

Daily walnut intake improves metabolic syndrome status and increases circulating adiponectin levels: randomized controlled crossover trial

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BACKGROUND/OBJECTIVES: Several previous studies have investigated whether regular walnut consumption positively changes heart-health-related parameters. The aim of this study was to investigate the effects of daily walnut intake on metabolic syndrome (MetS) status and other metabolic parameters among subjects with MetS.

SUBJECTS/METHODS: This study was a two-arm, randomized, controlled crossover study with 16 weeks of each intervention (45 g of walnuts or iso-caloric white bread) with a 6 week washout period between interventions. Korean adults with MetS (n = 119) were randomly assigned to one of two sequences; 84 subjects completed the trial. At each clinic visit (at 0, 16, 22, and 38 weeks), MetS components, metabolic parameters including lipid profile, hemoglobin A1c (HbA1c), adiponectin, leptin, and apolipoprotein B, as well as anthropometric and bioimpedance data were obtained.

RESULTS: Daily walnut consumption for 16 weeks improved MetS status, resulting in 28.6%-52.8% reversion rates for individual MetS components and 51.2% of participants with MetS at baseline reverted to a normal status after the walnut intervention. Significant improvements after walnut intake, compared to control intervention, in high-density lipoprotein cholesterol (HDL-C) ($P = 0.028$), fasting glucose ($P = 0.013$), HbA1c ($P = 0.021$), and adiponectin ($P = 0.019$) were observed after adjustment for gender, age, body mass index, and sequence using a linear mixed model.

CONCLUSION: A dietary supplement of 45 g of walnuts for 16 weeks favorably changed MetS status by increasing the concentration of HDL-C and decreasing fasting glucose level. Furthermore, consuming walnuts on a daily basis changed HbA1c and circulating adiponectin levels among the subjects with MetS. This trial is registered at ClinicalTrials.gov as NCT03267901.

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INTRODUCTION

Metabolic syndrome (MetS), which is comprised of central obesity, elevated blood pressure, increased levels of fasting blood glucose and triglycerides (TG), and decreased high-density lipoprotein cholesterol (HDL-C), has been well-established as a risk factor for type 2 diabetes (T2D) and cardiovascular disease [1]. Due to rapid transitions toward high energy intake and sedentary lifestyle in the last few decades, MetS has become a major health challenge globally. In South Korea, 31.3% of Korean adults have been affected according to the revised National Cholesterol Education Program (NCEP) Adult Treatment Panel III [2]. In particular, the percentage of the population with hypercholesterolemia has been gradually increasing. Prevalence of hypercholesterolemia among Koreans aged 30 and above has increased from 8.0% in 2005 to 15.7% in 2014 [3]. The

customary Korean diet is characterized by a high proportion of carbohydrate consumption compared to the average Western diet. Based on results from the 2014 Korea National Health and Nutrition Examination Survey, the proportion of carbohydrate consumption was 63.0% for males and 65.8% for females [4]. Carbohydrate is consumed not only through regular meals but also from snacks. The most popular carbohydrate originated snacks among Koreans include cereals, ramen, rice cakes, and bread.

Effects of walnut (*Juglans*) intake on various heart health markers, including reducing total cholesterol (TC), lowering low-density lipoprotein cholesterol (LDL-C) [5], decreasing blood pressure [6], and improving vascular function [7], have been investigated. A meta-analysis [8] reviewed the heart health benefits of walnuts on 365 participants, and compared to control diets, diets that were supplemented with walnuts (5%-10% of total calories) resulted in a significantly greater

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decrease in TC and LDL-C without any adverse effect on body weight. Compared to the favorable results for walnut intake on heart health, there are limited data for the effects of walnut consumption on MetS. A double-blinded, randomized, placebo-controlled study [9] found that consuming 48 g of walnuts each day for four days may help to control blood lipids among subjects with MetS. Additional research [10] reported that daily ingestion of 56 g of walnuts improves endothelial function in overweight adults with visceral adiposity, which is one of the components of MetS. Results showing the effects of nut consumption, including walnuts, on improving MetS components have been inconsistent. There were no significant improvement of MetS indicators among South African subjects after a walnut feeding study provided 20% of calories from walnut intake for 8 weeks [11], whereas improvement of insulin resistance has been reported among Spanish subjects with MetS after 12 weeks of daily mixed nut consumption, which included 15 g of walnuts [12].

With the need for longer-term studies in the area of MetS and to elucidate the effects of walnut consumption, we conducted a two-arm, randomized, controlled crossover trial to evaluate the effects of walnut consumption on the management of MetS components. In addition, we investigated lipid profiles and circulating markers of insulin resistance, adiponectin, and leptin in subjects with MetS.

