

## Chia seed does not promote weight loss or alter disease risk factors in overweight adults

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### Abstract

The objective of this study was to assess the effectiveness of chia seed (*Salvia hispanica* L) in promoting weight loss and altering disease risk factors in overweight adults. The hypothesis was that the high dietary fiber and  $\alpha$ -linolenic (ALA) contents of chia seed would induce a small but significant decrease in body weight and fat and improve disease risk factors. Subjects were randomized to chia seed (CS) and placebo (P) groups, and under single-blinded procedures, ingested 25 g CS or P supplements mixed in 0.25 L water twice daily before the first and last meal for 12 weeks. Ninety nondiseased, overweight/obese men and women between the ages of 20 and 70 years were recruited into the study, with 76 subjects ( $n = 39$  CS,  $n = 37$  P) completing all phases of the study. Pre- and poststudy measures included body mass and composition (dual energy x-ray absorptiometry), inflammation markers from fasting blood samples (C-reactive protein, interleukin 6, monocyte chemoattractant protein 1, and tumor necrosis factor  $\alpha$ ), oxidative stress markers (trolox equivalent antioxidant capacity and plasma nitrite), blood pressure, and a serum lipid profile. Plasma ALA increased 24.4% compared to a 2.8% decrease in CS and P, respectively (interaction effect,  $P = .012$ ). No group differences were measured for changes in plasma eicosapentaenoic acid and docosahexaenoic acid (interaction effects,  $P = .420$  and  $.980$ , respectively). Pre-to-post measures of body composition, inflammation, oxidative stress, blood pressure, and lipoproteins did not differ between CS and P for both sexes. In conclusion, ingestion of 50 g/d CS vs P for 12 weeks by overweight/obese men and women had no influence on body mass or composition, or various disease risk factor measures.

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### Keywords:

Body composition; Lipoproteins; Cytokines; Oxidative stress; C-reactive protein; Human

### Abbreviations:

ALA,  $\alpha$ -linolenic acid; CRP, C-reactive protein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; IL-6, interleukin 6; PUFA, polyunsaturated fatty acid; TNF- $\alpha$ , tumor necrosis factor  $\alpha$ .

### 1. Introduction

The essential fatty acid,  $\alpha$ -linolenic acid (ALA; 18:3n-3), is present in various seeds, nuts, and vegetable oils such as flaxseed, linseed, canola, and soy oils.  $\alpha$ -Linolenic acid can

be metabolically converted to long-chain n-3 polyunsaturated fatty acids (n-3 PUFAs), including eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), although the efficiency is poor [1,2].

The reluctance of adults in the United States to increase fish intake and concerns over heavy metal accumulation in fish have accelerated interest in botanical sources of n-3 PUFAs such as flaxseed, walnuts, and algae [3,4]. Plant n-3

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PUFAs are abundant and readily available, and are often contained in foods that are high in dietary fiber and other components with potential health value. The cardioprotective effect of ALA in humans, however, is uncertain, and studies differ widely regarding influences on blood lipid profiles and measures of inflammation [5-7].

Chia seed (*Salvia hispanica* L) is an oilseed native to southern Mexico and northern Guatemala [8-13]. Chia seed has 4.4 g ALA and 9.4 g of dietary fiber per 25 g serving [11]. In chickens fed chia seed, the ALA content of light and dark meat was increased and saturated fat decreased, with a reduction in overall poultry body mass [10]. In rats, consumption of chia seed and oil counters dyslipidemia and visceral adiposity and has been touted as an alternative n-3 source for vegetarians and people allergic to fish and fish products [8,9]. The high fiber content of chia seed may improve satiety, decrease energy intake, and promote weight loss [10,14]. Howarth et al [14] determined that although the influence of dietary fiber on energy regulation remains controversial, consumption of an additional 14 g/d soluble or insoluble dietary fiber when energy intake is ad libitum should promote a 10% decrease in energy intake and body weight loss of 1.9 kg over 3.8 months. The consumption of 37 g/d of chia seed in one human showed a decrease in C-reactive protein (CRP) and no change in body weight or blood lipid profiles over 12 weeks in 20 type 2 diabetic subjects [12].

