Clinical Characterization of Serum Docosahexaenoic Acid and Its Relationship With Inflammation Factors in Patients With Diabetic Nephropathy

Li Zhou,[1] Di Yao,[1] Songqing Zhao,[1] Yuzhang Jiang,[2] Weiping Lu[1]

[1]Department of Endocrinology and Metabolism, Huai'an First People's Hospital, Nanjing Medical University, Huaian, Jiangsu Province, China
[2]Department of Clinical Laboratory, Huai'an First People's Hospital, Nanjing Medical University, Huaian, Jiangsu Province, China

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Introduction. A variety of molecular pathways, such as generation of advanced glycation end products, inflammation, and oxidative stress, are involved in the development of diabetic nephropathy (DN). Recently, a protective effect of omega-3 polyunsaturated fatty acids on the kidney has been reported. This study aimed to determine serum docosahexaenoic acid (DHA) level and its association with inflammation factors in patients with DN.

Materials and Methods. One hundred patients with type 2 diabetic mellitus were divided into 3 groups of non-DN, early DN, and clinical DN, based on 24-hour urinary albumin levels. Hemoglobin A1c, biochemical indicators, β2-microglobulin, and 24-hour urine albumin levels were assessed. Enzyme linked immunosorbent assay was applied to determine the serum concentrations of DHA, advanced glycation end products, fractalkine, superoxide dismutase, and tumor necrosis factor-α.

Results. Lower serum DHA and superoxide dismutase and higher serum β2-microglobulin and 24-hour urine albumin levels were associated with clinical DN, compared to no DN and early DN. The reductions in serum DHA levels were different among the patients with early and clinical DN, stratified by sex, body mass index, and serum lipid levels. Serum DHA significantly correlated positively with superoxide dismutase and negatively with fractalkine and tumor necrosis factor-α in the patients with DN.

Conclusions. Docosahexaenoic acid may suppress the expression and secretion of fractalkine through inhibiting the tumor necrosis factor-α signaling pathway in DN patients, which improves inflammation and oxidative stress of the kidney, and in turn, delaying the development of DN.

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INTRODUCTION

As one of the chronic microvascular complications of diabetes mellitus, diabetic nephropathy (DN) is a major cause of death in patients with type 1 diabetes mellitus (T1DM). In type 2 diabetes mellitus (T2DM), the severity of DN is just below that of atherosclerotic lesions in coronary and cerebral arteries. In 2001, the prevalence of DN in hospitalized patients was recorded as 34.7% in China.[1] Epidemiological studies have shown an annual increase in the prevalence of DN, which has a significant negative impact on the quality of life of patients with diabetes mellitus. To facilitate the development of effective interventions in the early stages of DN, it is crucial to understand the pathogenesis of the disease and clarify the associated mechanisms.

The pathogenesis of DN remains to be established.

A variety of molecular pathways are reported to be involved in the occurrence of DN, such as generation of advanced glycation end products (AGE), activation of polyol and protein kinase C pathways, inflammation, and oxidative stress.[2,3] Studies to date have focused on the interactions between AGEs and their receptors (RAGE), which are prevalent in abnormal cellular reactions, such as inflammation, autophagy, and apoptosis. Accumulating evidence has shown that decreased formation of AGEs and suppression of RAGE activation can lead to improvement of kidney function.[4]

The transcription factor, nuclear factor-kappa B (NF-κB), present in various tissues and cell types, is one of the promoters that regulates expression of RAGE. Furthermore, RAGE has been shown to steadily induce activation of NF-κB,[5] which ultimately promotes expression of RAGE to enhance interactions that participate in inflammatory events.[6] Consequently, a variety of cytokines are produced and released that contribute to the occurrence of DN. Tumor necrosis factor-α (TNF-α), a pro-inflammatory cytokine, is also an agonist of NF-κB. Tumor necrosis factor-α impairs muscle oxidative metabolism through activation of the NF-κB pathway.[7]

