of Medical Sciences.

REFERENCES

1. Ding Y, Choi ME. Autophagy in diabetic nephropathy. J Endocrinol. 2015;224:R15-30.
2. Navarro-Gonzalez JF, Mora-Fernandez C. The role of inflammatory cytokines in diabetic nephropathy. J Am Soc Nephrol. 2008;19:433-42.
3. Calcutt NA, Cooper ME, Kern TS, Schmidt AM. Therapies for hyperglycaemia-induced diabetic complications: from animal models to clinical trials. Nat Rev Drug Discov. 2009;8:417-29.
4. Fang Q, Zhao L, Wang Y, et al. A novel chalcone derivative attenuates the diabetes-induced renal injury via inhibition of high glucose-mediated inflammatory response and macrophage infiltration. Toxicol Appl Pharmacol. 2015;282:129-38.
5. Kanwar YS, Sun L, Xie P, Liu FY, Chen S. A glimpse of various pathogenetic mechanisms of diabetic nephropathy. Annu Rev Pathol. 2011;6:395-423.
6. Soleimani A, Taghizadeh M, Bahmani F, Badroj N, Asemi Z. Metabolic response to omega-3 fatty acid supplementation in patients with diabetic nephropathy: A randomized, double-blind, placebo-controlled trial. Clin Nutr. 2015. doi: 10.1016/j.clnu.2015.11.003. [Epub ahead of print].
7. Lee SM, Chung SH, Park Y, et al. Effect of Omega-3 Fatty Acid on the Fatty Acid Content of the Erythrocyte Membrane and Proteinuria in Patients with Diabetic Nephropathy. Int J Endocrinol. 2015;2015:208121. doi: 10.1155/2015/208121. Epub 2015 May 18.
8. Derosa G, Cicero AF, Fogari E, et al. Effects of n-3 PUFAs on postprandial variation of metalloproteinases, and inflammatory and insulin resistance parameters in dyslipidemic patients: evaluation with euglycemic clamp and oral fat load. J Clin Lipidol. 2012;6:553-64.
9. Wei W, Chen M, Zhu Y, et al. Down-regulation of vascular HMGB1 and RAGE expression by n-3 polyunsaturated fatty acids is accompanied by amelioration of chronic vasculopathy of small bowel allografts. J Nutr Biochem. 2012;23:1333-40.
10. Kooshki A, Taleban FA, Tabibi H, Hedayati M. Effects of marine omega-3 fatty acids on serum systemic and vascular inflammation markers and oxidative stress in hemodialysis patients. Ann Nutr Metab. 2011;58:197-202.
11. Andric T, Weiss TW, Huber K, Arnesen H, Seljeflot I. Effects of diet and/or n-3 fatty acid supplementation on components of the interleukin-6 trans-signalling system in elderly men. Scand J Clin Lab Invest. 2015;75:646-51.
12. Turk HF, Chapkin RS. Membrane lipid raft organization is uniquely modified by n-3 polyunsaturated fatty acids. Prostaglandins Leukot Essent Fatty Acids. 2013;88:43-7.
13. Li H, Ruan XZ, Powis SH, et al. EPA and DHA reduce LPS-induced inflammation responses in HK-2 cells: evidence for a PPAR-gamma-dependent mechanism. Kidney Int. 2005;67:867-74.
14. Mbodji K, Charpentier C, Guerin C, et al. Adjunct therapy of n-3 fatty acids to 5-ASA ameliorates inflammatory score and decreases NF-kappaB in rats with TNBS-induced colitis. J Nutr Biochem. 2013;24:700-5.
15. Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes Care. 2005;28:164-76.
16. Ainsworth BE, Haskell WL, Whitt MC, et al. Compendium of physical activities: an update of activity codes and MET intensities. Med Sci Sports Exerc. 2000;32:S498-504.
17. Dabhi B, Mistry KN. Oxidative stress and its association with TNF-alpha-308 G/C and IL-1alpha-889 C/T gene polymorphisms in patients with diabetes and diabetic nephropathy. Gene. 2015;562:197-202.
18. Wang L, Chen K, Liu K, et al. DHA inhibited AGEs-

induced retinal microglia activation via suppression of the PPARgamma/NFkappaB pathway and reduction of signal transducers in the AGEs/RAGE axis recruitment into lipid rafts. Neurochem Res. 2015;40:713-22.

19. Wang Y, Lin QW, Zheng PP, Zhang JS, Huang FR. DHA inhibits protein degradation more efficiently than EPA by regulating the PPARgamma/NFkappaB pathway in C2C12 myotubes. Biomed Res Int. 2013;2013:318981. doi: 10.1155/2013/318981. Epub 2013 Jul 28.
20. Xie J, Mendez JD, Mendez-Valenzuela V, Aguilar-Hernandez MM. Cellular signalling of the receptor for advanced glycation end products (RAGE). Cell Signal. 2013;25:2185-97.
21. Harris WS, Dayspring TD, Moran TJ. Omega-3 fatty acids and cardiovascular disease: new developments and applications. Postgrad Med. 2013;125:100-13.
22. Chuah YK, Basir R, Talib H, Tie TH, Nordin N. Receptor for advanced glycation end products and its involvement in inflammatory diseases. Int J Inflam. 2013;2013:403460. doi: 10.1155/2013/403460. Epub 2013 Sep 11.
23. Kontogianni MD, Vlassopoulos A, Gatzieva A, et al. Flaxseed oil does not affect inflammatory markers and lipid profile compared to olive oil, in young, healthy, normal weight adults. Metabolism. 2013;62:686-93.
24. Dewell A, Marvasti FF, Harris WS, Tsao P, Gardner CD. Low- and high-dose plant and marine (n-3) fatty acids do not affect plasma inflammatory markers in adults with metabolic syndrome. J Nutr. 2011;141:2166-71.
25. Barcelo-Coblijn G, Murphy EJ, Othman R, Moghadasian MH, Kashour T, Friel JK. Flaxseed oil and fish-oil capsule consumption alters human red blood cell n-3 fatty acid composition: a multiple-dosing trial comparing 2 sources of n-3 fatty acid. Am J Clin Nutr. 2008;88:801-9.
26. Kaul N, Kreml R, Austria JA, et al. A comparison of fish oil, flaxseed oil and hempseed oil supplementation on selected parameters of cardiovascular health in healthy volunteers. J Am Coll Nutr. 2008;27:51-8.
27. Nelson TL, Stevens JR, Hickey MS. Inflammatory markers are not altered by an eight week dietary α-linolenic acid intervention in healthy abdominally obese adult males and females. Cytokine. 2007;38:101-6.
28. Perunicic-Pekovic GB, Rasic ZR, Pljesa SI, et al. Effect of n-3 fatty acids on nutritional status and inflammatory markers in haemodialysis patients. Nephrology (Carlton). 2007;12:331-6.
29. Al-Safi ZA, Liu H, Carlson NE, et al. Omega-3 Fatty Acid Supplementation Lowers Serum FSH in Normal Weight but not Obese Women. J Clin Endocrinol Metab. 2015:jc20152913.

Correspondence to:
Zatollah Asemi
Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, Iran
Tel: +98 31 5546 3378
Fax: +98 31 5546 3377
E-mail addresses: asemi_r@yahoo.com

Received January 2016
Revised March 2016
Accepted April 2016