Supplementation with n-3 PUFAs from both fish and plant products is a recent strategy to help control disease risk factors in overweight and obese individuals [15]. Given results from animal studies, we hypothesized that the high dietary fiber and ALA content of chia seed would induce a small but significant decrease in body weight and fat and improve disease risk factors. Specifically, our objective was to investigate the effectiveness of a large chia seed supplement (50 g/d split into 2 daily doses) compared to placebo in promoting weight loss, altering body composition, decreasing blood lipids, and modifying inflammation in 90 overweight men and women during a 12-week period. The 50-g chia seed supplement provided 19 g dietary fiber and 8.8 g ALA per day for 12 weeks. We reasoned that this quantity would be sufficient to test our hypothesis based on other research.

## 2. Methods and materials

Subjects included 90 overweight and obese men and women, aged 20 to 70 years, who were recruited through local advertising. To enter the study, subjects had to be healthy without known disease, have a body mass index of 25 kg/m<sup>2</sup> and higher, agreed to be randomized to the chia seed or placebo groups, and be willing to adhere to all aspects of the research design. Written informed consent was obtained from each subject, and the experimental procedures were approved by the institutional review board

of Appalachian State University. We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research.

Subjects were randomized to chia seed and placebo groups, and under single-blinded procedures, ingested chia seed or placebo supplements daily for 12 weeks. Diet records and questionnaire responses to assess potential adverse effects and adherence to the supplementation regimen were administered every 2 weeks of the study. Subjects agreed to follow their normal dietary and physical activity patterns during the 12-week study and make no special attempts to lose weight.

Chia seed or placebo supplements were prepared by Savory Sun Inc (Englewood, Fla). Subjects were told that they would be randomized to chia seed (either whole seed or ground seed in powder form) or placebo seed (either whole seed or ground seed). For this study, whole chia seeds were used and the placebo consisted of a powder. Supplements were ingested on an empty stomach before breakfast and dinner as a 0.25-L drink each day of the 12-week study. Subjects in the chia seed group were given two 25-g packets of chia seeds per day that also had small amounts of flavorings and aspartame. The placebo packet (2 per day) contained 25 g of solids, flavorings, and aspartame and was formulated to have similar amounts of protein, carbohydrate, and fat. Chia seed and placebo supplements were mixed with 0.25 L water in a tumbler and then consumed after sitting for 10 minutes. The 25-g serving of chia seeds had 540 kJ, with 7.7 g total fat, 3.9 g protein, 11.0 g carbohydrate, 9.4 g dietary fiber, 4.4 g ALA, 1.4 g linoleic acid, and 158 mg calcium. The placebo consisted of concentrated soy, sunflower oil, carrot fiber, and tapioca starch and contained 481 kJ, with 7.0 g total fat, 5.3 g protein, 7.7 g carbohydrate, 4.0 g dietary fiber, 4.0 g oleic acid, and 2.0 g linoleic acid.

### 2.1. Body composition

Stature and body mass were measured pre- mid-, and post-study using a stadiometer and balance beam scale. Body composition was measured pre- and post-study using dual energy x-ray absorptiometry and the Discovery QDR Series bone densitometer (Hologic Inc, Bedford, Mass).

### 2.2. Serum lipoproteins and blood pressure

Blood samples were drawn from an antecubital vein in overnight-fasted subjects in the seated position for at least 15 minutes. Blood samples were drawn at 7:00 to 9:00 AM, with all subjects having avoided food and beverage intake other than water for at least 9 hours. Blood samples were centrifuged in sodium heparin or EDTA tubes, and plasma was aliquoted and then stored at -80°C before analysis. A serum lipid panel was performed by our clinical hematology laboratory. Blood pressure was measured by technicians after a 15-minute seated rest after an overnight fast.

