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Blood biomarkers of various dietary patterns correlated with metabolic indicators in Taiwanese type 2 diabetes

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Popular scientific summary
- Dietary modification serves as an integral part of treatments for diabetes.
- We find that habitual consumption of diet pattern rich in fish and vegetables may contribute in part to better glycemic and lipid control in type 2 diabetes.

Abstract

Background: Metabolic alterations correlate with adverse outcomes in type 2 diabetes. Dietary modification serves as an integral part in its treatment.

Objective: We examined the relationships among dietary patterns, dietary biomarkers, and metabolic indicators in type 2 diabetes ($n = 871$).

Design: Diabetic patients ($n = 871$) who provided complete clinical and dietary data in both 2008 and 2009 were selected from a cohort participating in a diabetic control study in Taiwan. Dietary data were obtained using a short, semiquantitative food frequency questionnaires, and dietary pattern identified by factor analysis. Multiple linear regressions were used to analyze the association between dietary biomarkers (ferritin, folate, and erythrocyte n-3 polyunsaturated fatty acids [n-3 PUFAs]) and metabolic control upon adjusting for confounders.

Results: Three dietary patterns (high-fat meat, traditional Chinese food–snack, and fish–vegetable) were identified. Ferritin correlated positively with high-fat meat factor scores (P for trend <0.001). Erythrocyte n-3 PUFAs (eicosapentaenoic acid [EPA] + docosahexaenoic acid [DHA], n-3/n-6 PUFA ratio) correlated positively with fish–vegetable factor scores (all P for trends <0.001). Multiple linear regressions revealed a positive relationship between ferritin concentrations and fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), and triglycerides, but a negative relationship with high-density lipoprotein cholesterol (HDL-C). Erythrocyte n-3 PUFA, EPA+DHA, and n-3/n-6 PUFA ratio were negatively linked to FPG, HbA1c, and triglycerides (all $P < 0.05$) and positively with HDL-C (though n-3/n-6 ratio marginally correlated).

Conclusions: Ferritin and n-3 PUFA can serve as valid biomarkers for high-fat meat and fish–vegetable dietary patterns. Unlike ferritin, erythrocyte n-3 PUFA status was related to better glycemic and blood lipid profiles. Our results suggest that habitual consumption of diet pattern rich in fish and vegetables may contribute in part to a healthier metabolic profile in type 2 diabetes.

Keywords: *dietary pattern; ferritin; n-3 PUFA; Taiwan; type 2 diabetes*

To access the supplementary material, please visit the article landing page

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In the past two decades, the prevalence of diabetes mellitus (DM) in developing countries has been increasing (1). Based on a report by the International Diabetes Federation (IDF), approximately 366 million adults were diagnosed with diabetes in 2011 and 552 million adults are expected to be diabetic by 2030 (2). A Taiwan nationwide survey (2000–2009) found that the overall incidence of DM rose from 0.76 to 0.93% within 10 years, equivalent to a 25% increase (3). Type 2 diabetes is a well-established risk factor for cardiovascular diseases, and patients with diabetes frequently have metabolic abnormalities, including hypertriglyceridemia, elevated low-density lipoprotein cholesterol (LDL-C), or high-density lipoprotein cholesterol (HDL-C) (4).

Epidemiological studies have associated some dietary patterns with risk of type 2 diabetes (5–8). Generally, these studies suggest that the healthy patterns, including higher intakes of vegetables and fruits, whole grains, fish, and low-fat dairy, may decrease diabetes risk and that the unhealthy patterns, including frequent intakes of sugars, processed and red meats, and fried foods, may increase the risk. Dietary pattern analysis has emerged as an alternative method for assessing habitual dietary exposures, can be used to assess diet–disease relationships, and provides appropriate dietary recommendations more likely to succeed in real circumstances (9). Although most of the studies using this dietary pattern analysis to investigate the effect of dietary pattern on risk of diabetes have focused on Caucasians in the United States (6) and Canada (8), some have focused on Asians in Japanese and Singapore (5, 10). We previously reported that fish–vegetable dietary patterns correlated significantly with diabetic kidney diseases in Taiwan (11, 12). As an alternative to questionnaire data, biomarkers can serve as a surrogate measurement of past dietary intake and can possibly serve as an alternative to food intake questionnaires (13–16). Dietary biomarkers in blood can reflect both dietary consumption and biological processes such as absorption, incorporation, and metabolism. For example, ferritin has been correlated with higher red meat intake (13), carotenoids and folate with fruit and vegetables intakes (14, 15), and n-3 polyunsaturated fatty acids (n-3 PUFAs) with fish and seafood intakes (16).

