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Association of Serum Unsaturated Fatty Acid Patterns with the Risk of Diabetic Nephropathy

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Keywords
Diabetic nephropathy · Serum unsaturated fatty acid patterns · Factor analysis · Omega-3 fatty acids · Omega-6 fatty acids

Abstract
Introduction: Unsaturated fatty acids play an essential role in the progression of diabetic nephropathy (DN). However, previous studies were mainly focused on the role of individual unsaturated fatty acid. The serum unsaturated fatty acid patterns (FAPs) in patients with DN remain to be determined. ***Methods:*** A total of 135 patients with DN (DN group) and 322 patients with type II diabetes without nephropathy (non-DN group) were included in this study. Clinical data, serum levels of unsaturated fatty acids, and other laboratory indicators were collected. Multivariate logistic regression was applied to identify risk factors for serum unsaturated fatty acid level in both groups. Serum unsaturated fatty acids were subjected to factor analysis to identify distinct FAPs. Multivariable logistic regression was employed to assess the risk of DN associated with different serum FAPs. ***Results:*** After adjusting for confounders, three types of unsaturated fatty acid including C20:5 (eicosapentaenoic acid [EPA]), C22:6 (docosahexaenoic acid [DHA]), and C22:5 *n*-3 (docosapentaenoic acid *n*-3) were significantly associated with DN in the population. The odds ratios (ORs) (95% confidence interval [CI]) of DN were 0.583 (0.374, 0.908), 0.826 (0.716, 0.954), and 0.513 (0.298, 0.883), respectively. Factor analysis revealed five major FAPs, among which FAP2 (enriched with EPA and DHA) exhibited a significant inverse association with DN. In the multivariate-adjusted model, the OR (95% CI) was 0.678 (0.493, 0.933). Additionally, a combination of DHA and EPA enriched in FAP2 further decreased extracellular matrix production induced by transforming growth factor beta 1 in podocytes and tubular cells. ***Conclusions:*** Our findings suggest that FAP2 which is enriched with DHA and EPA is associated with a reduced risk of DN. This highlights the potential of targeting FAP2 for the patients with DN.

Introduction

Diabetic nephropathy (DN) characterized by glomerular basement membrane thickening, mesangial expansion, and nodular sclerosis on kidney biopsy is a common chronic microvascular complication of diabetes mellitus. It has become the major cause for patients with end-stage renal disease and uremia needing renal

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replacement therapy worldwide [1, 2]. The pathogenesis of DN is complicated, with metabolic abnormalities playing a pivotal role. In addition to hyperglycemia-induced metabolic changes, the disturbance in lipid metabolism may be associated with renal dysfunction in DN patients [3, 4]. On one side, abnormal lipid metabolism promotes the accumulation of ectopic lipid and their derivatives in the kidney, leading to renal cell damage through enhancing inflammation and oxidative stress [5, 6]. On the other side, some types of free fatty acid may prevent the development of DN. For instance, dietary fiber may prevent DN progression through increasing short-chain fatty acid production by modulating intestinal microbiota [7]. Additionally, long-chain fatty acids may downregulate the expression of fibronectin (FN), alpha-smooth muscle actin (α-SMA), and collagen IV in the kidneys of DN mice and podocytes cultured with high concentration of glucose [8].

Free fatty acids are classified into short-chain, medium-chain, and long-chain fatty acids based on their carbon chain length. Unsaturated fatty acids are long-chain fatty acids containing at least one carbon-carbon double bond [9], which can be categorized into monounsaturated fatty acids and polyunsaturated fatty acids (PUFAs). PUFAs can be further classified as *n*-3 PUFAs (ω-3 PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and *n*-6 PUFAs (ω-6 PUFAs) based on the location of carbon-carbon double bonds. Previous studies showed that saturated fatty acids and *n*-6 PUFAs possess pro-inflammatory properties and increase the risk of cardiovascular diseases [10]. Conversely, monounsaturated fatty acids and *n*-3 PUFAs exhibit anti-inflammatory effects and protect against cardiovascular diseases [11–13]. Alessandra Borsini et al. [14] proposed that *n*-3 PUFAs exhibit neuroprotective effects mediated by lipid oxidase and cytochrome P450. Takeshi Yamamoto et al. [15] suggested that EPA mitigates renal lipotoxicity by restoring autophagic flux. While clinical studies often focus on the effects of dietary unsaturated fatty acids on health and disease, the role of serum unsaturated fatty acids in DN remains to be further studied.

Considering the wide variety and structural similarities among unsaturated fatty acids [16], it becomes even more crucial to investigate their combined effects. Fatty acid pattern (FAP) analysis provides insight into the interactions between various fatty acids and elucidates their intricate relationship with disease progression [17]. Previous clinical studies have utilized factor analysis to explore the correlation between FAPs and various diseases, including obesity, metabolic syndrome, gestational diabetes, and diverse cancers [18, 19]. However, there is a lack of research investigating serum unsaturated FAPs in DN.

In this study, we conducted a retrospective analysis of serum unsaturated fatty acids in patients with diabetes and employed factor analysis to identify serum unsaturated FAPs in patients with DN. Subsequently, we used multivariate logistic regression analysis to establish the correlation between FAPs and the risk of DN. Finally, we verified the role of the major unsaturated fatty acids at the cellular level with the aim of providing potential insights for the prevention and treatment of DN.

Methods

Study Population

A total of 457 patients diagnosed with type II diabetes were enrolled in this study at the Second Affiliated Hospital of Nanjing Medical University between January 2021 and August 2022. The inclusion criteria comprised individuals over 18 years of age who had been diagnosed with type II diabetes. Exclusion criteria consisted of patients with non-type II diabetes (such as type I diabetes, gestational diabetes, and other special types of diabetes), a history of kidney transplantation, or individuals with infections or malignant tumors that could influence systemic conditions. All participants underwent serum unsaturated fatty acid testing. Following the 2022 KDIGO clinical practice guideline, the study population was categorized into two groups: 135 patients with DN (urine albumin/creatinine ratio [UACR] >30 mg/g, eGFR [calculated via the MDRD formula] >60 mL/min per 1.73 m^2, or both) and 322 patients with type II diabetes without nephropathy (UACR <30 mg/g and eGFR <60 mL/min per 1.73 m^2) [20].