SUBJECTS AND METHODS

Subjects

Participants were recruited through an online community platform and through local newspaper advertisements in Seoul, South Korea. Among 210 screened volunteers, 119 (aged 30-55 years) eligible volunteers were enrolled in the study. According to the revised NCEP criteria [2], each participant was diagnosed as having MetS if he or she had three or more of the following criteria: 1) waist circumference ≥ 90 cm for men and ≥ 80 cm for women; 2) triglycerides ≥ 150 mg/dL; 3) HDL cholesterol < 40 mg/dL for men and < 50 mg/dL for women; 4) blood pressure $\geq 130/85$ mmHg; and 5) fasting glucose ≥ 100 mg/dL. Exclusion criteria were: body mass index (BMI) greater than 35; the use of any medications to control blood pressure, lipid metabolism, or glucose; consumption of a hypocaloric diet within the past year and regular consumption of omega-3 supplements; and post-menopausal women and smokers. All study procedures were approved by the Ethics Committee of Sookmyung Women's University, Seoul, South Korea (SMWU-1508-BR-051-01). All of the participants provided written informed consent before the start of the interventions, and the study was conducted according to the principles of the Declaration of Helsinki.

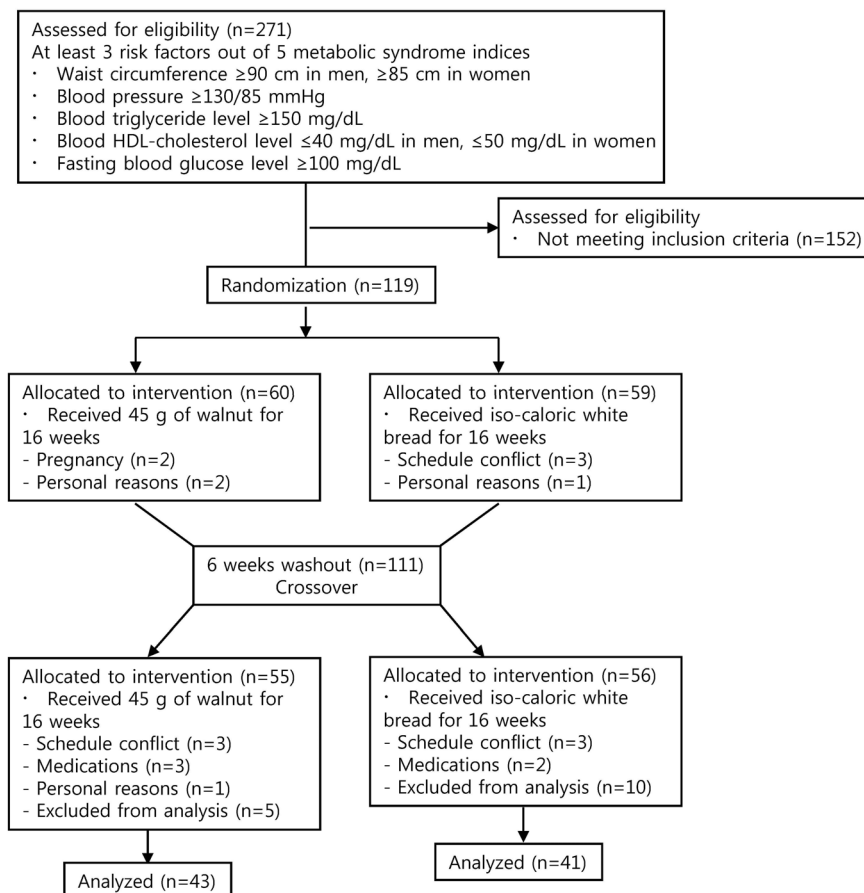


Fig. 1. CONSORT flow chart diagram of the crossover trial

Study design

A randomized, controlled, crossover, 16 week intervention was conducted using 45 g of walnut and iso-caloric white bread treatments. A 6 week washout period was included between the 16 week consumptions of each treatment. The subjects were randomly assigned to one of two groups by using a random-number generator program. Subjects in the first group ($n = 60$) were instructed to consume 45 g of walnut (containing 305.4 kcal, 6.4 g of carbohydrate, 28.9 g of fat, and 6.4 g of protein) per day for 16 weeks, as convenient during the daytime. After a 6 week washout period, those subjects consumed white bread (corresponding to 313.3 kcal, 60.5 g of carbohydrate, 3.4 g of fat, and 10.2 g of protein) per day for 16 weeks, as convenient during the daytime. The second group ($n = 59$) received white bread for 16 weeks followed by a 6 week washout period, after which they received walnuts for 16 weeks. Fig. 1 shows the process from screening to crossover randomization of the participants. Thirty subjects were unable to finish the study due to the length of the intervention period, pregnancy, or use of medication. Thus, a total of 84 volunteers completed the intervention.

Diet and physical activity assessment

Three-day dietary records that included two consecutive weekdays and one weekend day were completed once before each intervention and twice during the trial period. A registered dietitian provided detailed instructions to participants on how to fill out the diet record and report their diet by taking a picture and sending it via text messages. The dietary intake of the participants was assessed based on the three-day dietary record before the trial and one record during each period. The data were processed using CAN-Pro 4.0 software (The Korea Nutrition Society). Each participant was provided a consumption log sheet to self-report compliance with protocols. Subjects recorded how many packages they did not consume during the previous week and reported the number to care providers each Monday using a text message. Physical activity was estimated by using the international physical activity questionnaire short form. Maintaining a habitual diet and physical activity level were required to prevent intentional weight gain or loss during the study period.