Till date, very few studies in Asia have explored dietary patterns, multiple dietary biomarkers, and metabolic parameters in type 2 diabetes simultaneously. This study was aimed to investigate the relationships between various dietary patterns, the dietary biomarkers (ferritin, folate, and erythrocyte n-3 PUFAs), and measurement of glycemic control (fasting plasma glucose [FPG] and hemoglobin A1c [HbA1c]) and lipid control (triglyceride and HDL-C) in patients with type 2 diabetes in Taiwan.

Materials and methods

Study design and subjects
The participants for this cross-sectional study were enrolled from the Diabetes Management through Integrated Delivery System (DMIDS) Project (NCT00288678 ClinicalTrial.gov) previously conducted in Taiwan. The DMIDS project was undertaken to investigate the effect of implementing case management for patients with type 2 diabetes in primary care clinics. Patients were recruited for that project between August 2003 and December 2005 and followed from the beginning of 2008 to the end of December 2012. Data, including anthropometric measurements, diabetic self-management status, food frequency questionnaires (FFQ) to assess habitual intakes of various foods, and clinical parameter measurements, were collected yearly. Patients were excluded if they were pregnant or on dialysis; if they had ever been diagnosed for type 1 diabetes, myocardial infarction or cerebrovascular accident, comorbid blindness or systemic disease (e.g. cancer); or if they had ever undergone foot amputation on Taiwan's national insurance claim forms at baseline (2003–2005). The protocol of DMIDS project has been described previously (17).

For this cross-sectional study, a total of 871 type 2 diabetes patients aged 30–70 years, who provided complete dietary data in both 2008 and 2009 and who had provided complete demographic information and metabolic parameters measured in 2009, were included in the analysis. The study protocol was approved by the IRBs of both National Health Research Institutes/Taiwan and Kaohsiung Medical University Hospital. All patients provided written informed consent.

Measurement of clinical parameters
Hypertension was defined in a subject who had a measurement of blood pressure of ≥140/90 mmHg. Biochemical analysis was performed on blood samples collected at least 8 h overnight fasting. FPG, cholesterol, triglyceride, HDL-C, and creatinine were analyzed using an autoanalyzer (Beckman Coulter LX20, Fullerton, CA, USA). HbA1c assay was performed using high-performance liquid chromatography (The G7, Tosoh Kobe, Japan). All clinical parameters were sent to a laboratory (Union Clinical Laboratory, Taipei, Taiwan) certified by the College of American Pathology and the US Commission on Office Laboratory Accreditation.

Dietary assessment
Dietary data were collected by trained research assistants. A short, semiquantitative FFQ was used to assess dietary intake by face-to-face interviews in both 2008 and 2009. This FFQ was recently validated (18) for use with Taiwanese diabetes adults. The information surveyed included

intake items or groups, frequency of food group consumption, and serving sizes. Participants were asked to report how frequently they consumed certain foods over the 12 months prior to answering the survey questions. The nine frequency options ranged from 'almost never' to '4–6 times per day.' Portion sizes per meal or per day were recorded for some foods, such as milk, fruit, vegetable, meats, fish, egg, and Chinese staples. Factor analysis was used to identify the major dietary patterns derived from 44 food items or groups (18). Three dietary patterns were identified: (1) the high-fat meat dietary pattern, (2) the traditional Chinese food–snack pattern, and (3) the fish–vegetable pattern, similar to our previous report (11, 18). We identified dietary patterns in 2008 and 2009 first. Because similar patterns were obtained in 2008 and 2009, the factors scores were averaged, and those food items with factor loadings ≥0.2 (Supplementary Table 1) were considered to contribute importantly to any of the dietary pattern.

