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Association between fish and shellfish, and omega-3 PUFAs intake and CVD risk factors in middle-aged female patients with type 2 diabetes

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BACKGROUND/OBJECTIVES: This study was performed to investigate the association between the dietary intake of fish and shellfish, and omega-3 polyunsaturated fatty acids (PUFAs) and cardiovascular disease (CVD) risk factors in the middle-aged Korean female patients with Type 2 diabetes (T2D).
SUBJECTS/METHODS: A cross-sectional analysis was performed with 356 female patients (means age: 55.5 years), who were recruited from the Huh's Diabetes Clinic in Seoul, Korea between 2005 and 2011. The dietary intake was assessed by a validated semi-quantitative food frequency questionnaire and analyzed using the Computer Aided Nutritional Analysis program (CAN-Pro) version 4.0 software.
RESULTS: In a multiple regression analysis after the adjustment for confounding factors such as age, BMI, duration of diagnosed T2D, alcohol consumption, fiber intake, sodium intake, and total energy intake, fish and shellfish intake of the subjects was negatively associated with triglyceride and pulse wave velocity (PWV). Omega-3 PUFAs intake was negatively associated with triglyceride, systolic blood pressures, diastolic blood pressures, and PWV. The multiple logistic regression analysis with the covariates showed a significant inverse relationship between the omega-3 PUFAs consumption and prevalence of hypertriglyceridemia [OR (95% CI) for greater than the median compared to less than the median: 0.395 (0.207-0.753)].
CONCLUSIONS: These results suggest that the consumption of fish and shellfish, good sources of omega-3 PUFAs, may reduce the risk factors for CVD in the middle-aged female patients with T2D.

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INTRODUCTION

The prevalence of diabetes in the adults aged ≥ 30 years in South Korea has increased from 7.2% in 1990s to 11% (12.8% for males and 9.1% for females) in 2013, and the diabetic complications such as dyslipidemia, vascular stiffness, macro-vascular diseases have become leading causes of morbidity and death [1,2]. Despite decreased prevalence in females for Type 2 diabetes (T2D), female diabetics are reported to suffer from a higher risk of diabetic vascular complications than male patients in various populations in the world [3] including those in S. Korea [4].

Numerous observational studies and clinical trials have shown that a healthy diet including vegetables and fruits, whole grains and legumes, low-fat dairy products, lean meat, poultry, and fish is essential not only for the glycemic control but also for cardiovascular disease (CVD) risk factor management in T2D. Among various components of diet, considerable evidence indicates that the fish consumption has beneficial effects on the lipid profiles, blood pressure, and vascular stiffness, and it is also protective against vascular complications [5-11]. As to the mechanism, omega-3-polyunsaturated fatty acids (PUFAs) in the fish decrease the synthesis of hepatic very low-density lipoprotein (VLDL) and triglyceride and also promote triglyceride clearance from chylomicrons and VLDL particles [12-16]. Protective role of fish against CVD has been reported in various populations in the world including Asia such as Japan and coastal China [5,9,10] whose fish consumption is relatively high compared to the Western countries. However, to the best of our knowledge, no such study has been performed in S. Korea where the fish

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and shellfish consumption is considerably high [1].

Therefore, the aim of this study was to determine the relationship between fish and shellfish, and omega-3 PUFAs intake and CVD risk factors in the middle-aged Korean female patients with T2D.

SUBJECTS AND METHODS

Study subjects

The participants were female patients with T2D who visited Huh's Diabetes Clinic in Seoul, Korea. Among a total 854 patients from September 2005 to February 2011, 358 patients aged 40−65 years with a diagnosis of T2D were selected and interviewed for a baseline investigation; all the 358 patients had anthropometric data as well as dietary data. From the baseline samples, the data for two patients administering estrogen were excluded. The remaining 356 subjects had sufficient data for the blood profiles. Thus, a total of 356 female patients with T2D were ultimately eligible for this study. The research protocol was approved by the Institutional Review Board (IRB) of Yonsei University Medical Center (3-2006-0004), and all the subjects provided their written informed consent to participate.