Measurements

During the study, participants visited the clinical research unit four times: once at baseline, once after the washout period, and at the end of both intervention periods. Anthropometric measurements and blood sample collection were conducted during each visit.

Anthropometric and body composition measurement

Each of the anthropometric measurements was obtained by a single trained examiner while the participant was barefoot. Waist and hip circumference measurements were obtained by using a measuring tape (SECA-201; SECA, Hamburg, Germany). Body weight was measured and rounded to the nearest 0.1 kg, and height was obtained by using a stadiometer (BSM330; Biospace, Seoul, Korea) and rounded to the nearest 0.1 cm. Blood pressure was measured on the right arm using an up-load blood pressure monitor (BPBIO320S; Biospace, Seoul, Korea)

after at least a 5 min rest period. Body composition including body composition analysis (total body water, protein, mineral, and body fat mass), muscle-fat analysis (body weight, skeletal muscle mass, and body fat mass), obesity analysis [percentage body fat and BMI (calculated as weight (kg) divided by height in meters (m) squared)] was assessed by undertaking multi-frequency whole-body bioimpedance measurements using *InBody 620* (Biospace, Seoul, Korea).

Blood sampling and determination of general serum biochemical variables

Blood samples were collected in the fasting state and the samples were immediately centrifuged at 3,000 r/min for 15 min at 4°C. Serum and plasma specimens were frozen at -80°C until analysis. Serum TC and TG levels were measured by using an enzymatic-colorimetric method, HDL-C and LDL-C levels were determined via a homogeneous enzymatic-colorimetry method, and the high-sensitive C-reactive protein (hs-CRP) level was measured by applying a particle-enhanced immunoturbidimetric method using a Cobas 8000 c702 chemistry analyzer (Roche Diagnostics, Mannheim, Germany). Fasting blood glucose was measured using a Glucocard X-meter (Arkray, Kyoto, Japan). Serum insulin was measured with the commercially available, ultrasensitive insulin ELISA kit (80-INSHU-E01.1, E10.1; ALPCO, Salem, NH, USA) using an Epoch microplate spectrophotometer (BIOTEK, Winooski, VT, USA).

Serum leptin and adiponectin levels were measured using the quantikine human leptin ELISA kit (DLP00; R&D Systems, Minneapolis, MN, USA) and the quantikine human total adiponectin ELISA kit (DRP300; R&D Systems, Minneapolis, MN, USA), respectively. Hemoglobin A1c (HbA1c) separation and quantification were performed by using a high-performance liquid chromatography analyzer (Tosoh HLC-723 G8; Sysmex, Kobe, Japan). Serum apolipoprotein B (apoB) was measured by turbidimetric immunoassay using a HITACHI 7600 chemistry analyzer (Hitachi, Tokyo, Japan).

Plasma fatty acid composition

Total lipid extraction from plasma was performed by applying a modified Folch method [13], and fatty acid methyl esters (FAMES) were obtained via the modified method described by William *et al.* [14]. FAME samples were analyzed by an Agilent 7890B gas chromatograph (Agilent Technologies, Folsom, CA, USA) equipped with a flame ionization detector and a DB-WAX capillary column (J & W Scientific, Folsom, CA, USA) (30 m long, 0.25 μ m film thickness, and 0.25 mm inner diameter). For analysis, a 2 μ L sample was manually injected into the inlet at 250°C in a 20:1 pulsed split mode under 15 psi. Initial oven temperature was 150°C for 1 min followed by heating to 180°C at a rate of 10°C/min, then to 235°C at a rate of 2°C/min, finally to 245°C at a rate of 1°C/min, and then maintained for 1 min. Carrier gas (N_2), air, fuel gas (H_2), and make-up gas (N_2) flowed at speeds of 1 mL/min, 350 mL/min, 30 mL/min, and 20 mL/min, respectively. The detector was set at 270°C. Each FAME was identified by comparison with a multiple FAME mix standard and several single standards. FAMES were quantitated by applying a single point internal standard method using Agilent ChemStation software.

Statistical analysis

We estimated the appropriate sample number by taking into consideration the specific variances of methodology for MetS components with a type I error of $\alpha = 0.05$ and 80% power. After verifying the efficacy of the washout period by comparing the initial baseline and post-washout baseline data, we merged the results from both arms of the interventions ($n = 84$ each). To identify the significant differences between groups that started with either walnut ($n = 43$) or control (bread; $n = 41$) at baseline, an independent samples *t*-test was conducted. After verifying the efficacy of the washout period using a paired *t*-test, the initial baseline and post-washout baseline data were combined and the final data from each study arm ($n = 84$ each for the two interventions) were consolidated. Treatment effects for each intervention were determined using a paired *t*-test on each variable. Intervention effects were determined using a linear mixed-effects model with the intercept as a random effect and a covariance structure for repeated measures by time and treatment. The fixed effects included time as a categorical variable, interventions, age, sex, BMI, WC, energy intake, and

interactions between intervention and the arms. After 16 weeks of walnut consumption, we calculated the proportion of participants who no longer met the criteria for MetS (*i.e.*, reverted MetS). Values are presented as mean \pm SD and a $P < 0.05$ was considered significant. All statistical analyses were performed using SPSS (IBM SPSS version 23, IBM corp., Armonk, NY, USA).

