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Association between dietary flavanones intake and lipid profiles according to the presence of metabolic syndrome in Korean women with type 2 diabetes mellitus

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BACKGROUND/OBJECTIVES: This study was aimed at examining the association between dietary flavanones intake and lipid profiles according to the presence of metabolic syndrome (MetS) in Korean women with type 2 diabetes mellitus (T2DM).
SUBJECTS/METHODS: A cross-sectional analysis was performed among 502 female T2DM patients (non-MetS group; n = 129, MetS group; n = 373) 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 (FFQ) and the data was analyzed using the Computer Aided Nutritional Analysis program (CAN-Pro) version 4.0 software. The intake of flavanones was estimated on the basis of the flavonoid database.
RESULTS: In the multiple linear regression analysis after adjustment for confounding factors, daily flavanones intake was negatively associated with CVD risk factors such as total cholesterol, LDL-cholesterol, and apoB and apoB/apoA1 ratio only in the MetS group but not in the non-MetS group. Multiple logistic regression analysis revealed that the odds ratio for a higher apoB/apoA1 ratio above the median ($\geq$ 0.74) was significantly low in the 4th quartile compared to that in the 1st quartile of dietary flavanones intake [OR: 0.477, 95% CI: 0.255-0.894, *P* for trend = 0.0377] in the MetS group.
CONCLUSIONS: Dietary flavanones intake was inversely associated with the apoB/apoA1 ratio, suggesting a potential protective effect of flavanones against CVD in T2DM women with MetS.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is now a worldwide health problem and the prevalence of diabetes in subjects aged 30 years or older has increased from 8.6% in 2001 to 11.0% in 2013 among Koreans [1,2]. Cardiovascular disease (CVD) is a major complication and a main cause of death in people with T2DM [3], making prevention of CVD a primary goal of T2DM management. This is especially true for women because the risk of diabetes-associated CVD is reported to be higher in women than in men [4].

Metabolic syndrome (MetS) is also known to be a risk factor for CVD, T2DM, and all-cause mortality [4]. MetS is characterized as a cluster of risk factors including central obesity, hypertension, hyperglycemia, dyslipidemia such as elevated triglyceride (TG), apolipoprotein B (apoB)-containing lipoproteins and low levels of HDL-cholesterol (C) [5]. To reduce the risk of CVD, the normalization of serum levels of lipid such as total cholesterol (TC) or LDL-C is very important to the patients with MetS and T2DM.

Several studies have shown that a high consumption of plant foods such as whole grains, nuts, fruit and vegetables was inversely associated with the risk of CVD [6-10]. This beneficial effect of plant foods may be related to fiber [11,12], antioxidant vitamins [13,14], and bioactive components such as flavonoids [15,16] present in them. Recent studies suggest that among several flavonoids, flavanones with their antihypertensive [17,18], antioxidative [19,20], anti-inflammatory [21,22] and lipid-reducing [19,23,24] properties may have a protective role in CVD. Flavanones are found in citrus fruits either as free aglycone forms (naringenin, hesperetin and eriodictyol) or as glycoside forms (hesperidin, naringin, eriocitrin) [25,26]. Epidemiological studies reported that intake of flavanones-rich citrus fruits reduced the incidence of CVD in the general population [27]

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and the risk of stroke in women [28]. In animal studies, naringenin or hesperidin was shown to reduce TG and TC levels, and apoB secretion [29,30].

However, to our knowledge, no study has been done to investigate the association between flavanones intake and cardiovascular risk factors in Korean T2DM patients. Therefore, this study was aimed to examine the association between dietary flavanones intake and lipid profiles according to the presence of MetS in Korean women with T2DM.

SUBJECTS AND METHODS

Study subjects

The participants were female patients who visited Huh's Diabetes Clinic in Seoul, Korea. A total number of 854 patients were recruited from September 2005 to February 2011. Of the 854 subjects, patients with no diet information (n = 267) were excluded. From the remaining 587 patients, we excluded patients aged < 30 years (n = 4), non-T2DM patients (n = 60), those with energy consumption less than 500 kcal (n = 16) or more than 5,000 kcal (n = 1), estrogen medication users (n = 3) and those missing clinical data for MetS diagnosis (n = 1). Thus, a total of 502 female subjects with T2DM were ultimately eligible for this study, and were divided into two groups according to the presence of MetS.

