Chia Seed Supplementation and Disease Risk Factors in Overweight Women: A Metabolomics Investigation

Authors
David C. Nieman, Nicholas Gillitt, Fuxia Jin, Dru A. Henson, Krista Kennerly, **R. Andrew Shanely**, Brandon Ore, MingMing Su and Sarah Schwartz

Abstract

Objective/setting: This study assessed the effectiveness of milled and whole chia seed in altering disease risk factors in overweight, postmenopausal women using a metabolomics approach.
Design/intervention: Subjects were randomized to chia seed (whole or milled) and placebo (poppy seed) groups, and under double-blinded procedures ingested 25 g chia seed or placebo supplements each day for 10 weeks. Subjects: Subjects included 62 overweight (body–mass index 25 kg/m2 and higher), nondiseased, nonsmoking, postmenopausal women, ages 49–75 years, with analysis based on the 56 subjects who completed all phases of the study.
Outcome measures: Pre- and poststudy measures included body mass and composition, blood pressure and augmentation index, serum lipid profile, inflammation markers from fasting blood samples, plasma fatty acids, and metabolic profiling using gas chromatography–mass spectrometry with multivariate statistical methods including principal component analysis and partial least-square discriminant analysis (PLS-DA).
Results: Plasma a-linolenic acid (N = ALA) increased 58% (interaction effect, $p = 0.002$) and eicosapentaenoic acid (EPA) 39% ($p = 0.016$) in the milled chia seed group (N = 14) compared to nonsignificant changes in the whole chia seed (N = 16) and placebo (N = 26) groups. Pre-to-post measures of body composition, inflammation, blood pressure, augmentation index, and lipoproteins did not differ between chia seed (whole or milled) and placebo groups (all interaction effects, $p > 0.05$). Global metabolic difference scores for each group calculated through PLS-DA models were nonsignificant ($Q2Y < 0.40$), and fold-changes for 28 targeted metabolites associated with inflammation and disease risk factors did not differ between groups.
Conclusions: Ingestion of 25 g/day milled chia seed compared to whole chia seed or placebo for 10 weeks by overweight women increased plasma ALA and EPA, but had no influence on inflammation or disease risk factors using both traditional and metabolomics-based measures.

David C. Nieman, Nicholas Gillitt, Fuxia Jin, Dru A. Henson, Krista Kennerly, **R. Andrew Shanely**, Brandon Ore, MingMing Su and Sarah Schwartz (2012) "Chia Seed Supplementation and Disease Risk Factors in Overweight Women: A Metabolomics Investigation" *Journal of Alternative and Complementary Medicine* 18 pp. 700-708 Version of Record available @ (doi: 10.1089/acm.2011.0443)

Chia Seed Supplementation and Disease Risk Factors in Overweight Women: A Metabolomics Investigation

David C. Nieman, DrPH,[1] Nicholas Gillitt, PhD,[2] Fuxia Jin, PhD,[2] Dru A. Henson, PhD,[3] Krista Kennerly, MS,[3] R. Andrew Shanely, PhD,[1] Brandon Ore, MS,[4] MingMing Su, PhD,[4] and Sarah Schwartz, PhD[4]

Abstract

Objective/setting: This study assessed the effectiveness of milled and whole chia seed in altering disease risk factors in overweight, postmenopausal women using a metabolomics approach.
Design/intervention: Subjects were randomized to chia seed (whole or milled) and placebo (poppy seed) groups, and under double-blinded procedures ingested 25 g chia seed or placebo supplements each day for 10 weeks.
Subjects: Subjects included 62 overweight (body–mass index 25 kg/m^2 and higher), non-diseased, nonsmoking, postmenopausal women, ages 49–75 years, with analysis based on the 56 subjects who completed all phases of the study.
Outcome measures: Pre- and post-study measures included body mass and composition, blood pressure and augmentation index, serum lipid profile, inflammation markers from fasting blood samples, plasma fatty acids, and metabolic profiling using gas chromatography–mass spectrometry with multivariate statistical methods including principal component analysis and partial least-square discriminant analysis (PLS-DA).
Results: Plasma *a*-linolenic acid (N = ALA) increased 58% (interaction effect, $p = 0.002$) and eicosapentaenoic acid (EPA) 39% ($p = 0.016$) in the milled chia seed group ($N = 14$) compared to nonsignificant changes in the whole chia seed ($N = 16$) and placebo ($N = 26$) groups. Pre-to-post measures of body composition, inflammation, blood pressure, augmentation index, and lipoproteins did not differ between chia seed (whole or milled) and placebo groups (all interaction effects, $p > 0.05$). Global metabolic difference scores for each group calculated through PLS-DA models were nonsignificant ($Q^2Y < 0.40$), and fold-changes for 28 targeted metabolites associated with inflammation and disease risk factors did not differ between groups.
Conclusions: Ingestion of 25 g/day milled chia seed compared to whole chia seed or placebo for 10 weeks by overweight women increased plasma ALA and EPA, but had no influence on inflammation or disease risk factors using both traditional and metabolomics-based measures.

