

The effects of daily intake timing of almond on the body composition and blood lipid profile of healthy adults

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BACKGROUND/OBJECTIVES: Timing of almond intake during a day may result differently in the perspectives of body composition and changes of lipid profile. The current study was conducted to compare the effects of daily almond intake as a preload versus as a snack on body composition, blood lipid profile, and oxidative and inflammation indicators among young Korean adults aged 20-39 years old.

SUBJECTS/METHODS: Participants were randomly assigned to one of three groups: a pre-meal almond group (PM), a snack almond group (SN) in which participants were instructed to consume 56 g of almonds either as a preload before meals or as a snack between meals, respectively, and a control group (CL) in which participants were provided high-carbohydrate iso-caloric control food. Measurements were performed at baseline, weeks 8 and 16.

RESULTS: A total of 169 (M 77 / F 92) out of the 227 participants completed the study between June 2014 and June 2015 (n = 58 for PM; 55 for SN; and 56 for CL). A significant decrease in body fat mass was observed in the PM group at both weeks 8 and 16 compared with the CL. There were significant intervention effects on changes of body fat mass ($P = 0.025$), body fat percentages ($P = 0.019$), and visceral fat levels ($P < 0.001$). Consuming almonds as a daily snack reduced the levels of total cholesterol ($P = 0.043$) and low-density lipoprotein (LDL) cholesterol ($P = 0.011$) without changing high-density lipoprotein (HDL) cholesterol compared with the CL.

CONCLUSION: Almond consumption as a preload modified body fat percentages, whereas snacking on almonds between meals improved blood lipid profiles. This trial was registered at ClinicalTrials.gov as NCT03014531.

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INTRODUCTION

According to the USDA National Nutrient Database, 28 g of almonds contain 6 g of protein [12% of daily value (DV)], 12.44 g of unsaturated fat (88% of DV), 3.5 g of dietary fiber (14% of DV), and 7.27 g of vitamin E (36% of DV) [1]. Almonds are also known as an anti-oxidative and anti-inflammatory food that contains polyphenolic compounds including flavonoids and proanthocyanidins [2,3]. There have been various studies which examined the health benefits of almond intake in the fields of weight management, heart health, and diabetes. One of major results from the previous almonds studies might be improvement of blood lipid profiles, including the levels of triglycerides, total cholesterol, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol particularly among subjects with diet-related clinical symptoms such as obesity, diabetes, and hyperlipidemia [4-6]. Even with these positive

effects of almond intake, there have been continuous arguments whether regular consumption of almonds may increase the body weight or not due to fairly high calories like other nuts. However, previous studies have reported that almonds have no effect on weight changes in both healthy subjects who consumed 68 g of almonds [7] and hyperlipidemic subjects who consumed 73 g of almonds [8]. Even in the study consuming almonds over six months, subjects' body weight were not increased [9]. Daily almond intake might also help changing the body composition as reported in a weight reduction program study [10]. In this previous study, participants consuming 56 g of almonds per day decreased waist circumference by 14%, while a highly controlled carbohydrate diet decreased waist circumference by 9% ($P < 0.05$).

Another 10-weeks long study conducted by Hollis and Mattes found that the subjects in almond treatment groups showed similar energy intake compared to control group who followed

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their usual diet. According to the authors, the results suggest that almond intervention as a replacement for other food did not change overall calorie intakes. Authors stated that these compensations were caused by the fiber and protein in almonds, which both increase satiation [11]. Recent 5-arm randomized parallel study conducted by Tan and Mattes examined significant reductions in the daylong hunger ratings among the subjects who consumed 43 g of almonds as a morning or afternoon snack compared to the subjects who consumed same amount of almonds with breakfast or lunch. Authors stated that the results indicate potential different effects of almond intake based on the timing of consumption during a day [12].

These previous results led us to investigate the effects of daily almond consumption based on the different timing of almond intake during a day (as a preload versus as a snack between meals) compared with iso-caloric food among the free-living adults with no clinical symptoms. The current study is particularly focused on the changes of body composition using bioimpedance analyzer, lipid profiles, and oxidative and inflammation indicators with serial measurement during 16 weeks of almond intake.

SUBJECTS AND METHODS

Subjects

Participants were recruited through flyers and newspaper advertisements in Seoul, South Korea. Subjects were first screened using detailed and general medical questionnaires and trained nurse interviewed each subject for enrollment. Subjects were selected if they were non-smokers aged 20-39. Other inclusion criteria included a body mass index (BMI) of 17-30 kg/m² and a habitual diet close to that of a typical Korean diet. Subjects were excluded if they had any previous hypertension, arteriosclerosis, or metabolic diseases, had experienced any significant weight change in the last six months, were chronically ill or taking medications, consumed nuts frequently (more than twice per week), had erratic exercise habits, were smokers, or consumed alcohol more than twice per week. Women who had an irregular menstrual cycle, took birth control pills, or were pregnant or lactating were also excluded. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Committee of Sookmyung Women's University, Seoul, Korea (SMWU-1407-BR-006). Written informed consent was obtained from all study subjects.

