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Research Article

Fish Consumption with Adequate Fruit and Vegetables Decreases the Risk of Diabetes-Related Dyslipidemia Based on Clinical Measurement and Gas Chromatography

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Abstract

Background: The role of specific dietary patterns in the pathogenesis of diabetes is important. The purpose of this study was assessed the clinical association between lipid profiles and fish consumption in the present of adequate Fruit and Vegetables (FV) or not in Type 2 Diabetes Mellitus (T2DM) patients.

Methods: This was a two-stage cross-sectional study conducted at Keelung Hospital (KH) and Taipei Medical University Hospital (TMUH) in 2004. All participants were older than 18 years and patients' demographics, biochemical measurements as well as dietary data were collected. Using the 24-hour dietary recall conducted by the registered dietitian, we computed the total number of fish and FV intakes within two groupings as stage one (high fish and low fish) and four groupings as stage two (high fish-high FV, low fish-high FV, high fish-low FV, and low fish-low FV).Cutoff values for the fish and FV intake were based on the serving size and American Diabetes Association recommendations respectively. Moreover, plasma fatty acid compositions by gas chromatography were analyzed.

Results: Participants in high fish consumption with adequate FV corresponded to a significantly lower Triglyceride (TG). Furthermore, higher palmitic acid, sum of n-3 fatty acid and ratio of n-3 to n-6 fatty acid as well as lower linoleic acid, total polyunsaturated fatty acid and sum of n-6 fatty acid in the plasma fatty acid fractions were significantly found in high fish consumption with adequate FV than those subjects with low fish consumption.

Conclusion: Two or more servings of fish per week and at least 5 servings of FV daily eliminate diabetes-related dyslipidemia, especially the lower levels of TG.

Keywords: Diabetes mellitus; Fish; Fruit and vegetables; n-3 and n-6 fatty acid; Dyslipidemia; Gas chromatography

Introduction

The worldwide prevalence of Type 2 Diabetes Mellitus (T2DM) has been estimated to be about 285 million and been expected to increase consistently [1]. In accordance with the global diabetes trends, the increased prevalence rates of overall diabetes in Taiwan were 74% among men and 56% among women from 2000 to 2008 [2]. Diabetes is associated with a greater risk of mortality from Cardiovascular Disease (CVD) which is well known as dyslipidemia. The characteristic features of diabetic dyslipidemia are decreased High Density Lipoprotein Cholesterol (HDL-C), increased low density lipoprotein composition and Triglyceride (TG) levels [3]. There has been scores of interest in the effect of different diets on the prevention and management of T2DM [4]. Further understanding of the role of specific dietary patterns in the pathogenesis of DM is paramount importance.

The benefits of fish and fiber intake are well documented in diabetic dyslipidemia [5,6]. The American Diabetes Association (ADA) recommends not only two or more servings of fish per week (with the exception of commercially fried fish filets), but 20-35 grams of fiber a day from plant foods, including both soluble and insoluble fiber for T2DM [7]. Sheehan and colleagues investigated the effect of high fiber intake combined with fish oil treatment on the T2DM patients: during an 8-week fish-oil-treatment period (20 gram fish oil/day) with 15 gram pectin supplement, the cholesterol ester fraction of plasma lipids was reduced by 34% when compared with fish oil alone [8]. However, previous findings elucidated that fish oil supplements may have a more deleterious effect than fish-based diets on circulating inflammatory markers [9] or may increase the Low Density Lipoprotein Cholesterol (LDL-C) concentration, the risk factor of CVD, in T2DM patients [10,11].

The objective of this study was to evaluate the clinical association between the ADA-recommended fish consumption and lipid profile in T2DM patients in the present of adequate Fruit and Vegetables (FV) intake or not. We computed the total number of fish and FV intakes within two groupings as stage one (high fish and low fish) and four groupings as stage two (high fish-high FV, low fish-high FV,

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high fish-low FV, and low fish-low FV). Specifically, we examined the fatty acid profile of the plasma sample to further investigate the potential underlying function of combined dietary regimen on the diabetes-related dyslipidemia.