Dietary biomarker analysis

Ferritin and folate were analyzed by chemiluminescence (ADVIA CentaurXPT; Siemens, Tarrytown, NY, USA).

Erythrocyte lipids were extracted using a procedure modified from the Bligh and Dyer method (19). A derivative of fatty acid methyl esters (FAMEs) was extracted using boron trifluoride-methanol. The methylated fatty acids were quantified in a gas chromatography (Agilent 6890 N; Agilent Technologies, Santa Clara, CA, USA) equipped with a capillary column (30 m × 0.250 mm inner diameter × 0.25 mm film). The carrier gas was N2. The C17:0 (Sigma, St Louis, MO, USA) was added as internal standard. Twenty individual fatty acids were identified by comparing findings with known standards. The concentration of each fatty acid was quantified as a percentage of total area under the peaks (weight %) (17). Ferritin and erythrocyte fatty acids were analyzed in both 2008 and 2009, and their mean concentrations were used for statistical analysis. Due to the limited availability of plasma specimen, folate was only measured for 2009.

Statistical analysis

Descriptive data were expressed as mean ± SD for continuous or N (%) for categorical variables. Chi-square tests and one-way ANOVA were used to examine the differences in categorical and continuous variables among three dietary

Table 1. Correlations between quartiles of dietary patterns and demographic characteristic and metabolic parameters in type 2 diabetes ($N = 871$)

Patient characteristics	High-fat meat diet			Traditional Chinese food–snack diet			Fish–vegetable diet		
	Q1	Q4	P^a	Q1	Q4	P^a	Q1	Q4	P^a
Age (years)									
<65	116 (53.5)	175 (80.6)	<0.001	112 (51.6)	163 (74.8)	<0.001	152 (70.0)	121 (55.8)	0.012
≥65	101 (46.5)	42 (19.4)		105 (48.4)	55 (25.2)		65 (30.0)	96 (44.2)	
Gender									
Male	58 (26.7)	151 (69.6)	<0.001	103 (47.5)	98 (45.0)	0.935	113 (52.1)	100 (46.1)	0.155
Female	159 (73.3)	66 (30.4)		114 (52.5)	120 (55.0)		104 (47.9)	117 (53.9)	
Education year									
≤6	139 (64.1)	73 (33.6)	<0.001	155 (71.4)	100 (45.9)	<0.001	104 (47.9)	125 (57.6)	0.033
>6	78 (35.9)	144 (66.4)		62 (28.6)	118 (54.1)		113 (52.1)	92 (42.4)	
BMI (kg/m²)									
<24	74 (34.1)	64 (29.5)	0.773	67 (31.2)	66 (30.4)	0.936	65 (30.1)	69 (31.9)	0.941
≥24	143 (65.9)	153 (70.5)		148 (68.8)	151 (69.6)		151 (69.9)	147 (68.1)	
Diabetes duration (years)									
<7	64 (29.5)	83 (38.8)	0.103	48 (22.6)	79 (36.7)	0.006	73 (33.6)	61 (28.4)	0.379
≥7	153 (70.5)	131 (61.2)		168 (77.4)	135 (63.3)		144 (66.4)	154 (71.6)	
Smoking									
Yes	30 (13.8)	117 (53.9)	<0.001	64 (29.8)	76 (34.9)	0.410	85 (39.4)	76 (35.0)	0.003
No	187 (86.2)	100 (46.1)		151 (70.2)	142 (65.1)		131 (60.6)	141 (65.0)	
Alcohol drinking									
Yes	12 (5.5)	58 (26.7)	<0.001	20 (9.3)	30 (13.8)	0.225	25 (11.6)	33 (15.2)	0.726
No	205 (94.5)	159 (73.3)		195 (90.7)	188 (86.2)		191 (88.4)	184 (84.8)	

[a]Chi-square tests were used to examine differences in quartile 1 to quartile 4.
Data were expressed as N (%). $P < 0.05$ was considered significant.
BMI, Body mass index.