RESULTS

Preintervention characteristics and fatty acid profiles of subjects by group

Before the intervention, the average age of the participants who consumed walnut first (WAL) was greater than that of the subjects who consumed control food (bread) first (CON). In addition, the average body fat mass of WAL at baseline was significantly greater than that of CON. Consequently, TG, LDL-C, insulin, apoB, and hs-CRP levels were significantly higher in the WAL group, whereas the HDL-C level was significantly lower in the WAL group, as shown in Table 1.

Table 1. Baseline characteristics of metabolic syndrome components, body composition, and blood metabolic profile among the subjects with metabolic syndrome.

	ALL (n = 84)	CON (n = 41)	WAL (n = 43)
Age (yrs)	39.44 \pm 6.53	37.91 \pm 5.69	41.05 \pm 7.02*
Men, n (%)	42 (50)	20 (48.8)	22 (51.2)
BMI (kg/m ²)	27.08 \pm 3.61	26.29 \pm 3.31	27.92 \pm 3.75
MetS components			
Systolic blood pressure (mm Hg)	134.51 \pm 14.47	134.21 \pm 16.38	134.83 \pm 12.35
Diastolic blood pressure (mm Hg)	83.32 \pm 10.74	83.51 \pm 10.90	83.12 \pm 10.71
Waist circumference (cm)	89.87 \pm 7.75	90.06 \pm 6.31	89.67 \pm 9.15
Triglycerides (mg/dL)	135.9 \pm 65.09	111.60 \pm 53.71	162.08 \pm 66.71*
HDL cholesterol (mg/dL)	41.58 \pm 9.01	44.12 \pm 10.39	38.98 \pm 6.49*
Fasting glucose (mg/dL)	104.94 \pm 25.45	104.40 \pm 7.63	105.51 \pm 35.80
Body composition			
Body weight (kg)	76.09 \pm 14.85	73.70 \pm 12.20	78.61 \pm 16.99
Waist-hip ratio	0.92 \pm 0.06	0.93 \pm 0.05	0.92 \pm 0.06
Total body water (L)	37.65 \pm 7.82	37.25 \pm 7.11	38.08 \pm 8.57
Total body protein (kg)	10.15 \pm 2.13	10.07 \pm 1.95	10.24 \pm 2.34
Body mineral (kg)	3.53 \pm 0.75	3.44 \pm 0.69	3.62 \pm 0.81
Body fat mass (kg)	24.75 \pm 7.66	22.93 \pm 6.78	26.66 \pm 8.14*
Soft lean mass (kg)	48.42 \pm 10.09	47.91 \pm 9.17	48.96 \pm 11.06
Fat free mass (kg)	51.34 \pm 10.69	50.77 \pm 9.72	51.96 \pm 11.70
Skeletal muscle mass (kg)	28.65 \pm 6.47	28.36 \pm 5.90	28.95 \pm 7.08
Percent body fat (%)	32.4 \pm 6.85	31.09 \pm 6.93	33.80 \pm 6.57
Metabolic profile			
LDL cholesterol (mg/dL)	116.27 \pm 28.92	107.67 \pm 25.93	125.29 \pm 29.45*
Total cholesterol (mg/dL)	191.19 \pm 34.67	195.74 \pm 36.05	186.54 \pm 32.98
Insulin (pmol/L)	2.71 \pm 1.51	2.06 \pm 1.32	3.35 \pm 1.43*
HbA1c (%)	5.38 \pm 0.345	5.34 \pm 0.40	5.42 \pm 0.28
ApoB (mg/dL)	96.04 \pm 19.09	91.33 \pm 18.07	100.75 \pm 19.14*
hs-CRP (mg/L)	0.83 \pm 0.68	0.63 \pm 0.45	1.05 \pm 0.81*
Adiponectin (μ g/mL)	4.31 \pm 2.08	4.27 \pm 1.99	4.35 \pm 2.19
Leptin (μ g/mL)	15.30 \pm 6.72	13.78 \pm 5.67	16.78 \pm 7.37*

CON, subjects who consumed white bread first; WAL, subjects who consumed walnut first; BMI, body mass index; MetS, metabolic syndrome; HbA1c, hemoglobin A1c; ApoB, apolipoprotein B; hs-CRP, high sensitivity C-reactive protein. Data are mean \pm SD.

* indicates a significant difference between CON and WAL groups using independent *t*-test.

Table 2. Baseline blood fatty acid profiles (%) in subjects with metabolic syndrome.