General characteristics and anthropometric parameters

All the female patients were individually interviewed at the first visit to obtain information about their general characteristics and lifestyle behaviors. Age, duration of diagnosed T2D, family history of diabetes, medical information for diabetes, hypertension and dyslipidemia treatment were obtained from the medical records. Life-style behaviors such as alcohol drinking status, smoking, exercise, and nutritional supplement use were also obtained from the patients by the individual interviews. The standing height was measured using a standiometer (Seca Inc., Hamburg, Germany). The body weight and body composition such as skeletal muscle mass, fat mass, fat free mass, and percentage of body fat were measured using an In-body 4.0 (Biospace Co., Ltd, Seoul, Korea), and the body mass index (BMI, kg/m^2) was calculated as well.

Clinical parameters

Blood samples were drawn after a minimum 12-h overnight fast, collected in EDTA-containing tubes, and centrifuged at 3,000 rpm for 20 min at 4°C (Hanil Science Industrial Co., Ltd, Seoul, Korea). Fasting plasma levels of glucose, total cholesterol, triglyceride, and high-density lipoprotein (HDL)-cholesterol were assessed using an autoanalyzer (Cobas Mira Roche Autoanalyzer, Hoffmann-La Roche Ltd., Basel, Switzerland). The low-density lipoprotein (LDL)-cholesterol and atherogenic index (AI) were calculated using the following Equations described by Friedwald [17] and Lauer [18], respectively.

LDL-cholesterol
= Total cholesterol - HDL-cholesterol - (Triglyceride/5)

AI = (Total cholesterol - HDL-cholesterol)/HDL-cholesterol

Systolic and diastolic blood pressures were taken in the sitting position after a 10-min rest, using an automatic blood-pressure monitor (Biospace Co., Seoul, Korea). Brachial-ankle pulse wave velocity (baPWV) was calculated by measuring the moving velocity of pulse wave between the two different points (the brachial and ankles) of the artery using an automated analyzer (VP-1000; Colin Co. Ltd., Komaki, Japan). The transmission distance was measured by the subjects' height, and each side baPWV was calculated using the following Equation [19].

baPWV = transmission distance/transmission time

The average value of the right and left side baPWV was used for the analysis.

Dietary assessment

Dietary intake information was collected by trained dietitians using a validated semi-quantitative food frequency questionnaire (FFQ) designed to assess the average food intake over the previous year [20]. The FFQ consists of 144 food items with standard intake amount (below-standard, standard and over-standard) and a selection of nine frequency categories (such as never or less than once a month, once a month, 2-3 times a month, 1-2 times a week, 3-4 times a week, 5-6 times a week, once a day, twice a day and three times a day). Thirteen food items were selected as fish and shellfish sources including yellow croaker, pollack, mackerel, tuna, eel, mudfish, squid, anchovy, clam, shrimp, crab, Korean fishcake, and salted seafood. Participants were asked to report their frequency of consumption of each food item during the last 1 year. The dietary intake data was analyzed using the Computer Aided Nutritional Analysis program (CAN-Pro) version 4.0 software [21]. To increase coverage for fatty acid intake, fatty acids data from USDA [22], National Fisheries Research, and Development Institute [23] were applied to Can-Pro 4.0 database. We covered 83% of total fatty acid intake of the subjects.

Statistical analysis

Continuous values were expressed as means with standard deviation (SD), and categorical values were represented by the frequency and percentage. The blood profiles data were log-transformed to normalize their distributions before the analysis. A multiple regression analysis was used to investigate the relationship between fish and shellfish, and omega-3 PUFAs intake and CVD risk factors. The patients were divided into two groups based on their fish and shellfish (less than the median, 0.7-48.3 g/d; greater than the median, 48.6-498.8 g/d), and omega-3 PUFAs consumption (less than the median, 0.02-1.2 g/d; greater than the median, 1.2-13.6 g/d). The association among the risk for dyslipidemia, hypertension, and high PWV and their fish and shellfish, and omega-3 PUFAs intake level were identified using the multiple logistic regression analysis and expressed as odds ratios (ORs) with 95% confidence intervals (CIs). Potential confounders were included in a multiple regression and multiple logistic regression as covariates (age, BMI, duration of diagnosed T2D, alcohol consumption, fiber intake, sodium intake, and total energy intake). All the statistical analyses were performed using the SAS statistical package (SAS 9.2, SAS Institute Inc., Cary, NC, USA), and the level of significance was set at $P < 0.05$.