pattern score quartiles. Simple linear regression was used to analyze metabolic parameters and dietary biomarkers across dietary pattern quartiles. Multiple linear regression analysis was used to examine the independent association between dietary biomarkers and FPG, HbA1c, triglycerides, and HDL-C after adjusting for confounders. We adjusted for age (<65, ≥65 years), gender, body mass index (<24.0, ≥24.0 kg/m^2), education (≤6, >6 years), diabetes duration (<7, ≥7 years), energy intake (continuous), hypertension (yes, no), and hypoglycemic agent use (yes, no). Hypolipidemic agents use (yes, no) was further adjusted as the outcome variables were TG and HDL-C. All statistical operations were performed using IBM SPSS Version 22.0 (Chicago, IL, USA). A two-tailed P-value <0.05 was considered significant.

Results

Patients' characteristics analyzed by dietary pattern

As can been seen in Table 1, it presents the relations between study population characteristics across quartile of the three dietary pattern factor scores. The higher the quartile level of high-fat meat pattern, the greater the proportions of patients less than 65 years old, being males, patients with more than 6 years of education, and patients who had the habits of smoking and no drinking (all $P < 0.05$). The higher the traditional Chinese food–snack diet score quartile, the greater the proportions of patients aged less than 65 years old, patients with more than 6 years of education, and patients with diabetes more than 7 years (all $P < 0.05$). Moreover, the higher the fish–vegetable diet factor score quartile, the greater the proportions of patients more than 65 years old, less than 6 years of education, and patients who did not smoke ($P < 0.05$).

Metabolic parameters and dietary biomarkers analyzed by dietary pattern

Metabolic parameters and dietary biomarkers were analyzed against dietary pattern score quartiles (Table 2). The high-fat meat dietary pattern scores were positively correlated with diastolic blood pressure (DBP), FPG, HbA1c, ferritin, n-3 PUFAs, eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA), and n-3/n-6 PUFA ratio, and negatively correlated with HDL-C and folate in a dose responsive manner (all P for trends <0.05). The traditional Chinese food–snack pattern was positively associated with triglycerides, and negatively associated with n-3 PUFA, EPA+DHA, and n-3/n-6 PUFA ratio (all P for trends <0.05). The fish–vegetable pattern was significantly correlated with reduced concentrations of serum triglyceride, and increased levels of n-3 PUFA, EPA+DHA, and n-3/n-6 PUFA ratio (all P for trends <0.05) (Table 2).

Independent association between dietary biomarkers and metabolic parameters

As shown in Table 3, a summary of the results of our multiple liner regression analyses, ferritin was correlated positively with FPG, HbA1c, and triglycerides, and correlated negatively with HDL-C ($P < 0.001$). Folate was correlated positively with FPG ($P < 0.05$), but was not correlated with HbA1c. Three erythrocyte n-3 fatty acid biomarkers (n-3 PUFA, EPA+DHA, and n-3/n-6 PUFA ratio), however, seemed to be linked negatively to FPG, HbA1c, and triglycerides and positively to HDL-C, though n-3/n-6 PUFA ratio was only marginally associated with HDL-C ($P = 0.054$) (Table 3).

Discussion

This investigation, the first in Taiwan to study the possible association between dietary biomarkers of major dietary patterns and metabolic profiles among type 2 diabetes, found positive relationships between concentrations of the dietary biomarker ferritin and the metabolic parameters FPG, HbA1C, and triglycerides, and a negative relationship between this marker and HDL-C. It also linked three erythrocyte n-3 PUFA biomarkers (n-3 PUFA, EPA+DHA, n-3/n-6 PUFA ratio) to increases in HDL-C and decreases in FPG, HbA1c, and triglycerides.