	ALL (n = 84)	CON (n = 41)	WAL (n = 43)
Myristic acid	1.72 ± 0.76	1.81 ± 0.71	1.64 ± 0.81
Palmitic acid	56.85 ± 3.85	56.81 ± 4.17	56.90 ± 3.57
Stearic acid	21.73 ± 3.15	22.99 ± 3.39	20.54 ± 2.38*
Oleic acid	6.58 ± 1.99	6.34 ± 2.20	6.80 ± 1.77
Linoleic acid	7.54 ± 2.52	6.72 ± 2.21	8.32 ± 2.58*
Gamma-linolenic acid	0.8 ± 0.32	0.92 ± 0.36	0.69 ± 0.22*
Alpha-linolenic acid	0.25 ± 0.35	0.34 ± 0.40	0.17 ± 0.27*
Arachidonic acid	2.43 ± 1.01	2.16 ± 0.98	2.69 ± 0.98*
Eicosapentaenoic acid	0.55 ± 0.65	0.47 ± 0.76	0.62 ± 0.54
Docosahexaenoic acid	1.51 ± 0.96	1.42 ± 0.97	1.60 ± 0.94
Total SFAs	80.31 ± 5.03	81.61 ± 4.62	79.08 ± 5.14*
Total MUFAs	6.59 ± 1.98	6.34 ± 2.20	6.83 ± 1.73
Total PUFAs	13.19 ± 4.31	12.05 ± 3.85	14.26 ± 4.48*
Total n-3 PUFAs	2.34 ± 1.54	2.26 ± 1.46	2.43 ± 1.64
Total n-6 PUFAs	10.77 ± 3.25	9.79 ± 2.88	11.69 ± 3.35*

CON, subjects who consumed white bread first; WAL, subjects who consumed walnut first; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.

Fatty acid compositions are shown as a percentage of the total amount of fatty acids measured.

Data are mean ± SD.

* indicates a significant difference between CON and WAL groups using independent *t*-test.

Table 3. Dietary intake of the participants at baseline and after the 16 week walnut intake period

	Before walnut intervention (1)	During walnut intervention (2)	(2)-(1)	<i>P</i>
Energy (kcal)	1,683.22 ± 334.37	1,652.94 ± 409.79	-30.28 ± 51.58	0.559
Carbohydrate (g)	233.9 ± 48.74	217.35 ± 56.14	-16.55 ± 6.35	0.011
Total fat (g)	48.89 ± 15.93	55.25 ± 19.08	6.37 ± 2.55	0.015
Vegetable fat (g)	24.16 ± 9.08	33.04 ± 14.90	8.88 ± 1.74	< 0.0001
Animal fat (g)	24.73 ± 11.14	22.22 ± 10.66	-2.51 ± 1.61	0.122
Total protein (g)	69.58 ± 18.6	66.08 ± 17.34	-3.50 ± 2.50	0.164
Vegetable protein (g)	28.92 ± 7.93	29.42 ± 8.22	0.50 ± 1.16	0.665
Animal protein (g)	40.66 ± 15.76	36.66 ± 13.97	-4.00 ± 2.06	0.056
Fiber (g)	17.95 ± 5.75	17.21 ± 5.16	-0.74 ± 0.64	0.250
Cholesterol (mg)	370.11 ± 155.31	345.97 ± 137.67	-24.13 ± 21.93	0.274
Total fatty acid (g)	28.17 ± 14.4	35.11 ± 16.54	6.94 ± 2.34	0.004
SFAs (g)	8.97 ± 5.96	9.32 ± 5.41	0.35 ± 0.87	0.693
MUFAs (g)	11.18 ± 7.11	11.6 ± 5.72	0.42 ± 0.98	0.669
PUFAs (g)	9.31 ± 4.48	15.21 ± 9.42	5.90 ± 1.09	< 0.0001
Omega-3 fatty acid (g)	0.84 ± 0.46	1.66 ± 1.22	0.82 ± 0.14	< 0.0001

SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.

Data are mean ± SD.

Statistical significance set at *P* < 0.05 for the difference between dietary intake before consuming walnut and during walnut intake using a two-tailed paired *t*-test.

P-values for comparison between pre- and post-intervention using paired *t*-tests.

Blood fatty acid profiles at baseline are presented in Table 2. Subjects in the WAL group had significantly higher sums of polyunsaturated fatty acids (PUFAs) and n-6 fatty acids but a significantly lower sum of saturated fatty acids (SFAs) compared to those of the CON group.

Dietary analysis

Dietary analysis results before and during walnut intervention are presented in Table 3. Subjects maintained an iso-caloric diet during the walnut intake period. Compared with the pre-walnut intake, consumption of carbohydrate was significantly reduced and total fat intake, particularly vegetable fat intake, was

increased during the intervention period. Intake of total fatty acids during the intervention period was increased due to the significant increase in PUFAs intake. No changes in dietary intake, including calorie intake, were observed between pre- and post-control food intake (data not shown).