Infiltration of inflammatory cells in the glomerulus is an important step in the development of DN. The mechanism is reported to be associated with local high expression of chemokines.[8,9] Fractalkine, the only member of the CX3C family, works both as a chemoattractant and adhesion molecule. Fractalkine plays a regulatory role in protein overload and ischemic renal injury through combination with its cognate receptor chemokine (C-X3-C motif) receptor 1 (CX3CR1).[10]

Omega-3 poly-unsaturated fatty acids (omega-3 PUFAs) are essential for normal human growth. Alpha linolenic acid, eicosapentaenoic acid and docosahexaenoic acid (DHA) are the three main members of the omega-3 PUFA family.[11] Numerous studies have reported multiple physiological functions of omega-3 PUFA, such as resistance to inflammation, dilation of blood vessels, resistance to platelet aggregation, reduction of blood pressure, and improvement of metabolism. Additionally, omega-3 PUFA plays an important role in kidney protection. An epidemiological survey showed that omega-3 PUFA prevents the decrease in creatinine clearance ratio in healthy elderly individuals, reduces the risk of proteinuria in patients with T1DM, slows down the progress of proteinuria in elderly patients with T2DM, and delays the decline in renal function, although the associated mechanisms remain unclear at present.[12] The current study has focused on serum DHA and its relationship with inflammation factors in patients with DN.

MATERIALS AND METHODS

Study Population

One hundred patients with T2DM were selected from Huai'an First People's Hospital, Nanjing Medical University (2014 to 2015). The T2DM diagnosed was based on the 1999 World Health Organization criteria. According to 24-hour urinary albumin levels, patients were divided into non-DN (< 30 mg/24 h, n = 30), early DN (30 mg/24 h to 300 mg/24 h, n = 40), and clinical DN groups (> 300 mg/24 h, n = 30). The following were excluded: cases of T1DM, acute T2DM complications (including diabetic ketoacidosis, hyperglycemia hypertonic syndrome, and diabetic lactic acidosis), secondary diabetes mellitus (pancreatic exocrine disease, other endocrine diseases, and diabetes mellitus caused by drugs or chemicals), serious systemic disease (heart, lung, liver, or kidney failure), severe hypertension (systolic blood pressure > 180 mm Hg or diastolic blood pressure > 110 mm Hg), acute or chronic glomerular nephritis, nephrotic syndrome, lupus nephritis, obstructive nephropathy, gouty nephropathy, urinary tract infection, fever, chronic diarrhea, tuberculosis, administration of drugs (except angiotensin-converting enzyme inhibitors and angiotensin receptor antagonists), and other factors influencing urinary protein, routine urine examination for hematuria or tube urine, as well as mental disease, pregnancy, and nursing mothers. Patients taking drugs, health products, or food containing DHA were additionally excluded.

The study protocol followed with the principles outlined in the Declaration of Helsinki and informed consent was obtained from the study participants.

Anthropometric and Blood Pressure Measurements

Anthropometric evaluations included weight, height, and waist circumference. Weight was measured without heavy clothing using an

electronic scale. Height was assessed in patients without shoes with a settled wall distance meter. Body mass index (BMI) was calculated as weight divided by height squared. Waist circumference was measured at the midpoint between the edge of the ribs and the superior border of the ilium at the end of the expiration period. Daytime seated systolic blood pressure and diastolic blood pressure were measured using a mercury sphygmomanometer at 8 AM with 15-minute breaks. Every individual was measured 3 times with 2-minute intermission periods, and the mean values of the three blood pressure measurements were used for analysis.