Study design

This study was a randomized, 3-arms parallel, controlled 16-week trial. After being screened, eligible participants were simply randomized without any restriction into one of the three study arms using a computerized random number generator by the primary investigator. (1) In the pre-meal group (PM), participants were instructed to consume 56 g of almonds per day as a preload when having regular meals. They were directed to prepare the table first and consume nearly 1/3 amount of almond before each meal. (2) In the snack group (SN),

participants were instructed to consume 56 g of almonds between meals as snacks. A snack was defined as an eating event that occurred between participants' regular meals, specifically two hours before and after meals. All the participants in the PM and SN groups were provided daily portions of packaged almonds. (3) In the control group (CL), participants were provided with high-carbohydrate control food items (66 g of commercial cookies; 320 kcal, 18 g fat, 3.9 g protein, 1.2 g total dietary fiber and 0.79 mg α -tocopherol) that had a similar number of calories as 56 g of almonds (independent analyses indicates that they contain 340 kcal, 30 g fat, 12 g protein, 6.2 g total dietary fiber, and 14 mg α -tocopherol). All of the subjects were able to determine the frequency of ingestion per day as desired, if they keep the guidelines for their groups. Participants were instructed to maintain their habitual dietary intake and usual level of physical activity and avoid consuming any additional nuts or nut products throughout the study. Participants, care providers, and analysts for dietary record were not blinded because of obvious differences between treatments, whereas personnel who were in charge of assessing outcomes and data management were blinded.

Compliance and dietary assessment

Compliance was measured by self-report. A consumption log sheet was provided for participants to keep a record of their almond or cookies consumption. On every Monday, subjects reported to care providers, trained registered dietitians using text message how many packages they did not consume during previous week. Good compliance was defined as >80% adherence to the treatment. 80% compliance was defined as intake of almond or cookies over 90 times during 16 weeks (112 days). The three-day diet records, including two consecutive weekdays and one weekend day, were done once before the trial and twice during the trial. A registered dietitian provided detailed instructions of how to fill out a diet record and report their diet by taking pictures and sending these via text messages. All dietary records were analyzed to provide an estimation of the daily average energy and nutrient intake using CAN-Pro 4.0 software (Korean Nutrition Society, Seoul, Korea).

Measurements

All measurements were conducted with participants going barefoot and wearing light clothing for the baseline (week 0) and main-trial days (weeks 8 and 16). Body composition including body fat percentages was assessed through multi-frequency whole-body bioimpedance measurement using InBody 620 (Biospace Co., Ltd, Seoul, Korea). The results included body composition analysis (total body water, protein, and body fat mass), muscle-fat analysis (body weight, skeletal muscle mass, and fat free mass), obesity analysis (body fat percentage, BMI (calculated as weight (kg) divided by height in meters (m) squared)).

For measuring blood lipid profiles, after a 12-hour fast, blood samples were taken at the baseline (week 0) and at weeks 8 and 16 by standard venipuncture. Blood was centrifuged at 3,000 rpm for 15 min at 4°C. Aliquots of serum were prepared and stored at -80°C for following biochemical measurements.

The serum total cholesterol and triglycerides levels were measured by the enzymatic-colorimetric method using a Cobas 8000 c702 chemistry analyzer (Roche Diagnostics; Mannheim, Germany). HDL cholesterol and LDL cholesterol levels were determined via homogeneous enzymatic colorimetry. Non-HDL cholesterol levels were calculated from the total cholesterol and HDL cholesterol using the following formula: Non-HDL cholesterol (mg/dL) = total cholesterol - HDL cholesterol [13].

Waist and hip circumference measurements were measured by trained technical staff using a measuring tape (SECA-201, SECA Ltd., Hamburg, Germany). Waist circumferences were obtained at the mid-point between the lowest rib and the iliac crest to the nearest 0.1 cm after inhalation and exhalation. Hip circumferences were rounded to the nearest 0.1 cm at the widest part between the waist and knees. Weight while fasting was measured and rounded to the nearest 0.1 kg, and height was rounded to the nearest 0.1 cm using a stadiometer (BSM330, Biospace Co., Ltd, Seoul, Korea). 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 five-minute rest.

Serum Interleukin-6 (IL-6) was measured using an enzyme immunometric assay kit (ADI-900-033, Enzo Life Science, Farmingdale, NY, USA). Serum malondialdehyde (MDA) and oxidized LDL-C (cholesterol) were measured using commercially available kits, a human MDA ELISA kit (MBS724277, MyBioSource, San Diego, CA, USA) and oxidized LDL ELISA kit (10-1143-01, Mercodia AB, Uppsala, Sweden) with an Epoch microplate spectrophotometer (BIOTEK, Inc., Winooski, VT, USA). The coefficients of variation (CV) for all measurements were less than 4%.

Statistical analysis

The sample size was calculated based on the sample size estimation for clinical trials using OpenEpi version 3.01. Percentages of the subjects who may show the primary outcomes in the almond groups and snack group were set at 25 and 5%, respectively. In total, 45 subjects were required in each group to provide over 80% power. The significance of the differences between the baseline (week 0) and main-trial periods (weeks 8 and 16) with different interventions was assessed using the repeated measures analysis of variance (RMANOVA) with Duncan multi-comparison, and the model assumptions were checked. Differences in baseline and Δ (changes from the baseline at weeks 8 or 16) among the three groups were detected using one-way ANOVA. All data were expressed as means with standard deviations unless otherwise specified. All statistical analyses were performed using of SAS (version 9.3; SAS Institute, Cary, NC).

