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

Hyesook Kim1,*, Seokyung Park2,*, Hyesu Yang1, Young Ju Choi3, Kap Bum Huh3 and Namsoo Chang1§

1Department of Nutritional Science and Food Management, Ewha Womans University, 52, Ewhayeodae-gil, Seodaemun-gu, Seoul 120-750, Korea
2Department of Clinical Nutrition Science, The Graduate School of Clinical Health Sciences, Ewha Womans University, Seoul 120-750, Korea
3Huh’s Diabetes Clinic & the 21C Diabetes and Vascular Research Institute, Seoul 121-806, Korea

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.

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...
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 stadiometer (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.

\[
LDL-\text{cholesterol} = \frac{\text{Total cholesterol} - \text{HDL-cholesterol} - (\text{Triglyceride}/5)}{\text{Triglyceride}}
\]

\[
\text{AI} = \frac{\text{Total cholesterol} - \text{HDL-cholesterol}}{\text{HDL-cholesterol}}
\]

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].

\[\text{baPWV} = \frac{\text{transmission distance}}{\text{transmission time}}\]

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, 1-2 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)

| General characteristics                      | Mean ± SD or n (%) | Range |
|----------------------------------------------|--------------------|-------|
| 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 (32.2)         |       |
| Cholesterol medication users [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 (46.7)         |       |
| Anthropometric 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/m²)                      | 24.2 ± 3.4         | 16.4-39.0 |
| Clinical characteristics                     |                    |       |
| Triglyceride (mg/dL)                         | 145.0 ± 91.1       | 250-553.0 |
| Total cholesterol (mg/dL)                    | 197.9 ± 45.2       | 64-432.0 |
| HDL-cholesterol (mg/dL)                      | 51.1 ± 11.8        | 15-94.0 |
| LDL-cholesterol (mg/dL)                      | 117.9 ± 39.0       | 36-318.0 |
| Atherogenic index                            | 3.0 ± 1.1          | 0.7-6.9 |
| SBP (mmHg)                                   | 135.2 ± 18.2       | 80-200.0 |
| DBP (mmHg)                                   | 83.3 ± 11.1        | 50-122.0 |
| 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.

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

| Food group intakes (g/d)                  | Amount          | Range              |
|-------------------------------------------|-----------------|--------------------|
| Fish and shellfish                         | 63.9 ± 60.7     | 0.7-498.8          |
| Total animal food                          | 297.9 ± 227.1   | 4.9-2242.6         |
| Total fish intakes                         | 1,492.4 ± 68.2  | 271.4-4743.4       |

Nutrients intakes

| Energy (kcal/d)                            | 1,847.9 ± 750.1 | 508-4912.5         |
| 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/m². 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      | R²   | β (SE)  | P      | R²   |
| **Triglyceride**     |         |        |      |         |        |      |
| Unadjusted           | -0.0014 (0.0005) | 0.0061 | 0.0211 | -0.0689 (0.0205) | 0.0099 | 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) | 0.010 | 0.0300 |
| Adjusted (1)         | 0.0006 (0.0002) | 0.0059 | 0.0429 | 0.0269 (0.0081) | 0.010 | 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 | 0.0414 | -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           | -0.1344 (0.0093) | 0.2457 | 0.0038 | -0.6752 (0.3970) | 0.0899 | 0.0081 |
| Adjusted (1)         | -0.1344 (0.0093) | 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 (0.364-1.295) | 1 (ref) | 0.651 (0.429-0.989) |
glyceridemia and low HDL-cholesterol were lower in the 3rd tertile than the Hawaiian women. In Iranian female adults without T2D (average fish intake = 14.4 g/d), the adjusted ORs for hypertriglyceridemia were lower than the Hawaiian women. 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 makes 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.