Clinical and Laboratory Data

Demographic information and comorbidities were obtained from electronic medical records to ensure accuracy and consistency. The laboratory indicators used for the cross-sectional analysis were derived from the results obtained during the unsaturated fatty acid detection period. The serum unsaturated fatty acid measurements included C16:1 (palmitoleic acid [PA]), C18:1 (oleic acid [OA]), C18:2 (linoleic acid [LA]), α-C18:3 (alpha LA [ALA]), γ-C18:3 (gamma LA [GLA]), C20:1 (eicosenoic acid), C20:4 (arachidonic acid [AA]), C20:5 (EPA), C22:6 (DHA), C22:5 *n*-3 (docosapentaenoic acid [DPA] *n*-3), and C22:5 *n*-6 (DPA *n*-6). All samples were collected and assayed using standardized protocols to ensure consistency and reliability of the data.

Serum Unsaturated Fatty Acid Detection Methods

A 5-mL venous blood sample was aseptically collected without any additives or coagulants, and subsequently subjected to centrifugation at 1,200 rpm for 15 min. The resulting supernatant was carefully obtained to ensure a serum sample volume ranging from 0.5 to 1 mL. Liquid-liquid extraction methods were employed for serum

Table 1. Basic characteristics and serum unsaturated fatty acid level of diabetic patients

Parameter	Non-DN group (n = 322)	DN group (n = 135)	p value
Diabetes duration, years	7.70±6.73	13.5±7.51	<0.001
Age, years	58.94±11.50	65.04±11.82	<0.001
Male, *n* (%)	191 (59.3)	83 (61.5)	0.667
Smoking, *n* (%)	70 (21.7)	36 (26.7)	0.255
Drinking, *n* (%)	34 (10.6)	14 (10.4)	0.952
Hypertension, *n* (%)	175 (54.3)	108 (80.0)	<0.001
Coronary heart disease, *n* (%)	33 (10.2)	18 (13.3)	0.339
UACR, mg/g	8.31 (4.69, 14.39)	106.43 (51.58, 552.01)	<0.001
BUN, mmol/L	5.52±1.41	7.48±3.21	<0.001
SCr, μmol/L	65.34±15.70	92.32±51.12	<0.001
UA, μmol/L	294.49±82.16	341.43±104.06	<0.001
Alb, g/L	40.70±3.50	39.35±4.88	0.001
TC, mmol/L	4.36±1.14	4.40±1.23	0.759
TG, mmol/L	1.41 (1.01, 1.99)	1.64 (1.07, 2.74)	0.031
HDL-C, mmol/L	1.12±0.33	1.08±0.28	0.217
LDL-C, mmol/L	2.71±1.05	2.59±1.03	0.279
P, mmol/L	1.11±0.17	1.10±0.22	0.678
K, mmol/L	3.85±0.36	3.92±0.40	0.069
t-Ca, mmol/L	2.21±0.11	2.21±0.12	0.638
HbA1C, %	8.92±2.13	9.40±2.03	0.025
Hb, g/L	139.15±14.96	133.62±19.94	0.001
C16:1, μmol/L	11.70 (7.22, 17.63)	9.30 (6.04, 16.00)	0.014
C18:1, μmol/L	167.00 (111.80, 231.30)	140.00 (98.90, 207.00)	0.041
C18:2, μmol/L	126.50 (86.20, 170.00)	112.00 (76.00, 160.00)	0.129
α-C18:3, μmol/L	6.10 (4.13, 9.01)	5.90 (3.48, 7.98)	0.130
γ-C18:3, μmol/L	36.881 (0, 2.00)	1.29 (0.80, 1.87)	0.429
C20:1, μmol/L	1.20 (0.73, 1.83)	0.98 (0.69, 1.55)	0.045
C20:4, μmol/L	8.21 (6.11, 11.6)	7.64 (5.61, 10.40)	0.050
C20:5, μmol/L	0.65 (0.44, 1.01)	0.54 (0.36, 0.78)	0.001
C22:6, μmol/L	3.36 (2.47, 4.75)	2.86 (2.16, 3.80)	0.001
C22:5 *n*-3, μmol/L	0.73 (0.52, 1.15)	0.63 (0.47, 0.91)	0.011
C22:5 *n*-6, μmol/L	0.32 (0.23, 0.45)	0.29 (0.22, 0.42)	0.191
AA/DHA	2.53 (2.01, 3.39)	2.79 (2.03, 3.44)	0.228
AA/EPA	13.14 (9.04, 18.65)	14.59 (9.94, 20.19)	0.066
ω-6/ω-3	11.66 (9.71, 14.26)	11.94 (9.82, 15.01)	0.260

UACR, urine albumin/creatinine ratio; BUN, blood urea nitrogen; SCr, serum creatinine; UA, blood uric acid; Alb, serum albumin; TC, total cholesterol; TGs, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; P, serum phosphorus; K, serum potassium; t-Ca, serum total calcium; HbA1C, hemoglobin A1C; Hb, hemoglobin.

sample extraction, followed by the addition of derivatization reagents to facilitate free fatty acid derivatization. The samples were then analyzed using the AB SCIEX API 3200 Liquid Chromatograph Mass Spectrometer in multiple reaction monitoring mode. For the detection process, an unsaturated fatty acid detection kit (Jiangsu Haosi Biotech) was utilized. The liquid chromatography mass spectrometer was equipped with a dedicated free fatty acid analysis column, and the column temperature was maintained at 45°C. The chromatographic gradient conditions were optimized according to the instrument's specifications for optimal performance. In terms of mass spectrometry, the following conditions were employed: curtain gas (CUR) set at 25 psi, collision gas (CAD) at 3 psi, ion source gas1 (GS1) at 55 psi, and a temperature (TEMP) of 550°C. These parameters were carefully chosen to ensure accurate and reliable detection of unsaturated fatty acids in the serum samples.

Cell Culture and Treatment

The conditionally immortalized mouse podocyte cell line, generously provided by Peter Mundel from the Mount Sinai School of Medicine in New York, USA, was utilized for this study. The cells were cultured at a temperature of 33°C in RPMI-1640 medium (Gibco, Thermo Fisher Scientific), supplemented with 10% fetal bovine serum (Invitrogen, Grand Island, NY, USA) and recombinant interferon-γ (Invitrogen, Thermo Fisher Scientific).