RESULTS

Basic characteristics, compliance, and dietary analyses

In total, 169 (n = 58 for PM; 55 for SN; and 56 for CL) out of the 227 participants completed the study between June 2014 and June 2015. Fifty-eight participants dropped out due to schedule conflict, poor compliance, nausea and stomach pain, traveling, and pregnancy (Fig. 1). The basic characteristics of the participants are presented in Table 1. Age, BMI and blood lipid profiles at baseline indicated that the subjects were healthy and young. The results of the dietary analyses (Table 2) showed that at weeks 8 and 16, the carbohydrate intake of the PM and SN groups was lower than that of the CL group, while the intake of vegetable fat, vegetable protein, fiber, monounsaturated

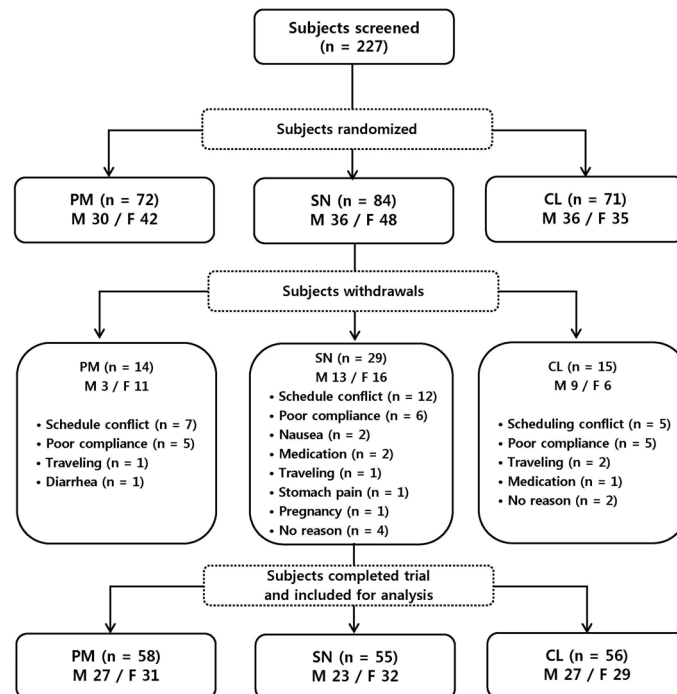


Fig. 1. Flow chart of the study. PM, pre-meal almond group; SN, snack almond group; CL, control group.

Table 1. Baseline characteristics of participants

Variable	
n (M / F)	169 (77 / 92)
Age (yrs)	26.33 ± 5.55 ¹⁾
Systolic blood pressure (mmHg)	124.2 ± 15.21
Diastolic blood pressure (mmHg)	73.21 ± 9.91
Waist (cm)	76.23 ± 8.59
Hip (cm)	96.98 ± 7.35
Height (cm)	167.71 ± 9.08
Weight (kg)	63.92 ± 12.31
BMI (kg/m ²)	22.59 ± 3.04
Total body water (L)	34.68 ± 8.00
Total body protein (kg)	9.34 ± 2.21
Body fat mass (kg)	16.54 ± 5.85
Body fat percentage (%)	26.06 ± 7.91
Fat free mass (kg)	47.38 ± 10.91
Skeletal muscle mass (kg)	26.19 ± 6.64
Basal metabolic rate (kcal)	1393 ± 236
Visceral fat level ²⁾	6.53 ± 3.02
Total cholesterol (mg/dL)	169.62 ± 29.74
HDL cholesterol (mg/dL)	59.36 ± 14.03
non-HDL cholesterol (mg/dL)	110.26 ± 30.24
LDL cholesterol (mg/dL)	104.24 ± 27.49
Triglycerides (mg/dL)	79.85 ± 40.42
IL-6 (pg/mL)	36.7 ± 25.53
Oxidized LDL-C (mU/L)	6.32 ± 2.18
MDA (ng/mL)	14.32 ± 5.37

¹⁾ Values are means ± SD.

²⁾ Indicator generated from bioimpedance analysis based on the estimated amount of fat surrounding internal organs in the abdomen [normal range: 1–9]. BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; IL, Interleukin; MDA, malondialdehyde.

fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) of the PM and SN groups was significantly higher than that of the CL group.

Changes from the baseline in body composition

Changes from baseline in body composition including total body protein, body water, body fat mass, skeletal muscle mass, and visceral fat levels are presented in Table 3(A). Body weight changes from the baseline did not differ significantly for each time point among the three groups, whereas significant intervention effect was observed for changes of body fat mass ($P = 0.025$, RMANOVA) as results with one-way ANOVA revealed that body fat mass of PM group was decreased compared to CL at both weeks 8 and 16. In addition, body fat percentages in both almond groups were significantly reduced compared to the control group at week 16 using one-way ANOVA and significant interaction effect ($P = 0.032$, RMANOVA) was also observed. The visceral fat level significantly decreased in the PM group compared to the control group at both 8 and 16 weeks. In summary, consuming almond as a preload before meals reduced body fat mass, body fat percentages, and visceral obesity without reducing body weight.

Changes from the baseline in blood biochemistries

Blood biochemistries including lipid profiles, inflammation and anti-oxidants markers were presented in Table 3(B). At baseline, CL group showed lower levels of triglycerides, LDL, and non-HDL cholesterol when compared to the PM and SN groups. Total blood cholesterol levels were significantly reduced in SN group compared to CL at week 8, not at week 16 due to the great reduction of cholesterol levels in the CL group. SN group showed significantly greater reductions of LDL cholesterol levels at week 16 than the CL and PM groups using one-way ANOVA. These results suggest that consuming almonds as a daily snack may reduce total cholesterol and LDL cholesterol levels. However, changes in blood HDL cholesterol levels were not observed throughout the trial. Due to the great reduction in the total cholesterol for the almond groups, a significant decrease in non-HDL cholesterol was observed in the SN group at both weeks 8 and 16 compared with the CL. Changes in

Table 2. Dietary assessment at baseline, weeks 8 and 16 of the control, pre-meal almond, snack almond interventions.