One recent meta-analysis of observational studies has found an independent correlation between high serum ferritin and risks of metabolic syndrome (20). Another meta-analysis including cross-sectional and prospective studies revealed that subjects with highest quartile of ferritin were at significantly higher relative risk of having or developing type 2 diabetes compared to those in the lowest quartile (21). In addition, one study comparing ferritin concentrations in healthy controls with subjects with impaired glucose tolerance and normal glucose-tolerant first-degree relatives of type 2 diabetes patients, found increases in both study subject groups and suggested that overexpression of ferritin occurs before elevations in plasma glucose (22). Similar to those observed in healthy populations (23), in subjects with type 2 diabetes, increased ferritin concentrations have been associated with worsened triglyceride, blood pressure, and liver enzyme profiles (24). Considered together, the results of the above studies (20–24) and those of the current investigation all demonstrated that there is a correlation between increased ferritin concentrations and worsening of several metabolic parameters, including blood glucose, triglycerides, and HDL-C in type 2 diabetes. Based on human studies, iron overload has been postulated to affect insulin secretion and resistance, leading to decreased insulin sensitivity (25) and contributing to earlier complications in diabetes (26).

Red meat or processed meat intake has been correlated with overexpression of ferritin, insulin resistance,

Table 2. Metabolic parameters in 871 patients with type 2 diabetes analyzed by quartile levels of dietary pattern score

	Quartiles of dietary patterns				P^a	P-trend[b]
	Q1	Q2	Q3	Q4		
High-fat meat diet						
Metabolic parameters						
SBP (mmHg)	135.8 ± 19.1	136.3 ± 20.0	134.5 ± 17.7	135.9 ± 16.4	0.766	0.659
DBP (mmHg)	76.1 ± 10.4	76.7 ± 11.1	78.9 ± 10.4	81.9 ± 9.3	<0.001	<0.001
Fasting plasma glucose (mg/dL)	146.6 ± 50.5	145.2 ± 51.8	153.4 ± 56.9	161.2 ± 60.0	0.009	<0.001
Hemoglobin A1c (%)	7.8 ± 1.5	7.7 ± 1.6	7.9 ± 1.6	8.0 ± 1.6	0.181	0.007
Triglyceride (mg/dL)	168.7 ± 108.9	149.7 ± 76.4	149.1 ± 115.2	181.2 ± 146.2	0.007	0.250
Cholesterol (mg/dL)	194.7 ± 39.1	196.9 ± 37.4	192.9 ± 35.7	194.7 ± 39.3	0.745	0.720
HDL-C (mg/dL)	49.1 ± 12.5	49.8 ± 12.1	49.4 ± 12.3	44.9 ± 11.4	<0.001	0.003