Effects of walnut intake on MetS components and its prevalence

Reversion rates for MetS after daily walnut intake for 16 weeks are presented in Table 4. At baseline, all 84 subjects exhibited MetS. However, after 16 weeks of walnut intervention, 43 subjects had reverted to normal status. The number of subjects whose MetS risk factors were reduced after walnut intake was

Table 4. Prevalence of metabolic syndrome and its components among study participants at baseline and after the 16 week walnut intake period¹⁾

MetS	
Baseline	84 (100.0)
PostW ²⁾ Reduced number of risk factors	54 (64.3)
Reversion rate ³⁾	43 (51.2)
Central obesity	
Baseline	69 (82.2)
PostW	37 (44.0)
Reversion rate	32 (46.4)
Elevated fasting glucose	
Baseline	56 (66.7)
PostW	47 (56.0)
Reversion rate	19 (33.9)
Elevated triglycerides	
Baseline	36 (42.9)
PostW	23 (27.4)
Reversion rate	19 (52.8)
Reduced HDL cholesterol	
Baseline	65 (77.4)
PostW	46 (54.8)
Reversion rate	22 (33.8)
Elevated systolic blood pressure	
Baseline	56 (66.7)
PostW	40 (47.6)
Reversion rate	16 (28.6)
Elevated diastolic blood pressure	
Baseline	46 (54.8)
PostW	26 (31.0)
Reversion rate	15 (32.6)

MetS: metabolic syndrome

¹⁾ Data are n (%).²⁾ PostW, the period after consuming walnut for 16 weeks.³⁾ Reversion rate indicates the proportion of participants who met the criterion at baseline but not after walnut consumption.

even greater than the number of reverted subjects. Of the participants, 64.3% experienced improvement in one or more MetS components. Central obesity and reduced HDL-C were the most common components among study participants at baseline (82.2% and 77.4%, respectively) and their reversion rates after walnut intake were 46.4 % and 33.8%, respectively. Over 60% of the study participants showed elevated fasting glucose level and blood pressure at baseline. However, about 30% of the subjects had reverted back to normal glucose level and blood pressure status after the walnut intervention. Subjects with an elevated TG level comprised 42.9% of the volunteers at baseline, which was the lowest prevalence among the five MetS components assessed. However, the TG reversion rate was the highest (52.8%).

Baseline characteristics before each intervention are summarized in Table 5; none of the parameters differed between the two baseline periods. There were no significant differences between groups in reported physical activity (data not shown). However, we detected significant intervention effects on HDL-C ($P = 0.028$) and fasting glucose ($P = 0.000$), compared to the control group after applying linear mixed model adjustments for age, gender, BMI, and sequence (Table 5). The HDL-C level was increased after walnut intervention but was stable after control intervention. A significant intervention effect on fasting blood glucose was observed after walnut intake compared with that after the control intervention, but this might be due to an increase in glucose level after the control period. Other MetS components including blood pressure, WC, and TG were reduced after walnut intake, although there were no significant intervention effects compared to those after the control intervention.

Table 5. Metabolic syndrome components and blood metabolic profiles in subjects with metabolic syndrome at baseline and after the 16 week walnut and white bread (control) interventions

	Before white bread intervention	After white bread intervention	Before walnut intervention	After walnut intervention	<i>P</i>
MetS components					
Systolic blood pressure (mm Hg)	133.93 ± 15.47	129.16 ± 14.72*	134.92 ± 15.11	131.85 ± 14.82*	0.054
Diastolic blood pressure (mm Hg)	83.46 ± 10.40	79.49 ± 11.36	81.32 ± 11.44	81.25 ± 11.47	0.813
Waist circumference (cm)	89.01 ± 7.70	85.76 ± 9.58*	88.42 ± 8.90	86.83 ± 9.11*	0.693
Triglycerides (mg/dL)	124.11 ± 56.47	135.21 ± 58.23	137.93 ± 64.83	117.74 ± 51.78*	0.219
HDL cholesterol (mg/dL)	41.61 ± 9.37	41.76 ± 9.55	42.36 ± 9.32	43.86 ± 10.03	0.028
Fasting glucose (mg/dL)	106.27 ± 24.62	111.58 ± 30.20	103.04 ± 27.44	101.37 ± 16.46	0.000
Metabolic profile					
LDL cholesterol (mg/dL)	116.38 ± 28.51	118.76 ± 29.71	121.74 ± 28.09	113.07 ± 23.63	0.866
Total cholesterol (mg/dL)	191.78 ± 34.61	187.15 ± 32.01	185.17 ± 30.63	178.28 ± 30.34*	0.633
Insulin (pmol/L)	2.67 ± 1.55	2.55 ± 1.17	2.77 ± 1.43	2.61 ± 1.46	0.510
HbA1c (%)	5.38 ± 0.37	5.43 ± 0.37*	5.39 ± 0.34	5.38 ± 0.35	0.021
ApoB (mg/dL)	93.57 ± 18.27	89.76 ± 20.49	94.23 ± 19.32	89.45 ± 19.17*	0.108
hs-CRP (mg/L)	0.81 ± 0.64	0.78 ± 0.66	0.90 ± 0.74	0.83 ± 0.66	0.185
Adiponectin (µg/mL)	4.10 ± 2.12	4.02 ± 1.88	4.51 ± 2.10	4.99 ± 1.91	0.019
Leptin (µg/mL)	16.18 ± 7.79	12.38 ± 8.17	14.65 ± 6.87	15.64 ± 6.96	0.853

MetS, metabolic syndrome; HbA1c, hemoglobin A1c; ApoB, apolipoprotein B; hs-CRP, high sensitivity C-reactive protein.

Data are mean ± SD.

P-values of significance between walnut and control determined using a linear mixed model and after adjustment for age, gender, BMI, and sequence.* indicates significant difference ($P < 0.05$) between pre- and post- of each intervention type using a paired *t*-test.