Nutrients	Baseline			Week 8			Week 16		
	PM ²⁾	SN	CL	PM	SN	CL	PM	SN	CL
Energy (kcal)	1,606.5 ± 321.4 ¹⁾	1,674.2 ± 463.2	1,623.4 ± 357.3	1,656.8 ± 362.0	1,758.3 ± 337.1	1,776.0 ± 358.3	1,703.5 ± 319.8	1,682.2 ± 320.3	1,810.5 ± 358.2
Carbohydrate (g)	218.2 ± 47.6	221.3 ± 60.6	222.1 ± 47.7	196.5 ± 52.7 ^a	216.3 ± 45.5 ^b	246.2 ± 51.9 ^c	199.1 ± 47.4 ^a	206.5 ± 47.3 ^a	247.8 ± 56.8 ^b
Total fat (g)	53.0 ± 17.3	57.2 ± 22.8	51.5 ± 16.3	70.0 ± 19.0 ^b	70.2 ± 17.8 ^b	59.4 ± 15.6 ^a	73.8 ± 15.3 ^b	66.5 ± 15.2 ^a	61.9 ± 16.3 ^a
Vegetable fat (g)	25.9 ± 10.8 ^{a3)}	31.2 ± 11.6 ^b	26.2 ± 9.7 ^a	47.0 ± 15.2 ^b	48.4 ± 13.4 ^b	34.5 ± 9.2 ^a	49.1 ± 10.5 ^c	44.8 ± 11.7 ^b	35.8 ± 11.0 ^a
Animal fat (g)	27.1 ± 13.3	26.0 ± 14.8	25.3 ± 12.0	23.0 ± 12.6	21.8 ± 10.5	24.9 ± 11.5	24.7 ± 12.0	21.8 ± 10.3	26.0 ± 12.0
Total protein (g)	61.9 ± 15.7	65.4 ± 21.5	62.9 ± 15.4	63.8 ± 16.6 ^{ab}	67.8 ± 16.8 ^b	60.5 ± 13.5 ^a	64.6 ± 15.2	63.9 ± 15.2	64.2 ± 15.5
Vegetable protein (g)	26.6 ± 7.3	29.2 ± 8.4	26.6 ± 6.2	31.8 ± 8.3 ^b	34.0 ± 7.2 ^b	27.0 ± 6.6 ^a	32.0 ± 6.0 ^b	32.7 ± 7.3 ^b	29.0 ± 8.2 ^a
Animal protein (g)	35.2 ± 14.7	36.2 ± 16.6	36.3 ± 13.1	32.0 ± 13.5	33.8 ± 14.0	33.5 ± 10.1	32.7 ± 13.3	31.2 ± 13.5	35.2 ± 12.5
Fiber (g)	13.8 ± 4.5	14.9 ± 4.7	15.5 ± 4.1	17.1 ± 4.3 ^b	18.1 ± 4.5 ^b	13.7 ± 3.9 ^a	17.4 ± 3.7 ^b	17.4 ± 4.5 ^b	14.3 ± 4.6 ^a
Cholesterol (g)	312.6 ± 111.7	352.1 ± 172.5	344.4 ± 159.9	268.5 ± 102.8 ^a	313.7 ± 138.7 ^{ab}	344.5 ± 137.5 ^b	305.9 ± 152.6	325.0 ± 136.6	356.4 ± 132.2
Total fatty acid (g)	28.7 ± 14.5	31.8 ± 18.2	30.7 ± 16.8	48.7 ± 17.6 ^b	46.9 ± 17.9 ^b	27.2 ± 12.5 ^a	53.3 ± 16.9 ^c	44.1 ± 16.5 ^b	29.7 ± 13.3 ^a
SFA (g)	9.6 ± 6.0	11.0 ± 7.8	10.0 ± 7.4	9.9 ± 5.9	9.9 ± 5.9	9.4 ± 5.6	11.1 ± 5.9	9.3 ± 5.4	9.7 ± 5.0
MUFA (g)	11.2 ± 6.2	12.8 ± 8.6	12.4 ± 8.6	25.5 ± 9.1 ^b	24.8 ± 10.0 ^b	11.1 ± 6.8 ^a	28.1 ± 8.5 ^c	23.6 ± 9.6 ^b	11.7 ± 5.7 ^a
PUFA (g)	8.1 ± 3.2	9.6 ± 5.3	9.8 ± 4.3	13.3 ± 4.8 ^b	13.1 ± 4.4 ^b	8.5 ± 4.0 ^a	14.1 ± 3.9 ^c	12.2 ± 4.1 ^b	8.7 ± 3.8 ^a

¹⁾ Values are means ± SD.

²⁾ PM, pre-meal almond group; SN, snack almond group; CL, control group; SFA, saturated fatty acid; MUFA, Monounsaturated fatty acid; PUFA, Polyunsaturated fatty acid.

³⁾ The different letters (a, b, and c) within a row indicate significant differences ($P < 0.05$) determined by Duncan's multiple range test.

Table 3. Changes from baseline in body composition (A) and blood biochemistries (B)