### 2.3. Fatty acid analyses

After addition of 500  $\mu\text{g}$  butylated hydroxytoluene and 20  $\mu\text{g}$  of heptadecanoic acid methyl ester (NU-Chek Prep, Inc, Elysian, Minn) internal standard to 100  $\mu\text{L}$  thawed plasma, lipids were extracted and methyl esters were formed after the mixture was added to 2 mL of methanolic 5% HCl and incubated at 80°C for 2 hours in an OLS200 Shaking Waterbath (Grant Instruments Ltd, Shepreth, Cambridge-shire, England). The samples were cooled to room temperature upon completion of the incubation and the methyl esters were extracted twice with 2 mL of *n*-hexane. The top layer of the supernatant was combined and dried with N-EVAP116 Nitrogen Evaporator (Organomation Associates, Inc, Berline, Mass). The dried extract was then reconstituted in 200  $\mu\text{L}$  of *n*-hexane that contained 0.05% butylated hydroxytoluene, of which 1  $\mu\text{L}$  was injected into an HP 6890N gas chromatograph (Agilent Technologies, Palo Alto, Calif) equipped with a 5975B Inert XL MSD mass spectrometer detector. A DB-WAX GC column (30 m  $\times$  320  $\mu\text{m}$   $\times$  0.25  $\mu\text{m}$ ) purchased from J & W Scientific (Agilent Technologies) was used to separate the methyl esters of the extracted fatty acids.

### 2.4. Plasma cytokines

Total plasma concentrations of interleukin 6 (IL-6), monocyte chemotactic protein 1, and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) were determined using quantitative sandwich enzyme-linked immunosorbent assay kits purchased from R & D Systems, Inc (Minneapolis, Minn). All samples and standards were analyzed in duplicate. High sensitivity kits were used to analyze TNF- $\alpha$  and IL-6.

### 2.5. Serum CRP

Serum CRP was analyzed in accordance with the manufacturer's guidelines using an enzyme-linked immunosorbent assay kit (catalog no. 1000) obtained from Alpha Diagnostic International (San Antonio, Tex). All samples and provided standards were analyzed in duplicate.

### 2.6. Food records and analysis

During orientation, a nutritionist instructed the subjects regarding completion of the 24-hour food record using food models and record samples. Subjects recorded food intake on a biweekly basis on random days. The food records were analyzed using a computerized dietary assessment program (Food Processor, ESHA Research, Salem, Ore).

### 2.7. Symptom logs

Symptom logs were recorded in conjunction with the food records on a biweekly basis on random days. The symptom log consisted of measures of digestive health (constipation, heartburn, bloating, diarrhea, and nausea), hunger levels (morning, afternoon, and evening), energy levels (morning, afternoon, and evening), sickness (fever, cough, sore throat,

stuffy nose, runny nose, and headache), pain (joint, muscle, and back), allergies, dry eyes, fingernail growth, stress level, focus/concentration, and overall well-being. The intensity of the above symptoms was measured on a scale from 1 to 12 (none to very high).

### 2.8. Statistical analyses

Data were analyzed using a 2 (group)  $\times$  2 (time) repeated-measures analysis of variance between subjects model for each sex separately, with pre- to post-supplementation changes calculated and compared using a Student *t* test. Dietary and symptom log data were compared between groups using Student *t* tests. Data are reported as means  $\pm$  SE.

## 3. Results and discussion

Subject characteristics for the 28 men and 48 women completing all phases of the study did not differ significantly between chia seed and placebo groups for both sexes. Subjects consumed all of the chia seed and placebo supplied to them for the study as assessed by biweekly e-mail inquiries. The most common reason for dropping out of the study ( $n = 14$ ) was difficulty in adhering to the supplementation regimen. A post-study questionnaire revealed that 41% of subjects in the chia group thought they were on chia, whereas 59% responded they were on placebo or did not know. Of subjects in the placebo group, 62% thought they were on the placebo, whereas 38% reported that they were on chia or did not know. These group differences were statistically significant ( $P < .05$ ).