The diagnosis of MetS was determined following the Korean Diabetes Association's Guideline [31]. MetS was diagnosed if the patient had three or more risk determinants. The determinants were as follows: 1) a waist circumference > 80 cm; 2) a plasma TG concentration $\geq$ 150 mg/dL; 3) a plasma HDL-C concentration < 50 mg/dL or hyperlipidemia medication use; 4) a systolic blood pressure (SBP) $\geq$ 130 mmHg and diastolic blood pressure (DBP) $\geq$ 85 mmHg or hypertension medication use; 5) a fasting blood sugar (FBS) concentration $\geq$ 100 mg/dL or diabetes medication use.

The research protocol was approved by the Institutional Review Board of Yonsei University Medical Center (3-2006-0004), and all subjects provided their written informed consent to participate in the study.

General characteristics

All patients were individually interviewed during their first visit to obtain information about their general characteristics and lifestyle behaviors. Age, duration after being diagnosed with T2DM, family history of diabetes, medication use for diabetes, hypertension and dyslipidemia treatment, education, employment status, and family income were obtained from the patients' medical records. Lifestyle behaviors such as smoking, alcohol drinking status, exercise, and nutritional supplement use were also obtained from the patients during the individual interviews.

Anthropometric variable and clinical characteristics

The anthropometric measurements were performed using standard techniques. The standing height was measured with a standiometer (Seca Inc., Hamburg, Germany). Body weights were measured with an In-body 4.0 (Biospace Co., Ltd, Seoul, Korea), and BMIs (kg/m^2) were calculated. Waist and hip circumferences were measured midway between the lowest rib and the iliac crest with a tapeline (Tanita anthropometric tape, Seoul, Korea) and the waist hip ratios were calculated from the measured values.

Blood samples were drawn after a minimum 12-hour overnight fast, collected in EDTA-containing tubes, and centrifuged at 3,000 rpm for 20 minutes at 4°C (Hanil Science Industrial Co., Ltd, Seoul, Korea). Fasting plasma levels of glucose, TC, TG and high density lipoprotein (HDL)-C were assessed with an autoanalyzer (Cobas Mira Roche Autoanalyzer, Hoffmann-La Roche Ltd., Basel, Switzerland). Low density lipoprotein (LDL)-C levels were calculated using the following equation put forward by both Friedwald [32] and Lauer [33].

LDL-C = TC - HDL-C - (TG/5)

SBP and DBP were taken in the sitting position after a 10-minute rest, using an automatic blood-pressure monitor (Biospace Co., Seoul, Korea). Glycosylated hemoglobin (HbA1c) was measured using a HLD-723 G7 (Tosoh Corporation, Tokyo, Japan). The apolipoprotein A1 (apoA1) and apolipoprotein B (apoB) analyses were performed at the Seoul Medical Science Institute.

Dietary intake and flavanones intake 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 (last 12 months) [34]. The FFQ consisted of 144 food items with standard serving sizes and a selection of 9 frequency categories ranging from never to 3 times per day. Dietary intake data was analyzed using the Computer Aided Nutritional Analysis program (CAN-Pro) version 4.0 software (Korean Nutrition Society, Seoul, Korea). The intake of flavanones was estimated on the basis of the flavonoid database by Yang *et al*. [35,36], which was created for the Korean population. The total flavanone intake was the sum of naringenin, hesperetin and eriodictyol intakes.

Statistical analysis

General characteristics of the subjects were expressed as means with standard deviations (continuous data), or as numbers with percentages (categorical data). Biochemical markers and dietary intakes data were log-transformed in order to normalize their distributions before analysis. Subjects were divided into two groups (non-MetS group / MetS group) according to the presence of MetS in patients. The Student's t-test and the Chi-square test, Fisher's exact test were applied to determine differences in means and distribution of general, anthropometric, and clinical characteristics and dietary intakes between the MetS group and the non-MetS group. Potential confounders in this study included age, total energy intake, smoking status, alcohol drinking status, and exercise.