Table 2. Multivariate logistic regression models of serum unsaturated fatty acids associated with the risk of diabetic nephropathy

Parameter	B	OR	95% CI	*p* value
C16:1, μmol/L				
Crude model	−0.025	0.975	(0.951, 1.000)	0.048
Adjusted model	−0.025	0.975	(0.944, 1.007)	0.126
C18:1, μmol/L				
Crude model	−0.002	0.998	(0.996, 1.000)	0.122
Adjusted model	−0.002	0.998	(0.995, 1.001)	0.184
C18:2, μmol/L				
Crude model	−0.002	0.998	(0.996, 1.001)	0.275
Adjusted model	−0.002	0.998	(0.994, 1.001)	0.219
α-C18:3, μmol/L				
Crude model	−0.038	0.963	(0.913, 1.015)	0.162
Adjusted model	−0.067	0.935	(0.871, 1.004)	0.063
γ-C18:3, μmol/L				
Crude model	−0.071	0.931	(0.792, 1.095)	0.387
Adjusted model	−0.142	0.868	(0.703, 1.071)	0.186
C20:1, μmol/L				
Crude model	−0.186	0.830	(0.660, 1.044)	0.111
Adjusted model	−0.106	0.899	(0.693, 1.166)	0.423
C20:4, μmol/L				
Crude model	−0.048	0.953	(0.909, 0.999)	0.045
Adjusted model	−0.026	0.975	(0.921, 1.031)	0.374
C20:5, μmol/L				
Crude model	−0.656	0.519	(0.328, 0.822)	0.005
Adjusted model	−0.540	0.583	(0.374, 0.908)	0.017
C22:6, μmol/L				
Crude model	−0.188	0.828	(0.726, 0.945)	0.005
Adjusted model	−0.191	0.826	(0.716, 0.954)	0.009
C22:5 *n*-3, μmol/L				
Crude model	−0.535	0.586	(0.372, 0.922)	0.021
Adjusted model	−0.667	0.513	(0.298, 0.883)	0.016
C22:5 *n*-6, μmol/L				
Crude model	−0.633	0.531	(0.192,1.470)	0.223
Adjusted model	−0.658	0.518	(0.148, 1.818)	0.305
AA/DHA				
Crude model	0.083	1.086	(0.926, 1.275)	0.309
Adjusted model	0.141	1.151	(0.933, 1.420)	0.190
AA/EPA				
Crude model	0.024	1.025	(1.000, 1.050)	0.050
Adjusted model	0.025	1.026	(0.997, 1.055)	0.077
ω-6/ω-3				
Crude model	0.033	1.034	(0.988, 1.082)	0.150
Adjusted model	0.026	1.026	(0.967, 1.089)	0.391

Crude model: not adjusted for confounders. Adjusted model: adjusted for diabetes duration, gender, age, smoking, drinking, hypertension, coronary heart disease, BUN, UA, Alb, TC, TG, HDL-C, LDL-C, P, K, t-Ca, HbA1C, Hb. BUN, blood urea nitrogen; UA, blood uric acid; Alb, serum albumin; TC, total cholesterol; TGs, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; P, serum phosphorus; K, serum potassium; t-Ca, serum total calcium; HbA1C, hemoglobin A1C; Hb, hemoglobin.

To induce cellular differentiation, the podocytes were cultured at 37°C in the absence of interferon-γ. Once the podocytes reached a mature state, they were cultured in serum-free medium and treated with DHA (200 nM) (catalog number: E6627, Sigma-Aldrich), EPA (200 nM) (catalog number: D2534, Sigma-Aldrich), or a combination of DHA (200 nM) and EPA (200 nM) for 30 min. Subsequently, they were stimulated with transforming growth factor beta 1 (TGFβ1) (2 ng/mL) (catalog number: 240-B-010, Bio-Techne) for 48 h without removing DHA or EPA. Then, the cells were collected for further analysis. Kidneys collected from C57BL/6J mice were planted on 50-μm mesh. The tissues filtered through the 50-μm mesh but not 150-μm mesh were digested with 2 mg/mL collagenase I for 30 min at 37°C with gentle stirring. The digested cells were cultured in Dulbecco's modified Eagle's medium-F12 (catalog number: 12400024, Gibco, Grand Island, NY, USA) supplemented with 10% FBS, 0.5X insulin-transferrin-selenium (Gibco, Grand Island, NY, USA), 36 ng/mL hydrocortisone, 4 pg/mL tri-iodothyronine, 10 ng/mL epidermal growth factors (Sigma-Aldrich, St. Louis, MO, USA), and 1% penicillin-streptomycin at 5% CO_2, 37°C. The primary cultured tubular cells (PTCs) were treated with DHA, EPA, and TGFβ1.

Western Blot Analysis

The cultured podocytes and PTCs were harvested using 1× SDS sample buffer. Equal amounts of protein were loaded onto 10% SDS-PAGE gels and subsequently transferred onto PVDF membranes. The primary antibodies used in this analysis were as follows: anti-FN (catalog number: F3648, Sigma-Aldrich, diluted 1:5,000), anti-α-SMA (catalog number: A5228, Sigma-Aldrich, diluted 1:1,000), and anti-tubulin (catalog number: T9026, Sigma-Aldrich, diluted 1:10,000). The membranes were then subjected to appropriate secondary antibody incubation and detection methods to visualize the target proteins.

Immunofluorescence Staining

Podocytes and PTCs cultured on coverslips were carefully washed with 1× PBS and fixed with 4% paraformaldehyde for 15 min. After three thorough washes with 1× PBS, the cells were treated with 0.1% Triton X-100 for 7 min to facilitate permeabilization. To prevent nonspecific binding, the cells were blocked with 2% normal donkey serum in 1× PBS buffer for 45 min at room temperature. The primary antibodies used for staining were anti-FN (catalog number: F3648, Sigma-Aldrich, diluted 1:200) and anti-α-SMA (catalog number: A5228, Sigma-Aldrich, diluted 1:200). Subsequently, the cells were stained with FITC-conjugated secondary antibodies for visualization. Additionally, DAPI staining was performed to visualize the nuclei of the cells. The stained slides were observed using a Nikon Eclipse 80i Epifluorescence Microscope equipped with a digital camera to capture the fluorescence signals.