Dietary biomarkers						
Ferritin (ng/mL)	165.8 ± 150.4	172.2 ± 131.7	190.6 ± 168.6	247.5 ± 222.5	<0.001	<0.001
Folate (ng/mL)	11.6 ± 12.3	8.5 ± 6.4	8.7 ± 5.8	8.7 ± 10.2	0.002	0.006
n-3 PUFA	9.5 ± 2.3	10.2 ± 1.4	10.3 ± 1.6	10.7 ± 1.8	<0.001	<0.001
EPA+DHA	7.2 ± 2.1	7.9 ± 1.3	8.0 ± 1.4	8.3 ± 1.6	<0.001	<0.001
n-3/n-6 PUFA ratio	0.31 ± 0.09	0.34 ± 0.06	0.34 ± 0.7	0.34 ± 0.7	<0.001	<0.001
Traditional Chinese food–snack diet						
Metabolic parameters						
SBP (mmHg)	137.5 ± 18.9	135.2 ± 18.2	134.9 ± 19.0	135.0 ± 17.2	0.378	0.026
DBP (mmHg)	77.6 ± 10.2	77.8 ± 11.4	78.2 ± 10.9	80.1 ± 9.4	0.046	0.057
Fasting plasma glucose (mg/dL)	154.9 ± 58.3	143.4 ± 45.5	147.4 ± 52.7	160.7 ± 61.8	0.005	0.370
Hemoglobin A1c (%)	8.0 ± 1.6	7.6 ± 1.4	7.8 ± 1.6	8.1 ± 1.6	0.014	0.539
Triglyceride (mg/dL)	149.3 ± 75.5	151.9 ± 86.5	169.0 ± 133.9	178.2 ± 145.9	0.024	0.022
Cholesterol (mg/dL)	194.9 ± 36.7	193.4 ± 37.5	194.4 ± 38.3	196.5 ± 39.2	0.864	0.936
HDL-C (mg/dL)	48.9 ± 11.6	49.1 ± 13.2	47.9 ± 12.7	47.4 ± 11.3	0.410	0.051
Dietary biomarkers						
Ferritin (ng/mL)	191.7 ± 162.8	185.4 ± 131.4	201.5 ± 198.0	198.9 ± 199.7	0.806	0.695
Folate (ng/mL)	8.8 ± 6.2	10.1 ± 12.9	9.3 ± 9.8	9.2 ± 6.2	0.577	0.848
n-3 PUFA	11.1 ± 1.7	10.3 ± 1.7	10.1 ± 1.7	9.5 ± 1.9	<0.001	<0.001
EPA+DHA	8.7 ± 1.4	8.0 ± 1.5	7.7 ± 1.5	7.2 ± 1.8	<0.001	<0.001
n-3/n-6 PUFA ratio	0.37 ± 0.07	0.34 ± 0.07	0.32 ± 0.07	0.29 ± 0.07	<0.001	<0.001
Fish–vegetable diet						
Metabolic parameters						
SBP (mmHg)	135.4 ± 18.5	136.0 ± 18.5	134.5 ± 17.9	136.7 ± 18.4	0.629	0.700
DBP (mmHg)	78.2 ± 10.8	78.2 ± 10.2	78.1 ± 10.7	79.2 ± 10.5	0.649	0.577
Fasting plasma glucose (mg/dL)	148.4 ± 52.7	154.2 ± 56.7	154.3 ± 54.1	149.4 ± 57.4	0.554	0.901
Hemoglobin A1c (%)	7.9 ± 1.5	7.9 ± 1.7	7.9 ± 1.4	7.8 ± 1.6	0.989	0.397
Triglyceride (mg/dL)	176.9 ± 132.5	168.2 ± 138.8	153.1 ± 87.7	150.4 ± 89.9	0.050	<0.001
Cholesterol (mg/dL)	196.1 ± 39.1	195.2 ± 36.9	193.0 ± 35.9	194.9 ± 39.5	0.855	0.399
HDL-C (mg/dL)	47.3 ± 11.7	48.8 ± 12.6	48.9 ± 12.0	48.3 ± 12.6	0.552	0.128
Dietary biomarkers						
Ferritin (ng/mL)	197.4 ± 180.8	199.9 ± 202.9	186.6 ± 162.5	193.3 ± 149.5	0.888	0.350
Folate (ng/mL)	9.7 ± 13.1	9.1 ± 6.4	8.9 ± 5.9	9.6 ± 9.5	0.799	0.892
n-3 PUFA	9.6 ± 1.7	9.8 ± 1.7	10.1 ± 1.6	11.3 ± 1.8	<0.001	<0.001
EPA+DHA	7.3 ± 1.5	7.5 ± 1.5	7.8 ± 1.4	8.9 ± 1.6	<0.001	<0.001
n-3/n-6 PUFA ratio	0.31 ± 0.07	0.32 ± 0.07	0.33 ± 0.06	0.37 ± 0.07	<0.001	<0.001