A general linear model (GLM) was performed to analyze the differences in anthropometric and clinical characteristics and dietary intakes among the two groups after adjustment for confounding factors. Anthropometric, clinical and dietary values were displayed as least square (LS) means with standard errors of mean (SEM). Multiple linear regression analysis was used to investigate the association between flavanones intake and

cardiovascular risk factors within each group. Multiple logistic regression analysis was also performed to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) for the risk of hyperlipidemia, high apoB, and high apoB/apoA1 ratios across dietary flavanones intake level (quartiles) in the MetS group. All statistical analyses were performed using the SAS statistical package (SAS 9.3, SAS Institute Inc., Cary, NC, USA), and the level of significance was set at $P < 0.05$.

RESULTS

General characteristics according to the presence of MetS

The mean age of subjects was significantly higher in the MetS group (60.0 ± 9.2 years) than in the non-MetS group (55.8 ± 9.5 years). The proportion of patients taking diabetes, hypertension, and dyslipidemia medication, was significantly higher in the MetS group than in the non-MetS group. The percentage of subjects with less than a college education was higher in the MetS group (70.5%) than in the non-MetS group (51.9%). There were no significant differences between the non-MetS group and the MetS group in the mean duration of T2DM, family history of T2DM, lifestyle behaviors (including current smoking, current alcohol drinking, regular exercise, and nutritional supplement use), employment status and family income (Table 1).

Anthropometric, clinical and dietary data according to the presence of MetS

Table 1. General characteristics according to the presence of MetS in patients

	Non-MetS (n = 129)	MetS (n = 373)	*P*-value[2]
Age (yrs)	55.8 ± 9.5[1]	60.0 ± 9.2	< 0.0001
Duration of T2DM (yrs)	7.6 ± 7.6	9.0 ± 7.2	0.0856
Family history of T2DM	88 (68.2)	223 (60.1)	0.1018
Medication usage			
Diabetes medication	73 (62.4)	278 (79.2)	0.0003
Hypertension medication	29 (24.8)	150 (42.7)	0.0005
Cholesterol medication	0 (0.0)	129 (36.8)	< 0.0001[3]
Lifestyle behavior			
Current smoker	5 (4.0)	9 (2.5)	0.5608
Current alcohol drinker	13 (10.2)	53 (14.3)	0.3728
Regular exercise	89 (70.1)	239 (65.3)	0.3255
Nutritional supplement user	66 (56.4)	174 (49.9)	0.2196
Education			0.0006[3]
< 12 yrs	67 (51.9)	263 (70.5)	
12-16 yrs	38 (29.5)	58 (15.5)	
> 16 yrs	3 (2.3)	10 (2.7)	
No-response	21 (16.3)	42 (11.3)	
Employed	42 (38.2)	136 (39.7)	0.7838
Family monthly income (US $)			0.2331
< 20,000	36 (27.9)	122 (32.7)	
> 5,000	21 (16.3)	41 (11.0)	
No-response	38 (29.4)	108 (29.0)	

MetS: metabolic syndrome, Non-MetS: non metabolic syndrome, T2DM: type 2 diabetes mellitus
[1] Values are presented as mean ± SD or n (%).
[2] Student's t-test or chi-square test
[3] Fisher's exact test

The means for BMI, waist circumference and waist hip ratio were significantly higher in the MetS group than in the non-MetS group after adjustment for age, total energy intake, smoking status, alcohol drinking status and exercise. With regard to clinical characteristics, the means for SBP, DBP, TG, TG/HDL-C ratio, TC/HDL-C ratio and apoB/apoA1 ratio were significantly higher in the MetS group than in the non-MetS group. The average HDL-C and apoA1 levels in the MetS group were significantly lower than that in the non-MetS group.