Introduction

The essential fatty acid, *a*-linolenic acid (ALA; 18:3n-3), is present in various seeds, nuts, and vegetable oils and can be metabolically converted to long-chain *n*-3 polyunsaturated fatty acids (*n*-3 PUFAs), including eicosapentaenoic acid (EPA; 20:5n-3), docosapentaenoic acid (DPA; 22:5n-3, and docosahexaenoic acid (DHA; 22:6n-3).[1,2] U.S. male and female adults consume about 1.7 and 1.3 g ALA, respectively, slightly above the Adequate Intake recommendations of 1.6 and 1.1 g/day.[3,4]

Consumption of long-chain *n*-3 PUFAs EPA and DHA from fish and fish-oil supplements reduces disease risk factors, counters the metabolic syndrome, and decreases all-cause mortality, cardiac and sudden death, and possibly stroke.[5–8] These health benefits have prompted the U.S. Food and Drug Administration to support a qualified health claim status for EPA and DHA.

The reluctance of U.S. adults to increase fish intake, and concerns over fish farming and accumulation of pollutants in fish have accelerated interest in botanical sources of *n*-3 PUFAs such as flaxseed, chia seed, walnuts, and algae.[9–11]

Additionally, estimates are that the ratio of omega-6 (n-6) linoleic acid (LA) to omega-3 (*n*-3) ALA increased from 6.4 to 10.0 during the 20th century, resulting in decreased tissue concentrations of EPA and DHA.[12] Plant *n*-3 PUFAs are abundant and readily available, and are often contained in foods that are high in dietary fiber, minerals, polyphenols, and other components with potential health value. A growing number of epidemiologic studies support a variety of potential health benefits from a higher than normal chronic ALA intake including decreased incidence of diabetes, bone fracture, and depression.[13–15] The cardioprotective effect of ALA in humans, however, is unclear, and studies differ widely regarding influences on disease risk factors such as blood lipid profiles, blood pressure, insulin resistance, fibrinogen, endothelial function, and systemic inflammation.[7–9,16–18] About one third of ingested ALA is oxidized, and enzymatic conversion to EPA and DHA is relatively inefficient in humans.[1,2]

Chia seed (*Salvia hispanica L.*) is an oilseed native to southern Mexico and northern Guatemala, and contains 4.4 g ALA (57% of total fat) per 25 g serving.[19–24] In rats, consumption of chia seeds and chia seed oil counters dyslipidemia and visceral adiposity, improves insulin sensitivity and glucose tolerance, and induces tissue lipid redistribution associated with cardioprotection.[19,25,26] These animal studies support the strategy of using *n*-3 PUFAs supplements to help control chronic inflammation and disease risk factors in overweight/obese individuals, but human data are limited and conflicting. In a previous study with 76 overweight men and women, it was shown that daily ingestion of 50 g whole chia seed (soaked in water for 10 minutes) over a 12-week period increased plasma ALA (24%) but not EPA, with no change in body mass, inflammation, or disease risk factors.[20] Research with fleed suggests that ingestion of milled flaxseed or flaxseed oil results in higher plasma ALA levels than whole flaxseed, and beneficial changes in risk factors.[9,21–23] Thus, one purpose of this second randomized community trial was to compare ingestion of milled and whole chia seed on the plasma ALA and EPA response, and potential effects on traditional biomarkers of cardiovascular disease. Additionally, ALA ingestion may be associated with subtle changes in inflammation and disease risk factors that are best captured through global and targeted metabolomics profiling, the primary outcome for this study.[24] Correspondingly, it was hypothesized that ingestion of milled chia seed would increase plasma ALA and EPA, resulting in a significant change in metabolites related to influence on and disease risk factors.