Statistical Analysis

Statistical analyses of the collected data were conducted using IBM SPSS version 25. Quantitative variables were presented as either the mean ± standard deviation for normally or approximately normally distributed variables, or as median (interquartile range) for variables that exhibited abnormal distribution. Categorical variables were reported as counts and percentages.

To assess differences between the two groups, independent sample *t* tests and Mann-Whitney U tests were employed for normally distributed and abnormally distributed variables, respectively. For

Table 3. The Spearman's rank correlation coefficients between serum unsaturated fatty acids

	C16:1	C18:1	C18:2	α-C18:3	γ-C18:3	C20:1	C20:4	C20:5	C22:6	C22:5 *n*-3	C22:5 *n*-6
C16:1	1.00	0.85*	0.81*	0.74*	0.68*	0.55*	0.42*	0.51*	0.67*	0.75*	0.58*
C18:1		1.00	0.93*	0.74*	0.62*	0.69*	0.42*	0.45*	0.70*	0.80*	0.61*
C18:2			1.00	0.75*	0.64*	0.60*	0.39*	0.43*	0.68*	0.76*	0.58*
α-C18:3				1.00	0.67*	0.54*	0.30*	0.50*	0.61*	0.72*	0.45*
γ-C18:3					1.00	0.42*	0.42*	0.53*	0.56*	0.67*	0.46*
C20:1						1.00	0.38*	0.35*	0.57*	0.63*	0.60*
C20:4							1.00	0.54*	0.59*	0.52*	0.64*
C20:5								1.00	0.73*	0.69*	0.40*
C22:6									1.00	0.83*	0.74*
C22:5 *n*-3										1.00	0.66*
C22:5 *n*-6											1.00

*Correlation is significant at the 0.05 level (2-tailed).

Table 4. Eigenvalues and principal component contribution rate

Principal component	Original eigenvalues			Rotation sums of squared loadings		
	eigenvalues	contribution rate, %	cumulative contribution rate, %	eigenvalues	contribution rate, %	cumulative contribution rate, %
FAP1	6.808	61.891	61.891	3.501	31.83	31.83
FAP2	1.172	10.652	72.542	2.051	18.648	50.477
FAP3	0.784	7.123	79.665	1.938	17.618	68.096
FAP4	0.688	6.259	85.924	1.39	12.632	80.728
FAP5	0.552	5.016	90.941	1.123	10.212	90.941
FAP6	0.29	2.636	93.577			
FAP7	0.232	2.109	95.686			
FAP8	0.19	1.728	97.414			
FAP9	0.13	1.184	98.598			
FAP10	0.102	0.93	99.528			
FAP11	0.052	0.472	100			

Extraction method: principal component analysis. Rotation method: varimax Kaiser normalization.

categorical variables, the χ^2 test or Fisher's exact test was utilized to evaluate frequencies. Spearman correlation analysis was performed to estimate the correlation between unsaturated fatty acids, enabling the exploration of potential associations among these variables.

Factor analysis was conducted to consolidate serum PUFAs into several independent common factors, denoted as FAPs. Initially, the Kaiser-Meyer-Olkin (KMO) test and Bartlett's sphericity test were performed to assess the suitability of factor analysis on serum PUFAs. A KMO value greater than 0.5 and a significant result ($p < 0.001$) from Bartlett's sphericity test indicated a strong association between serum PUFAs and their potential for factor analysis. Then, the principal component factor analysis was employed, and varimax rotation was used to derive serum FAPs to obtain a simpler structure with greater interpretability. FAPs were extracted considering eigenvalue (>1.0) and variance contribution. Finally, we applied two multivariable logistic regression models to estimate the odds ratios (ORs) and 95% confidence interval (CI) between FAPs and the risk of DN. $p < 0.05$ was considered statistically significant, indicating strong evidence of associations or differences within the analyzed data.

Results

Basic Characteristics and Serum Unsaturated Fatty Acid Level of the Study Population

A total of 457 patients diagnosed with type II diabetes were included in the study, consisting of 135 patients with DN (DN group) and 322 patients with type II diabetes without nephropathy (non-DN group). Table 1 presents the

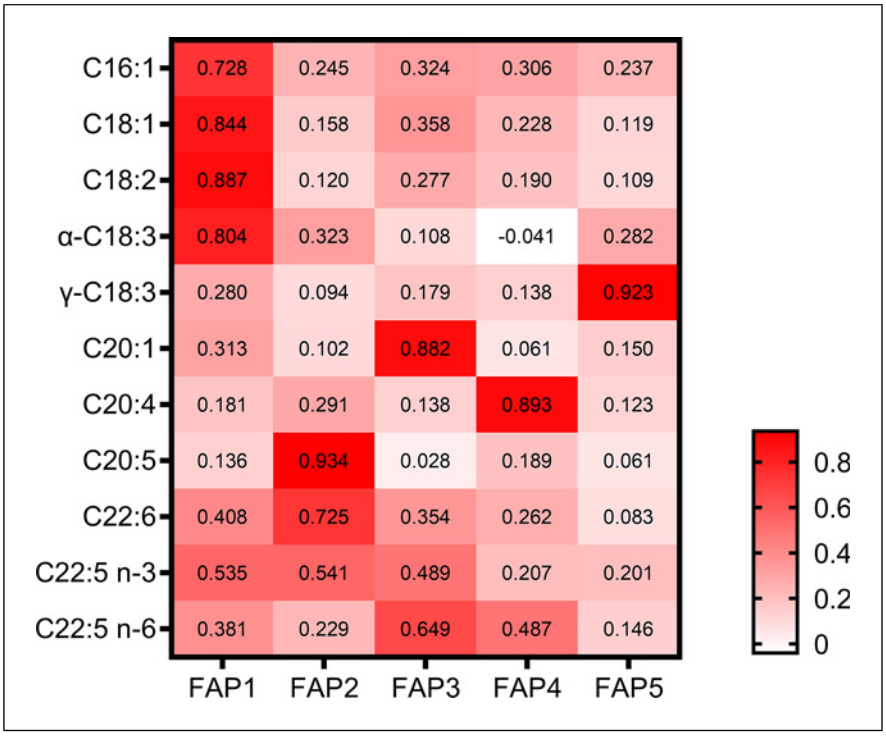


Fig. 1. Principal component eigenvalue load matrix after rotation. Extraction method: principal component analysis. Rotation method: varimax Kaiser normalization. C16:1 (PA), C18:1 (OA), C18:2 (LA), α-C18:3 (ALA), γ-C18:3 (GLA), C20:1 (eicosenoic acid), C20:4 (AA), C20:5 (EPA), C22:6 (DHA), C22:5 *n*-3 (DPA *n*-3), and C22:5 *n*-6 (DPA *n*-6).

