Dietary sesame reduces serum cholesterol and enhances antioxidant capacity in hypercholesterolemia

Pey Rong Chen^{a,b,f}, Kuo Liong Chien^{c,d}, Ta Chen Su^c, Chee Jen Chang^e,
Tsuei-Ling Liu^a, Hsiuching Cheng^g, Chingmin Tsai^{a,g,*}

^a*Department of Nutrition and Food Sciences, Fu Jen University, Taipei 242, Taiwan*

^b*Department of Dietetics, National Taiwan University Hospital and National Taiwan University, Taipei 100, Taiwan*

^c*Department of Internal Medicine, National Taiwan University Hospital and National Taiwan University, Taipei 100, Taiwan*

^d*Institute of Preventive Medicine, National Taiwan University Hospital and National Taiwan University, Taipei 100, Taiwan*

^e*Department of Medical Research, National Taiwan University Hospital and National Taiwan University, Taipei 100, Taiwan*

^f*Department of Nutrition and Health Science, College of Public Health and Nutrition, Taipei Medical University, Taipei 110, Taiwan*

^g*Department of Bioscience Technology, Chung Yuan University, Chungli 320, Taiwan*

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Abstract

The purpose of the study was to investigate the effects of sesame on lowering serum lipids and enhancing antioxidant capacity in 21 hyperlipidemic patients. Subjects were instructed to consume their regular diet for 2 weeks before starting the experimental diet. The experimental diet with 40 g roasted sesame was consumed for 4 weeks and the regular diet followed for another 4 weeks. Body weights and fasting blood were determined at weeks 0, 4, and 8. The results showed that the diet with sesame significantly decreased the levels of serum total cholesterol and low-density lipoprotein (LDL) cholesterol. The time for erythrocyte hemolysis and the lag phase of LDL oxidation were significantly increased by sesame ingestion. A diet containing sesame slightly reduced the levels of thiobarbituric acid–reactive substances in LDL. The beneficial effects of sesame disappeared when patients returned

* Corresponding author. Department of Bioscience Technology, Chung Yuan University, Chungli 320, Taiwan. Tel.: +886 3 265 3500; fax: +886 3 265 3599.

E-mail address: jtsai@cycu.edu.tw (C. Tsai).

to their regular diets. Sesame in this study appeared to exert beneficial effects on serum lipids and to improve antioxidant capacity in hypercholesterolemic patients.

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1. Introduction

Sesame (*Sesamum indicum* L.) is a traditional health food and has been used to improve nutritional status and prevent various diseases in Asian countries for thousands of years. Sesame seeds are not only rich in oil (about 50%) and protein (about 20%), but also in lignans (eg, sesamin and sesamol) (up to 1.5%) [1,2]. Some nutraceutical characteristics of sesame seeds have been identified, including antioxidant, hypocholesterolemic, and hepatoprotective effects, as well as prevention of hypertension [3–6]. Two types of antioxidants have been isolated and identified from sesame seeds: fat soluble and water soluble. The fat-soluble antioxidants are tocopherols, sesaminol [7], sesamol [8], and pinoretinol [9]. During the industrial bleaching process of unroasted sesame oil, sesamol is chemically converted to sesaminol [10]. Sesaminol inhibits unsaturated oil peroxidation in low-density lipoprotein (LDL) induced by 2,2'-azobis(2,4-dimethylvaleronitrile) (AMVN), CuSO₄, or 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) in vitro [11,12]. Data indicate that the presence of sesaminol effectively reduces the oxidative modification of LDL, suggesting that sesaminol may have a preventive role in atherogenesis [13]. Sesaminol glucosides are the main water-soluble antioxidants in sesame seed and include sesaminol monoglucoside, sesaminol diglucoside, and sesaminol triglucoside. These glucosides act as precursors of fat-soluble antioxidative lignans. In the rat gastrointestinal tract, the glucosides are hydrolyzed by β -glucosidase to yield glucose and aglycone [14]. Kang et al. [15] reported that sesaminol was detected in abundant quantities in the serum and liver of rabbits fed sesaminol glucosides. This suggests that sesaminol is the principal metabolite of sesaminol glucosides.