Methods

Subjects

Subjects included 24 overweight (body–mass index [BMI] 25–29.9 kg/m^2) and 38 obese (BMI 30 kg/m^2 and higher), non-diseased, nonsmoking, postmenopausal women, ages 49–75 years, who were recruited through local advertising. Postmenopausal women were selected as subjects to remove the potential confounding effect of the menstrual cycle on the outcome measures in this study. Subjects were apparently healthy with no overt chronic disease (specifically, coronary heart disease, stroke, cancer [other than skin], diabetes mellitus, rheumatoid arthritis), and not taking dietary supplements or medications known to influence inflammation (in particular, nonsteroidal anti-inflammatory drugs). Subjects agreed to maintain normal dietary and physical activity patterns during the 10-week study, make no formal attempts to lose body weight, and avoid flaxseed, flaxseed oil, and fish oil. Fish and seafood were limited to no more than one serving per week, and subjects were instructed to limit intake of omega-6 fatty acids by lowering use of corn, soybean, safflower, sunflower, and similar oils, and substituting moderate amounts of olive and canola oil. Subjects also agreed to be randomized to chia seed or placebo groups. Written informed consent was obtained from each subject, and the experimental procedures were approved by the institutional review board of Appalachian State University.

Research design

Subjects were randomized to chia seed (whole or milled) and placebo groups, and under double-blinded procedures ingested chia seed or placebo supplements daily for 10 weeks. Body composition, blood pressure, augmentation index, and blood samples were taken from all subjects pre- and post-study after an overnight fast. Diet records and questionnaire responses to assess potential adverse effects and adherence to the supplementation regimen were administered prestudy, and after 5- and 10-week supplementation. The food records were analyzed using a computerized dietary assessment program (Food Processor, ESHA Research, Salem, OR). The symptom logs included questions on 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, stress level, focus/con- centration, and overall well-being. Subjects indicated responses using a 12-point Likert scale, with 1 relating to ''none at all,'' 6 ''moderate,'' and 12 ''very high.'' Subject compliance was monitored by incorporating three laboratory visits during the study and regular e-mail correspondence.

Chia seed and placebo supplements

Chia seed (whole and milled) and placebo supplements were prepared by Dole Packaged Foods, LLC (Westlake Village, CA). Whole poppy seed was used as the placebo because it is similar to chia seed in appearance, energy, dietary fiber, protein, and mineral content, but has no ALA. Subjects were told that supplements could be whole or mil- led seed, and received chia seed or poppy seed supplements in 25-g packets (opaque packaging material). Subjects used one 25-g packet each day and ingested the chia or poppy seed supplements throughout each day for 10 weeks within fruit juice and other beverages, yogurt, on salads and cooked vegetables, or on breakfast cereal. Subjects were instructed to consume the supplement raw and not heat it in any way, including cooking, baking, boiling, or microwaving.

Body composition

Height was measured using a stadiometer, while body mass and body composition were measured using a Tanita bioelectrical impedance scale (Tanita Corporation of America, Inc., Arlington Heights, IL). Testing took place between 7:00 am and 9:30 am both prestudy and after 10 weeks

Table 1. Subject Characteristics (Mean – Standard Error)

Variable	*Whole chia seed (N = 16)*	*Milled chia seed (N = 14)*	*Placebo (poppy seed) (N = 26)*	p-*Value*
Age (years)	60.4 – 1.6	57.2 – 1.7	58.5 – 1.1	0.363
Stature (m)	1.65 – 0.01	1.63 – 0.01	1.63 – 0.01	0.355
Body mass (kg)	90.0 – 4.5	88.8 – 3.3	87.3 – 2.5	0.829
BMI (kg/m^2)	32.9 – 1.3	33.5 – 1.5	33.1 – 0.9	0.940

BMI, body mass index.

supplementation. Subjects were measured while they were standing erect, wearing light clothing, with bare feet on the analyzers' foot pads.