Subjects and Methods

Research design

This was a two-stage cross-sectional study conducted at Keelung Hospital (KH) as stage 1 and at Taipei Medical University Hospital (TMUH) as stage 2 respectively. Patients' demographics and biochemical measurements were collected at outpatient visits in each stage. We also used the 24-hour dietary recall to evaluate the amount of fish and FV consumption. At stage 2, sixteen patients who volunteered to the further analysis of plasma fatty acid compositions were investigated.

Subjects

Patients with T2DM were recruited from the Diabetes Shared Care Program in KH and from the division of endocrinology & metabolism of TMUH. This study was approved by the ethics committee of Institutional Review Board of Taipei Medical University (no. P930039) and conducted in 2004. The recruitment criteria required participants be 18 or older, and good blood sugar control with oral hypoglycemic agents. Those patients with history of acute complications of diabetes such as diabetic ketoacidosis or hyperglycemic hyperosmolar non-ketotic coma and with supplementary antioxidant such as vitamin E were excluded. The patients were enrolled in this study after signing an informed consent form.

Demographics and biochemical measurements

We collected the demographic data such as age, gender, medical and drug use history from the medical records. The 8-hour preprandial blood samples were collected at outpatient visits and were subsequently analyzed in the clinical laboratories of KH or TMUH. The analysis items included Glycosylated Hemoglobin (HbA1c), Total Cholesterol (TC), TG, HDL-C, and LDL-C. The non HDL-C (TC-HDL-C) [12], the ratio of LDL-C to HDL-C, atherogenic index (TC /HDL-C) [13] and atherogenic index of plasma ($\log_{10}$ TG/HDL-C) [14] were also calculated. No further medicate change during the study period.

Dietary data

A 24-hour dietary recall was conducted by the same registered dietitians face-to-face with the patients. Serving size was analyzed by using the standardized food model and Taiwan Food and Nutrition Guide to confirm the dietary data. We define one serving of fish as 30-40 gram according to the most consumed type of fish via the self-report dietary recall. The cutoff values of 75 gram fish (2 servings) per week was used to stratified participants into two groups: at least 75 gram of fish consumption per week or less than 75 gram of fish per week. We also defined adequate FV intake as daily intake of at least 5 servings of fruit and vegetables according to the diabetes food pyramid from ADA [15].

Plasma fatty acid analysis

The fatty acid composition of the plasma lipid fractions was analyzed by gas chromatography with a Restek capillary column (Stabilwax DA™, 30 min x 0.53 mm ID) after lipid class separation by silica-fused column chromatograpy. Briefly, a mixture of 200 μL plasma sample and internal standards was extracted with chloroform, n-Hexane, and NaCl buffer using a modification of the methods of Folch et al. (1957) and Morrison and Smith (1964) [16,17]. The

Table 1: HbA1C and lipid level in 107 participants from Keelung Hospital stratified by amount of fish consumption[1].

	High fish intake group (≥ 75 g fish /wk)	Low fish intake group (< 75 g fish /wk)	*P*
No.	63	44	
Male/female	29/34	25/19	
Age (years)	65.0±8.5	63.0±8.1	0.07
History of DM (years)	7.0±1.6	7.1±1.3	0.12
Servings of fish per week	7.0±1.7	0.8±0.7	< 0.0001
Height (cm)	155.0±6.7	158.0±6.6	0.84
Weight (kg)	62.2±7.7	62.0±5.9	0.23
BMI (kg/m^2)	25.9±2.8	24.8±1.3	0.31
HbAlc (% Hb)	8.3±1.7	7.9±1.8	0.25
TC (mg/dL)	212.9±46.9	202.4±35.4	0.24
TG (mg/dL)	161.4±134.5	170.6±120.1	0.18
HDL-C (mg/dL)	41.0±8.8	41.1±11.5	0.61
LDL-C (mg/dL)	143.5±43.9	137.6±31.7	0.45
Non HDL-C (mg/dL)	171.9±49.2	158.9±31.6	0.17
LDL-C/HDL-C	3.7±1.4	3.4±1.1	0.37
Atherogenic index[2]	5.4±1.7	5.2±1.4	0.44
Atherogenic index of plasma[3]	0.1±0.3	0.2±0.3	0.64

[1]DM: Diabetes Mellitus; BMI: Body Mass Index; HbA1c: Glycosylated Hemoglobin; TC: Total Cholesterol; TG: Triglyceride; HDL-C: High Density Lipoprotein Cholesterol; LDL-C: Low Density Lipoprotein Cholesterol

[2]The atherogenic index defined as the ratio of TC to HDL-C.