Table 5. Component score coefficient matrix

	FAP1	FAP2	FAP3	FAP4	FAP5
C16:1	0.255	−0.093	−0.095	0.129	0.006
C18:1	0.393	−0.170	−0.063	0.057	−0.187
C18:2	0.478	−0.194	−0.164	0.045	−0.199
α-C18:3	0.403	0.104	−0.287	−0.295	0.108
γ-C18:3	−0.200	−0.056	−0.081	−0.037	1.120
C20:1	−0.249	−0.061	0.877	−0.289	−0.016
C20:4	−0.087	−0.138	−0.249	0.991	−0.015
C20:5	−0.161	0.724	−0.156	−0.136	0.003
C22:6	−0.066	0.426	0.097	−0.087	−0.103
C22:5 *n*-3	−0.023	0.244	0.216	−0.155	0.007
C22:5 *n*-6	−0.142	−0.114	0.414	0.297	−0.066

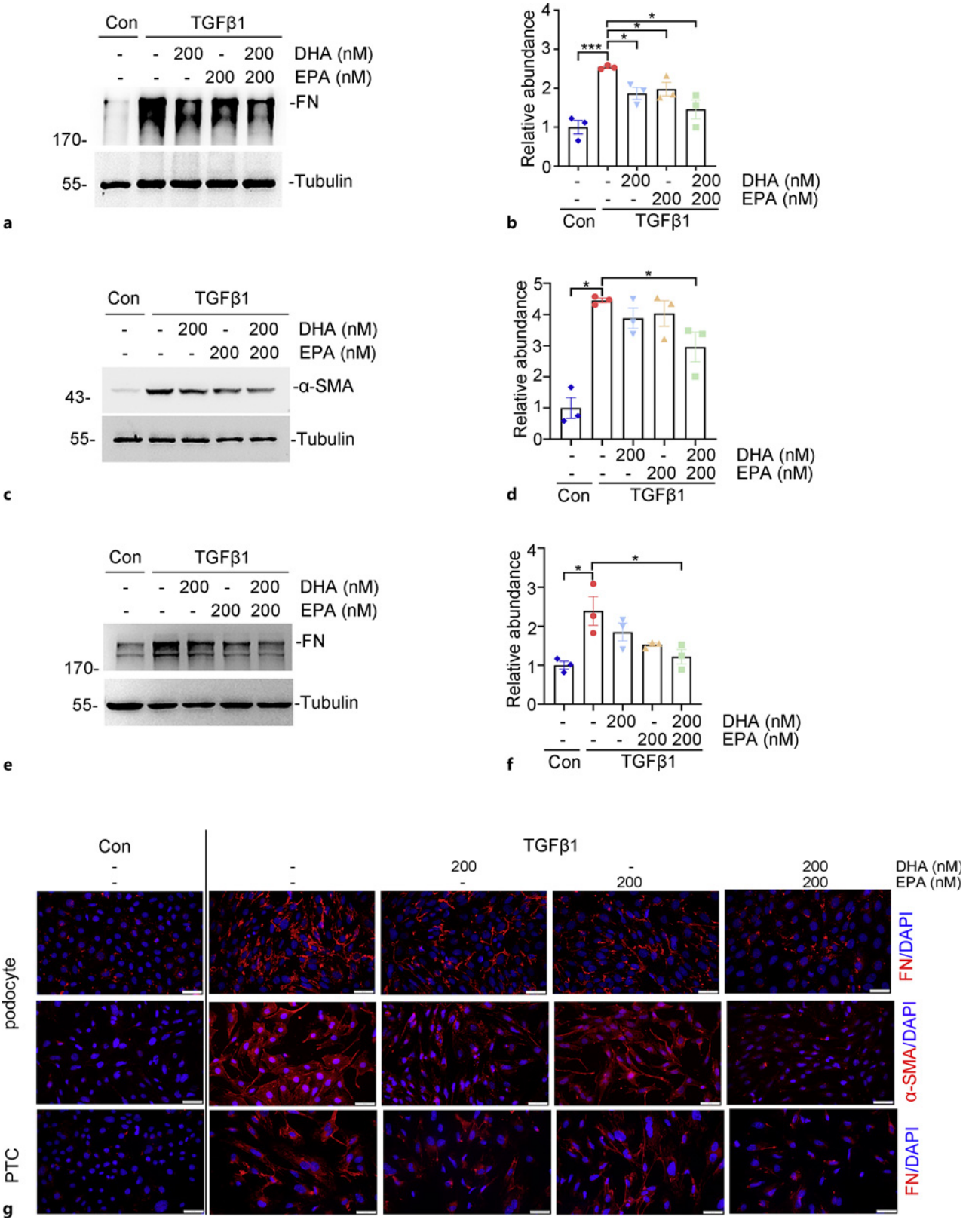
Extraction method: principal component analysis. Rotation method: varimax Kaiser normalization.

basic characteristics of the participants. Patients with DN were characterized by advanced age, longer diabetes duration, and a higher prevalence of hypertension. Laboratory findings indicated elevated levels of UACR, blood urea nitrogen, serum creatinine, uric acid, triglycerides, and hemoglobin A1C in patients with DN. Conversely, they exhibited lower levels of serum albumin and hemoglobin. Moreover, DN group showed significantly lower levels of six unsaturated fatty acids (PA, OA, eicosenoic acid, DHA, EPA, and DPA *n*-3) compared to non-DN group. However, there