Blood measures, lipoproteins, blood pressure, augmentation index

Blood samples were drawn from an antecubital vein with subjects in the seated position for at least 15 minutes. Blood samples were drawn at 7:00 am – 9:30 am, with all subjects having avoided food and beverage intake other than water for at least 9 hours. A serum lipid panel and comprehensive diagnostic chemistry panel was performed by the authors' clinical hematology laboratory. Blood pressure was measured by technicians following a 15-minute seated rest after an overnight fast. The SphygmoCor Central Blood Pressure System and Pulse Wave Velocity Assessment System (AtCor Medical, Atasca, IL) was used to measure arterial stiffness and the augmentation index. The SphygmoCor system de- rives a calibrated blood pressure waveform at the ascending aorta from a peripheral pressure waveform, recorded non- invasively at the radial artery using a high-fidelity pressure transducer. Augmentation index was calculated as the ratio of amplitude of the pressure wave above its systolic shoulder to the total pulse pressure, and then normalized to a resting heart rate of 75 beats per minute.

Plasma ALA, EPA, DPA, and DHA

Plasma ALA, EPA, DPA, and DHA were analyzed as previously described.[20] The HP 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a 5975B Inert XL MSD mass spectrometer detector and a DB-23 GC column (60 m - 250 *l*m - 0.25 *l*m) from J&W Scientific (Agilent Technologies) was used to separate the methyl esters of the extracted fatty acids. Fatty-acid concentrations were calculated in relation to the heptadecanoic acid methyl ester internal standard peak.

Plasma Cytokine Measurements and C-Reactive Protein

Total plasma concentrations of nine inflammatory cytokines (interleukin-6 [IL-6], tumor necrosis factor *a* [TNF*a*], granulocyte-macrophage colony-stimulating factor [GM- CSF], interferon gamma *c* (IFN*c*), IL-1*b*, IL-2, IL-8, IL-10, IL-12p70) were determined using an electrochemiluminescence- based solid-phase sandwich immunoassay (Meso Scale Discovery, Gaithersburg, MD). All samples and provided standards were analyzed in duplicate, and the intra-assay coefficient of variation (CV) ranged from 1.7% to 7.5%, and the interassay CV ranged from 2.4% to 9.6%, for all cytokines measured. The minimum detectable concentration of IL-6 was 0.27 pg·mL^{-1}, TNF*a* 0.50 pg·mL^{-1}, GM-CSF 0.20 pg·mL^{-1}, IFN*c* 0.53 pg·mL^{-1}, IL-1*b* 0.36 pg·mL^{-1}, IL-2 0.35 pg·mL^{-1}, IL-8 0.09 pg·mL^{-1}, IL-10 0.21 pg·mL^{-1}, and IL-12p70 1.4 pg·mL^{-1}. Pre- and post-exercise samples for the cytokines were analyzed on the same assay plate to decrease interkit assay variability. High- sensitivity C-reactive protein (CRP) was measured using an LX- 20 clinical analyzer (Beckman Coulter Electronics, Brea, CA).

Metabolomics procedures

All samples (both plasma extracts and standards for the internal library) were analyzed on an Agilent 7890A GC system coupled to an Agilent 5975C EI/CI Mass Selective Detector (Foster City, CA). The raw data files generated by gas chromatography-mass spectrometry (GC-MS) were converted to NetCDF format. The converted data were processed using ChromaTOF software (v4.24, Leco Co., CA) including baseline de-nosing, smoothing, peak picking, and peak signal alignment (signal-to-noise ‡ 30). Metabolite annotation was performed by comparing unknown signal patterns from the study samples to those of reference standards from an internal library containing approximately 600 human metabolites (Sigma-Aldrich, St. Louis, MO) established on the GC-MS system. Commercial libraries including the NIST library 2008 and LECO/Fiehn Metabolomics Li- brary for GC-MS metabolome data (similarity threshold of 70%) were also used for additional compound annotation. Heptadecanoic acid was added to the study samples as an internal standard to monitor analytical variations during the entire sample preparation and analysis processes, and precision was calculated by injecting six randomly selected samples five times. The average CV for heptadecanoic acid was less than 5%. The mean CV of the internal standard across the entire sample analysis (158 injections) was 15.3%.