The intakes of energy and macronutrients such as carbohydrates, proteins, fats and cholesterol showed no significant differences between the two groups. Dietary fiber intake was significantly higher in the MetS group than in the non-MetS

Table 2. Anthropometric, clinical and dietary values according to the presence of MetS in patients[1]

	Non-MetS (n = 129)	MetS (n = 373)	*P*-value	
			Unadjusted	Adjusted[2]
Anthropometric variables				
Height (cm)	155.2 ± 0.8[3]	155.3 ± 0.6	0.2447	0.8022
Weight (kg)	54.2 ± 1.3	61.4 ± 1.1	< 0.0001	< 0.0001
BMI (kg/m^2)	22.5 ± 0.5	25.4 ± 0.4	< 0.0001	< 0.0001
Waist circumference (cm)	75.0 ± 1.1	83.2 ± 1.0	< 0.0001	< 0.0001
Waist hip ratio	0.90 ± 0.01	0.94 ± 0.01	< 0.0001	< 0.0001
Clinical Characteristics				
SBP (mmHg)	127.0 ± 2.6	139.3 ± 2.3	< 0.0001	< 0.0001
DBP (mmHg)	77.9 ± 1.7	84.6 ± 1.4	< 0.0001	< 0.0001
FBS (mg/dL)	155.5 ± 1.0	149.1 ± 1.0	0.2094	0.2127
HbA1c (%)	8.1 ± 1.0	8.0 ± 1.0	0.7859	0.6617
TG (mg/dL)	85.4 ± 1.1	141.7 ± 1.0	< 0.0001	< 0.0001
TC (mg/dL)	203.5 ± 1.0	198.4 ± 1.0	0.4827	0.2727
HDL-C (mg/dL)	56.8 ± 1.0	47.2 ± 1.0	< 0.0001	< 0.0001
LDL-C (mg/dL)	126.8 ± 0.1	118.3 ± 1.0	0.1241	0.0523
TG/HDL-C ratio	1.5 ± 1.1	3.0 ± 1.1	< 0.0001	< 0.0001
TC/HDL-C ratio	3.6 ± 1.0	4.2 ± 1.0	< 0.0001	< 0.0001
ApoA1 (mg/dL)	138.3 ± 1.0	128.6 ± 1.0	< 0.0001	< 0.0001
ApoB (mg/dL)	96.1 ± 1.0	99.8 ± 1.0	0.1571	0.2385
ApoB/ApoA1 ratio	0.7 ± 1.1	0.8 ± 1.0	0.0005	0.0017
Nutrients				
Energy (kcal)	1,375.1 ± 0.1	1,352.6 ± 1.1	0.2007	0.6933
Carbohydrate (g)	184.6 ± 1.0	189.3 ± 1.0	0.6360	0.0940
Protein (g)	61.1 ± 1.0	60.1 ± 1.0	0.1154	0.2860
Fat (g)	37.3 ± 1.0	36.0 ± 1.0	0.0549	0.1841
Cholesterol (mg)	232.3 ± 1.1	218.9 ± 1.1	0.0561	0.1736
Fiber (g)	21.4 ± 1.0	22.9 ± 1.0	0.6966	0.0042
Flavanones (mg)	2.05 ± 1.21	2.93 ± 1.18	0.0381	0.0080
Naringenin (mg)	1.45 ± 1.17	1.87 ± 1.14	0.0475	0.0164
Hesperetin (mg)	1.5 ± 1.23	1.78 ± 1.19	0.4643	0.2225
Eriodictyol (mg)	0.04 ± 1.1	0.04 ± 1.09	0.4470	0.6405

MetS, metabolic syndrome, Non-MetS: non metabolic syndrome, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, FBS: fasting blood sugar, HbA1c: glycosylated hemoglobin, TG: triglyceride, TC: total cholesterol, HDL-C: HDL-cholesterol, LDL-C: LDL-cholesterol, apoA1: apolipoprotein A1, apoB: apolipoprotein B
[1] Values are log transformed except systolic blood pressure and diastolic blood pressure.
[2] Adjusted for age, total energy intake (log transformed), smoking status, alcohol drinking status and exercise
[3] Values are LS mean ± SEM.