[3]The atherogenic index of plasma defined as logarithm of the ratio of plasma concentration of TG to HDL-C.

organic layer was dried and extracted with boron trifluoride in methanol: n-Hexane: H_2O (1:2:1, by vol). The sample (5 μL) was applied to a silica-fused column and the plasma lipid fractions were eluted by using nitrogen as the carrier gas, and the flow rate for the analysis was 10 mL per minute. The quantitation was portrayed from standard curves of fatty acids analyzed on the same day.

Statistical methods

Values are represented as the mean ± standard deviation. SAS software version 9.2 was adopted for the statistical analyses; the Shapiro-Wilk test was used to assess normality, the Student's t test or Wilcoxon rank sum test was used to compare the low- and high-fish consumption group as appropriate. A value of *P* less than 0.05 indicated statistical significance.

Results

Of the 186 T2DM patients who participated in this study, 107 were from KH and 79 were from TMUH. The age range of the study group was 50-80 years old. Table 1 showed the demographics and biochemical measurements in 107 patients from KH. The HbAlc, TC, TG, HDL-C, LDL-C and other calculated cholesterol-related formula of the high fish consumption (at least 2 servings per week) group subjects were not significant different from those with low fish consumption. More than half of subjects did not intake adequate FV and 75% of them in high fish consumption group, whereas 23.4% in less fish consumption group. Therefore, we further investigated the results of adequate FV on diabetes-related dyslipidemia to explain this related dependence.

Of the 79 participants in TMUH, only 8.9% (n=7) subjects did not intake adequate FV (3 in high fish-low FV group, and 4 in low fish-low FV group). Because of the small sample size for comparison, we only compared the low- and high- fish consumption group in present of adequate FV (Table 2). The TG in high fish-high FV group were significantly lower than those of the low fish-high FV group subjects (143.3 ± 122.6 mg/dL vs. 169.5 ± 123.0 mg/dL, p=0.039). No significant different existed in the HbAlc, TC, HDL-C, LDL-C and other calculated cholesterol-related formula between high and low fish-high FV consumption. Given these results, plasma lipid fractions were additionally analyzed by gas chromatography among 16 individuals who volunteered to participate (9 from high fish consumption group, 7 from low fish consumption group).

As a result of gas chromatographic analyses (Table 3), palmitic acid (16:0), Linoleic Acid (LA, 18:2), total Polyunsaturated Fatty Acids (PUFA), sum of n-3 and n-6 fatty acid as well as ratio of n-3 to n-6 fatty acid were significantly different between high fish-high FV and low fish-high FV group. The ratio of polyunsaturated to monounsaturated to saturated fatty acids (P:M:S) were 2.5:1:2.2 and 2.5:1:2.6 in the high fish-high FV and low fish-high FV group respectively.

Discussion

In comparison with individuals in low fish-high FV group, the results of this study revealed that subjects with high fish consumption and adequate FV corresponded to a significantly lower TG (Table 2) and different fatty acids composition in the plasma lipid fractions (Table 3).

Fish intake has been of particular interest given the advantageous effects on the multiple risk factors associated with diabetes, such as blood pressure, inflammation, coronary heart disease and stroke as

Table 2: HbA1C and lipid level in 72 participants from Taipei Medical University Hospital stratified by amount of fish consumption with adequate fruit and vegetables[1].

