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Research Article

Daily Walnut Consumption Favourably Changed Lipid Profiles among Korean Subjects with Higher Waist Circumference

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Abstract

Even though many studies have shown that walnuts have beneficial effects on lipid profiles in various populations, there have been limited data on the effects of walnuts in Korean populations. We examined not only the effects of walnut intake on lipid profiles among Korean adults but also focused on the sub-classification by waist circumference (WC). 89 subjects out of 119 completed trial with daily consumption of 45g of walnuts for 16 weeks. Blood lipid profiles including triglycerides (TG), non-HDL cholesterol (non-HDL-C), LDL cholesterol (LDL-C), total cholesterol (TC), and HDL cholesterol (HDL-C), apolipoprotein B, anthropometric measurements (WC, weight, body mass index (BMI) and blood pressure) and glucose metabolism parameters including fasting blood sugar and insulin levels were assessed. Whose WC was greater than 85 cm for female and 90 cm for male were classified as higher WC group (n = 48) and others were classified as normal WC group (n = 41). Blood levels of non-HDL-C, LDL-C, TC and apolipoprotein B were improved after daily consumption of 45g of walnuts (P = 0.003, P = 0.011, P = 0.002, and P = 0.012, respectively) compared to baseline levels. Systolic blood pressure, TG, non-HDL-C, LDL-C and TC were significantly decreased in the higher WC groups (P = 0.048, P = 0.002, P = 0.002 and P = 0.001, respectively) compared to normal WC group. The results suggest that consuming 45 g of walnuts daily for 16 weeks had beneficial effects on lipid profiles in general, and these results were even much stronger among the subjects with abdominal obesity as waist circumference compared to those with non-abdominal obesity. This trial was registered at ClinicalTrials.gov as NCT03267901.

Keywords: Walnut; Triglycerides; Total Cholesterol; HDL Cholesterol; LDL Cholesterol; Waist Circumference

Abbreviations

TC: Total Cholesterol; LDL-C: Low Density Lipoprotein Cholesterol; Non-HDL-C: Non-high Density Lipoprotein Cholesterol; BMI: Body Mass Index; WC: Waist Circumference; TG: Triglyceride; HDL-C: High Density Lipoprotein Cholesterol; FBS: Fasting Blood Sugar; HOMA-IR: Homeostatic Model Assessment Insulin Resistance; HOMA-β: Homeostatic Model Assessment Beta Cell; QUICKI: Quantitative Insulin Sensitivity Check Index; GIR: Glucose Insulin Ratio; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; CVD: Cardiovascular Disease

Introduction

Walnuts (*Juglans regia Chandler*) are composed of monounsaturated and polyunsaturated fatty acids including alpha-linolenic acid (ALA). ALA are found in walnuts at a concentration of 9.1 g/100 g of walnuts which are more than eight times of the amount found in other nuts (i.e. almonds, pecans, pistachios and peanuts).

Health effects of walnut intake have been investigated in various populations and subjects with certain clinical conditions. According to a meta-analysis, total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) levels after consumption of 48 g of walnuts were significantly decreased compared with levels after consumption of control diets [1,2]. Both healthy people and participants with diseases putting them at risk for cardiovascular disease (i.e. diabetes mellitus, hypercholesterolemia and obesity) experienced significantly decreased TC levels [2]. Even though many studies have shown that walnuts have beneficial effects on lipid profiles in various populations, there have been limited data on the effects of walnuts in Korean populations. In a randomized controlled crossover trial, consuming 43g of walnuts daily for 8 weeks significantly decreased non-high density lipoprotein cholesterol (non-HDL-C) and apolipoprotein B in healthy Caucasian men and postmenopausal women [3]. Young and healthy male adults (n = 15 for white and n = 3 for Asian) showed significantly improved TC and LDL-C after consuming 28g of walnuts for 4 weeks [4]. 15 obese American subjects with metabolic syndrome (n = 9 men; n = 6 women; mean for body mass index (BMI): 36.6 ± 1.7 kg/m2; and mean for waist circumference (WC): 112 cm) were shown to experience significantly beneficial effects on their lipid profiles after consuming 48g of walnuts for 4 days [5]. Another study also reported a reduction of LDL-C levels among insulin-sensitive and overweight women (various race; ≥ 70% white non-hispanic) after the daily intake of 42g of walnuts for 2 weeks [6]. As well know, each population has different cutoff point of WC for central obesity. The cutoff point of WC for central obesity in the United States was defined as ≥ 102 cm for men and ≥ 88 cm for women [7] whereas, for Europeans, the cutoff was ≥ 94 cm for men and ≥ 80 cm for women [8]. Asians are more prone to obesity-related co-morbidities than Caucasians, even at lower BMI and/or smaller WC values. Therefore, cutoff values of ≥ 90 cm for men and ≥ 80 cm for women were adopted for South Asians [9], and the Chinese used cutoff points of ≥ 90 cm for men and ≥ 85 cm for women [10] which are same for Korean adults [11].