	Intervention groups	Baseline	Changes from baseline at week 8	Changes from baseline at week 16	P-values for time effect	P-values for intervention effect	P-values for time by intervention interaction ³⁾
(A)							
Weight (kg)	PM ¹⁾	65.46 ± 12.641	0.45 ± 1.18	0.57 ± 1.75	0.091	0.839	0.899
	SN	63.91 ± 12.81	0.41 ± 1.28	0.57 ± 1.65			
	CL	62.33 ± 11.47	0.51 ± 1.07	0.74 ± 1.36			
BMI (kg/m ²)	PM	22.96 ± 2.86	0.14 ± 0.43	0.19 ± 0.65	0.029	0.840	0.700
	SN	22.88 ± 3.06	0.16 ± 0.41	0.23 ± 0.58			
	CL	21.92 ± 3.15	0.15 ± 0.39	0.27 ± 0.46			
Total body water (L)	PM	35.04 ± 8.28	0.20 ± 0.55	0.31 ± 0.83	0.122	0.463	0.726
	SN	34.38 ± 8.13	0.09 ± 0.76	0.25 ± 0.88			
	CL	34.58 ± 7.70	0.07 ± 0.81	0.10 ± 0.91			
Total body protein (kg)	PM	9.43 ± 2.28	0.05 ± 0.22	0.09 ± 0.18	0.481	0.893	0.393
	SN	9.27 ± 2.26	0.07 ± 0.23	0.09 ± 0.26			
	CL	9.30 ± 2.12	0.07 ± 0.18	0.05 ± 0.24			
Body fat mass (kg)	PM	17.56 ± 5.50	-0.06 ± 1.08 ^{a4)}	-0.10 ± 1.26 ^a	0.714	0.025	0.206
	SN	16.91 ± 5.84	0.28 ± 0.93 ^{ab}	0.17 ± 1.53 ^{ab}			
	CL	15.12 ± 6.02	0.35 ± 0.87 ^b	0.60 ± 1.20 ^b			
Body fat percentage (%)	PM	27.12 ± 7.60	-0.13 ± 1.48	-0.35 ± 1.60 ^a	0.498	0.019	0.032
	SN	26.68 ± 7.74	0.22 ± 1.19	-0.15 ± 1.98 ^a			
	CL	24.37 ± 8.24	0.35 ± 1.33	0.70 ± 1.82 ^b			
Fat free mass (kg)	PM	47.90 ± 11.31	0.29 ± 0.90	0.50 ± 1.16	0.115	0.647	0.550
	SN	47.00 ± 11.08	0.17 ± 1.04	0.38 ± 1.21			
	CL	47.21 ± 10.48	0.22 ± 1.09	0.23 ± 1.26			
Skeletal muscle mass (kg)	PM	26.46 ± 6.86	0.19 ± 0.48	0.32 ± 0.55	0.114	0.783	0.178
	SN	26.01 ± 6.81	0.13 ± 0.58	0.27 ± 0.66			
	CL	26.09 ± 6.36	0.20 ± 0.63	0.15 ± 0.76			
Basal metabolic rate (kcal)	PM	1405 ± 244	5.44 ± 18.60	9.74 ± 24.08	0.149	0.635	0.531
	SN	1385 ± 239	3.73 ± 22.28	8.19 ± 25.78			
	CL	1390 ± 226	3.94 ± 22.26	3.67 ± 26.61			
Visceral fat level ²⁾	PM	7.10 ± 3.03	-0.19 ± 0.74 ^a	-0.25 ± 0.71 ^a	0.166	0.000	0.235
	SN	6.62 ± 2.90	-0.02 ± 0.57 ^{ab}	0.12 ± 0.75 ^b			
	CL	5.86 ± 3.05	0.20 ± 0.41 ^b	0.38 ± 0.70 ^b			
(B)							
Total cholesterol (mg/dL)	PM	169.86 ± 29.86	3.62 ± 16.53 ^{ab}	-10.84 ± 20.33	< 0.001	0.043	0.955
	SN	175.35 ± 29.69	-1.02 ± 19.25 ^a	-16.55 ± 20.50			
	CL	163.75 ± 29.06	7.52 ± 21.12 ^b	-7.81 ± 23.69			
HDL cholesterol (mg/dL)	PM	56.60 ± 12.84	3.32 ± 7.85	-2.93 ± 8.54	< 0.001	0.856	0.565
	SN	58.69 ± 14.01	2.93 ± 8.43	-3.25 ± 9.25			
	CL	62.88 ± 14.70	4.65 ± 10.07	-3.24 ± 12.01			
non-HDL cholesterol (mg/dL)	PM	113.26 ± 29.96 ^b	0.14 ± 14.19 ^{ab}	-7.32 ± 16.71 ^{ab}	< 0.001	0.013	0.910
	SN	116.65 ± 31.45 ^b	-4.83 ± 17.86 ^a	-13.68 ± 19.99 ^a			
	CL	100.88 ± 27.43 ^a	3.36 ± 17.32 ^b	-4.68 ± 18.10 ^b			
LDL cholesterol (mg/dL)	PM	105.55 ± 26.75 ^{ab}	-0.62 ± 14.60 ^{ab}	-9.05 ± 17.75 ^b	< 0.001	0.011	0.610
	SN	110.78 ± 28.27 ^b	-4.42 ± 17.44 ^a	-16.02 ± 18.52 ^a			
	CL	96.45 ± 26.01 ^a	3.91 ± 17.46 ^b	-6.82 ± 18.61 ^b			
Triglycerides (mg/dL)	PM	88.97 ± 48.63 ^b	-4.81 ± 26.21	-8.43 ± 30.38	0.769	0.267	0.542
	SN	82.76 ± 41.76 ^{cb}	-5.08 ± 30.72	-4.53 ± 31.38			
	CL	67.55 ± 24.01 ^a	0.45 ± 23.11	1.78 ± 29.87			
IL-6 (pg/dL)	PM	38.82 ± 22.78	-7.34 ± 24.44	-0.57 ± 14.54	0.004	0.206	0.319
	SN	32.72 ± 22.63	-0.99 ± 16.88	1.36 ± 13.10			
	CL	38.33 ± 30.40	2.61 ± 16.49	5.30 ± 12.74			
Oxidized LDL-C (mU/L)	PM	5.94 ± 2.04 ^a	-0.95 ± 1.77	-0.27 ± 1.99	< 0.001	0.599	0.812
	SN	6.91 ± 2.40 ^b	-1.25 ± 1.79	-0.49 ± 2.07			
	CL	6.13 ± 2.01 ^{ab}	-0.84 ± 1.68	-0.28 ± 1.96			
MDA (ng/dL)	PM	14.09 ± 3.57	0.59 ± 4.63	4.50 ± 5.56	< 0.001	0.316	0.944
	SN	14.43 ± 4.51	-0.84 ± 4.47	-3.35 ± 5.81			
	CL	14.45 ± 7.43	-0.60 ± 3.93	3.60 ± 6.33			