Statistical procedures

Data are reported as mean – standard error. Table 1 data were analyzed using one-way analysis of variance (ANOVA). Data in Tables 2 and 3, and Figure 1A,B were analyzed using a 3 (group) - 2 (time) repeated-measures ANOVA between-subjects model, with pre-to-post-supplementation changes calculated and compared using Student's *t*-test. Diet record and symptom log data were analyzed in a similar fashion using a 3 - 3 repeated-measures ANOVA. For the metabolomics data (Fig. 2), all initial mathematical calculations including peak signal compensations, normalization to internal standards, and univariate analyses (nonparametric Mann-Whitney-Wilcoxon test) were performed using custom scripts in MATLAB R2010a (MathWorks, Inc., Natick, MA). The data set was mean-centered and autoscaled (scaled to

Table 2. Pre- and Poststudy Body Mass and Composition, Serum Glucose, Cholesterol, C-Reactive Protein, Systolic Blood Pressure, and Vascular Augmentation Index[a] in Female Subjects Consuming Whole or Milled Chia Seed, or Placebo Supplements for 10 Weeks (Mean−Standard Error)

Variable	*Whole chia seed (N=16)*	*Milled chia seed (N=14)*	*Placebo (poppy seed) (N=26)*	*Interaction* p-*value*
Body mass (kg)				
Baseline	90.0 – 4.4	88.8 – 3.3	87.3 – 2.5	0.281
10 weeks	90.8 – 4.4	88.5 – 3.0	87.1 – 2.5	
Body fat (%)				
Baseline	37.1 – 1.8	37.3 – 1.2	37.2 – 0.9	0.668
10 weeks	38.8 – 1.9	39.0 – 1.2	38.0 – 1.0	
Serum glucose (mmol/L)				
Baseline	5.09 – 0.15	5.32 – 0.22	5.23 – 0.08	0.629
10 weeks	5.29 – 0.16	5.45 – 0.25	5.31 – 0.10	
Serum cholesterol (mmol/L)				
Baseline	5.46 – 0.26	5.35 – 0.34	5.56 – 0.18	0.805
10 weeks	5.48 – 0.19	5.25 – 0.29	5.48 – 0.19	
Serum C-reactive protein (mg/L)				
Baseline	4.26 – 0.95	4.04 – 0.88	5.71 – 1.15	0.975
10 weeks	4.12 – 0.84	3.84 – 0.75	5.66 – 0.90	
Systolic blood pressure (mm Hg)				
Baseline	140 – 3.8	140 – 5.2	140 – 4.9	0.746
10-weeks	140 – 4.5	136 – 4.7	141 – 4.2	
Augmentation index (mm Hg)[a]				
Baseline	30.8 – 2.2	31.1 – 2.2	28.6 – 1.8	0.448
10 weeks	33.4 – 2.1	31.4 – 2.2	33.4 – 1.8	

[a]Augmentation index normalized to a heart rate of 75 beats/min.

unit variance) prior to statistical analysis. Multivariate statistical analyses including principal component analysis (PCA) and partial least square–discriminant analysis (PLS-DA) were performed using SIMCA-P 12.0.1 + (Umetrics, Umeå , Sweden). PCA was initially used to detect data outliers, clustering, and classification trends without *a priori* knowledge of the sample set. PLS-DA was used to visualize the difference between global metabolic profiles for the three groups. In PCA and PLS-DA, each spatial dot in the *K*- dimensional space represents an individual observation with color-coded grouping information. R^2X and R^2Y represent the fraction of the variance of *X* matrix and *Y* matrix, respectively, while Q^2Y suggests the predictive accuracy of the model. Cumulative values of R^2X, R^2Y, and Q^2Y close to 1 indicate an excellent model with a reliable predictive ability. In practice, Q^2Y values of 0.4 or greater indicate a reliable model. To validate the model against overfitting, a default seven-round cross-validation in SIMCA-P software package was carried out with 1/7th of the data being excluded from the modeling in each round. Using this method, the Y value for each subject was predicted using a model from which that subject was excluded during the model building and all the predictions were collated. Fold changes on selected metabolites related to inflammation and disease risk factors

Table 3. Pre- and Post-study Plasma Cytokine Levels in Female Subjects Consuming Whole or Milled Chia Seed, or Placebo Supplements for 10 Weeks (Mean−Standard Error)