Therefore, our study investigates not only the effects of walnuts on lipid profiles among Korean adults but also the effects among the subjects with abdominal obesity.

Materials and Methods

Subjects

Participants were recruited with flyers and SNS advertisements in Seoul and Ulsan, South Korea. Subjects were selected if they were between the ages of 25 and 55 years. Subjects were excluded if they took medications, consumed nuts frequently (> 2 times/week), used nutritional supplements, drank alcohol > 2 times a week, or smoked. Women who had irregular menses, took birth control pills, or were pregnant, lactating, or menopausal were also excluded.

This study was conducted according to the guidelines laid out in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Committee of Sookmyung Women's University, Seoul, South Korea (SMWU-1508-BR-051-01). Written informed consent was obtained from all study subjects.

Study design

It was a self-controlled intervention trial, and all subjects consumed 45g of walnuts daily for 16 weeks. All subjects were able to determine the frequency of ingestion per day as desired. Participants were instructed to maintain their habitual dietary intake and usual level of physical activity and to avoid consuming any additional nuts, nut products, or omega-3 foods including canola oil, salmon, flaxseed oil and vitamin supplements, throughout the study.

Compliance and dietary assessment

Compliance was measured by self-report. A consumption log sheet was provided for participants to keep a record of their walnut consumption. Each Monday, subjects reported to care providers, who were trained registered dietitians, using a text message to indicate how many packages they did not consume during previous week. Good compliance was defined as > 80%: intake of walnuts ≥ 87 times during 108 days, and moderate compliance was defined as > 60%: intake of walnuts > 66 times during 108 days. Three-day diet records that included 2 consecutive weekdays and 1 weekend day were done once before the trial and two times during the trial period. A registered dietitian provided detailed instructions to participants on how to fill out a diet record and report their diet by taking a picture and sending it via text messages. All dietary records were analyzed to provide an estimation of daily average energy and nutrient intake using the CAN-Pro 4.0 software (Korean Nutrition Society, Korea).

Anthropometric measurements

All subjects visited and were measured in the Seoul and Ulsan centers. During all baseline (week 0) and final period (week 16) measurements, participants were barefoot and wore light clothing. Trained technical staff members measured WC and hip circumferences using a measuring tape (SECA-201, SECA Ltd., Hamburg, Germany). WC were obtained at the midpoint between the lowest rib and the iliac crest and rounded to the nearest 0.1 cm after inhalation and exhalation. Hip circumferences were measured at the widest part between the waist and knees and rounded to the nearest 0.1 cm. Weight while fasting was measured and rounded to the nearest 0.1 kg, and height was determined and rounded to the nearest 0.1 cm using a stadiometer (BSM330, Biospace Co., Ltd, Seoul, Korea). BMI was calculated as weight (kg) divided by height in meters (m) squared. Blood pressure was measured on the right arm using an up-load blood pressure monitor (BPBIO320S, Biospace Co., Ltd, Seoul, Korea) with participants in a comfortably seated position after at least a 5-minute rest.