The primary limitation of this study relates to the challenge of finding a suitable placebo supplement. To get around the difficulty of finding an appropriate seed placebo, subjects were told that they would be randomized to chia seed (either whole seed or ground seed in powder form) or placebo seed (either whole seed or ground seed). About 6 in 10 subjects in the placebo group answered correctly in a post-study questionnaire that felt they were ingesting the placebo supplement compared to 4 in 10 for the chia group. The placebo was formulated to have similar amounts of protein, carbohydrate, and fat as the chia seed supplement and thus contained ingredients such as soy, sunflower oil, carrot fiber, and tapioca starch. The net difference in dietary fiber intake was about 11 g of dietary fiber per day for the chia group. The placebo supplement did not contain any ALA. Thus, the chia seed supplement added significant dietary fiber and ALA above placebo levels, and all but 14 of 90 subjects adhered strictly to the supplementation regimen.

Macro- and micronutrient intake did not differ between groups during the 12-week study. Subjects in the chia group added 18.8 g dietary fiber to their intake each day of the study compared with 8.0 g/d for the placebo group. Ingestion of 50 g/d chia seed increased ALA intake by 8.8 g/d, substantially above the 1.5 g/d US adult average [16].

Symptoms for digestive health, hunger, energy level, illness, pain, allergies, stress, focus/concentration, and overall well-being, and physical activity patterns as assessed by biweekly symptoms logs did not differ significantly between chia seed and placebo groups.

Body mass did not change during the 12-week study for either the chia or placebo group for men (time effect,  $P = .112$ ; interaction effect,  $P = .862$ ) and women (time effect,  $P = .471$ ; interaction effect,  $P = .987$ ). Body composition as measured with dual energy x-ray absorptiometry also did not change during the study for either the chia or the placebo group for men (time effect,  $P = .830$ ; interaction effect,  $P = .474$ ) and women (time effect,  $P = .798$ ; interaction effect,  $P = .146$ ). Chia seed dietary fiber is 95% insoluble, has a high water-holding capacity, and should have induced a sense of fullness before the morning and evening meals [13]. In rats, consumption of chia seed counters visceral adiposity [8]. Despite the hypothesized link between increased intake of chia seed ALA, dietary fiber, and weight loss, our data showed no differences in total body mass or body composition after 12 weeks between the chia seed and placebo group. These findings are in contrast to the results from 2 chia seed supplementation studies in rats and poultry [8,10] but in agreement with one other human study [12].

Table 1 summarizes the serum lipoprotein, serum glucose, and systolic blood pressure data for the subjects. For both men and women, the pattern of change over time did not differ between chia seed and placebo groups for each of these variables (interaction effects, all  $P > .05$ ). Serum CRP and plasma cytokine levels are listed in Table 2. For both men

Table 1

Pre- and post-study serum lipoproteins and glucose, and systolic blood pressure in male and female subjects consuming chia seed or placebo supplements for 12 weeks

Variable	Men		Women	
	Chia seed (n = 14)	Placebo (n = 14)	Chia seed (n = 25)	Placebo (n = 23)
Serum glucose (mmol/L)				
Baseline	5.17 ± 0.16	5.43 ± 0.23	5.26 ± 0.17	5.26 ± 0.23
12 wk	4.95 ± 0.15	5.04 ± 0.14	4.91 ± 0.18	5.06 ± 0.26
Serum cholesterol (mmol/L)				
Baseline	4.38 ± 0.35	4.64 ± 0.36	4.84 ± 0.18	5.00 ± 0.18
12 wk	4.58 ± 0.31	4.53 ± 0.36	4.92 ± 0.16	5.00 ± 0.18
Serum LDL cholesterol (mmol/L)				
Baseline	2.80 ± 0.26	3.29 ± 0.34	3.13 ± 0.21	3.32 ± 0.21
12 wk	3.06 ± 0.28	3.29 ± 0.34	3.03 ± 0.16	3.11 ± 0.18
Serum HDL cholesterol (mmol/L)				
Baseline	0.98 ± 0.05	1.09 ± 0.08	1.33 ± 0.06	1.33 ± 0.06
12 wk	0.99 ± 0.05	1.33 ± 0.06	1.32 ± 0.03	1.42 ± 0.09
Serum triglycerides (mmol/L)				
Baseline	1.62 ± 0.25	1.83 ± 0.34	1.39 ± 0.11	1.50 ± 0.16
2 wk	1.79 ± 0.28	1.79 ± 0.29	1.71 ± 0.23	1.64 ± 0.16
Systolic blood pressure (mm Hg)				
Baseline	129 ± 3.0	127 ± 3.0	122 ± 3.0	124 ± 2.0
12 wk	128 ± 4.0	131 ± 4.0	124 ± 2.0	125 ± 3.0

Values are means ± SE. No significant 2 × 2 interaction effects within sex. LDL indicates low density lipoprotein; HDL, high-density lipoprotein.