The hypocholesterolemic effect of sesamin, a major lignan in sesame seeds, was found to inhibit the absorption of cholesterol in the intestinal tract, increase the excretion of cholesterol in bile, and decrease the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase in rats [16]. Dietary sesamin reduces serum total cholesterol and LDL cholesterol concentrations in hypercholesterolemic patients [4].

There are various lignans with multiple functions in sesame, but previous studies are generally restricted to substances extracted from sesame and reports of using whole seeds for nutritional studies are limited. Therefore, the aim of this study was to evaluate the influence of sesame seed supplementation on the prevention of atherosclerosis. Measurements included lipid profiles and antioxidant capacity in patients with hypercholesterolemia.

2. Methods and materials

2.1. Subjects

Subjects were recruited from hyperlipidemic outpatients at the National Taiwan University Hospital and were enrolled after providing written informed consent. Patients were defined as

having total plasma cholesterol levels of greater than 240 mg/dL and being enrolled for dietary control or medical treatment for more than 3 months. Twenty-eight subjects enrolled, but only 21 were available for data analysis because some of them could not complete the entire protocol. The 21 patients were composed of 6 men and 15 women. The mean age was 50.9 ± 3.7 years. Body weights of most subjects were in a normal range and the mean body mass index (BMI) was 22.9 ± 2.8 kg/m². Only one subject was obese (BMI >27 kg/m²). The health conditions of subjects were evaluated on the basis of clinical examination, routine hematologic assay, and pertinent questionnaires. Subjects with malignant disease, diabetes mellitus, or renal, hepatic, endocrine, or gastrointestinal disorders were excluded. None of the patients used tobacco products, but 6 were concurrently undergoing medication-based lipid-lowering therapy.

2.2. Experimental design

All subjects were instructed to maintain dietary patterns similar to those in the guidelines of the National Cholesterol Education Program Step I diet [17] throughout the study. In the preexperimental period (weeks –2 to 0), the dietary stabilization phase, subjects maintained a regular diet and blood samples were taken for baseline measurements. In the experimental period (weeks 1–4), subjects replaced calories in their daily dietary intake with 40 g sesame supplement per day. In the postexperimental period (weeks 5–8), the recovery phase, subjects suspended the sesame intake and resumed their regular diet. At the end of the experimental and postexperimental periods (week 4 and week 8), fasting blood samples were collected for biochemical analysis. Throughout the observation period, the subjects did not consume alcohol, tea, vitamin-mineral supplements, or any commercial health foods, and they maintained their daily physical activities. The Institutional Review Board of the Health Food Society of Taiwan approved the study and all protocols.

2.3. Sesame consumption

The sesame diet supplement was prepared by roasting seeds that were then ground into powder to enhance digestion and absorption by the subjects. The powder was then processed

Table 1

The ingredients and nutritional compositions of sesame meal and sesame balls

Component	Sesame meal (40 g per serving)	Sesame ball (10 g per serving)
Ingredients		
Sesame powder (g)	20	5
Wheat flour (g)	13	—
Sucrose (g)	5	—
Maltodextrin (g)	1.9	—
Saccharin (g)	0.057	—
Maltose (g)	—	5
Nutritional composition^a		
Total energy (kcal/kJ)	200.4/839.0	47.9/200.5
Carbohydrate (g)	22.9	5.5
Protein (g)	6.5	1.3
Fat (g)	9.2	2.3

^a Based on Taiwan Food Composition Data Base [18].

into sesame meal or balls. One packet of sesame meal contained 20 g of sesame powder as well as sugar, wheat flour, maltodextrin, and saccharin. Each sesame ball contained 5 g of sesame powder and 5 g of maltose (Table 1). Subjects consumed one packet of sesame meal and 4 sesame balls daily to replace portions of breakfast food and snacks. Compliance was monitored by interviewing subjects and counting the number of empty packets returned at each follow-up visit. To ensure that the total daily energy intake remained the same throughout the study, 390 kcal of equivalent foods was removed from each subjects' daily intake to compensate for the supplementation of 390 kcal from sesame meal and sesame balls each day.

2.4. Lipid determination

Venous blood samples were collected after an overnight fast and processed for serum lipid analysis (total cholesterol, high-density lipoprotein [HDL] cholesterol, and triacylglycerol [TG]), as determined by enzymatic methods. Low-density lipoprotein cholesterol was calculated using the Friedewald formula [19]. These studies were performed in the National Taiwan University Hospital Laboratory using standard clinical laboratory procedures.