Blood analyses

All subjects visited and had blood collected at the Seoul and Ulsan centers, and blood was analyzed at the Seoul center. After a 12-hour fasting period, the blood samples were taken at a baseline time point (week 0) and at week 16 by standard venipuncture. Blood was centrifuged at 3000 rpm for 15 minutes at 4°C. Aliquots of serum were prepared and stored at -80°Cuntil the following biochemical measurements were done. Serum TC and triglycerides (TG) levels were measured by an enzymatic-colorimetric method using the Cobas 8000 c702 chemistry analyzer (Roche Diagnostics; Mannheim, Germany). High density lipoprotein cholesterol (HDL-C) and LDL-C levels were determined by a homogeneous enzymatic-colorimetric method. Non-HDL-C levels were calculated from TC and HDL-C values using the following formula: non-HDL-C (mg/dL) = TC–HDL-C [3]. Serum insulin was measured with a commercially available kit, the ultrasensitive insulin ELISA kit (80-INSHUU-E01.0, E10, ALPCO, Salem, NH, USA) using an Epoch microplate spectrophotometer (BIOTEK, Inc., Winooski, VT, USA). Fasting blood sugar (FBS) was measured using a Glucocard X-meter (Arkray, Kyoto, Japan). Homeostatic model insulin resistance (HOMA-IR), homeostatic model beta cell function (HOMA-β) and quantitative insulin sensitivity check index (QUICKI) levels were calculated from fasting blood glucose and serum insulin values using the following formula: HOMA-IR = FBS (mg/dL) × serum insulin (μU/mL)/405; HOMA-β = [20 × fasting plasma insulin (IU/mL)]/[FBS (mmol/L)-3.5]; and QUICKI = 1/[log(serum insulin μU/mL)+log(FBS mg/dL)]. Serum apolipoprotein B was measured by turbidimetric immunoassay using the HITACHI 7600 chemistry analyzer (Hitachi, Inc., Tokyo, Japan). The glucose insulin ratio (GIR) was calculated with the formula: GIR = FBS level (mg/dL)/fasting insulin level (μIU/L).

Statistical analysis

The study participants were categorized according to WC. According to the WC criteria for Asian and Pacific populations, participants were classified into the normal WC group (non-obesity is defined as a WC under 90 cm for men and 85 cm for women) or the high WC group (obesity defined as a WC greater than or equal to 90 cm for men and 85 cm for women).

Participants, care providers, and analyses of dietary records were not blinded because of obvious differences between treatments. However, personnel who were responsible for assessing outcomes and data management were blinded. Data were analyzed using SPSS (IBM SPSS version 23, IBM corp., Armonk, NY, USA). All data are expressed as the means and standard deviations. The significance of differences between baseline (week 0) and final (week 16) outcomes within groups were assessed using paired t-test, and those between groups were assessed using independent t-test. P values were considered statistically significant at $P < 0.05$.

Results and Discussion

The current self-controlled pre-post intervention trial showed that daily walnut intake improved TG, non-HDL-C, LDL-C, TC and apolipoprotein B levels among Koreans. Subjects in the high WC group showed much more favorable improvements in lipid profile indices and insulin levels than those in the normal WC group.

Of the 119 subjects, a total of 89 (n = 44 for men; n = 45 for women) completed the study. 22 subjects dropped out due to personal reasons (n = 5), scheduling conflicts (n = 7), pregnancy (n = 2), or medication (n = 6) (Figure 1). In addition, two smokers were dropped out. Compliance of the subjects was 100% for 5 male subjects and 3 female subjects during the 108 days. 54 subjects (n = 27 for men; n = 28 for women) were in good compliance, consuming walnuts on 75 - 99% of days, and 16 subjects (n = 12 for men; n = 14 for women) ate walnuts on 50 - 74% of days during the study.