REFERENCES

1. Ministry of Health and Welfare, Korea Centers for Disease Control and Prevention. Korea Health Statistics 2010: Korea National Health and Nutrition Examination Survey (KNHANES V-1). Cheongwon: Korea Centers for Disease Control and Prevention; 2011.
2. Statistics Korea. Annual Report on the Cause of Death Statistics. Daejeon: Statistics Korea; 2012.
3. Huxley R, Barzi F, Woodward M. Excess risk of fatal coronary heart disease associated with diabetes in men and women: meta-analysis of 37 prospective cohort studies. BMJ 2006;332:73-8.
4. Kim HK, Kim CH, Kim EH, Bae SJ, Choe J, Park JY, Park SW, Yun YD, Baek SJ, Mok Y, Jee SH. Impaired fasting glucose and risk of cardiovascular disease in Korean men and women: the Korean Heart Study. Diabetes Care 2013;36:328-35.
5. Zhang J, Wang C, Li L, Man Q, Meng L, Song P, Freyland L, Du ZY. Dietary inclusion of salmon, herring and pampomo as oily fish reduces CVD risk markers in dyslipidaemic middle-aged and elderly Chinese women. Br J Nutr 2012;108:1455-65.
6. Mozaffarian D, Rimm EB. Fish intake, contaminants, and human health: evaluating the risks and the benefits. JAMA 2006;296:1885-99.
7. Saravanan P, Davidson NC, Schmidt EB, Calder PC. Cardiovascular effects of marine omega-3 fatty acids. Lancet 2010;376:540-50.
8. Siscovick DS, Raghunathan T, King I, Weinmann S, Bovbjerg VE, Kushi L, Cobb LA, Copass MK, Psaty BM, Lemaitre R, Retzlaff B, Knopp RH. Dietary intake of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. Am J Clin Nutr 2000;71:2085-212S.
9. Mizushima S, Moriguchi EH, Ishikawa P, Helman P, Nara Y, Mimura G, Moriguchi Y, Yamori Y. Fish intake and cardiovascular risk among middle-aged Japanese in Japan and Brazil. J Cardiovasc Risk 1997;4:191-9.
10. Iso H, Kobayashi M, Ishihara J, Sasaki S, Okada K, Kita Y, Kokubo Y, Tsugane S. JPHC Study Group. Intake of fish and n3 fatty acids and risk of coronary heart disease among Japanese: the Japan Public Health Center-Based (JPHC) Study Cohort I. Circulation 2006;113:195-202.
11. Hu FB, Cho E, Rexrode KM, Albert CM, Manson JE. Fish and long-chain omega-3 fatty acid intake and risk of coronary heart disease and total mortality in diabetic women. Circulation 2003;107:1852-7.
12. Moore CS, Bryant SP, Mishra GD, Krebs JD, Browning LM, Miller GJ, Jebb SA. Oily fish reduces plasma triacylglycerols: a primary prevention study in overweight men and women. Nutrition 2006;22:1012-24.
13. Kris-Etherton PM, Harris WS, Appel LJ; American Heart Association. Nutrition Committee. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 2002;106:2747-57.
14. Harris WS, Miller M, Tighe AP, Davidson MH, Schaefer EJ. Omega-3 fatty acids and coronary heart disease risk: clinical and mechanistic perspectives. Atherosclerosis 2008;197:12-24.
15. Harris WS, Ginsberg HN, Arunakul N, Shachter NS, Windsor SL, Adams M, Berglund L, Osmundsen K. Safety and efficacy of Omecor in severe hypertriglyceridemia. J Cardiovasc Risk 1997;4:385-91.
16. Maas AH, Franke HR. Women’s health in menopause with a focus on hypertension. Neth Heart J 2009;17:68-72.
17. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.
18. Lauer RM, Lee J, Clarke WR. Factors affecting the relationship between childhood and adult cholesterol levels: the Muscatine Study. Pediatrics 1988;82:309-18.
19. Yamashina A, Tomiyama H, Takeda K, Tsuda H, Arai T, Hirose K, Koji Y, Hori S, Yamamoto Y. Validity, reproducibility, and clinical significance of noninvasive brachial-ankle pulse wave velocity measurement. Hypertens Res 2002;25:359-64.
20. Oh SY, Kim EM, Shin MH, Lee SH, Kim JE, Lee HS, Jo JS, Kim WY. Development and validation of a food frequency questionnaire for adults. Proceedings of the Korean Society of Health Promotion Annual Spring Conference; 2007 May 19; Seoul. Seoul: Korean Society of Health Promotion; 2007.
21. The Korean Nutrition Society. Nutritional assessment program ‘CAN-Pro 4.0’ [CD-ROM]. Seoul: The Korean Nutrition Society; 2011.
22. United State Department of Agriculture, Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 26 [Internet]. Washington, D.C.: Agricultural Research Service; 2013. Available from: http://www.ars.usda.gov/Services/docs.htm?docid=24936.
23. National Fisheries Research & Development Institute (KR). Fatty acid composition of fisheries products in Korea. Busan: National Fisheries Research & Development Institute; 2012.
24. Dewaily E E, Blanchet C, Gingras S, Lemieux S, Sauvé L, Bergeron J, Holub BJ. Relations between n-3 fatty acid status and cardiovascular disease risk factors among Quebecers. Am J Clin Nutr 2001;74:603-11.
25. Okuda N, Ueshima H, Okayama A, Saitoh S, Nakagawa H, Rodriguez BL, Sakata K, Choudhury SR, Curb JD, Stamler J. INTERLIPID Research Group. Relation of long chain n-3 polyunsaturated fatty acid intake to serum high density lipoprotein cholesterol among Japanese men in Japan and Japanese-American men in Hawaii: the INTERLIPID study. Atherosclerosis 2005;178:371-9.