2.5. Isolation and oxidation of LDL

The procedure for preparation and oxidation of LDL was adapted from the method of Esterbauer et al [20]. Fresh serum was adjusted to density 1.019 g/mL with sodium bromide (density = 1.097 g/mL) and then ultracentrifuged at 453 000g at 4°C for 3.5 hours. The very low-density lipoprotein fraction was removed, and the remaining solution was mixed with sodium bromide (density = 1.28 g/mL) to achieve a final density of 1.063 g/mL. Low-density lipoprotein was isolated by ultracentrifugation at 453 000g at 4°C for another 3.5 hours. Therefore, LDL cholesterol was adjusted with phosphate-buffered saline to a concentration of 50 µg/mL. Oxidation was triggered by adding 50 µmol/L CuSO₄ to the LDL solution. Diene conjugation was monitored at 234 nm at 15-minute intervals using a Beckman counter DU640 spectrophotometer (Beckman Instruments, Fullerton, CA). Susceptibility of LDL to oxidation was assayed by measuring the lag time of the oxidation curve.

The unsaturated lipid peroxidation of LDL was evaluated by measuring the formation of thiobarbituric acid–reactive substances (TBARS). Thiobarbituric acid–reactive substances were calculated as malondialdehyde (MDA) equivalents using standard freshly diluted 1,1,3,3-tetramethoxypropane (Sigma Chemical, St Louis, MO). Low-density lipoprotein (100 mg/L protein, diluted with phosphate-buffered saline) and 5 µmol/L CuSO₄ were coincubated at 37°C for 2 hours in a thermostatically controlled water bath to form oxidized LDL. Butylated hydroxytoluene (2%, dissolved in ethanol) and 400 µL TCA-TBA-HCl stock solution (15% trichloroacetic acid, 0.375% thiobarbituric acid, 0.25 N hydrochloric acid) were added to 200 µL oxidized LDL solution, then completely mixed at 100°C for 10 minutes. Contents of TBARS were estimated from absorbance (532 nm) after centrifugation at 10 000g for 10 minutes. The protocol was modified from Buege and Aust [21].

2.6. Measurement of erythrocyte hemolysis

Red blood cell hemolysis was assessed on fresh red blood cells collected in vacutainer tubes containing EDTA disodium salt dehydrate. Whole blood (0.1 mL) was added to 1 mL phosphate buffer solution, then incubated at 37°C for 5 minutes before AAPH was added.

AAPH (Wako Pure Chemical Co, Osaka, Japan) was dissolved in 10 mmol/L disodium hydrogen phosphate and incubated with the erythrocyte suspension at 37°C in a shaking water bath placed in a dark room. After 5 minutes of incubation, 0.2 mL erythrocyte suspension was added to 2.5 mL distilled water, centrifuged at 1500g for 10 minutes, and hemolysis was estimated by measuring absorbance (540 nm) of the hemoglobin released into the supernatant. The absorbance values were detected at 30, 60, 90, 120, and 180 minutes. The results were expressed as percentage of hemolysis. Hemolysis was taken to be 100% when the buffer was replaced by distilled water. Fifty percent hemolysis time was the definition for fragility of the erythrocyte [22].

2.7. Statistical analysis

Values were expressed as mean $\pm$ SD. For repeated measures at 3 time points (weeks 0, 4, and 8), a mixed-effects model was used to handle the dependency among continuous variables treating time as a random effect. The treatment effects on lipid profiles and antioxidant status were estimated. The interaction by sex or drug treatment in the lipid parameters and the changes in antioxidation status were also tested under a mixed-effects model. The SAS statistical software package was used for data analysis and the level of significance was established at $P < .05$.

3. Results

The results for lipid parameters, antioxidant capacity of LDL, and damage to erythrocytes are presented in Tables 2 and 3, respectively. The subjects displayed no adverse health effects after 4 weeks of sesame supplementation. Body weights and BMI did not change over the study period.