Table 6. Multivariate logistic regression models of FAPs associated with the risk of diabetic nephropathy

	B	OR	95% CI	*p* value
FAP1				
Crude model	−0.064	0.938	(0.764, 1.150)	0.537
Adjusted model	−0.105	0.900	(0.687, 1.179)	0.445
FAP2				
Crude model	−0.443	0.642	(0.465, 0.886)	0.007
Adjusted model	−0.388	0.678	(0.493, 0.933)	0.017
FAP3				
Crude model	−0.098	0.907	(0.705, 1.165)	0.444
Adjusted model	−0.023	0.977	(0.749, 1.274)	0.864
FAP4				
Crude model	−0.094	0.910	(0.740, 1.120)	0.374
Adjusted model	0.031	1.031	(0.800, 1.330)	0.812
FAP5				
Crude model	−0.026	0.974	(0.788, 1.204)	0.808
Adjusted model	−0.132	0.877	(0.674, 1.141)	0.327

Crude model: not adjusted for confounders. Adjusted model: adjusted for diabetes duration, gender, age, smoking, drinking, hypertension, coronary heart disease, BUN, UA, Alb, TC, TG, HDL-C, LDL-C, P, K, t-Ca, HbA1C, Hb. BUN, blood urea nitrogen; UA, blood uric acid; Alb, serum albumin; TC, total cholesterol; TGs, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; P, serum phosphorus; K, serum potassium; t-Ca, serum total calcium; HbA1C, hemoglobin A1C; Hb, hemoglobin.



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were no significant differences observed for the remaining five fatty acids between the two groups. Additionally, no significant variations were found in the AA/DHA, AA/EPA, and *n*-6/*n*-3 PUFAs ratios between the two populations.

Association between Serum Unsaturated Fatty Acids and the Risk of DN

Table 2 presents the results of multivariate logistic regression analysis evaluating the association between unsaturated fatty acids and the risk of DN. Initially, without adjusting for confounding factors, PA, DHA, EPA, and DPA *n*-3 showed significant associations with the risk of DN. However, after adjusting for all confounders, only DHA, EPA, and DPA *n*-3 remained significantly associated with the risk of DN. The ORs (95% CI) were 0.583 (0.374, 0.908), 0.826 (0.716, 0.954), and 0.513 (0.298, 0.883), respectively. No significant associations were found between the other unsaturated fatty acids and the risk of DN.

FAPs in the Study Population Based on Factor Analysis

Correlation between Serum Unsaturated Fatty Acids

The Spearman rank correlation coefficients among serum unsaturated fatty acids are presented in Table 3. All 55 pairs (100%) demonstrated significant correlations with unsaturated fatty acids ($|r| \geq 0.3$), including 5 strongly correlated pairs ($|r| \geq 0.8$), 37 moderately correlated pairs ($0.5 \leq |r| < 0.8$), and 13 weakly correlated pairs ($0.3 \leq |r| < 0.5$). The results of the KMO test indicated a KMO value of 0.887, surpassing the recommended threshold of 0.5, and the Bartlett's sphericity test yielded a significant result with $p < 0.001$. These findings collectively suggest a robust correlation among unsaturated fatty acids, validating their suitability for factor analysis.

FAPs Extraction

Principal component analysis extracted a total of 11 principal components, representing 11 FAPs (Table 4). Five FAPs were extracted when the cumulative variance contribution rate exceeded 90%. Subsequently, the varimax method was applied to perform orthogonal rotation on these principal components. Following rotation, the characteristic roots of the five FAPs were greater than 1, specifically 3.501, 2.051, 1.938, 1.390, and 1.123. The factor loading matrix after rotation (Fig. 1) reflects the significance of unsaturated fatty acids within each FAP, with larger absolute values indicating higher significance. Based on a criterion of factor loadings exceeding 0.6 in absolute value, FAP1 exhibited higher concentrations of PA, OA, LA, and ALA; FAP2 showed elevated levels of DHA and EPA; FAP3 displayed increased concentrations of eicosenoic acid and DPA *n*-6; FAP4 demonstrated a higher concentration of AA, while FAP5 exhibited relatively more content of GLA.

Association between the FAP Scores and the Risk of DN

The FAP scores, calculated using the score coefficients for each unsaturated fatty acid in the FAPs (Table 5), were utilized to assess their association with the risk of DN. Multivariate logistic regression analysis was performed on the factor scores and the risk of DN. The results (Table 6) revealed a significant association between FAP2 and the occurrence of DN, without adjusting for confounding factors. The OR (95% CI) was 0.642 (0.465, 0.886). In the adjusted model, which accounted for variables such as diabetes duration, gender, age, smoking, drinking, hypertension, coronary heart disease, blood urea nitrogen, uric acid, serum albumin, total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, serum phosphorus, serum potassium, serum total calcium, hemoglobin A1C, and hemoglobin, FAP2 remained significantly associated with the risk of DN. The OR (95% CI) was 0.678 (0.493, 0.933). However, there was no significant association observed between the remaining FAPs and the risk of developing DN. These findings suggest that FAP2, characterized by elevated levels of DHA and EPA, serves as an independent protective factor against DN.

Effect of DHA and EPA on TGFβ1-Induced Extracellular Matrix Production in Podocytes and PTCs

To investigate the role of DHA and EPA in DN, podocytes and PTCs were treated with DHA, EPA, or a combination of DHA and EPA for 30 min, followed by TGFβ1 stimulation for 48 h without removing DHA or EPA. The production of extracellular matrix was then evaluated. The results of western blot analysis and immunofluorescence staining (Fig. 2) demonstrated that both DHA and EPA tended to reduce the production of extracellular matrix

Fig. 2. DHA and EPA synergistically reduce FN and α-SMA expression in podocytes and PTCs. **a**, **c** Western blotting analyses for FN and α-SMA abundance in podocytes among groups as indicated. **b**, **d** Quantitative analysis for FN and α-SMA in podocytes from different groups. *$p < 0.05$, $n = 3$. **e** Western blotting analyses for FN abundance in PTCs among groups as indicated. **f** Quantitative analysis for FN in PTCs from different groups. *$p < 0.05$, $n = 3$. **g** Representative micrographs of immunofluorescence staining showing FN and α-SMA-positive area in podocytes and FN-positive area in PTCs among groups as indicated. Scale bar, 50 μm. PTCs, primary cultured tubular cells; FN, fibronectin; α-SMA, alpha-smooth muscle actin.

induced by TGFβ1. Moreover, combination of both further suppressed the production of FN and α-SMA in podocytes, as well as reduced the production of FN in PTCs.