Table 3. Association between flavanones intake and cardiovascular risk factors according to the presence of MetS in patients[1)]

	Flavanones (mg/d)							
	Non-MetS (n = 129)				MetS (n = 373)			
	Unadjusted		Adjusted[2)]		Unadjusted		Adjusted[2)]	
	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value
TC (mg/dL)	0.0173 (0.0147)	0.2425	0.0180 (0.0164)	0.2726	-0.0192 (0.0092)	0.0376	-0.0199 (0.0099)	0.0445
TG (mg/dL)	0.0093 (0.0286)	0.7447	0.0223 (0.0312)	0.4771	-0.0117 (0.0227)	0.6067	-0.0128 (0.0244)	0.6003
HDL-C (mg/dL)	0.0071 (0.0135)	0.6010	-0.0011 (0.0146)	0.9407	-0.0056 (0.0100)	0.5787	-0.0080 (0.0107)	0.4542
LDL-C (mg/dL)	0.0235 (0.0228)	0.3038	0.0285 (0.0252)	0.2615	-0.0339 (0.0140)	0.0159	-0.0317 (0.0149)	0.0343
TG/HDL-C ratio	0.0023 (0.0337)	0.9462	0.0224 (0.0360)	0.5171	-0.0061 (0.0276)	0.8243	-0.0048 (0.0295)	0.8724
TC/HDL-C ratio	0.0102 (0.0173)	0.5546	0.0191 (0.0191)	0.3185	-0.0136 (0.0119)	0.2531	-0.0119 (0.0127)	0.3518
ApoA1 (mg/dL)	0.0182 (0.0104)	0.0824	0.0164 (0.0115)	0.1565	0.0021 (0.0075)	0.7798	-0.0007 (0.0080)	0.9287
ApoB (mg/dL)	0.0208 (0.0194)	0.2845	0.0273 (0.0217)	0.2104	-0.0304 (0.0133)	0.0224	-0.0349 (0.0140)	0.0129
ApoB/ApoA1 ratio	0.0026 (0.0205)	0.8983	0.0109 (0.0228)	0,6343	-0.0319 (0.0144)	0.0271	-0.0339 (0.0152)	0.0264

MetS: metabolic syndrome, Non-MetS: non metabolic syndrome, TC: total cholesterol, TG: triglyceride, HDL-C: HDL-cholesterol, LDL-C: LDL-cholesterol, apoA1: apolipoprotein A1, apoB: apolipoprotein B
[1)] Values are log transformed.
[2)] Adjusted for age, total energy intake (log transformed), smoking status, alcohol drinking status and exercise

Table 4. Odds ratio (OR) and 95% confidence interval (CI) for the risk of hyperlipidemia, high apoB, and high apoB/apoA1 ratios across quartiles of dietary flavanones intake in the MetS group

	Flavanones (mg/d)				*P* for trend
	Q1 (< 1.18)	Q2 (1.18-3.37)	Q3 (3.37-6.44)	Q4 (> 6.44)	
OR (95% CI) for TC ≥ 200 mg/dL					
Unadjusted	1.00 (ref)	0.861 (0.484, 1.533)	1.044 (0.587, 1.856)	0.672 (0.375, 1.206)	0.2928
Adjusted[1)]	1.00 (ref)	0.915 (0.507, 1.651)	1.175 (0.645, 2.141)	0.660 (0.350, 1.244)	0.3717
OR (95% CI) for LDL-C ≥ 100 mg/dL					
Unadjusted	1.00 (ref)	0.668 (0.366, 1.219)	0.952 (0.513, 1.765)	0.527 (0.289, 0.959)	0.1006
Adjusted[1)]	1.00 (ref)	0.726 (0.392, 1.344)	1.027 (0.542, 1.949)	0.537 (0.281, 1.024)	0.1546
OR (95% CI) for apoB ≥ 97.5 mg/dL (median)					
Unadjusted	1.00 (ref)	0.693 (0.389, 1.234)	0.771 (0.433, 1.374)	0.595 (0.333, 1.063)	0.1210
Adjusted[1)]	1.00 (ref)	0.694 (0.385, 1.249)	0.777 (0.427, 1.413)	0.583 (0.313, 1.088)	0.1359
OR (95% CI) for apoB/apoA1 ratio ≥ 0.74 (median)					
Unadjusted	1.00 (ref)	0.608 (0.341, 1.085)	0.648 (0.363, 1.157)	0.521 (0.291, 0.934)	0.0443
Adjusted[1)]	1.00 (ref)	0.604 (0.335, 1.088)	0.678 (0.373, 1.233)	0.477 (0.255, 0.894)	0.0377