Table 2
Body weights and serum lipid levels before and after dietary sesame treatment

	Week 0 ^a	Week 4 ^a	Week 8 ^a
Body weight (kg)			
Male	67.6 $\pm$ 9.4	67.6 $\pm$ 9.5	67.4 $\pm$ 9.4
Female	53.0 $\pm$ 5.7	52.7 $\pm$ 5.9	52.6 $\pm$ 5.5
BMI (kg/m ²)	22.9 $\pm$ 2.8	22.8 $\pm$ 2.8	22.7 $\pm$ 2.8
Total cholesterol (mg/dL)	262.2 $\pm$ 24.8 (250.9-273.5)	245.5 $\pm$ 22.3* (235.3-255.6)	255.2 $\pm$ 28.3 (242.4-268.1)
TG (mg/dL)	101.0 $\pm$ 35.1 (84.96-116.9)	100.9 $\pm$ 43.3 (81.1-120.6)	120.2 $\pm$ 56.3** (94.6-145.8)
LDL cholesterol (mg/dL)	182.9 $\pm$ 27.9 (170.1-195.6)	165.6 $\pm$ 29.6* (152.2-179.1)	179.5 $\pm$ 24.1*** (168.6-190.5)
HDL cholesterol (mg/dL)	59.2 $\pm$ 11.2 (54.1-64.3)	59.2 $\pm$ 15.8 (52.0-66.4)	56.7 $\pm$ 13.5*** (50.5-62.8)

Values are expressed as raw mean $\pm$ SD or mean $\pm$ SD (95% confidence interval). All the comparisons were based on a mixed-effects linear model.

^a Regular diets consumed before week 0 and during weeks 5 to 8 and diet with sesame supplement consumed during weeks 1 to 4.

* $P < .05$ for differences between W4 and W0.

** $P < .05$ for differences between W8 and W0.

*** $P < .05$ for differences between W8 and W4.

Table 3
The antioxidant capacity of erythrocytes and LDL cholesterol from subjects before and after dietary sesame treatment

Parameter	Week 0 ^a	Week 4 ^a	Week 8 ^a
Erythrocyte hemolysis time (min)	163.7 ± 19.4 (154.9-172.5)	168.9 ± 20.3* (159.7-178.2)	159.9 ± 13.7**
LDL lag phase (min)	163.1 ± 48.4 (141.0-185.1)	192.2 ± 71.6* (159.6-224.8)	142.6 ± 42.1** (123.4-161.7)
LDL TBARS (nmol MDA/mg LDL protein)	30.5 ± 13.3 (24.4-36.5)	24.6 ± 15.7*** (17.4-31.8)	33.9 ± 12.3** (28.3-39.5)

Values are expressed as raw mean ± SD (95% confidence interval). All the comparisons were based on a mixed-effects linear model.

^a Regular diets consumed before week 0 and during weeks 5 to 8 and diet with sesame supplement consumed during weeks 1 to 4.

* *P* < .05 for differences between W4 and W0.

** *P* < .05 for differences between W8 and W4.

*** *P* < .10 for differences between W4 and W0.

In patients with hypercholesterolemia supplemented with sesame for 4 weeks, mean serum total cholesterol and LDL cholesterol concentrations decreased significantly by 6.4% and 9.5%, respectively (Table 2). There were no differences between baseline and week 4 values for HDL cholesterol and TG levels. The hypolipidemic effect of sesame disappeared after cessation of the treatment. Serum total and LDL cholesterol concentrations were 4.0% and 8.4% (*P* < .05) higher, respectively, at week 4 compared with week 8, but there were no differences between week 8 and baseline values (Table 2). Because the TG levels of subjects in the study were in the normal range at baseline, it was assumed that there would be no lowering effect of sesame supplementation on serum TG concentrations; however, it was higher at week 8 compared to baseline (120.2 ± 56.3 vs 101.0 ± 35.1, *P* < .05). This unusual increase in serum TG might be explained by a discrepancy in reporting food intake. The HDL cholesterol level at the end of week 8 was lower compared with the value at week 4 (56.7 ± 13.5 vs 59.2 ± 11.2, *P* < .05) and could not be explained from the data collected.

The erythrocyte hemolysis time increased 3.2% (*P* < .05), LDL oxidation increased 17.8% (*P* < .05), and LDL TBARS levels decreased 19.3% (*P* < .05) in samples taken from subjects given sesame for 4 weeks (Table 3). All benefits from sesame of greater antioxidant capacity in hypercholesterolemic patients were abolished at week 8 or after 4 weeks of withdrawal of the dietary sesame treatment.