26. Friday KE, Childs MT, Tsunehara CH, Fujimoto WY, Bierman EL, Ensink JW. Elevated plasma glucose and lowered triglyceride levels from omega-3 fatty acid supplementation in type II diabetes. Diabetes Care 1989;12:276-81.

27. Rivellese AA, Maffettone A, Iovine C, Di Marino L, Annuzzi G, Mancini M, Riccardi G. Long-term effects of fish oil on insulin resistance and plasma lipoproteins in NIDDM patients with hypertriglyceridemia. Diabetes Care 1996;19:1207-13.

28. Woodman RJ, Mori TA, Burke V, Puddley IB, Watts GF, Beilin LJ. Effects of purified eicosapentaenoic and docosahexaenoic acids on glycemic control, blood pressure, and serum lipids in type 2 diabetic patients with treated hypertension. Am J Clin Nutr 2002;76:1007-15.

29. Lankinen M, Kolehmainen M, Jääskeläinen T, Paananen J, Joukamo L, Kangas AJ, Soininen P, Poutanen K, Mykkänen H, Gylling H, Orešič M, Jauhiainen M, Ala-Korpela M, Uusitupa M, Schwab U. Effects of whole grain, fish and bilberries on serum metabolic profile and lipid transfer protein activities: a randomized trial (Sydmet). PLoS One 2014;9:e90352.

30. Nilsen DW, Albrektsen G, Landmark K, Moen S, Aarsland T, Woie L. Effects of a high-dose concentrate of n-3 fatty acids or corn oil introduced early after an acute myocardial infarction on serum triacylglycerol and HDL cholesterol. Am J Clin Nutr 2001;74:50-6.

31. Harris WS. n-3 fatty acids and serum lipoproteins: human studies. Am J Clin Nutr 1997;65:1645S-1654S.

32. Park Y, Harris WS. Omega-3 fatty acid supplementation accelerates chylomicron triglyceride clearance. J Lipid Res 2003;44:455-63.

33. Rustan AC, Nosseen JO, Christiansen EN, Drevon CA. Eicosapentaenoic acid reduces hepatic synthesis and secretion of triacylglycerol by decreasing the activity of acyl-coenzyme A:1,2-diacylglycerol acyltransferase. J Lipid Res 1988;29:1417-26.

34. Morris MC, Sacks F, Rosner B. Does fish oil lower blood pressure? A meta-analysis of controlled trials. Circulation 1993;88:523-33.