Discussion

In this study, we employed factor analysis to reveal the role for serum FAPs in diabetic patients for the first time. Among the five FAP patterns, FAP2, characterized by high concentrations of DHA and EPA, and low concentration of the other unsaturated fatty acids, is associated with low risk of DN. This study reveals that FAP analysis may provide a new strategy to predict the risk of DN in patients.

DN is pathologically characterized by the excessive accumulation of extracellular matrix, thickening of the basement membrane, and increased deposition of mesangial matrix. TGF-β, vascular endothelial growth factors, and tumor necrosis factor alpha have been implicated in the pathogenesis of DN, as they activate transcription factors involved in the accumulation of extracellular matrix within kidney [21, 22]. TGF-β, in particular, plays a crucial role in podocyte injury and interstitial fibrosis, leading to the progression of DN [23, 24]. In this study, both DHA and EPA exhibited a tendency to decrease the generation of extracellular matrix caused by TGFβ1. Additionally, their combined effect resulted in further suppression of FN and α-SMA production in podocytes, along with a reduction in FN production in PTCs. These findings support the protective effect of DHA and EPA against DN. It should be noted that FAP2 includes not only DHA and EPA, but also other non-significant fatty acids. The more the pattern of unsaturated fatty acids in the body resembles FAP2, the less the risk of developing DN. However, the situation in vivo is more complex and difficult to simulate in vitro. We only verified that the combined effect of DHA and EPA with factor loads more than 0.6 is better than the effect of either one.

The *n*-3 PUFAs possess significant potential in the prevention and treatment of DN through their anti-inflammatory, anti-angiogenic, and antioxidant properties [25–27]. The results of a randomized controlled trial by Eugene Han et al. [28] showed that dietary supplementation with *n*-3 PUFAs reduced urinary microalbumin excretion and slowed the decline in renal function, thereby exerting a protective effect on DN. Yazhuo Liu et al. [29] observed relatively low serum concentrations of DHA and C24:1 (nervonic acid) in patients with DN, along with a negative correlation between DHA and UACR. Despite conducting a correlation analysis of serum fatty acids and DN, the study did not consider the interaction between fatty acids. Notably, the main objective of this study was to summarize the interrelationships between fatty acids in an interpretable manner. Factor analysis allows the derivation of distinct FAPs from correlated unsaturated fatty acids.

It is important to acknowledge the limitations of this study. Dietary intake of fatty acids was not assessed, which may influence the observations. The sample size was relatively small, and further data from patients with diabetes are necessary to validate these conclusions. Additionally, as a single-center retrospective analytical study, conducting a prospective multicenter study would enhance our comprehensive understanding of the association between FAPs and the risk of DN. Despite these limitations, our findings align with previous research and suggest potential intervention measures for preventing DN [14, 30–33].

In conclusion, FAP2, characterized by high concentrations of DHA and EPA, exhibits a protective effect against DN in patients with type II diabetes. These findings provide insights into potential intervention strategies for reducing the risk of developing DN.

Statement of Ethics

This study protocol was reviewed and approved by the Committee of Ethics for Human Research of the Second Affiliated Hospital of Nanjing Medical University, approval number [2020] KY 009-01. All patients gave written informed consent to participate in this study.

Conflict of Interest Statement

Chunsun Dai is Associate Editor of the journal of "Kidney Diseases." The authors declare that they have no conflicts of interest with the contents of this article.

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Author Contributions

S. Xu, X. Li, Q. Hou, N. Xu, Q. Lu, and S. Wang conducted the experiments. S. Xu analyzed the data and wrote the manuscript. C. Dai designed the study, provided supervision throughout the research process, and revised the manuscript for intellectual content.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