For the 6 male and 15 female patients in this study, there were no significant interactions between sex or medical treatment and any of the lipid variables and antioxidation parameters measured (data not shown).

4. Discussion

The salient findings of this investigation were that 40 g of sesame dietary supplementation reduced the levels of serum lipid parameters and enhanced the antioxidant capacity of LDL and erythrocyte fragility in hypercholesterolemic patients. The serum total and LDL cholesterol levels were reduced after 4 weeks of sesame supplementation in this study. The

hypolipidemic effect from sesame might be related to the amounts of sesamin, L-arginine [23], dietary fiber, or unsaturated fatty acids in the food. Hirata et al [4] reported that daily oral intake of sesamin in hypercholesterolemic patients for 4 weeks significantly decreased total and LDL cholesterol concentrations. In hyperlipidemic rats, dietary supplementation with sesamin reduced plasma and liver total cholesterol and LDL cholesterol concentrations [24]. In the rat, the mechanism for the hypocholesterolemic effect of sesamin is believed to be related to inhibition of intestinal absorption of cholesterol, increased excretion of cholesterol into bile, and decreased activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase [16].

Previous studies have shown that dietary plant seeds or nuts, which have favorable fatty acid and nutrient profiles, can improve serum lipids in hyperlipidemia [25–30]. Sesame shares some functional components with nuts such as low levels of saturated fatty acids and high levels of monounsaturated and polyunsaturated fatty acids. In addition, there are other bioactive molecules in nuts and sesame that prevent hypercholesterolemia. These include plant protein, dietary fiber, phytochemicals, and lignans. Kris-Etherton et al [31] reviewed 9 nut-feeding studies that incorporated various nuts into test diets to determine their plasma lipid-lowering effects. The actual response was compared with the predicted response derived from the current predictive equations for blood cholesterol levels [32]. They reported that when subjects consumed test diets including nuts, there was an additional 25% cholesterol-lowering response compared with the level predicted by the equation. These findings suggest that there are non-fatty acid components in nuts that have powerful cholesterol-lowering effects.

The current data suggest that sesame supplementation might be a viable adjunctive therapy in treating hypercholesterolemia. The established guidelines advocate stepwise dietary control as the primary therapy for hyperlipidemia. Evidence that diet can be an effective means to lower blood levels of total and LDL cholesterol [33,34] reinforces the importance of the current findings with sesame. Before drug therapy is prescribed to reverse the blood lipid risk factors for coronary heart disease, nutraceutical foods may offer an adjunct to total therapy.

Another purpose of the study was to determine the effect of daily intake of 40 g roasted sesame on the antioxidant capacity in hypercholesterolemic patients. Evidence suggests that oxidatively modified LDL plays an important role in the development of atherosclerotic lesions [35]. Determinations of LDL conjugated diene formation enhanced by CuSO_4 and TBARS measurements reflect LDL oxidation. Free radical attack of erythrocyte membrane components such as protein and lipid results in greater fragility contributing to hemolysis [36,37]. In our study, we investigated the effects of peroxy radicals initiated by AAPH on erythrocytes collected from the subjects. After 4 weeks of sesame supplementation, we observed that sesame significantly increased the antioxidant capacity in hypercholesterolemic patients by prolonging the time before LDL oxidation, decreasing MDA generation in LDL, and delaying the hemolysis of erythrocytes. The benefits of antioxidation in hypercholesterolemic patients disappeared after withdrawal of the daily sesame supplementation regimen. The functional components contributing to these antioxidative effects might be tocopherols and various sesame lignans, as previously demonstrated by *in vitro* or animal studies [11,12,15]. Sesamin also might play a role in antioxidation by inhibiting the catabolism of tocopherol and resulting in enhanced accumulation of tocopherols in plasma and tissues [38].

In the current clinical study, the effect of dietary sesame (40 g/day) supplementation resulted in a 6.4% lower serum total cholesterol and 9.5% lower LDL cholesterol. We also

found that sesame supplementation increased the antioxidant capacity, which was confirmed in LDL from the subjects. These beneficial effects of sesame may contribute to a protective action to counter atherosclerosis in hypercholesterolemic patients.

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