Indicator Measurements

Serum samples were collected into dry tubes from each patient in the morning after overnight fasting. Urine specimens (24-hour samples) were obtained in disposable urine cups. Both serum and urine samples were centrifuged at 2000 g for 15 minutes and stored at -80°C. Fasting plasma glucose, 2-hour postmeal plasma glucose, total cholesterol, total triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, blood uric acid, blood urea nitrogen, serum creatinine, blood calcium and phosphorus, and 24-hour urine albumin were measured using auto-analyzers according to the manufacturer's instructions (Hitachi 7600, Ibaraki Prefecture, Japan). Hemoglobin A1c was determined via ion chromatography in keeping with the manufacturer's instructions (hlc73g8, Tosoh AIA, Tokyo, Minato, Japan). Chemiluminescence was applied to measure the concentration of β2-microglobulin (Architect i4000 sr, Abbot, USA). Estimated glomerular filtration rate (GFR) was calculated using the serum creatinine-based Chronic Kidney Disease Epidemiology Collaboration equation.[13] Serum levels of DHA and superoxide dismutase (SOD) were measured using an enzyme-linked immunosorbent assay kit (USCN Life Science, Hankou, Wuhan, China), and serum AGEs, fractalkine, and TNF-α were assessed using an enzyme-linked immunosorbent assay kit (Signalway Antibody LLC, College Park, Maryland, USA) according to the manufacturers' protocols. All samples were measured in triplicate.

Statistical Analysis

Data were analyzed using the SPSS software (Statistical Package for the Social Sciences, version 17.0, SPSS Inc, Chicago, IL, USA). Quantitative data were presented as mean ± standard deviations. Normal distribution of data was analyzed with the independent-samples *t* test or the 1-way analysis of variance, and nonnormal distribution data with the Kruskal-Wallis Rank sum test. Correlations of all the indicators were explored using the Pearson correlation coefficient. The chi-square test was applied to compare the component ratios. Differences at a *P* value less than .05 were considered significant.

RESULTS

Clinical Data and Diabetic Nephropathy

We observed no significant differences in age, sex ratio, course of the disease, systolic blood pressure, diastolic blood pressure, BMI, or waist circumference among the three groups of non-DN, early DN, and clinical DN. Compared with patients in the non-DN group, those with early DN contained lower serum DHA and higher serum AGEs, fractalkine, TNF-α, β2-microglobulin, and 24-hour urine albumin levels. Lower concentrations of serum DHA, SOD, calcium, and estimated GFR and higher concentrations of blood urea nitrogen, serum creatinine, serum β2-microglobulin, and 24-hour urine albumin were observed in the clinical DN group relative to the other two groups (Table 1).

Docosahexaenoic Acid and Diabetic Nephropathy

The patients that showed no significant differences in serum DHA levels (Figure 1) were

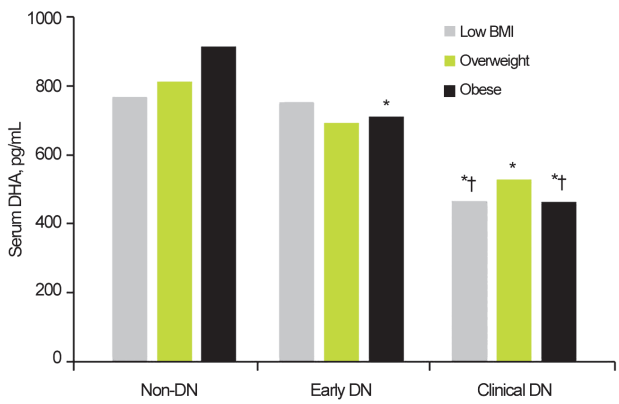

Figure 1. Comparison of serum docosahexaenoic acid (DHA) levels of patients with and without diabetic nephropathy (DN) stratified by body mass index (BMI).
*$P < .05$ compared to the group without diabetic nephropathy
†$P < .05$ compared to the early diabetic nephropathy group