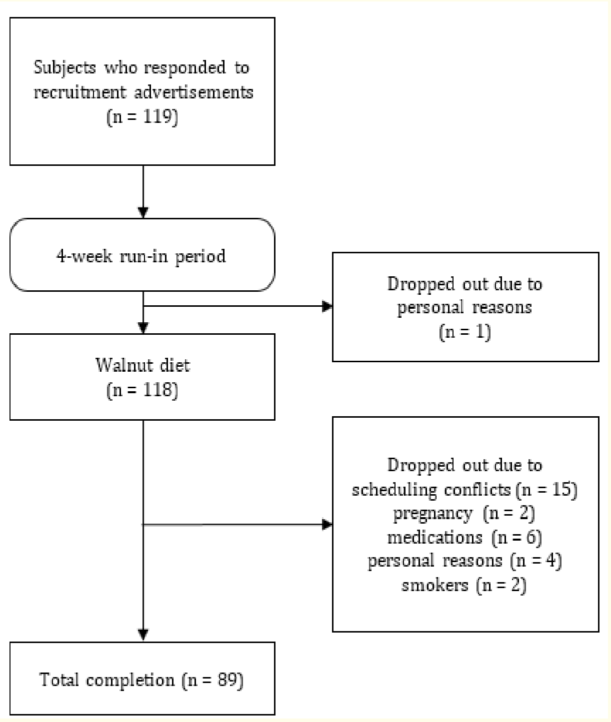

Figure 1: Flow chart of the study.

Changes in circulating biochemical outcomes from baseline to week 16 included a significantly decreased systolic blood pressure (SBP) at week 16 after walnut intake (P = 0.004) and a slightly decreased diastolic blood pressure (DBP) at week 16 (Table 1). Regarding the lipid profile, non-HDL-C, LDL-C, TC and apolipoprotein B levels significantly decreased after daily consumption of 45g of walnuts (P = 0.003, P = 0.011, P = 0.002, and P = 0.012, respectively). TG and HDL-C levels slightly decreased from baseline to week 16. However, FBS and insulin levels did not significantly change. The findings of the current study are consistent with previous studies that reported beneficial effects of walnut consumption on lipid profiles among adults of normal weight and those who are overweight or obese [12-17]. According to a meta-analysis, consuming walnuts as 10 - 24% of energy intake significantly decreased TC and LDL-C levels, while the effect on HDL-C levels was not significant [2]. 87 subjects with normal to moderately high plasma total cholesterol exhibited improvements in their lipid profiles, particularly TG and TC levels in subjects with high TC, after consuming 28 - 64 g/day of walnuts for 6 months [18].

	Baseline (n = 89)	Changes from baseline at week 16 (n = 89)[2)]	Within group *P*-value[3)]
Waist circumference (cm)[1)]	88.61 ± 9.98	-1.13 ± 5.18	0.043
Weight (kg)	75.68 ± 14.89	0.16 ± 2.11	0.489
BMI (kg/m^2)	26.80 ± 3.59	0.00 ± 0.77	0.956
WHR	0.91 ± 0.06	0.00 ± 0.02	0.070
SBP (mmHg)	134.64 ± 14.60	-3.24 ± 10.36	0.004
DBP (mmHg)	81.47 ± 11.21	-0.20 ± 8.12	0.815
Lipid profile			
TG (mg/dL)	145.96 ± 97.66	-12.02 ± 70.87	0.113
HDL-C (mg/dL)	43.07 ± 11.07	-0.67 ± 6.89	0.359
Non-HDL-C (mg/dL)	142.96 ± 31.96	-7.98 ± 24.62	0.003
LDL-(mg/dL)	121.29 ± 28.31	-6.18 ± 22.39	0.011
TC (mg/dL)	186.02 ± 30.11	-8.65 ± 24.92	0.002
Apolipoprotein B (mg/dL)	94.75 ± 20.22	-3.82 ± 14.05	0.012
Glucose and insulin profile			
FBS (mg/dL)	101.33 ± 25.79	1.49 ± 21.27	0.509
Insulin (μIU/L)	2.87 ± 1.72	0.37 ± 1.52	0.024
GIR (mg/μIU)	48.22 ± 30.21	2.54 ± 27.84	0.391
HOMA-IR	0.65 ± 0.44	0.09 ± 0.04	0.045
HOMA-β	31.95 ± 26.88	4.17 ± 27.24	0.152
QUICKI	0.42 ± 0.05	0.00 ± 0.04	0.436

Table 1: Effects of daily walnut intake for 16 weeks on biochemical indices.

1) BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; TG: Triglyceride; HDL-C: High Density Lipoprotein-Cholesterol; Non-HDL-C: Non-High Density Lipoprotein-Cholesterol; LDL-C: Low Density Lipoprotein-Cholesterol; TC: Total Cholesterol; FBS: Fasting Blood Sugar; HOMA-IR: Homeostatic Model Insulin Resistance; HOMA-β: Homeostatic Model Beta Cell Function; QUICKI: Quantitative Insulin Sensitivity Check Index; GIR: Glucose Insulin Ratio.