[a]ANOVA was used to examine differences among quartile 1, 2, 3, and 4 levels.
[b]Simple linear regression was used to examine the trend between metabolic parameters or dietary biomarkers against four quartile levels of three major dietary patterns.
Data were expressed as mean ± SD and $P < 0.05$ was considered significant.

Table 3. Association between dietary biomarkers and metabolic parameters after adjustment for confounders by multiple liner regression

	Ferritin (ng/mL)		Folate (ng/mL)		n-3 PUFA		EPA+DHA		n-3/n-6 PUFA ratio	
	β (SE)	P	β (SE)	P	β (SE)	P	β (SE)	P	β (SE)	P
Fasting plasma glucose (mg/dL)										
Crude model	0.055 (0.011)	<0.001	0.465 (0.211)	0.028	−1.737 (1.226)	0.157	−1.924 (1.347)	0.154	−49.002 (30.307)	0.106
Model 1[a]	0.061 (0.012)	<0.001	0.432 (0.210)	0.040	−2.903 (1.309)	0.027	−3.124 (1.431)	0.029	−64.715 (31.980)	0.043
Model 2[b]	0.062 (0.012)	<0.001	0.456 (0.212)	0.032	−2.943 (1.310)	0.025	−3.224 (1.432)	0.025	−66.494 (31.947)	0.038
Hemoglobin A1c × 10 (%)										
Crude model	0.007 (0.003)	0.037	0.067 (0.060)	0.263	−0.877 (0.343)	0.011	−0.895 (0.377)	0.018	−25.211 (8.464)	0.003
Model 1[a]	0.007 (0.003)	0.027	0.056 (0.060)	0.351	−1.308 (0.365)	<0.001	−1.333 (0.399)	0.001	−33.048 (8.901)	<0.001
Model 2[b]	0.008 (0.003)	0.020	0.068 (0.061)	0.265	−1.296 (0.367)	<0.001	−1.336 (0.401)	0.001	−32.991 (8.927)	<0.001
Triglyceride (mg/dL)										
Crude model	0.213 (0.021)	<0.001	0.699 (0.419)	0.095	−12.028 (2.541)	<0.001	−13.104 (2.794)	<0.001	−177.51 (63.629)	0.005
Model 1[a]	0.231 (0.022)	<0.001	0.800 (0.424)	0.060	−14.300 (2.772)	<0.001	−15.390 (3.032)	<0.001	−200.81 (68.704)	0.004
Model 3[c]	0.240 (0.022)	<0.001	0.637 (0.424)	0.134	−15.952 (2.766)	<0.001	−17.064 (3.032)	<0.001	−236.70 (68.851)	0.001
HDL-C (mg/dL)										
Crude model	−0.015 (0.002)	<0.001	0.066 (0.047)	0.162	0.469 (0.276)	0.090	0.545 (0.303)	0.073	8.407 (6.833)	0.219
Model 1[a]	−0.010 (0.002)	<0.001	0.006 (0.046)	0.888	0.704 (0.284)	0.014	0.736 (0.311)	0.018	13.245 (6.961)	0.058
Model 3[c]	−0.010 (0.002)	<0.001	0.011 (0.046)	0.819	0.726 (0.290)	0.013	0.756 (0.318)	0.018	13.723 (7.113)	0.054

[a]Model 1 was adjusted for demographic characteristic (age, gender, body mass index, diabetes duration, education, and energy intake).
[b]Model 2 was adjusted for confounders in model 1 plus hypertension and hypoglycemic agents use.
[c]Model 3 was adjusted for confounders in model 1 plus hypertension, hypolipidemic, and hypoglycemic agents use.
Data were expressed as beta (SE) and P < 0.05 was considered significantly different.

and hyperglycemia in healthy populations in the United States (13) and Sweden (27) as well as in European subjects with diabetes (28). The relationship between these adverse metabolic conditions and the Western dietary pattern, which is typically high in red meat or fatty meat, may be mediated through several mechanisms, including intake of saturated fat, foods with different glycemic loads, nitrites, and heme iron (13). In mouse models, iron excess has been shown to cause increased beta-cell oxidative stresses and decreased insulin secretory secondary to beta-cell apoptosis, loss of beta-cell mass, and desensitization of glucose-induced insulin secretion (29). Iron can also interfere with insulin extraction in the liver, and thereby contribute to peripheral hyperinsulinemia (30). Insulin has been postulated to induce a rearrangement of transferrin receptors to the cell surface where they mediate uptake of extracellular iron, activation of oxidative stress, and release of inflammatory cytokines in the subendothelial space, which, in turn, upregulate transcription and translation of ferritin mRNA in macrophages (31). Higher vascular iron deposition and accompanying superoxide release were observed in mice fed high-fat diet with iron administration (32). This observation offers a plausible explanation of the link between high-fat diets and development of cardiovascular disease, possibly occurring through fatty acid–iron extracellular oxidative stress and subsequent membrane oxidative injury. Based on the above observation, insulin resistance seems to be one pathological basis for the development of diabetes as well as risk of cardiovascular diseases. Excess body iron can impose oxidative injury, which has been reported to be correlated with several cardiovascular risk factors including dyslipidemia.