RESULTS

General, anthropometric, and clinical characteristics

As shown in Table 1, the average age of the subjects was

Table 1. General, anthropometric, and clinical characteristics of subjects (n = 356)

	Mean ± SD or n (%)	Range
General characteristics		
Age (year)	55.5 ± 5.9	40-64
Duration of type 2 diabetes (year)	7.8 ± 6.6	0-36
Family history of type 2 diabetes [n (%)]	241 (67.7)	
Medication usage		
Diabetes medication users [n (%)]	242 (71.2)	
Hypertension medication users [n (%)]	113 (33.2)	
Cholesterol medication useres [n (%)]	95 (27.9)	
Health behavior		
Current smokers [n (%)]	12 (3.5)	
Current alcohol drinker [n (%)]	49 (13.9)	
Regular exercise [n (%)]	231 (66.0)	
Nutritional supplement user [n (%)]	164 (48.7)	
Athropometric characteristics		
Height (cm)	156.6 ± 4.8	143.0-171.0
Weight (kg)	59.3 ± 9.1	35.4-105.0
Body mass index (kg/m^2)	24.2 ± 3.4	16.4-39.0
Clinical characteristics		
Triglyceride (mg/dl)	145.0 ± 91.1	25.0-553.0
Total cholesterol (mg/dl)	197.9 ± 45.2	64.0-432.0
HDL-cholesterol (mg/dl)	51.1 ± 11.8	15.0-94.0
LDL-cholesterol (mg/dl)	117.9 ± 39.0	36.6-318.0
Atherogenic index	3.0 ± 1.1	0.7-6.9
SBP (mmHg)	135.2 ± 18.2	88.0-200.0
DBP (mmHg)	83.3 ± 11.1	50.0-122.0
baPWV (cm/sec)*	1,568.1 ± 269.4	1046.5-2338.0

Abbreviations: SD, standard deviation; HDL-cholesterol, high density lipoprotein cholesterol; LDL-cholesterol, low density lipoprotein cholesterol; AI, atherogenic index; SBP, systolic blood pressure; DBP, diastolic blood pressure; baPWV, brachial-ankle pulse wave velocity
* Data were available for 166 women

Table 2. Daily foods and nutrients intakes of subjects (n = 356)

	Amount	Range
Food group intakes (g/d)		
Fish and shellfish	63.9 ± 60.7	0.7-498.8
Total animal food	297.9 ± 227.1	4.9-2242.6
Total food intakes	1,492.4 ± 68.2	271.4-4743.4
Nutrients intakes		
Energy (kcal/d)	1,847.9 ± 750.1	508.0-4912.5
Carbohydrate (g/d)	283.9 ± 99.3	77.8-687.2
Protein (g/d)	77.8 ± 40.8	14.3-360.0
Fat (g/d)	46.9 ± 31.7	2.1-265.5
SFAs	12.3 ± 9.5	0.2-63.6
MUFAs	15.1 ± 12.2	0.3-88.7
PUFAs	12.0 ± 9.2	0.4-70.2
Omega-3	1.7 ± 1.5	0.1-13.6
Omega-6	9.5 ± 6.9	0.4-52.7
Fiber (g/d)	28.8 ± 13.1	5.6-87.3
Sodium (mg/d)	5,280.1 ± 2,848.5	738.4-21629.1

Values are mean ± SD, which were calculated by Can-pro 4.0
Abbreviations: SFAs, Saturated fatty acids; MUFAs, Monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids

55.5 years, and the average number of years with T2D was 7.8 years. The proportion of the patients with a family history of T2D was 67.7%. With regard to health behavior, the proportion of current smoker, current alcohol drinker, regular exercise and nutritional supplement user among the patients were 3.5%, 13.9%, 66.0%, and 48.7%, respectively. The mean BMI of the subjects was 24.2 kg/m^2. The average plasma concentrations of triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, AI, and baPWV were 145.0, 197.9, 51.1, 117.9 mg/dL, 3.0, and 1568.1 cm/s, respectively.