Variable	*Whole chia seed (N=16)*	*Milled chia seed (N=14)*	*Placebo (poppy seed) (N=26)*	*Interaction* p-*Value*
Plasma IL-6 (pg/mL)				
Baseline	2.18 – 0.38	2.31 – 0.53	2.91 – 0.74	0.785
10 weeks	2.17 – 1.58	2.38 – 0.66	2.47 – 0.44	
Plasma TNF-*a* (pg/mL)				
Baseline	6.00 – 0.41	7.55 – 0.63	6.92 – 0.45	0.543
10 weeks	5.82 – 0.38	7.47 – 0.71	7.15 – 0.68	
Plasma IL-8 (pg/mL)				
Baseline	5.55 – 1.24	5.17 – 0.72	4.87 – 0.43	0.871
10 weeks	4.65 – 0.59	4.77 – 0.41	4.43 – 0.31	
Plasma IL-10 (pg/mL)				
Baseline	2.98 – 0.85	4.75 – 0.98	2.92 – 0.75	0.444
10 weeks	2.69 – 0.48	4.20 – 0.67	2.90 – 0.52	

IL, interleukin; TNF, tumor necrosis factor.

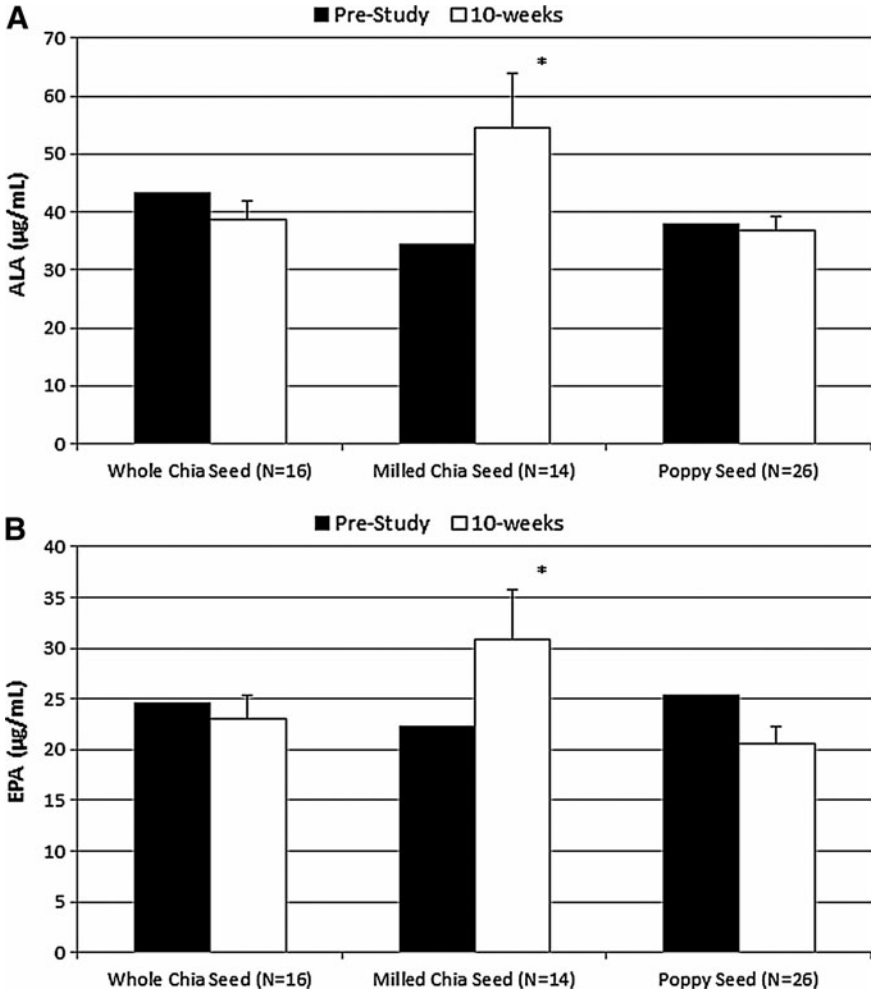

FIG. 1. Plasma *a*-linolenic acid (ALA) (A) and eicosapentaenoic acid (EPA) (B) pre- and post-10 weeks supplementation with chia seed (whole or milled) or placebo (poppy seed). *represents $P < 0.025$.