References

1 DeFronzo RA, Reeves WB, Awad AS. Pathophysiology of diabetic kidney disease: impact of SGLT2 inhibitors. Nat Rev Nephrol. 2021;17(5):319–34.
2 Tervaert TW, Mooyaart AL, Amann K, Cohen AH, Cook HT, Drachenberg CB, et al. Pathologic classification of diabetic nephropathy. J Am Soc Nephrol. 2010;21(4):556–63.
3 Kim Y, Lim JH, Kim MY, Kim EN, Yoon HE, Shin SJ, et al. The adiponectin receptor agonist AdipoRon ameliorates diabetic nephropathy in a model of type 2 diabetes. J Am Soc Nephrol. 2018;29(4):1108–27.
4 Sun Y, Cui S, Hou Y, Yi F. The updates of podocyte lipid metabolism in proteinuric kidney disease. Kidney Dis. 2021;7(6):438–51.
5 Opazo-Ríos L, Mas S, Marín-Royo G, Mezzano S, Gómez-Guerrero C, Moreno JA, et al. Lipotoxicity and diabetic nephropathy: novel mechanistic insights and therapeutic opportunities. Int J Mol Sci. 2020;21(7):2632.
6 Su W, Cao R, He Y, Guan Y, Ruan X. Crosstalk of hyperglycemia and dyslipidemia in diabetic kidney disease. Kidney Dis. 2017;3(4):171–80.
7 Li YJ, Chen X, Kwan TK, Loh YW, Singer J, Liu Y, et al. Dietary fiber protects against diabetic nephropathy through short-chain fatty acid-mediated activation of G protein-coupled receptors GPR43 and GPR109A. J Am Soc Nephrol. 2020;31(6):1267–81.
8 Wei TT, Yang LT, Guo F, Tao SB, Cheng L, Huang RS, et al. Activation of GPR120 in podocytes ameliorates kidney fibrosis and inflammation in diabetic nephropathy. Acta Pharmacol Sin. 2021;42(2):252–63.
9 Kimura I, Ichimura A, Ohue-Kitano R, Igarashi M. Free fatty acid receptors in health and disease. Physiol Rev. 2020;100(1):171–210.
10 Lee D, Sevits K, Battson M, Wei Y, Cox-York K, Gentile C. Monounsaturated fatty acids protect against palmitate-induced lipoapoptosis in human umbilical vein endothelial cells. PLoS One. 2019;14(12):e0226940.
11 Yang Z, Inoue S, Taniguchi Y, Miyahara H, Iwasaki Y, Takeo J, et al. Long-term dietary supplementation with saury oil attenuates metabolic abnormalities in mice fed a high-fat diet: combined beneficial effect of omega-3 fatty acids and long-chain monounsaturated fatty acids. Lipids Health Dis. 2015;14:155.
12 Hu F, Stampfer M, Manson J, Rimm E, Colditz G, Rosner B, et al. Dietary fat intake and the risk of coronary heart disease in women. N Engl J Med. 1997;337(21):1491–9.
13 Rimm E, Appel L, Chiuve S, Djoussé L, Engler M, Kris-Etherton P, et al. Seafood long-chain n-3 polyunsaturated fatty acids and cardiovascular disease: a science advisory from the American heart association. Circulation. 2018;138(1):e35–e47.
14 Borsini A, Nicolaou A, Camacho-Muñoz D, Kendall AC, Di Benedetto MG, Giacobbe J, et al. Omega-3 polyunsaturated fatty acids protect against inflammation through production of LOX and CYP450 lipid mediators: relevance for major depression and for human hippocampal neurogenesis. Mol Psychiatry. 2021;26(11):6773–88.
15 Yamamoto T, Takabatake Y, Minami S, Sakai S, Fujimura R, Takahashi A, et al. Eicosapentaenoic acid attenuates renal lipotoxicity by restoring autophagic flux. Autophagy. 2021;17(7):1700–13.
16 Dyall SC, Balas L, Bazan NG, Brenna JT, Chiang N, da Costa Souza F, et al. Polyunsaturated fatty acids and fatty acid-derived lipid mediators: recent advances in the understanding of their biosynthesis, structures, and functions. Prog Lipid Res. 2022;86:101165.
17 Choi E, Ahn S, Joung H. Association of dietary fatty acid consumption patterns with risk of hyper-LDL cholesterolemia in Korean adults. Nutrients. 2020;12(5):1412.
18 Hu C, Lin Z, Liu Z, Tang X, Song J, Lin J, et al. Dietary fatty acid patterns and risk of oesophageal squamous cell carcinoma. PeerJ. 2022;10:e13036.
19 Warensjö E, Sundström J, Lind L, Vessby B. Factor analysis of fatty acids in serum lipids as a measure of dietary fat quality in relation to the metabolic syndrome in men. Am J Clin Nutr. 2006;84(2):442–48.
20 Rossing P, Caramori ML, Chan JCN, Heerspink HJL, Hurst C, Khunti K, et al. KDIGO 2022 clinical practice guideline for diabetes management in chronic kidney disease. Kidney Int. 2022;102(5):S1–127.
21 Li S, Huang P, Yang A, Tarng D, Yang W, Lin C, et al. Matrix metalloproteinase-9 deficiency attenuates diabetic nephropathy by modulation of podocyte functions and dedifferentiation. Kidney Int. 2014;86(2):358–69.
22 Wei L, Xiao Y, Li L, Xiong X, Han Y, Zhu X, et al. The susceptibility genes in diabetic nephropathy. Kidney Dis. 2018;4(4):226–37.
23 Herman-Edelstein M, Thomas MC, Thallas-Bonke V, Saleem M, Cooper ME, Kantharidis P. Dedifferentiation of immortalized human podocytes in response to transforming growth factor-β: a model for diabetic podocytopathy. Diabetes. 2011;60(6):1779–88.
24 Ibrahim S, Rashed L. Estimation of transforming growth factor-beta 1 as a marker of renal injury in type II diabetes mellitus. Saudi Med J. 2007;28(4):519–23.
25 Al-Shabrawey M, Bartoli M, El-Remessy AB, Platt DH, Matragoon S, Behzadian MA, et al. Inhibition of NAD(P)H oxidase activity blocks vascular endothelial growth factor overexpression and neovascularization during ischemic retinopathy. Am J Pathol. 2005;167(2):599–607.
26 Calder PC. Long-chain fatty acids and inflammation. Proc Nutr Soc. 2012;71(2):284–9.
27 Koto T, Nagai N, Mochimaru H, Kurihara T, Izumi-Nagai K, Satofuka S, et al. Eicosapentaenoic acid is anti-inflammatory in preventing choroidal neovascularization in mice. Invest Ophthalmol Vis Sci. 2007;48(9):4328–34.
28 Han E, Yun Y, Kim G, Lee YH, Wang HJ, Lee BW, et al. Effects of omega-3 fatty acid supplementation on diabetic nephropathy progression in patients with diabetes and hypertriglyceridemia. PLoS One. 2016;11(5):e0154683.
29 Liu Y, Li Y, Shen H, Li Y, Xu Y, Zhou M, et al. Association between the metabolic profile of serum fatty acids and diabetic nephropathy: a study conducted in northeastern China. Ther Adv Endocrinol Metab. 2022;13:20420188221118750.
30 Li X, Bi X, Wang S, Zhang Z, Li F, Zhao A. Therapeutic potential of ω-3 polyunsaturated fatty acids in human autoimmune diseases. Front Immunol. 2019;10:2241.
31 Boyd J, LoCoco P, Furr A, Bendele M, Tram M, Li Q, et al. Elevated dietary ω-6 polyunsaturated fatty acids induce reversible peripheral nerve dysfunction that exacerbates comorbid pain conditions. Nat Metab. 2021;3(6):762–73.
32 Montecillo-Aguado M, Tirado-Rodriguez B, Antonio-Andres G, Morales-Martinez M, Tong Z, Yang J, et al. Omega-6 polyunsaturated fatty acids enhance tumor aggressiveness in experimental lung cancer model: important role of oxylipins. Int J Mol Sci. 2022;23(11):6179.
33 Calder PC. Omega-3 fatty acids and inflammatory processes. Nutrients. 2010;2(3):355–74.