MetS: metabolic syndrome, Non-MetS: non metabolic syndrome, TC: total cholesterol, LDL-C: LDL-cholesterol, apoA1: apolipoprotein A1, apoB: apolipoprotein B
[1)] Adjusted for age, total energy intake (log transformed), smoking status, alcohol drinking status and exercise

group. Flavanone intake was significantly higher in the MetS group (LS mean; 2.933 mg/d) than in the non-MetS group (LS mean; 2.051 mg/d). The intake of naringenin among the flavanones subgroups was significantly higher in the MetS group (LS mean; 1.872 mg/d) than in the non-MetS group (LS mean; 1.445 mg/d), but the intake of the other flavanone subgroups (hesperetin and eridictyol) was not significantly different between the two groups (Table 2). Dietary intake of antioxidative micronutrients such as vitamin C, vitamin E, and β-carotene also did not differ between the two groups (data not shown).

Association between flavanones intake and lipid profiles according to the presence of MetS

In a multiple linear regression analysis after adjustment for confounding factors, daily flavanones intake was negatively associated with TC (β = -0.0199, *P* = 0.0445), LDL-C (β = -0.0317, *P* = 0.0343), apoB (β = -0.0349, *P* = 0.0129) and the apoB/apoA1 ratio (β = -0.0339, *P* = 0.0264); this association only existed in the MetS group but not in the non-MetS group (Table 3). The fiber intake was not associated with blood lipid profiles in both the non-MetS and MetS groups (data not shown).

Relationship between dietary flavanones intake and the risk of hyperlipidemia, high apoB, and high apoB/apoA1 ratios in the MetS group

As shown in Table 4, the multiple logistic regression analysis with covariates revealed that the odds ratio for a higher apoB/apoA1 ratio above the median (≥ 0.74) was significantly low in the 4th quartile compared to that in the 1st quartile of dietary flavanones intake [OR: 0.477, 95% CI: 0.255-0.894, *P* for trend = 0.0377].

DISCUSSION

The purpose of this study was to investigate the association between dietary flavanones intake and lipid profiles according

to the presence of MetS in Korean women with T2DM. We found that there was a negative association between the intake of flavanones and CVD risk factors such as TC, LDL-C, apoB and apoB/apoA1 ratio only in the MetS group but not in the non-MetS group. The odds ratio for a higher apoB/apoA1 ratio above the median was significantly low in the 4th quartile compared to that in the 1st quartile of dietary flavanones intake in the MetS group.

An inverse association between flavanones and CVD risk factors that we had observed in our study is supported by the results of other studies including clinical and prospective epidemiological studies. In a clinical study, naringin supplementation decreased plasma TC, LDL-C and apoB levels in hypercholesterolemic subjects [19], and hesperidin supplementation significantly reduced TC, apoB concentrations in patents with MetS [21]. In epidemiological studies, Yamada *et al*. [27] reported that citrus fruit intake was associated with a lower incidence of CVD in general Japanese population and Mink *et al*. [37] reported that flavanone-rich grapefruit consumption was related to a reduced risk of coronary heart disease in postmenopausal women. A recent prospective study showed that an increased intake of flavanones was related to a reduction in the risk of stroke among female participants [28].

In our study, there was a statistically significant inverse relationship between flavanones intake and high apoB/apoA1 ratio in the MetS group. The apoB/apoA1 ratio has been suggested as a good marker for LDL-C, myocardial infarction, and MetS in several studies. Willdius *et al*. [38] reported that the apoB/apoA1 ratio was strongly and positively related to an increased risk of fatal myocardial infarction in a prospective study and apoB was a stronger predictor of risk than LDL-C. A case-control study [39] revealed that there was a significant relationship between raised apoB/apoA1 ratio and the risk of myocardial infarction in 52 countries. In an intervention study [40], the consumption of soy protein reduced the apoB/apoA1 ratio compared with milk protein consumption in adults with T2DM, but did not affect apoB and apoA1. Kim *et al*. [41] reported that the apoB/apoA1 ratio was independently associated with the risk of MetS in Korean patients with T2DM. Jing *et al*. [42] in a Chinese cross-sectional study reported that the apoB/apoA1 ratio was a better MetS marker than traditional biomarkers such as TC, TG and HDL-C because measurements of apoB and apoA1 didn't need fasting samples and had internationally standardized methods.