Table 1. Clinical Characteristic of Diabetic Participants

	Diabetic Patients			
Characteristic	**No Nephropathy**	**Early Nephropathy**	**Clinical Nephropathy**	***P***
Age, y	56.07 ± 13.66	59.25 ± 13.52	58.50 ± 10.80	.58
Sex,%				
Male	43.3	55.0	53.3	.60
Female	56.7	45.0	46.7	
Course, y	8.01 ± 9.02	8.03 ± 7.39	10.77 ± 6.50	.10
Systolic blood pressure, mm Hg	135.50 ± 8.67	136.10 ± 11.54	140.47 ± 9.74	.12
Diastolic blood pressure, mm Hg	75.47 ± 6.74	78.20 ± 9.46	78.17 ± 9.69	.37
Body mass index, kg/m^2	24.54 ± 3.40	26.17 ± 3.77	24.88 ± 4.46	.18
Waist circumference, cm	86.13 ± 9.27	87.03 ± 9.29	87.57 ± 8.44	.83
Hemoglobin A1c, %	8.37 ± 1.56	8.48 ± 1.71	7.95 ± 1.32	.35
Fasting blood glucose, mmol/L	7.96 ± 2.02	8.50 ± 2.21	8.59 ± 2.48	.49
2-hour plasma glucose, mmol/L	11.56 ± 3.06	12.09 ± 2.98	11.86 ± 3.14	.77
Total cholesterol, mmol/L	4.78 ± 0.88	4.38 ± 1.06	4.82 ± 1.37	.19
Triglyceride, mmol/L	1.85 ± 0.80	1.70 ± 0.94	1.87 ± 1.52	.42
High-density lipoprotein cholesterol, mmol/L	1.23 ± 0.16	1.15 ± 0.26	1.30 ± 0.29	.06
Low-density lipoprotein cholesterol, mmol/L	2.62 ± 0.53	2.54 ± 0.75	2.85 ± 0.80	.20
Calcium, mmol/L	2.32 ± 0.13	2.31 ± 0.11	2.21 ± 0.17*†	.003
Phosphorus, mmol/L	1.32 ± 0.20	1.28 ± 0.19	1.32 ± 0.25	.64
β2-microglobulin, μg/mL	1.57 ± 0.32	2.03 ± 0.57*	3.05 ± 1.18*†	< .001
Uric acid, μmol/L	267.45 ± 63.81	294.73 ± 84.46	348.32 ± 105.36*	.002
Blood urea nitrogen, mmol/L	5.84 ± 1.80	6.03 ± 1.91	10.46 ± 6.11*†	.001
Serum creatinine, μmol/L	57.81 ± 9.89	67.22 ± 28.26	161.96 ± 118.77*†	< .001
Glomerular filtration rate, mL/min/1.73 m^2	102.61 ± 9.32	97.61 ± 23.76	67.77 ± 32.00*†	< .001
24 hour urine protein, g/24 h	0.28 ± 0.05	0.32 ± 0.14	2.37 ± 1.77*†	< .001
24 hour urine albumin, mg/24 h	14.26 ± 6.39	108.43 ± 72.12*	1598.48 ± 1357.39*†	< .001
Superoxide dismutase, ng/mL	1.23 ± 0.42	1.21 ± 0.40	0.65 ± 0.37*†	< .001
Advanced glycation end products, ng/mL	2.85 ± 1.17	4.00 ± 1.19*	4.83 ± 0.96*†	< .001
Docosahexaenoic acid, pg/mL	812.96 ± 216.27	716.83 ± 183.85*	484.24 ± 185.65*†	< .001
Fractalkine, ng/mL	1.00 ± 0.42	1.44 ± 0.71*	1.98 ± 0.74*†	< .001
Tumor necrosis factor-α, pg/mL	9.72 ± 2.07	17.15 ± 2.93*	41.15 ± 4.43*†	< .001