One prospective study (33) used 10 dietary biomarkers to explore the relationship between diet and type 2 diabetes independent of typical risk factors for type 2 diabetes and cardiovascular disease. Two biomarkers of fish intake, EPA ($P = 0.041$) and 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid ($P = 0.002$), were found to be inversely associated with future development of type 2 diabetes (33). Plasma levels of the long chain n-3 PUFA, EPA, have been correlated with decreases in fasting glucose and increases in HDL-C in patients with type 2 diabetes (34), and n-3 PUFA has been correlated with decreases in triglycerides (35). Increases in some n-3 erythrocyte fatty acids have been correlated with the consumption of certain foods including fish (36). One randomized crossover trial has reported that a 4-week fish-based diet intervention reduced blood triglyceride concentrations (16). We observed a significant association between higher fish–vegetable factor scores and higher erythrocyte n-3 PUFA levels (n-3 PUFA, EPA+DHA, n-3/n-6 PUFA ratio). Our study, as well as those of others (16, 37), found a clear association between frequent fish

intake and/or subsequent increases in blood n-3 PUFA and better glucose and lipid profiles in patients with type 2 diabetes. However, a significant and positive association between fish consumption and fasting blood glucose were also observed (38). Omega-3 has been found to have a beneficial effect on insulin sensitivity and glucose metabolism in obese and insulin-resistant animals (39). However, in humans this relationship may be more complicated, and there is a need to also consider the effect of age, disease progression, and medication use as well as underlying comorbidities.

One systematic review of intervention studies found that consuming oily fish led to significant improvements in two important biomarkers of cardiovascular risk, such as triglycerides and HDL levels (40). Epidemiological studies have also found an association between high fish consumption and reduced risk of stroke (41) and heart disease (42, 43). However, another review of seven randomized clinical trials (RCTs) and one single-arm study (2002–2010) concluded that capsule supplementation with n-3 PUFA did not improve glycemic control (44). In contrast, that review cited one prospective cohort study (45) focused on whole food omega-3 intake and observed an inverse relationship between baseline marine omega-3 fatty acid intake and triglycerides. The American Diabetes Association (ADA) recommends that people with diabetes eat fish, particularly fatty fish, at least 2 times (two servings) per week to obtain optimal levels of long-chain n-3 PUFA (EPA and DHA) because observational studies have found these fatty acids to have beneficial effects on lipoproteins and positive health outcomes (46).

Results of a meta-analysis study including 763 participants with metabolic diseases from 16 randomized controlled trials revealed that folate supplementation significantly leads to decreases in insulin levels and HOMA-IR, but does not seem to affect FPG and HbA1c levels (47). In the current study, plasma folate concentrations were correlated positively and significantly with FPG, but not with other metabolic parameters. Even though there has been evidences showing increased blood folate and better glycemic control in diabetic patients (47), we did not have clear evidence to confirm previous findings. There are many factors could have affected the relationships, such as diabetes duration, medication use, and comorbidities. Thus, relations between blood folate and glycemic control may need further investigations.

The study was strengthened by the administration of face-to-face interviews by trained research personnel using a validated semi-quantitative FFQ (18) and two consecutive measurements of dietary biomarkers (except for folate) in blood were performed instead of single-time-point measurement. Thus, variabilities derived from assessment of food consumption can be minimized. Correlations between food consumption pattern and dietary biomarkers can, thus, be ascertained. This study has some limitations. One limitation was that our study sample size was small and may not be applied to other Asian populations with different eating habits. Another limitation of this study was that it had a cross-sectional design, and thus we could not confidently determine a cause–effect relationship between dietary patterns, dietary biomarkers, and metabolic profiles. Still another limitation was that there may be other unmeasured or residual confounding effects, not ruled out by the confounders we adjusted for in our multivariable regression analyses.

Conclusions

In conclusion, our results suggest that ferritin and n-3 PUFA in blood can serve as valid dietary biomarkers for high-fat meat and fish–vegetable dietary patterns, respectively. Unlike ferritin, erythrocyte n-3 PUFA indicators were associated with better glycemic and blood lipid levels. Thus, habitual consumption of a fish–vegetable diet may help improve metabolic profiles in people with type 2 diabetes.

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