¹⁾ PM, pre-meal almond group; SN, snack almond group; CL, control group; BMI, Body Mass Index.
²⁾ Indicator generated from bioimpedance analysis based on the estimated amount of fat surrounding internal organs in the abdomen [normal range: 1–9].
³⁾ ANOVA for repeated measures.
⁴⁾ Between-group comparisons at the same time point were conducted using one-way ANOVA. The different letters (a, b) within a column indicate significant differences ($P < 0.05$) determined by Duncan's multiple range test.
 BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; IL, Interleukin; MDA, malondialdehyde.

the blood triglycerides levels did not significantly differ among the three groups based on the RMANOVA. There were significant intervention effects in changes of total cholesterol ($P=0.043$), LDL cholesterol ($P=0.011$), and non-HDL cholesterol ($P=0.013$). Most of the blood chemistries also showed significant time effects as well, whereas there was no interaction between time and intervention. None of the changes in the oxidative and inflammation indicators were not significantly different among groups.

DISCUSSION

Our results indicate that the daily consumption of almonds (56 g) as a snack had positive effects on blood lipid profiles, including decreased non-HDL cholesterol, LDL cholesterol, and total cholesterol levels. In addition, it was found that consuming almonds as a preload before a meal reduced body fat mass, visceral fat level, and body fat percentage. These results show that young free-living adults who do not have any clinical manifestations, such as hyperlipidemia or obesity might also obtain positive effects from the daily consumption of almonds.

One finding in the current study was that consuming almonds each day affected body fat mass and percentage without changing body weight significantly. In particular, the subjects in the PM group experienced decreased visceral fat levels, which measure the amount of fat in surrounding organs. The visceral fat cross-sectional area can be measured only by computed tomography (CT) scan. Indices for visceral fat level by bioelectrical impedance analysis (BIA) is an estimate based on the regression equation of visceral fat cross-sectional area measure by CT [14]. Visceral fat is potentially lipolytic; its lipotoxicity brings hepatic insulin resistance and affects insulin metabolism, which causes hyperinsulinemia, peripheral insulin resistance, and diabetes mellitus [15]. This finding is supported by the study done by Salas-Salvado *et al.* [16], which claimed that a diet of mixed nuts helped their subjects decrease their central adiposity or metabolic syndrome without changing their body weight. In addition, one cross-sectional study conducted among elderly subjects with high cardiovascular risk living in a Mediterranean country examined the inverse relationship between central adiposity and nut consumption [17]. The results of a 10-week crossover study demonstrated that when subjects consumed a 56 g of almonds each day, they compensated for the energy provided by the almonds and reduced their food intake from other sources [11]. The effect on visceral body fat among the subjects in the PM group might suggest that almond consumption as a preload before the meal may be related with the insulin metabolism, which could be a topic of further studies, because body fat reduction in the abdominal region facilitates glycemic control [18].

Other nuts have also been shown to have cardioprotective effects, especially in lowering serum lipids. The results of a Bayesian meta-analysis indicated that hazelnut-enriched diet is associated with a decrease of total and LDL cholesterol, while HDL cholesterol, triglycerides unchanged [19]. A recent meta-analysis of 27 randomized controlled trials intervening almonds by Musa-Veloso *et al.* [20] indicated that almond consumption was associated with a significant reduction in total and chole-

sterols, triglycerides, and it had no effects on HDL cholesterol. This tendency appears to be greater in hyperlipidemic patients [7,8,21]. Our participants, especially those in the SN group, experienced decreased non-HDL, LDL, and total cholesterol compared with those in the CL group which were consistent with previous studies which reported lipid-improving effects of almond consumption among normolipidemic patients [7,22]. The fatty acid profiles (rich in oleic and linoleic acids [23]) and plenty amount of protein in almonds might change the fatty acid profile and protein contents of the habitual Korean diet, which contains a large amount of carbohydrates and saturated fat [24]. When saturated fat was replaced by unsaturated fat, an LDL cholesterol-lowering effect was observed in several previous studies [25-27]. In addition, when people have replaced the carbohydrate in their diet with protein, both normolipidemic [28] and hypercholesterolemic [29] subjects showed beneficial effects on LDL cholesterol levels.

In previous studies, almonds were mostly added to snack foods such as cereals or muffins [7,8]. The lipid-lowering effects of almond in our study were consistent with previous studies that having almond as a snack decreases LDL cholesterol [4,30]. However, LDL cholesterol level of PM group was not significantly decreased compared with the control group. As Tan and Mattes reported [12], consuming almonds with meals did not have significant effects on lipid profiles. Since the effects of consuming almond as a preload have not been frequently investigated, these results suggest that potential different lipid-improving effects of almond intake based on the timing of consumption during a day. Future studies would be needed to elucidate the mechanism of how the timing of almond consumption influence lipid profiles.

One possibility for why the timing of almond consumption affected the body fat composition and lipid profiles of young adults differently might be related to the fluctuation of blood glucose concentration after meals or snacks compared with the effect of the control food. One previous study suggested that the ingestion of 28 g of almonds before a high-starch meal lowered postprandial glycemia by 30% in individuals with type 2 diabetes [31]. Even though we did not measure any parameters for glucose metabolism, almonds may act as a lower-glycemic index (GI) food compared with the control food. Consuming snacks such as cookies and candy that contain a high amount of glucose significantly increases the glucose and insulin levels in the blood. Blood glucose levels rapidly drop due to excessively increased insulin secretion, and the individual feels hungry again [32]. However, the intake of low-GI food helps the body digest the food more slowly, which generates lower glycemic and insulinemic responses, resulting in longer satiety and lower food intake. In other words, low-GI diets metabolically result in lower diabetes incidence and heart disease risk by decreasing postprandial glucose excursions [33]. Almond intake as a preload may decrease the consumption of habitual high-carbohydrate meals, and snacking on almonds between meals may affect the blood glucose and lipid metabolism.