*P < .05 compared to the group without diabetic nephropathy
†P < .05 compared to the early diabetic nephropathy group

divided into 3 groups according to their BMI. Among the patients with normal or low weight (BMI < 24 kg/m^2), serum DHA levels in the clinical DN group were significantly lower than those in the other two groups (F = 8.727, P= .001; non-DN versus clinical DN, P = .001; early DN versus clinical DN, P = .002). Overweight patients (BMI, 24 kg/m^2 to 27.99 kg/m^2) in the clinical DN group contained markedly lower concentrations of serum DHA than those in the non-DN group (F = 5.304, P = .01; non-DN versus clinical DN, P = .003). Serum DHA levels were significantly higher in the non-DN group than the other two groups among obese patients (BMI ≥ 28 kg/m2; F = 10.793, P < .001; non-DN versus early DN, P = .02; non-DN versus clinical DN, P = .005). Furthermore, serum DHA in the clinical DN group was clearly lower than that in the early DN group (P = .006).

In the patients with normal or low levels of serum triglyceride, serum DHA concentrations in the non-DN and early DN groups were significantly higher than those in the clinical DN group, which was also evident in the patients with normal or low total cholesterol levels, high triglyceride level, normal or high high-density lipoprotein cholesterol level, and normal or low –low-density lipoprotein cholesterol level. Among the patients with normal or high high-density lipoprotein cholesterol level and normal or low low-density lipoprotein cholesterol level, serum DHA was lower in the early DN than the non-DN group (Table 2).

Stratifying patients in the three groups by sex, serum levels of DHA in the women were lower than those in the men in both of the early DN and clinical DN groups (non-DN, P = .66; early DN, P = .003; clinical DN, P = .04; Figure 2).

Table 2. Comparison of Serum Docosahexaenoic Acid (DHA) Levels of Patients With and Without Diabetic Nephropathy (DN) By Serum Lipid Parameters

Lipid Profile	non-DN		Early DN		Clinical DN		*P*
	n	Mean DHA, pg/mL	n	Mean DHA, pg/mL	n	Mean DHA, pg/mL	
Total cholesterol, mg/dL							
Normal or low	26	808.32 ± 224.01	38	723.91 ± 186.00	26	484.62 ± 188.74*†	< .001
High	3	840.80 ± 220.97	1	572.38	4	481.77 ± 190.56	0.16
Triglyceride, mg/dL							
Normal or low	15	760.80 ± 264.51	24	713.17 ± 174.44	17	459.35 ± 191.22*†	< .001
High	14	866.20 ± 150.64	15	730.98 ± 206.94	13	516.78 ± 180.32*†	< .001
High-density lipoprotein cholesterol, mg/dL							
Low	0	…	4	657.8 ± 73.81	1	719.41	…
Normal or high	20	817.65 ± 174.74	26	704.32 ± 199.26*	23	488.69 ± 187.81*†	< .001
Low-density lipoprotein cholesterol, mg/dL							
Normal or low	19	829.74 ± 170.71	29	702.80 ± 188.73*	19	524.88 ± 202.28*†	< .001
High	1	587.82	1	562.17	5	397.30 ± 80.65	…

*P < .05 compared to the group without diabetic nephropathy
†P < .05 compared to the early diabetic nephropathy group

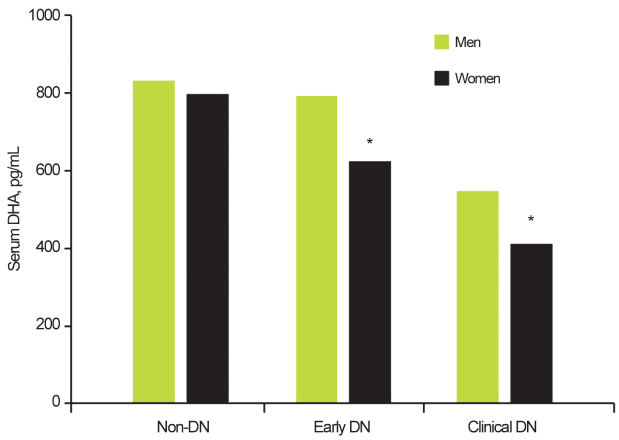

Figure 2. Comparison of serum docosahexaenoic acid (DHA) levels of men and women with and without diabetic nephropathy (DN).
*P < .05 compared to the men