Dietary intake

The average daily intakes of fish and shellfish were 63.9 g (Table 2). Total energy and fat intakes of the subjects were 1847.9 kcal and 46.9 g, respectively. The average intakes of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) were 12.3, 15.1, and 12.0 g, respectively. Omega-3 and omega-6 PUFAs intakes were 1.7 and 9.5 g, respectively.

Association between fish and shellfish, and omega-3 PUFAs intakes and CVD risk factors

In a multiple regression analysis, significant association between fish and shellfish, and omega-3 PUFAs intakes and CVD risk factors was found after the adjustment for confounding factors (Table 3). The fish and shellfish intake of the subjects was negatively associated with triglyceride ($P = 0.0126$) and baPWV ($P = 0.0324$). Similar results were also observed in the Omega-3 PUFAs intake. Omega-3 PUFAs intake was negatively associated with triglyceride ($P = 0.0016$), SBP ($P = 0.0049$), DBP ($P = 0.0060$), and baPWV ($P = 0.0372$).

The multiple logistic regression analysis (Table 4) with covariates showed a significant inverse relationship between the omega-3 PUFAs consumption and prevalence of hypertriglyceridemia [OR (95% CI) for the greater than the median compared to the less than the median: 0.395 (0.207-0.753)].

DISCUSSION

The intake of fish and shellfish was found to be negatively associated with plasma triglyceride levels and PWV in the middle-aged female patients with T2D. The omega-3 PUFAs intake was negatively associated with plasma triglyceride levels, SBP, DBP, and PWV. The women whose intakes of omega-3 PUFAs were greater than the median were at lower risk for hypertriglyceridemia than those with intakes less than the median.

The results of this study indicate an inverse association between fish and shellfish, and omega-3 PUFAs intake and dyslipidemia reported among the healthy non-T2D subjects in several countries by other investigators [24,25]. Dewailly *et al.* [24] showed that the concentrations of EPA and DHA in plasma phospholipids were reflected by fish consumption (average fish intake = 95 g/wk), and EPA was positively associated to the plasma HDL-cholesterol concentration. Another cross-sectional study by Okud *et al.* [25] revealed that Japanese women with a higher intake of fish products than their Hawaiian counterpart had lower serum TG levels and similar HDL-cholesterol levels

Table 3. Coefficients from multiple regression analysis between fish and shellfish, and omega-3 PUFAs intakes and CVD risk factors (n = 356)