Table 6. Blood fatty acid profiles (%) in subjects with metabolic syndrome at baseline and after the 16 week walnut and white bread interventions

	Before white bread intervention	After white bread intervention	Before walnut intervention	After walnut intervention	<i>P</i>
Myristic acid	2.51 ± 1.74	2.40 ± 1.69	2.23 ± 1.98	2.00 ± 1.50	0.000
Palmitic acid	58.22 ± 3.90	54.23 ± 4.67*	56.70 ± 4.44	56.35 ± 3.10	0.103
Stearic acid	23.13 ± 2.93	24.87 ± 2.67	22.92 ± 3.69	24.25 ± 2.54*	0.088
Oleic acid	6.13 ± 2.44	6.33 ± 2.09	6.25 ± 1.94	6.61 ± 2.35	0.375
Linoleic acid	5.48 ± 2.13	5.98 ± 2.59	6.09 ± 3.04	5.64 ± 2.46	0.340
Gamma-linolenic acid	0.87 ± 0.28	0.58 ± 0.32*	0.80 ± 0.22	0.67 ± 0.27*	0.431
Alpha-linolenic acid	0.21 ± 0.33	0.22 ± 0.25	0.23 ± 0.30	0.29 ± 0.28*	0.012
Arachidonic acid	1.66 ± 0.89	2.22 ± 1.49	1.90 ± 1.12	1.61 ± 1.05	0.743
Eicosapentaenoic acid	0.80 ± 1.01	1.56 ± 1.08	1.63 ± 1.17	1.40 ± 0.96	0.967
Docosahexaenoic acid	0.99 ± 0.88	1.52 ± 1.36	1.23 ± 0.87	1.17 ± 0.72	0.716
Total SFAs	83.86 ± 4.82	81.50 ± 5.00	81.85 ± 5.22	82.60 ± 4.05	0.431
Total MUFAs	6.13 ± 2.44	6.33 ± 2.09	6.27 ± 1.93	6.61 ± 2.35*	0.354
Total PUFAs	10.01 ± 3.63	12.17 ± 4.28	11.97 ± 4.24	10.79 ± 3.62	0.485
Total n-3 PUFAs	2.01 ± 1.42	3.38 ± 1.64	3.11 ± 1.55	2.86 ± 1.27	0.459
Total n-6 PUFAs	8.00 ± 2.83	8.79 ± 3.81	8.79 ± 3.97	7.93 ± 3.30	0.582

SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.

Fatty acid compositions are shown as a percentage of the total amount of fatty acids measured.

Data are mean ± SD.

P-values of significance between walnut and control determined using a linear mixed model and adjusting for age, gender, BMI, and sequence.

Effects of walnut intake on metabolic profiles

Baseline and final levels of the MetS components and metabolic profiles are presented in Table 5. None of the parameters were significantly different between the pre-walnut and pre-control periods. When analyzed using a paired *t*-test, it was noticed that TC and apoB had decreased after walnut consumption for 16 weeks. Significant differences in treatment effect between the two interventions on HbA1c (*P* = 0.021), and adiponectin (*P* = 0.019) were observed after linear mixed model adjustment for gender, age, BMI, and sequence. These results indicate that walnut consumption for 16 weeks might affect glucose metabolism by decreasing HbA1c and fasting blood glucose and by increasing circulating adiponectin.

Plasma fatty acids profile

Baseline fatty acid profiles before each intervention are summarized in Table 6. None of the blood fatty acid profiles differed between the two intervention groups. However, there was a significant difference in myristic acid levels (*P* = 0.000) between walnut and control interventions (Table 6). Notably, alpha-linolenic acid (ALA) level after the walnut intervention was significantly increased over that after the control intervention (*P* = 0.012). The changes in ALA constitution is reflective of the fatty acid composition of walnuts and confirmed the self-reported changes in fatty acid intake. Walnut intervention did not affect other fatty acids such as arachidonic, eicosapentaenoic, docosapentaenoic and docosahexaenoic acids.

DISCUSSION

Our study results show that supplementing a subject's habitual diet with 45 g of walnuts daily for 16 weeks improved MetS status with 28.6%-52.8% reversion rates for individual MetS components. Consequently, 51.2% of participants with MetS at baseline reverted back to normal status after the walnut

intervention. Based on the results from linear mixed model adjustments for gender, age, BMI, and sequence, walnut consumption favorably changed fasting blood glucose, HbA1c, HDL-C, adiponectin, and ALA in MetS subjects.