Design of the current study has several strengths: a robust sample size, sufficient study duration, and comparison between almond and control food consumption. In addition to providing standard amounts of almonds, our subjects were healthy

free-living young adults whose results are applicable to ordinary living situations. However, there are still several limitations to the study. Iso-caloric food contains high percentages of carbohydrates compared with almonds, which means it does not match the macronutrient composition of almonds. Additionally, the nutrients and bioactive substances generated from almonds were substituted with carbohydrate. Another limitation would be that body composition has not been carried out using dual-energy X-ray absorptiometry, or other imaging techniques (CT, magnetic resonance imaging). Further studies providing a control food item with matched macronutrient and fatty acid composition are needed.

In conclusion, regular almond consumption may help improve serum lipid profiles. Snacking almonds between meals may be more effective for improving lipid profile. Consuming almonds before meals may lower the visceral fat level and body fat mass and percentages without changing the body weight compared with iso-caloric high-carbohydrate cookies. Since the timing of almond consumption seems to be crucial regarding its effects, further study on glucose metabolism after almond consumption would provide important information.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interests.

ABBREVIATIONS

The following abbreviations are used in this manuscript:

DV: daily value

LDL cholesterol: low-density lipoprotein cholesterol

HDL cholesterol: high-density lipoprotein cholesterol

PM: pre-meal almond group

SN: snack almond group

CL: control group

BMI: body mass index

SFA: saturated fatty acid

MUFA: Monounsaturated fatty acid

PUFA: Polyunsaturated fatty acid

BIA: bioelectrical impedance analysis

CT: computed tomography

GI: glycemic index

REFERENCES

1. U.S. Department of Agriculture. USDA National Nutrient Database for standard reference. Release 28 [Internet]. Washington, D.C.: U.S. Department of Agriculture; 2016 [cited 2016 November 12]. Available from: <http://ndb.nal.usda.gov>.
2. Bolling BW, Chen CY, McKay DL, Blumberg JB. Tree nut phytochemicals: composition, antioxidant capacity, bioactivity, impact factors. A systematic review of almonds, Brazils, cashews, hazelnuts, macadamias, pecans, pine nuts, pistachios and walnuts. *Nutr Res Rev* 2011;24:244-75.
3. Bolling BW, Dolnikowski G, Blumberg JB, Oliver Chen CY. Quantification of almond skin polyphenols by liquid chromatography-mass spectrometry. *J Food Sci* 2009;74:C326-32.
4. Abazarfard Z, Salehi M, Keshavarzi S. The effect of almonds on anthropometric measurements and lipid profile in overweight and obese females in a weight reduction program: a randomized controlled clinical trial. *J Res Med Sci* 2014;19:457-64.
5. Li SC, Liu YH, Liu JF, Chang WH, Chen CM, Chen CY. Almond consumption improved glycemic control and lipid profiles in patients with type 2 diabetes mellitus. *Metabolism* 2011;60:474-9.
6. Spiller GA, Jenkins DA, Bosello O, Gates JE, Cragen LN, Bruce B. Nuts and plasma lipids: an almond-based diet lowers LDL-C while preserving HDL-C. *J Am Coll Nutr* 1998;17:285-90.
7. Sabaté J, Haddad E, Tanzman JS, Jambazian P, Rajaram S. Serum lipid response to the graduated enrichment of a Step I diet with almonds: a randomized feeding trial. *Am J Clin Nutr* 2003;77:1379-84.
8. Jenkins DJ, Kendall CW, Marchie A, Parker TL, Connelly PW, Qian W, Haight JS, Faulkner D, Vidgen E, Lapsley KG, Spiller GA. Dose response of almonds on coronary heart disease risk factors: blood lipids, oxidized low-density lipoproteins, lipoprotein(a), homocysteine, and pulmonary nitric oxide: a randomized, controlled, crossover trial. *Circulation* 2002;106:1327-32.
9. Fraser GE, Bennett HW, Jaceldo KB, Sabaté J. Effect on body weight of a free 76 Kilojoule (320 calorie) daily supplement of almonds for six months. *J Am Coll Nutr* 2002;21:275-83.
10. Wien MA, Sabaté JM, Iklé DN, Cole SE, Kandeel FR. Almonds vs complex carbohydrates in a weight reduction program. *Int J Obes Relat Metab Disord* 2003;27:1365-72.
11. Hollis J, Mattes R. Effect of chronic consumption of almonds on body weight in healthy humans. *Br J Nutr* 2007;98:651-6.
12. Tan SY, Mattes RD. Appetitive, dietary and health effects of almonds consumed with meals or as snacks: a randomized, controlled trial. *Eur J Clin Nutr* 2013;67:1205-14.
13. Wu L, Piotrowski K, Rau T, Waldmann E, Broedl UC, Demmelmair H, Koletzko B, Stark RG, Nagel JM, Mantzoros CS, Parhofer KG. Walnut-enriched diet reduces fasting non-HDL-cholesterol and apolipoprotein B in healthy Caucasian subjects: a randomized controlled cross-over clinical trial. *Metabolism* 2014;63:382-91.
14. Ogawa H, Fujitani K, Tsujinaka T, Imanishi K, Shirakata H, Kantani A, Hirao M, Kurokawa Y, Utsumi S. InBody 720 as a new method of evaluating visceral obesity. *Hepatogastroenterology* 2011;58:42-4.
15. Hardy OT, Czech MP, Corvera S. What causes the insulin resistance underlying obesity? *Curr Opin Endocrinol Diabetes Obes* 2012;19:81-7.
16. Salas-Salvadó J, Fernández-Ballart J, Ros E, Martínez-González MA, Fitó M, Estruch R, Corella D, Fiol M, Gómez-Gracia E, Arós F, Flores G, Lapetra J, Lamuela-Raventós R, Ruiz-Gutiérrez V, Bulló M, Basora J, Covas MI; PREDIMED Study Investigators. Effect of a Mediterranean diet supplemented with nuts on metabolic syndrome status: one-year results of the PREDIMED randomized trial. *Arch Intern Med* 2008;168:2449-58.
17. Ibarrola-Jurado N, Bulló M, Guasch-Ferré M, Ros E, Martínez-González MA, Corella D, Fiol M, Wärnberg J, Estruch R, Román P, Arós F, Vinyoles E, Serra-Majem L, Pintó X, Covas MI, Basora J, Salas-Salvadó J; PREDIMED Study Investigators. Cross-sectional assessment of nut consumption and obesity, metabolic syndrome and other cardiometabolic risk factors: the PREDIMED study. *PLoS One* 2013;8:e57367.
18. Pereira EV, Costa Jde A, Alfenas Rde C. Effect of glycemic index on obesity control. *Arch Endocrinol Metab* 2015;59:245-51.