The mechanism for protective effects of flavanones in CVD has been investigated in several animal studies. Jung *et al*. [29] reported that both hesperidin and naringenin decreased plasma TG, TC levels in T2DM mice and these results might be because both flavanones decrease the hepatic activity of hepatic 3-hydroxy-3-methlyglutaryl-coenzyme (HMG-CoA) reductase, the limiting enzyme of cholesterogenesis. Another animal study reported that naringenin decreased plasma TG level, TC level, apoB secretion in LDL-receptor-null mice with diet-induced insulin resistance [30].

Interestingly, in our study, the significant association between flavanones intake and lipid profiles was revealed only in the MetS group but not in the non-MetS group. We do not know the reason for this differential association. However, similar results have been reported in a recent case-control study of patients with MetS whose baseline characteristics (plasma glucose and TG levels) were different from those of the control group [43]. In their study, after consumption of citrus-based juice for 6 months, plasma TC and LDL-C were significantly decreased in patients with MetS but not in the control groups, suggesting that there may be some variability within subjects with the same pathology [43]. In a cross-sectional study comparing levels of oxidative stress biomarker that are related to the pathogenesis of atherosclerosis, subjects with MetS were found to have higher levels than those without MetS [44]. The T2DM patients with MetS in our study may be more sensitive to the intake of flavanones and their antioxidant properties than those without MetS.

In our study, fiber and flavanones intakes were significantly higher in the MetS group than in the non-MetS group. Some studies [45-47] reported that the T2DM patients with the MetS had a lower fiber intake than the T2DM patients without the MetS. Yoo *et al*. [48] showed that the fiber consumption of those with MetS was higher than those without MetS in nondiabetic Korean subjects and there were no difference of fiber intake according to MetS in other studies [49,50]. These disparities may be explained by the differences in races, gender, meal patterns, and national food environment among the study subjects. To our knowledge, there was no study conducted on the difference of flavanones intake according to MetS in T2DM patients. Oh *et al*. [51] reported that there was no significant difference in flavanones intake between the MetS group and the control group among the Korean women with polycystic ovary syndrome.

CVD is a major complication of T2DM, and is the main cause of death in people with T2DM [3]. MetS has been studied as a predictor of T2DM and CVD and is considered an important risk factor for CVD [52]. Thus, it is important to prevent CVD in T2DM patients. Due to the higher risk of CVD in females than in males [53], studies such as ours that focus on female subjects are needed. But, as we know, there are very few studies or reports on CVD risk factor based on the presence of MetS in women with T2DM. Furthermore, there are no reports on the relationship between flavonoids intake and CVD risk factor in these subjects.

The limitations of our study are the following. The first, being a cross-sectional study, it is impossible to determine whether flavanone intake is a cause or a consequence of CVD risk factors. The second, the recall bias in the FFQ may have affected dietary assessment despite the use of a validated FFQ and well-trained dietitians following standard protocols. Additionally, FFQ used in this study couldn't evaluate the flavanones' major sources. According to Yang's study using the KNHANES (Korea National Health and Nutrition Examination Survey) data, the sources of food contributing to dietary flavanones intake in the first order was satsuma mandarin, next was orange [54]. Maybe, when we analyzed flavanone intake in female T2DM subjects aged 30 years and higher who completed the 24-h dietary recall of the 4th and the 5th KHANENS, the mean value (5.08 mg/d) of flavanone intake was similar to that obtained in our study (4.8 mg/d - data not shown). The third, we did not have the data on the status of menopause, which could influence the association between dietary flavanone intake and lipid profiles. However,

mean ages of our subjects were 58.9 years (range; 31-85 years) and the proportion (15.3 %) of subjects aged < 50 years was relatively low (data not shown). Nevertheless, our study has several strengths as well. This study is the first study conducted to investigate the association between flavanone intake and CVD risk factor according to the presence of MetS in female patients with T2DM.

In conclusion, we found that dietary flavanones may be inversely associated with apoB/apoA1 ratio in the MetS group among women with T2DM. But, yet the protective mechanisms of flavanones in CVD is not fully known. Therefore, more studies are needed in the future to determine the factors that play a role in CVD prevention. To evaluate the CVD protective effects of flavanones, an intervention study should be conducted in Korean female patients with T2DM.

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