	Fish and shellfish intake			Omega-3 PUFAs intake		
	β (SE)	*P*	R^2	β (SE)	*P*	R^2
Triglyceride						
Unadjusted	-0.0014 (00005)	0.0061	0.0211	-0.0689 (0.0205)	0.0009	0.0310
Adjusted[1)]	-0.0025 (0.0005)	0.0017	0.1271	-0.0700 (0.0194)	0.0004	0.1342
Adjusted[2)]	-0.0016 (0.0001)	0.0126	0.1500	-0.0956 (0.0301)	0.0016	0.1597
Total cholesterol						
Unadjusted	0.0000 (0.0002)	0.8926	0.0001	-0.0031 (0.0082)	0.7078	0.0004
Adjusted[1)]	0.0000 (0.0002)	0.8423	0.0133	-0.0022 (0.0082)	0.7857	0.0133
Adjusted[2)]	-0.0001 (0.0002)	0.4551	0.6314	-0.0122 (0.0128)	0.3386	0.0398
HDL-cholesterol						
Unadjusted	0.0005 (0.0002)	0.0070	0.0204	0.0270 (0.0082)	0.0010	0.0300
Adjusted[1)]	0.0006 (0.0002)	0.0059	0.0429	0.0269 (0.0081)	0.0010	0.0517
Adjusted[2)]	0.0001 (0.0003)	0.7123	0.0753	0.0084 (0.0128)	0.5109	0.0762
LDL-cholesterol						
Unadjusted	-0.0001 (0.0003)	0.6750	0.0005	-0.0094 (0.0121)	0.4368	0.0017
Adjusted[1)]	-0.0001 (0.0002)	0.7700	0.0056	-0.0080 (0.0125)	0.5080	0.0066
Adjusted[2)]	-0.0002 (0.0004)	0.6172	0.0283	-0.0127 (0.0188)	0.4993	0.0289
AI						
Unadjusted	-0.0008 (0.0004)	0.0245	0.0143	-0.0432 (0.0136)	0.0016	0.0278
Adjusted[1)]	-0.0008 (0.0003)	0.0225	0.0638	-0.0419 (0.0133)	0.0018	0.0759
Adjusted[2)]	-0.0004 (0.0005)	0.4362	0.0764	-0.0318 (0.0209)	0.1288	0.0812
SBP						
Unadjusted	-0.0111 (0.0160)	0.4889	0.0014	-0.6657 (0.6536)	0.3091	0.0029
Adjusted[1)]	-0.0113 (0.0151)	0.4547	0.1170	-0.6115 (0.6182)	0.3233	0.1181
Adjusted[2)]	-0.0319 (0.0197)	0.1074	0.1348	-2.7005 (0.9532)	0.0049	0.1489
DBP						
Unadjusted	-0.0113 (0.0097)	0.2457	0.0038	-0.6752 (0.3970)	0.0899	0.0081
Adjusted[1)]	-0.1344 (0.0094)	0.1543	0.0755	-0.6824 (0.3847)	0.0770	0.0783
Adjusted[2)]	-0.0224 (0.0123)	0.0696	0.0740	-1.6468 (0.5956)	0.0060	0.0861
baPWV						
Unadjusted	-0.0006 (0.0002)	0.0025	0.0542	-0.0213 (0.0080)	0.0083	0.0417
Adjusted[1)]	-0.0005 (0.0002)	0.0065	0.2256	-0.0180 (0.0073)	0.0151	0.2184
Adjusted[2)]	-0.0004 (0.0001)	0.0324	0.3089	-0.0228 (0.0108)	0.0372	0.3079

From multiple regression analysis of log transformed CVD risk factors
Abbreviations: PUFAs, polyunsaturated fatty acids; CVD, cardiovascular disease; HDL-cholesterol, high density lipoprotein cholesterol; LDL-cholesterol, low density lipoprotein cholesterol; AI, atherogenic index; SBP, systolic blood pressure; DBP, diastolic blood pressure; baPWV, brachial-ankle pulse wave velocity
[1)] Adjusted for age, and BMI
[2)] Adjusted for age, BMI, duration of diagnosed T2D, alcohol consumption, fiber intake, sodium intake, and total energy intake

Table 4. Odds ratio (OR) and 95% confidence interval (CI) of CVD risk factors according to the intakes of fish and shellfish, and omega-3 PUFAs (n = 356)

	Fish and shellfish intake		Omega-3 PUFAs intake	
	Below the median	Above the median	Below the median	Above the median
Median intake (g/d)	28.5 (0.7-48.3)	99.0 (48.6-498.8)	0.77 (0.02-1.2)	2.53 (1.2-13.6)
OR (95% CI) for triglyceride ≥ 150 mg/dl				
Unadjusted	1 (ref)	0.688 (0.443-1.067)	1 (ref)	0.507 (0.325-0.791)
Adjusted[1)]	1 (ref)	0.626 (0.392-1.000)	1 (ref)	0.467 (0.292-0.746)
Adjusted[2)]	1 (ref)	0.621 (0.343-1.127)	1 (ref)	0.395 (0.207-0.753)
OR (95% CI) for HDL-cholesterol < 40 mg/dl				
Unadjusted	1 (ref)	0.950 (0.509-1.776)	1 (ref)	0.775 (0.413-1.453)
Adjusted[1)]	1 (ref)	1.029 (0.543-1.948)	1 (ref)	0.783 (0.415-1.476)
Adjusted[2)]	1 (ref)	1.164 (0.524-2.583)	1 (ref)	0.859 (0.367-2.012)
OR (95% CI) for AI > 2.87 (median)				
Unadjusted	1 (ref)	0.854 (0.564-1.295)	1 (ref)	0.651 (0.429-0.989)