As stated in a health claim made by the U.S. Food and Drug Administration in 2004, among tree nuts, walnuts have been found to be particularly promising in improving blood lipid level [15]. Several intervention and cross-sectional studies have demonstrated that PUFA intake through walnuts can improve TC, LDL-C, and HDL-C levels [16-18]. Walnuts not only contain a high level of PUFAs, but also a low amount of saturated fatty acids and high amounts of dietary fiber, antioxidants, and phytosterols [19,20]. Plant phenols, which are richer in walnuts (1,625 mg gallic acid equivalents per 100 g of walnuts) than in other nuts, may possess various bioactive health benefits including the capacity to lower serum LDL cholesterol and/or promote detoxification. Thus, as a consequence, various actual health benefits might be observed. In particular, ALA in walnuts may work in synergy with other nutrients such as mono- and polyunsaturated fatty acids, proteins, and fiber [20]. Such components of walnuts can lead to a positive effect on various aspects of health. Cross-sectional and cohort studies have shown that nut consumption was related to a low risk of insulin secretion [21] and glucose homeostasis [22], which may explain the inverse association between habitual nut consumption and the risk of T2D. Moreover, a previous intervention study reported the consumption of 56 g of mixed nuts every day, as a replacement for carbohydrate food items, improved glycemic control in patients with T2D [23]. Similar to those previous results, our findings also show improved fasting blood glucose levels after walnut intervention compared with that after control food intervention. In addition, compared to our control results, there was a significant decrease in HbA1c detected after the walnut intervention, in contrast to the results from a previous observational study [5]. Those authors reported that

a 43 g daily walnut intake for eight weeks increased HbA1c among healthy subjects, whereas their blood lipid parameters improved. Subjects in both studies did not have severe diabetes and their HbA1c levels were within the normal range before and after the walnut interventions. Further studies may be needed to investigate the effects of walnut intake on glucose metabolism among subjects with clinical T2D.

One of the major outcomes of the current study is that regular walnut intake among adults with MetS may have desirable effects, not only on fasting glucose and HbA1c but also on circulating adiponectin. The increased adiponectin level among the subjects with MetS might be evidence of a potential anti-diabetic effect of walnuts. Adiponectin is an adipocyte-derived anti-atherogenic and anti-diabetic hormone and is abundant in human plasma [24]. Blood levels of adiponectin are negatively regulated by an increase in visceral fat [25] and therefore are lower in obese individuals [26]. Moreover, clinical studies showed hypoadiponectinemia in the pathogenesis of T2D [27], coronary artery disease [28], and hypertension [29]. Also, there have been previous reports showing a significant inverse relationship between adiponectin and insulin resistance. In particular, HbA1c has been shown to have a negative correlation with serum adiponectin [30]. Hypoadiponectinemia seems to increase the risk of developing diabetes, and patients with MetS are at high risk of developing T2D [31]. Since the current study was performed with free-living subjects that added walnuts without other dietary controls or restriction, changes of adiponectin after walnut consumption for 16 weeks along with changes of glucose and HbA1c might provide strong evidence for an anti-diabetic effect of walnuts. A previous study evaluated the effects of ALA on adiponectin in T2D patients and indicated that ALA supplementation can improve glucose homeostasis and is associated with an increase in adiponectin [32]. In healthy humans, SFA intake was correlated negatively with circulating adiponectin levels, whereas omega-3 PUFA was positively associated with circulating adiponectin levels [33]. In our study, after the walnut intervention, the subjects increased their intakes of PUFA ($P < 0.0001$) and ALA ($P < 0.0001$). In addition, blood ALA after walnut consumption was significantly increased. The data suggest that the intake of walnut on a regular basis may have a direct influence on plasma fatty acid composition. A previous crossover study also showed a significant increase in blood ALA after the study's walnut diet period [34]. Our results suggest that the increased ALA intake from walnut consumption was principally responsible for increasing the levels of adiponectin, even though the potential mechanisms for the effect are still not fully described. Further studies are needed to elucidate the changes in glucose and adiponectin metabolism induced by walnut consumption among subjects with clinical T2D.

Walnut consumption for 16 weeks also improved other parameters, despite the lack of detection of statistically significant intervention effects. Results from paired *t*-tests showed that blood levels of TG, TC, and apoB were reduced during the walnut intake period. Lipid-lowering effects of walnut have been investigated for decades; however, the mechanism by which walnuts reduce atherogenic lipoproteins is still unclear. As Wu *et al.* [34] pointed out, there are no available data to

determine whether an improved lipid profile is a result of decreased production or increased clearance of apoB or a combination of both. Our results also showed an inverse relationship between walnut consumption and one of the atherogenic lipoprotein levels, although our results did not reveal a significant intervention effect.

Among the major strengths of this study is its randomized, crossover design with an addition of a washout period. The study systematically assessed the effect of walnut consumption on fasting lipid and glucose metabolism parameters, circulating adiponectin and leptin levels, as well as blood pressure and anthropometric measurements among free-living individuals. In addition, our study had an adequate sample size and appropriate intervention period. Our results have identified lipid-improving effects of a walnut dietary supplement in individuals with MetS. However, there is still a limitation to the study. Iso-caloric white bread, the control intervention, does not match the macronutrient content of walnuts due to its high percentage of carbohydrates. However, our results still showed that consuming walnuts as a substitute for a high carbohydrate snack (which is popular among Koreans) could be a healthy choice when managing MetS components.

In conclusion, our study shows that a daily dietary supplement of 45 g of walnuts for 16 weeks can favorably change MetS status by increasing the concentration of HDL cholesterol and decreasing the fasting glucose level. Furthermore, consuming walnuts on a daily basis also favorably changed HbA1c and circulating adiponectin levels, which may explain its potential anti-diabetic effects.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interests.

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