Correlation Analysis

Serum DHA positively correlated with SOD and negatively correlated with fractalkine and TNF-α in the DN patients (Table 3). The correlations of the four other indicators were additionally analyzed. Figure 2 depicts the differences in serum DHA levels between the male and female DN patients. Correlation analyses of DHA with SOD, AGEs, fractalkine, and TNF-α by sex with DN are presented in Table 4. Serum DHA was positively correlated with SOD in the male patients and negatively correlated with fractalkine and TNF-αin both of the male and the female patients.

DISCUSSION

Diabetic nephropathy is the most severe

Table 3. Correlation Analysis of the Measured Parameters in Patients With Diabetic Nephropathy*

	Docosahexaenoic Acid	Superoxide Dismutase	Advanced Glycation End Products	Fractalkine	Tumor Necrosis Factor-A
Docosahexaenoic acid	…	0.375 (.001)	-0.137 (.26)	-0.34 (.004)	-0.512 (< .001)
Superoxide dismutase	0.375 (.001)	…	-0.213 (.08)	-0.02 (.005)	-0.58 (< .001)
Advanced glycation end products	-0.137 (.256)	-0.213 (.08)	…	0.444 (.000)	0.364 (.002)
Fractalkine	-0.34 (.004)	-0.02 (.005)	0.444 (< .001)	…	0.354 (.003)
Tumor necrosis factor-α	-0.512 (< .001)	-0.58 (< .001)	0.364 (.002)	0.354 (.003)	…

*Values are correlation coefficient (*P* value).

Table 4. Correlation Analysis of Docosahexaenoic Acid With Other Parameters by Sex*

	Superoxide Dismutase	Advanced Glycation End Products	Fractalkine	Tumor Necrosis Factor-A
Docosahexaenoic acid				
Men	0.388 (.02)	-0.044 (.79)	-0.199 (.23)	-0.574 (< .001)
Women	0.343 (.06)	-0.257 (.16)	-0.68 (< .001)	-0.501 (.003)

*Values are correlation coefficient (*P* value).

complication of diabetes that is the leading cause of end-stage renal disease worldwide.[14] Characteristics of early DN include microalbuminuria, subsequent enhancement of albuminuria, and increased levels of serum creatinine that eventually leads to kidney failure.[1] A number of medications have been used to protect kidney function and delay progression of DN to date. Over the past few years, a series of randomized controlled trials have been conducted to explore new treatments for DN. However, these studies have provided uncertain or negative data or even been terminated due to lack of efficacy and safety considerations.[15] Therefore, the development of safe and efficacious therapy for patients with DN remains an urgent unmet medical need.

The pathogenesis of DN is complex and details of the underlying mechanisms are yet to be elucidated. Both in vivo and in vitro studies have demonstrated that AGEs play key roles in DN. Interactions between AGEs and RAGE trigger inflammation, oxidative stress, and fibrotic reactions, causing progressive changes in kidney structure and loss of kidney function in patients with diabetes mellitus.[16] In our experiments, serum AGE levels in the two DN groups were significantly higher than those in the non-DN group, with increasing AGE concentrations in line with deterioration of DN. Correlation analysis with 4 other indicators revealed that serum AGEs were positively correlated with fractalkine and TNF-α, important factors in the inflammatory response in DN patients. These results support the potential of AGE-induced inflammation as a therapeutic target for DN.