	High fish intake group (≥ 75 g fish /wk)	Low fish intake group (< 75 g fish /wk)	P
No.	40	32	
Male/female	18/22	19/13	
Age (years)	59.9±5.7	59.2±5.4	0.55
History of DM (years)	5.2±1.3	5.5±1.2	0.3
Servings of fish per week	6.5±1.2	0.8±0.6	< 0.0001
Height (cm)	162.4±8.4	162.0±6.0	0.79
Weight (kg)	67.9±9.0	63.1±7.5	0.23
BMI (kg/m^2)	25.8±3.6	24.1±3.1	0.31
HbAlc (% Hb)	8.2±1.7	8.1±1.8	0.86
TC (mg/dL)	213.5±53.3	203.7±34.6	0.48
TG (mg/dL)	143.3±122.6	169.5±123.0	0.04
HDL-C (mg/dL)	42.0±9.5	42.2±11.2	0.95
LDL-C (mg/dL)	145.3±51.6	137.1±31.8	0.44
Non HDL-C (mg/dL)	171.4±56.2	155.0±28.4	0.17
LDL-C/HDL-C	3.7±1.6	3.3±1.1	0.4
The atherogenic index[2]	5.4±1.9	5.0±1.4	0.38
Atherogenic index of plasma[3]	0.1±0.4	0.2±0.3	0.17

[1]DM: Diabetes Mellitus; HbA1c: Glycosylated Hemoglobin; TC: Total Cholesterol; TG: Triglyceride; HDL-C: High Density Lipoprotein Cholesterol; LDL-C: Low Density Lipoprotein Cholesterol

[2]The atherogenic index defined as the ratio of TC to HDL-C.

[3]The atherogenic index of plasma defined as logarithm of the ratio of plasma concentration of TG to HDL-C.

Table 3: Plasma fatty acid composition in 16 participants from Taipei Medical University Hospital stratified by amount of fish consumption with adequate fruit and vegetables.

	High fish intake group (≥ 75 g fish /wk)	Low fish intake group (< 75 g fish /wk)	P
No.	9	7	
Male/female	2/7	4/3	
Age (years)	63.67±9.22	63.14±8.82	0.92
History of DM	6.44±1.42	6.57±1.51	0.81
Servings of fish per week	5.7±1.3	0.3±0.2	0.01
Height (cm)	155.28±6.33	158.00±7.14	0.46
Weight (kg)	61.11±7.61	62.14±6.28	0.83
BMI (kg/m^2)	25.36±2.9	24.86±1.36	1
SFA			
Lauric (12:0)	0.23±0.13	0.62±1.16	1
Myristic (14: 0)	1.55±0.28	1.31±0.72	0.07
Palmitic (16: 0)	33.35±3.57	31.55±1.17	0.02
Stearic (18:0)	8.84±1.12	8.62±1.12	0.83
MUFA			
Palmitoleatic (16:1)	1.73±0.99	1.09±0.56	0.11
Oleic (18:1)	16.11±2.20	15.67±1.82	0.67
PUFA			
Linoleic (18:2)	29.96±3.59	34.23±2.66	0.03
Linolenic (18:3)	0.72±0.26	0.68±0.27	0.63
Arachidonic (20:4)	5.10±1.19	5.7±0.88	0.17
Eicosapentaenoic (20:5)	0.71±0.38	0.57±0.25	0.6
Docosahexaenoic (22:6)	2.68±0.60	2.07±0.75	0.07
Total SFA	43.97±3.39	42.09±3.04	0.11
Total MUFA	17.84±2.61	16.76±2.16	0.53
Total PUFA	39.17±2.85	43.23±3.08	0.03
PUFA+MUFA/SFA	18.73±2.61	17.79±2.16	0.53
Σ n-3 FA	4.11±0.80	3.31±0.78	0.04
Σ n-6 FA	35.06±3.27	39.92±2.81	0.01
n-3/n-6 FA	0.12±0.03	0.08±0.02	0.02

[1]The unit of blood fatty acids is g/100g total fatty acids.
DM: Diabetes Mellitus; BMI: Body Mass Index; SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids

well as the lipid profile [18]. McEwen and colleagues reviewed articles that addressed diabetes, CVD, or n-3 fatty acid and summarized that n-3 fatty acid, from fish-based diets or supplementary, can improve the lipid profile in patients with CVD with or without T2DM by lowering TG, TC, and LDL-C as well as slightly elevating HDL-C [19]. The results of this study (Table 1) were not consistent with McEwen and colleagues unless subjects consumed adequate FV (Table 2).