(Table 4) were compared between groups using Mann-Whitney-Wilcoxon.

Results

Subject characteristics for the three study groups are listed in Table 1, with no group differences found for age, stature, body mass, and BMI. Of the 62 subjects enrolled into the study, 6 (all from the placebo group) failed to complete all study requirements. Each of the 6 subjects leaving the study had difficulty consuming the poppy seeds, and 4 left the study within the first week. The primary problem reported by subjects in the poppy seed group was mouthfeel and seeds lodging between the teeth. Subjects completing the study consumed all of the chia seed and placebo supplied to them for the study as assessed during laboratory visits at weeks 5 and 10. Responses to a post-study questionnaire revealed that 35 of 56 subjects did not know what supplement they were ingesting (chia seed or poppy seed), with 14 subjects guessing correctly and 7 guessing incorrectly.

Macronutrient and micronutrient intake assessed from prestudy and 5- and 10-week 3-day food records did not differ between whole chia seed, milled chia seed, and placebo groups during the study (data not shown, all interaction *p*-values > 0.05). Symptoms for digestive health, hunger, energy level, illness,pain,stress, focus/concentration, and overall well-being as assessed from pre-study and 5- and 10- week symptoms logs did not differ significantly between chia seed and placebo groups (data not shown).

Plasma ALA increased 58.4% (interaction effect, $p = 0.002$), and EPA 38.6% ($p = 0.016$), in the milled chia seed group compared to nonsignificant changes in the whole chia seed and placebo groups (Fig. 1A and B). Plasma DPA and DHA increased 21.1% and 16.5% in the milled chia seed group, but these changes were not significant compared to placebo (data not shown).

Body mass and composition remained stable during the 10-week study, and pre-to-post-study values did not differ between groups (Table 2). The pattern of change over time did not differ between groups for serum glucose, cholesterol, CRP, systolic blood pressure, and augmentation index (Table 2), and for low-density lipoprotein (LDL)-cholesterol, high- density lipoprotein (HDL)-cholesterol, serum triglycerides, diastolic blood pressure, and all components in the comprehensive diagnostic chemistry panel (data not shown, all interaction *p*-values > 0.05).

Data on four of the nine plasma cytokines measured in this study are summarized in Table 3. The pattern of change over time did not differ between whole chia seed, milled chia seed, and placebo groups for each of these variables, or for GM-CSF, IFN*c*, IL-1*b*, IL-2, or IL-12p70) (data not shown, interaction effects, all $p > 0.05$).