2) The "Change" data represent differences from baseline to week 16

3) The significance of the differences between baseline and week 16 were assessed using a paired t-test, and P- values were considered statistically significant at $p < 0.05$.

4) Data are presented as mean ± SD

SBP in the normal WC group significantly decreased after walnut intake (P = 0.012), while a slight decrease in SBP was observed in the high WC group (Table 2). Regarding the lipid profile, TG, non-HDL-C, LDL-C and TC in the high WC group significantly decreased after walnut intake (P = 0.048, P = 0.002, P = 0.002 and P = 0.001, respectively), while non-HDL-C and TC levels in the normal WC group slightly decreased. The differences between groups in the changes in non-HDL-C, LDL-C and TC were significant (P = 0.015, P = 0.006 and P = 0.010, respectively). However, FBS levels in the normal WC group were significantly increased from baseline, with the difference being 3.78 mg/dL (P = 0.017).

	Normal-WC[2)] (n = 41)		**Higher WC[3)] (n = 48)**		**Between group *P*-value [4)]**
	Baseline	Δ	**Baseline**	Δ	
Waist (cm) [1)]	80.39 ± 5.29**	-	95.64 ± 7.31	-	-
Weight (kg)	65.50 ± 8.01**	0.17 ± 1.62	84.37 ± 13.89	0.14 ± 2.46	0.955
BMI (kg/m^2)	24.06 ± 1.88**	0.05 ± 0.59	29.14 ± 3.01	-0.03 ± 0.90	0.628
WHR	0.88 ± 0.03**	0.00 ± 0.02	0.94 ± 0.07	0.01 ± 0.02	0.557
SBP (mmHg)	135.15 ± 16.51	-4.37 ± 10.58†	134.21 ± 12.90	-2.27 ± 10.19	0.345
DBP (mmHg)	80.83 ± 12.24	0.24 ± 8.50	82.02 ± 10.35	-0.58 ± 7.85	0.635
Lipid profile					
TG (mg/dL)	107.85 ± 52.28**	1.83 ± 53.88	178.50 ± 114.82	-23.85 ± 81.38†	0.088
HDL-C (mg/dL)	46.90 ± 11.57*	-0.22 ± 7.69	39.79 ± 9.58	-1.06 ± 6.19	0.568
Non-HDL-C (mg/dL)	133.90 ± 30.05*	-1.17 ± 17.27	150.69 ± 31.80	-13.79 ± 28.40†	0.015
LDL-C (mg/dL)	116.76 ± 24.62	0.73 ± 15.74	125.17 ± 30.84	-12.08 ± 25.51†	0.006
TC (mg/dL)	180.81 ± 27.86	-1.39 ± 17.68	190.48 ± 31.51	-14.85 ± 28.49†	0.010
Apolipoprotein B (mg/dL)	89.51 ± 19.02*	0.41 ± 12.33	99.23 ± 20.32	-7.44 ± 14.54†	0.008
Glucose and insulin profile					
FBS (mg/dL)	98.90 ± 12.04	3.78 ± 9.73†	103.40 ± 33.36	-0.46 ± 27.54	0.352
Insulin (μIU/L)	1.93 ± 0.74**	0.07 ± 0.73	3.66 ± 1.92	0.62 ± 1.93†	0.087
GIR (mg/μIU)	60.55 ± 30.47**	6.28 ± 33.92	37.69 ± 25.94	-0.65 ± 21.19	0.244
HOMA-IR	0.42 ± 0.17**	0.04 ± 0.17	0.84 ± 0.51	0.13 ± 0.52	0.276
HOMA-β	21.08 ± 9.69**	-2.16 ± 8.67	41.23 ± 32.90	9.57 ± 35.51	0.031
QUICKI	0.45 ± 0.04**	0.00 ± 0.04	0.40 ± 0.04	-0.01 ± 0.04	0.454

Table 2: Effects of daily walnut intake for 16 weeks on biochemical indices among the subjects classified using waist circumference.

1) BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; TG: Triglyceride; HDL-C: High Density Lipoprotein-Cholesterol; Non-HDL-C: Non-High Density Lipoprotein-Cholesterol; LDL-C: Low Density Lipoprotein-Cholesterol; TC: Total Cholesterol; FBS: Fasting Blood Sugar; HOMA-IR: Homeostatic Model Insulin Resistance; HOMA-β: Homeostatic Model Beta Cell Function; QUICKI: Quantitative Insulin Sensitivity Check Index; GIR: Glucose Insulin Ratio.

2) Normal-WC: non-obese state defined as WC values ≤90 cm for men and ≤85 cm for women.

3) High-WC: obesity defined as WC values >90 cm for men and >85 cm for women.

4) P-value was assessed using Student's t-test for differences between groups at week 16 and was considered statistically significant at P < 0.05.

Data are presented as mean ± SD

*P-value was assessed using Student's t-test for differences between groups at baseline and was considered statistically significant at P < 0.05.

**P-value was assessed using Student's t-test for differences between groups at baseline and was considered statistically significant at P < 0.001.

†P-value was assessed using a paired t-test for differences between baseline and week 16 and was considered statistically significant at P < 0.05.

††P-value was assessed using a paired t-test for differences between baseline and week 16 and was considered statistically significant at P < 0.05.

According to the analyses in this study, TG, non-HDL-C, LDL-C and apolipoprotein B levels were more improved in the obesity group as classified by WC. The National Institutes of Health guidelines indicate that within each BMI category, men and women with high WC values are at a greater health risk than those with normal WC values [19]. Moreover, among Asians with obesity, WC criteria are lower than those for Americans, Europeans, South Asians or even Chinese individuals because being below the world health organization (WHO) cutoff point for overweight decreased the high risk for cardiovascular disease (CVD) [11]. In a randomized controlled crossover study, when healthy Japanese individuals (mean for BMI: for women; 20.7 kg/m^2; for men; 22.2 kg/m^2) consumed habitual Japanese diets with 44 - 58g of walnuts for 4 weeks, multiple lipid indices (TC, apolipoprotein B, LDL-C and the ratio of LDL-C to HDL-C) significantly decreased [12] compared to those observed in those consuming a reference diet. In our study, changes in lipid profiles and insulin levels were not significant in the normal WC group, while such changes were significant in the obese group. Notably, non-HDL-C, LDL-C and TC levels significantly decreased for men in the high WC group as determined using WC, and TG levels significantly decreased for women. The mechanisms by which walnuts reduce CVD risks are related to fat composition characteristics, such as degree of unsaturation. Other mechanisms may involve dietary fiber or nitric oxide formation due to the increase of arginine in proteins, which could influence endothelial function and inhibit platelet aggregation, monocyte adherence, chemotaxis and vascular smooth muscle cell proliferation. Moreover, the antioxidant actions of vitamin E can also reduce LDL oxidation [20].

Dietary analyses using 3-day diet records were performed at baseline and during the walnut intake period. Energy and macronutrient intake were not significantly changed and were not different among groups classified using WC, except for the change in total lipid intake, which was significantly increased during the walnut consumption period among subjects in the normal WC group (data not shown).

The strengths of this study include the sufficient study duration, the similar energy intakes among groups and the fact that all subjects were freely living their daily lives. Moreover, all circulating biochemical levels were consistently analyzed. Another strength of the study was the large number of individuals in the sample population and evenly distributed number of subjects in normal and higher WC groups. However, there are also some limitations to this study. Current study is a self-controlled pre-post intervention design which was not able to provide the comparison between walnut and control. In addition, post-menopausal female and smokers were not included for recruitment. Because these are major factors for abnormal lipid profiles, further studies which include those population would be needed.

Conclusion

In conclusion, adding 45g of walnuts into a habitual diet resulted in improvements in the levels of TC, non-HDL-C, LDL-C and apolipoprotein B among Koreans. These results were even stronger among subjects with abdominal obesity determined using WC.

Acknowledgements

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Conflict of Interest

No conflicts of interest, financial or otherwise, are declared by the author(s).

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