Dietary fiber has been focus on the management of diabetes in the past decade because of the beneficial effect on control of glucose and circulating lipid levels. The mechanism may possible through delayed gastric emptying, altered transit time of chyme in the small intestine, and inhibited the activities of digestive enzyme [20]. Chandalia and colleagues investigated a high intake of dietary fiber reduced10.2%TG (P=0.02) in T2DM patients in comparison with a diet containing moderate amounts of fiber [6]. Of the patients who consumed at least 5 servings of FV a day, subjects in high fish consumption had significant lower TG than those with low fish consumption group, suggesting that the additive or synergistic interaction of combined dietary regimen on the diabetes-related hypertriglyceridemia. However, due to the cross-sectional design in this study, the cause and effect relationship must be confirmed in future studies.

The fatty acid composition of plasma can be used as not only a biomarker of fat quality, but also an indicator of disease risk [21]. In this study, the percentage of saturated fatty acid of the most consumed type of fish according to the single 24-hour dietary recall was 36.8%, and most of them were palmitic acid, on a par with the gas chromatographic analyses. Furthermore, subjects in high fish-high FV group had lower n-6 fatty acids such as LA and Arachidonic Acid (AA) than those in low fish-high FV group. Prescott and colleagues investigated diabetic patients had significantly greater LA in all LDL subfractions compared to control subjects [22]. Hlavaty and colleagues discovered obese participants who supplemented

with n-3 PUFA in diet decreased sum of n-6 fatty acids in plasma [23]. Previous findings have elucidated that large amounts of LA will prompt excessive production of AA that result in the synthesis of pro-inflammatory cytokines subsequently [24-27]. At present, diabetic-induced inflammation may be effectively alleviated with increased fish and FV intakes. However, we did not recruit the healthy subjects and analyze the inflammatory cytokines in this study. The effect of combined dietary regimen on plasma fatty acid composition and inflammation in T2DM patients merits further investigation.

Some existing studies have used dietary questionnaires to estimate the consumption of fatty acid in T2DM patients [28-31], which may be tendency towards estimation error due to the complicated dietary patterns. The measurement of circulating fatty acids provides an objective measurement for reflecting the complex interrplay of diet and metabolism, particularly the n-3 fatty acid [32]. Interesting, the total PUFA in high fish consumption with adequate FV group was significant lower than subjects in low fish consumption. In this study, revisited participants were asked to fast at least for 8 hours prior to the blood collection. Individual issues of usual intakes may contradict the findings, requiring an extended evaluation and observation period.

There were several strengths and limitations to this study. The application of the gas chromatography was an objective and reliable measurements. However, we did not separate the different fractions of plasma such as cholesterol ester, phospholipids, and TG. Analysis of each fraction is sophisticated and time consuming that may be more prone to increased unexpected error from measurement. Moreover, we cannot completely exclude the possible unmeasured or residual confounding like geographical differences of dietary patterns, regimens of diabetic control, types of fish consumption (e.g. lean or fatty) and a relatively small number of patients due to the study design. These may result in insufficient statistical power to find associations fully in this study. For this reason, we enrolled subjects from different area with similar demographics.

Conclusion

In conclusion, many studies have investigated that DM is fully related disorders of lipid metabolism. This two stage cross-sectional study suggests approach for achieving two or more servings of fish per week and at least 5 servings of FV a day recommended by ADA promote the better control of dyslipidemia, especially the lower levels of TG in T2DM patients, according to the biochemical and authoritative plasma fatty acid analysis. Additional large-scale observational studies and randomized controlled trials are warranted to investigate to support our findings.

Practical Application

Diet pattern and health are highly related, especially in the T2DM patients. The recent version of ADA nutrition recommendations for diabetics elucidates that people with diabetes should consume not only at least two times (two servings) a week of fish rather than fish oil supplements, but also at least the amount of fiber recommended for the general public. Considering these observations, it may be appropriate for diabetes educators and health professionals to recommend a higher fish intake with the adequate FV for patients who are at high risk of diabetes-related dyslipidemia.

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