19. Perna S, Giacosa A, Bonitta G, Bologna C, Isu A, Guido D, Rondanelli M. Effects of hazelnut consumption on blood lipids and body weight: a systematic review and Bayesian meta-analysis. *Nutrients* 2016;8:E747.
20. Musa-Veloso K, Paulionis L, Poon T, Lee HY. The effects of almond consumption on fasting blood lipid levels: a systematic review and meta-analysis of randomised controlled trials. *J Nutr Sci* 2016;5:e34.
21. Damasceno NR, Pérez-Heras A, Serra M, Cofán M, Sala-Vila A, Salas-Salvadó J, Ros E. Crossover study of diets enriched with virgin olive oil, walnuts or almonds. Effects on lipids and other cardiovascular risk markers. *Nutr Metab Cardiovasc Dis* 2011;21 Suppl 1:S14-20.
22. Jambazian PR, Haddad E, Rajaram S, Tanzman J, Sabaté J. Almonds in the diet simultaneously improve plasma alpha-tocopherol concentrations and reduce plasma lipids. *J Am Diet Assoc* 2005;105:449-54.
23. Sathe SK, Seeram NP, Kshirsagar HH, Heber D, Lapsley KA. Fatty acid composition of California grown almonds. *J Food Sci* 2008;73: C607-14.
24. Park SH, Lee KS, Park HY. Dietary carbohydrate intake is associated with cardiovascular disease risk in Korean: analysis of the third Korea National Health and Nutrition Examination Survey (KNHANES III). *Int J Cardiol* 2010;139:234-40.
25. Mustad VA, Etherton TD, Cooper AD, Mastro AM, Pearson TA, Jonnalagadda SS, Kris-Etherton PM. Reducing saturated fat intake is associated with increased levels of LDL receptors on mononuclear cells in healthy men and women. *J Lipid Res* 1997;38:459-68.
26. Berglund L, Lefevre M, Ginsberg HN, Kris-Etherton PM, Elmer PJ, Stewart PW, Ershow A, Pearson TA, Dennis BH, Roheim PS, Ramakrishnan R, Reed R, Stewart K, Phillips KM; DELTA Investigators. Comparison of monounsaturated fat with carbohydrates as a replacement for saturated fat in subjects with a high metabolic risk profile: studies in the fasting and postprandial states. *Am J Clin Nutr* 2007;86:1611-20.
27. Egert S, Kratz M, Kannenberg F, Fobker M, Wahrburg U. Effects of high-fat and low-fat diets rich in monounsaturated fatty acids on serum lipids, LDL size and indices of lipid peroxidation in healthy non-obese men and women when consumed under controlled conditions. *Eur J Nutr* 2011;50:71-9.
28. Appel LJ, Sacks FM, Carey VJ, Obarzanek E, Swain JF, Miller ER 3rd, Conlin PR, Erlinger TP, Rosner BA, Laranjo NM, Charleston J, McCarron P, Bishop LM, OmniHeart Collaborative Research Group; OmniHeart Collaborative Research Group. Effects of protein, monounsaturated fat, and carbohydrate intake on blood pressure and serum lipids: results of the OmniHeart randomized trial. *JAMA* 2005;294:2455-64.
29. Wolfe BM, Giovannetti PM. Short-term effects of substituting protein for carbohydrate in the diets of moderately hypercholesterolemic human subjects. *Metabolism* 1991;40:338-43.
30. Berryman CE, West SG, Fleming JA, Bordi PL, Kris-Etherton PM. Effects of daily almond consumption on cardiometabolic risk and abdominal adiposity in healthy adults with elevated LDL-cholesterol: a randomized controlled trial. *J Am Heart Assoc* 2015;4:e000993.
31. Cohen AE, Johnston CS. Almond ingestion at mealtime reduces postprandial glycemia and chronic ingestion reduces hemoglobin A(1c) in individuals with well-controlled type 2 diabetes mellitus. *Metabolism* 2011;60:1312-7.
32. Thomas DE, Elliott EJ, Baur L. Low glycaemic index or low glycaemic load diets for overweight and obesity. *Cochrane Database Syst Rev* 2007:CD005105.
33. Radulian G, Rusu E, Dragomir A, Posea M. Metabolic effects of low glycaemic index diets. *Nutr J* 2009;8:5.