Inflammation of glomeruli and tubulointerstitial regions is a stage in the progression of DN, suggesting an important status of inflammation in the pathogenesis of the disease. Fractalkine, a unique chemokine, plays a critical role in inflammation, vascular remodeling and fibrosis in the kidney via chemotaxis of mononuclear macrophages, T cells, and other inflammatory cells.[17] A recent meta-analysis showed that serum TNF-α concentrations were significantly increased in T2DM patients and even higher in T2DM patients with DN, suggesting that DN enhanced the inflammatory burden.[18] Similar to data obtained with AGEs, concentrations of fractalkine and TNF-α were increased in DN patients. Furthermore, fractalkine was positively correlated with TNF-α, indicating that fractalkine might exert its effects through interactions with TNF-α.

Oxidative stress, another important mechanism in the pathogenesis of DN, is also closely associated with inflammation. Increased serum urotensin-II may play a role in the development of DN in view of its correlation with high levels of oxidative stress parameters.[19] Experimental models indicate that oxidative stress and inflammation, which supplement each other in visceral fat and blood vessels, are key processes that induce the initiation, progression, and subsequent rupture of atherosclerotic lesions.[20] Data from our study showed significantly lower levels of serum SOD, a classic marker of oxidative stress, in clinical DN than the other two groups. Superoxide dismutase was negatively correlated with both fractalkine and TNF-α. Accordingly, we speculate that interactions between fractalkine and TNF-α aggravate oxidative stress in patients with DN.

Omega-3 PUFAs, especially DHA and eicosapentaenoic acid, exert considerable beneficial effects to maintain physiological homeostasis in the human body, which may help to decrease the incidence of obesity and its complications.[11] The positive functions of omega-3 PUFAs on the cardiovascular system in T2DM patients are well documented.[21] Supplementation of DHA, a natural peroxisome proliferator-activated receptor ligand in metabolic pathways, is reported to prevent and reduce the risk of cardiovascular disease and control complications caused by diabetes mellitus.[22] Supplementation of omega-3 PUFA in DN patients has been shown to exert beneficial effects on AGEs and RAGE.[23] Eicosapentaenoic acid and DHA suppress activation of NF-κB, the prototype inflammatory transcription factor.[24] In an earlier study, treatment with fish oil rich in DHA and eicosapentaenoic acid suppressed the serum concentrations of TNF-α, interleukin-6, interleukin-1β, and nitric oxide metabolites, compared with the placebo group.[25] Omega-3 PUFA supplementation led to successful reduction of serum pro-inflammatory cytokine levels. The triad of oxidative stress, inflammation, and immune cell aging involves important mechanisms triggering pre-disease that may be improved through nutritional intervention.[26] One study showed that higher erythrocyte omega-3 PUFA status protected against development of T2DM in overweight women.[27] In the present study, levels of serum DHA in female patients were lower than

those in male patients with DN.

To date, most research on the relationship between omega-3 PUFA and T2DM has concentrated on dietary intake of omega-3 PUFA,[2] with limited focus on serum omega-3 PUFA levels. In the current study, we examined serum levels of DHA in diabetes patients in addition to correlations with inflammatory factors and indicators of oxidative stress. Notably, levels of serum DHA were decreased in DN patients and further differed between early and clinical DN patients stratified by sex, BMI, and serum lipid levels. Serum DHA was correlated positively with SOD and negatively with fractalkine and TNF-α in DN patients. Due to the small sample size, we did not explore the factors influencing DHA via multiple linear regression analysis to determine the underlying reasons for the observed differences. The next logical step to solve this problem is to expand the sample size in future studies.

CONCLUSIONS

Docosahexaenoic acid may suppress expression and secretion of fractalkine by inhibiting the TNF-α signaling pathway in DN patients, and in turn, improving inflammation and oxidative stress of kidney and delaying the development of DN. Further research is warranted to confirm this theory, both in vitro and animal models, and elucidate sex-dependent differences.

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CONFLICT OF INTEREST

None declared.

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Correspondence to:
Weiping Lu
Huanghe Road West, Huaian 223300, Jiangsu Province, China
Tel: +86 135 1155 6318
E-mail: 429536336@qq.com

Li Zhou and Di Yao contributed equally to this work.

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