Table 4. continued

	Fish and shellfish intake		Omega-3 PUFAs intake	
	Below the median	Above the median	Below the median	Above the median
Adjusted[1)]	1 (ref)	0.885 (0.577-1.356)	1 (ref)	0.659 (0.432-1.006)
Adjusted[2)]	1 (ref)	1.091 (0.644-1.848)	1 (ref)	0.756 (0.433-1.320)
OR (95% CI) for SBP ≥ 140 mmHg				
Unadjusted	1 (ref)	1.156 (0.751-1.777)	1 (ref)	1.156 (0.751-1.777)
Adjusted[1)]	1 (ref)	1.188 (0.756-1.867)	1 (ref)	1.165 (0.745-1.821)
Adjusted[2)]	1 (ref)	0.892 (0.508-1.566)	1 (ref)	0.744 (0.408-1.357)
OR (95% CI) for DBP ≥ 90 mmHg				
Unadjusted	1 (ref)	0.895 (0.563-1.421)	1 (ref)	0.715 (0.449-1.139)
Adjusted[1)]	1 (ref)	0.834 (0.516-1.346)	1 (ref)	0.691 (0.429-1.111)
Adjusted[2)]	1 (ref)	0.787 (0.435-1.423)	1 (ref)	0.520 (0.274-0.988)
OR (95% CI) for baPWV > 1532.5 cm/sec (median)				
Unadjusted	1 (ref)	0.604 (0.368-0.993)	1 (ref)	0.883 (0.541-1.441)
Adjusted[1)]	1 (ref)	0.705 (0.422-1.179)	1 (ref)	0.928 (0.560-1.537)
Adjusted[2)]	1 (ref)	0.749 (0.402-1.395)	1 (ref)	1.146 (0.596-2.204)

Abbreviations: PUFAs, polyunsaturated fatty acids; CVD, cardiovascular disease; HDL-cholesterol, high density lipoprotein cholesterol; AI, atherogenic index; SBP, systolic blood pressure; DBP, diastolic blood pressure; baPWV, brachial-ankle pulse wave velocity

[1)] Adjusted for age, and BMI

[2)] Adjusted for age, BMI, duration of diagnosed T2D, alcohol consumption, fiber intake, sodium intake, and total energy intake

than the Hawaiian women. In Iranian female adults without T2D (average fish intake = 14.4 g/d), the adjusted ORs for hypertriglyceridemia and low HDL-cholesterol were lower in the 3rd tertile than the 1st tertile of fish intake [26].

Although the cross-sectional study data on the fish oil consumption and lipid profiles among the T2D subjects are limited, several randomized-controlled studies revealed that the fish oil supplementation improve lipid profiles in the T2D subjects in several western countries [5,27-30]. After consuming 8 g of daily omega-3 PUFAs supplementation for eight weeks, the serum TG level showed a consistent decrease of 42% among the US patients with T2D [27]. The plasma TG concentration decreased after the consumption of fish oil supplement for six months in the T2D patients in Italy and Australia [28].

The possible mechanisms of protective CVD by fish and shellfish and omega-3 PUFAs intake are as follows. Omega-3 PUFAs in the fish is known to decrease the serum TG concentration by accelerating chylomicron TG clearance [12,31]. EPA and DHA supplementations lower the half-lives and particle size of chylomicron TG and enhance preheparin lipoprotein lipase activities [32]. Omega-3 PUFAs (EPA) are also known to inhibit hepatic synthesis of TG by decreasing the diacylglycerol acyltransferase [33]. Further, various studies showed convincing evidences that EPA and DHA lower the synthesis of hepatic very-low-density-cholesterol (VLDL) and TG and promote the TG clearance from chylomicrons and VLDL particles [12-16].