Table 2

Pre- and post-study serum CRP and plasma cytokines in male and female subjects consuming chia seed or placebo supplements for 12 weeks

Variable	Men		Women	
	Chia seed (n = 14)	Placebo (n = 14)	Chia seed (n = 25)	Placebo (n = 23)
Serum CRP (mg/L)				
Baseline	5.2 ± 1.4	3.7 ± 1.4	7.0 ± 1.0	8.0 ± 0.9
12 wk	4.9 ± 1.4	3.9 ± 0.8	7.4 ± 1.1	8.7 ± 1.0
Plasma IL-6 (pg/dL)				
Baseline	2.66 ± 0.81	2.03 ± 0.46	2.87 ± 0.87	2.35 ± 0.26
12 wk	2.93 ± 1.06	2.14 ± 0.60	3.16 ± 0.85	2.49 ± 0.31
Plasma TNF-α (pg/dL)				
Baseline	1.63 ± 0.30	1.59 ± 0.30	1.48 ± 0.25	1.40 ± 0.20
12 wk	1.52 ± 0.27	1.39 ± 0.14	1.60 ± 0.25	1.41 ± 0.16
Plasma MCP (pg/dL)				
Baseline	72.4 ± 4.08	78.9 ± 6.38	58.8 ± 4.49	58.2 ± 2.79
12 wk	77.4 ± 6.49	76.9 ± 4.35	62.4 ± 3.95	59.4 ± 3.34

Values are means ± SE. No significant 2 × 2 interaction effects within sex. MCP-1 indicates monocyte chemotactic protein 1.

and women, the pattern of change over time did not differ between chia seed and placebo groups for each of these variables (interaction effects, all  $P > .05$ ). Most other human studies using flaxseed or other ALA supplements have failed to show significant or meaningful changes in measures of inflammation or the blood lipid profile, in concert with our findings and in contrast to results from studies using fish oil supplements [3,5-7,17]. Nelson et al [7] showed no effect of 8 weeks of supplementation with flaxseed oil capsules (increasing ALA to 5% of total energy intake) on serum CRP or plasma IL-6 in obese men and women.

Plasma ALA increased 24.4% ( $67.3 ± 5.6$  to  $83.7 ± 8.5$  μg/mL) compared to a 2.8% ( $64.1 ± 5.2$  to  $62.3 ± 6.5$  μg/mL) decrease in chia and placebo groups, respectively (interaction effect,  $P = .012$ ). No group differences were measured for changes in plasma EPA and DHA (interaction effects,  $P = .420$  and  $.980$ , respectively). Our results are similar to those of Austria et al [18] who showed an increase in ALA but not EPA or DHA after 3 months supplementation with milled flaxseed or flaxseed oil in healthy male and female subjects. Upon ingestion, 15% to 35% of ALA is catabolized to carbon dioxide for energy, with less than 1% converted to DHA [1,2]. The fractional conversion of ALA to EPA is 0.3% to 8% in men and up to 21% in women [2].

In summary, despite ingestion of 50 g of chia seeds per day and a significant increase in plasma ALA, overweight men and women experienced no health benefits compared to placebo when measuring body composition and disease risk factors such as serum CRP, plasma cytokines, blood lipoproteins, and blood pressure. These data do not support the strategy of using 50 g/d chia seed supplements high in insoluble dietary fiber and ALA to induce weight loss, attenuate inflammation, increase blood antioxidant capacity, or improve the blood lipid profile in overweight men and women.

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