35. Kasim SE, Stern B, Khilnani S, McClain P, Bacioworski S, Jen KL. Effects of omega-3 fish oils on lipid metabolism, glycemic control, and blood pressure in type II diabetic patients. J Clin Endocrinol Metab 1988;67:1-5.

36. Margolin G, Huster G, Glueck CJ, Speirs J, Vandegrift J, Illig E, Wu J, Streicher P, Tracy T. Blood pressure lowering in elderly subjects: a double-blind crossover study of omega-3 and omega-6 fatty acids. Am J Clin Nutr 1991;53:562-72.

37. Banaa KH, Bjerve KS, Straume B, Gram IT, Thelle D. Effect of eicosapentaenoic and docosahexaenoic acids on blood pressure in hypertension. A population-based intervention trial from the Tromsø study. N Engl J Med 1990;322:795-801.

38. Needleman P, Raz A, Minkes MS, Ferrendelli JA, Sprecher H. Triene prostaglandins: prostacyclin and thromboxane biosynthesis and unique biological properties. Proc Natl Acad Sci U S A 1979;76:944-8.

39. Yin K, Chu ZM, Beilin LJ. Blood pressure and vascular reactivity changes in spontaneously hypertensive rats fed fish oil. Br J Pharmacol 1991;102:991-7.

40. Hashimoto M, Hossain S, Yamazaki H, Yazawa K, Masumura S. Effects of eicosapentaenoic acid and docosahexaenoic acid on plasma membrane fluidity of aortic endothelial cells. Lipids 1999;34:1297-304.

41. Lund BK, Harvey LJ, Ladha S, Clark DC, Johnson IT. Effects of dietary fish oil supplementation on the phospholipid composition and fluidity of cell membranes from human volunteers. Ann Nutr Metab 1999;43:290-300.

42. Hamazaki T, Urakaze M, Sawazaki S, Yamazaki K, Taki H, Yano S. Comparison of pulse wave velocity of the aorta between inhabitants of fishing and farming villages in Japan. Atherosclerosis 1988;73:157-60.

43. Sekikawa A, Shin C, Masaki KH, Barinas-Mitchell EJ, Hirooka N, Wilcox BJ, Choo J, White J, Evans RW, Fujiyoshi A, Okamura T, Miura K, Muldoon MF, Ueshima H, Kuller LH, Sutton-Tyrrell K; ERA JUMP Study Group. Association of total marine fatty acids, eicosapentaenoic and docosahexaenoic acids, with aortic stiffness in Koreans, Whites, and Japanese Americans. Am J Hypertens 2013;26:1321-7.

44. Fukudo Y, Nuruji N, Amiya S, Tofuku K, Aosaki S, Tsubouchi H. Effects of a fish-based diet and administration of pure eicosapentaenoic acid on brachial-ankle pulse wave velocity in patients with cardiovascular risk factors. J Cardioil 2014;63:211-7.

45. Payne RA, Wilkinson IB, Webb DJ. Arterial stiffness and hypertension: emerging concepts. Hypertension 2010;55:9-14.

46. Jia Y, Turek JJ. Altered NF-kappaB gene expression and collagen formation induced by polyunsaturated fatty acids. J Nutr Biochem 2005;16:500-6.

47. McEnery CM, McDonnell BJ, So A, Aitken S, Bolton CE, Munney M, Hickson SS, Yasin, Maki-Petaja KM, Cockcroft JR, Dixon AK, Wilkinson IB; Anglo-Cardiff Collaboration Trial Investigators. Aortic calcification is associated with aortic stiffness and isolated systolic hypertension in healthy individuals. Hypertension 2009;53:524-31.

48. Abedin M, Lim J, Tang TB, Park D, Demer LL, Tintut Y. N-3 fatty acids inhibit vascular calcification via the p38-mitogen-activated protein kinase and peroxisome proliferator-activated receptor-gamma pathways. Circ Res 2006;98:727-9.

49. Health Insurance Review & Assessment Service; National Health Insurance Service. National Health Insurance Statistical Yearbook 2011. Seoul: National Health Insurance Service, 2012.