Hypertension is widely known as an important CVD risk factor. The intake of omega-3 PUFAs was negatively associated to SBP and DBP, confirming the results of the earlier studies by other investigators [24,34]. Although the study subjects were non-T2D, increased consumption of omega-3 PUFAs was associated with decreased SBP [34], and EPA and DHA were inversely associated to SBP and DHA, respectively [24]. For T2D [35] and hypertensive subjects [36,37], several randomized- controlled studies reported a reduction in the SBP and DBP with the omega-3 PUFAs fish oil supplementation. Omega-3 PUFAs stimulate the release of nitric oxide (NO), ADP, and vasoactive prostanoids such as thromboxane A2 and prostacyclin I2, and possibly an endothelium-derived hyperpolarizing factor; all of which play a significant role in endothelial dysfunction and blood pressure [38,39]. They are often incorporated into the plasma and cellular membranes, leading to physiochemical changes in the membrane structure associated with less reactive platelets and a decreased response to hormones that increase blood pressure [39-41].

In our study, intakes of fish and shellfish and omega-3 PUFAs were negatively associated with PWV. Similar results showing a benefit of high fish or omega-3 PUFA consumption on PWV have been reported by other investigators, though the subjects were non-T2D. A cross-sectional study on Japanese women indicated that the PWV of the aorta was significantly slower in fishing villages than in the farming villages where people usually eat less fish [42]. The serum levels of total marine omega-3 PUFAs, EPA, and DHA were higher in the Korean middle-aged men than in the Caucasians and Japanese American counterparts, and that total marine omega-3 PUFAs had a significant inverse association with carotid-femoral PWV in Korean men only [43]. Among the Japanese patients with a CVD risk factor, a fish-based diet (> 1.0 g/d omega-3 PUFAs derived from fish) was effective against increased baPWV only in the patients with a low CVD risk [44].

There are several possible mechanisms in which omega-3 PUFAs from fish and shellfish protect against aortic stiffness. It is widely known that stiffer collagen plays a significant role in the formation of aortic stiffness along with the degradation of elastin fibers [45], whereas healthy collagen production is associated with the preservation of the structure of the aorta. Omega-3 PUFAs have been linked to the nuclear factor-kappa-B pathway, ultimately leading to the stimulation of healthy collagen production [46]. Further, aortic calcification is known to be associated with aortic stiffness [47]. Omega-3 PUFAs directly inhibit vascular calcification by activating the p-38-

mitogen-activated-protein kinase and peroxisome proliferator-activated receptor-γ pathway [48].

Our study has the following limitations. The cross-sectional study design mades it impossible to determine whether the intake of fish and shellfish, and omega-3 PUFAs is a cause or consequence of the improvement of CVD risk factors. The recall bias in the FFQ may have affected the dietary assessment, although the FFQ is a more powerful method for assessing a typical intake than other dietary assessment methods. Moreover, FFQ is limited to analyze the accurate omega-3 PUFAs content, depending on the species of fish and shellfish. Finally, although menopausal status has an association with CVD risk factor, this study did not apply the effect of menopause because of the cause of deficient menopause information. However, this disadvantage is likely to be reduced because ~16% of the subjects were younger than 49 years (the mean age at menopause in Korea [49]). Despite these limitations, to the best of our knowledge, this is the first study investigating the relationship of fish and omega-3 PUFAs intake and CVD risk factor in the Korean female patients with T2D. The results of this study may be helpful for the female patients with T2D with a need to lower their blood pressure and PWV including serum lipid levels to prevent diabetic CVD complications.

In conclusion, the intake of fish and shellfish, and omega-3 PUFAs among the middle-aged Korean female patients with T2D may be protective against CVD risk factors. Therefore, a moderately high intake of fish and shellfish should be recommended for the middle-aged Korean females with T2D to prevent CVD, which are serious diabetic complications.

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