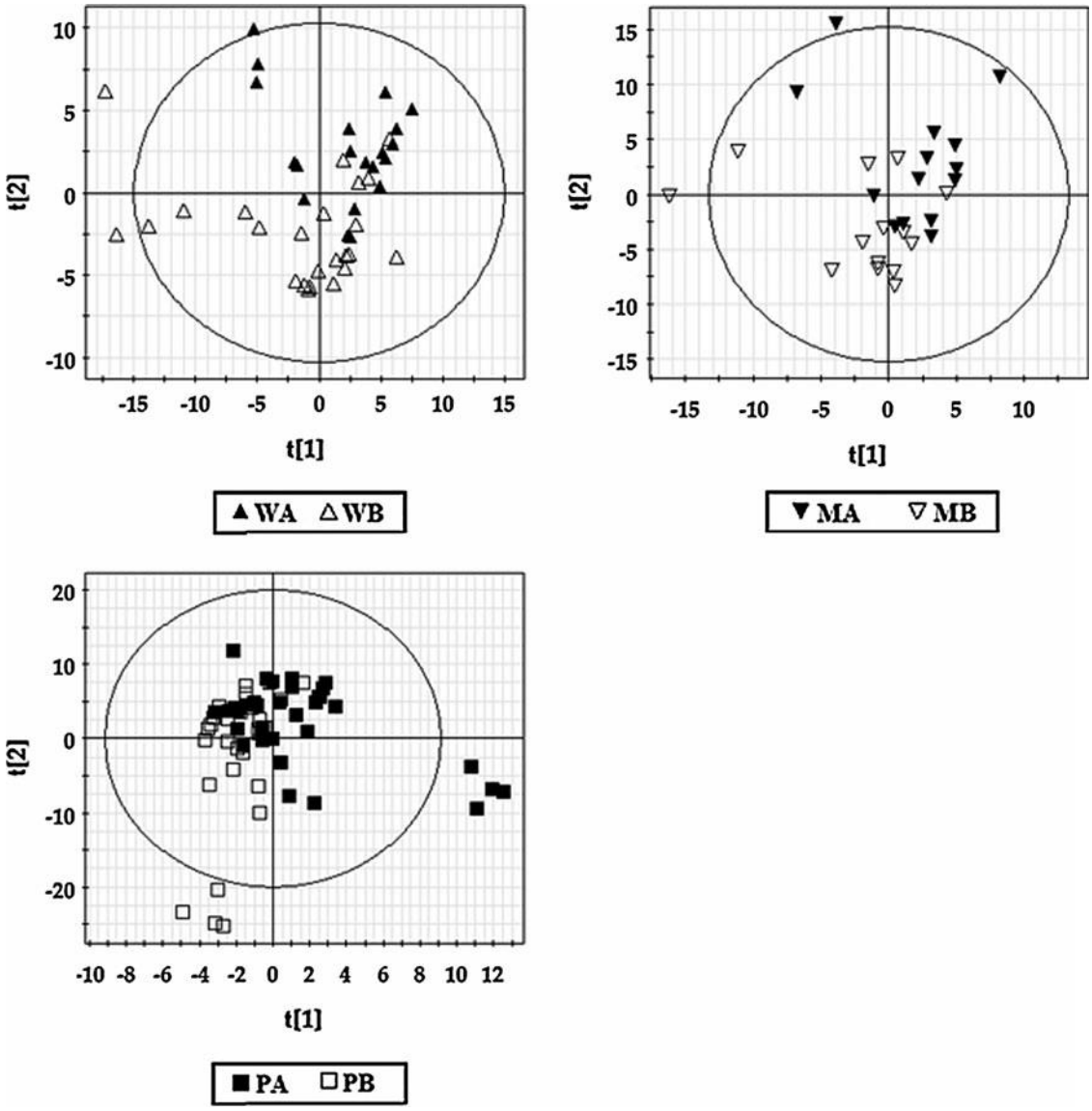

FIG. 2. Score plots from partial least square–discriminant analysis model visualize the global metabolic differences between pre-supplementation and post-supplementation in the whole chia seed group (WA, whole chia seed pre-supplementation; WB, whole chia seed post-supplementation), and for the milled chia seed group (MA and MB), and placebo (PA and PB). Differences between groups were not significantly different ($Q^2Y \pounds 0.40$).

Score plots from the PLS-DA model visualize the global metabolic differences between pre-supplementation and post- supplementation in the whole chia seed group (WA and WB), the milled chia seed group (MA and MB), and placebo (PA and PB) as shown in Figure 2. The Q^2Y scores of the two PLS-DA models were below 0.4, which indicates that the global metabolic profile difference of the three groups was non-significant. Fold-change scores for 28 selected metabolites related to inflammation and disease risk factors did not differ between groups (Table 4).

Discussion

Chia seed supplements are a rich source of ALA, and were consumed without adverse digestive or mental symptoms by our female, postmenopausal subjects for 10 weeks. Of the 62 original subjects, 56 complied with all phases of the research project, with difficulty in ingesting the poppy seed placebo emerging as the primary reason why 6 individuals dropped out of the study. Ingestion of 25 g of milled but not whole chia seeds per day for 10 weeks resulted in a significant increase in plasma ALA and EPA relative to placebo, but no group differences were measured for inflammation and various measures of disease risk factors as determined by both traditional biomarkers and global and targeted metabolic profiling.

Ingestion of 25 g/day chia seed increased dietary ALA intake by 4.4 g/day, substantially above the 1.1 g/day U.S. adult female average.[3] However, plasma ALA and EPA were elevated only with milled chia seed intake, with nonsignificant changes measured following ingestion of whole chia seed. In the previous study, plasma ALA increased 24% in

Table 4. Fold Change for Metabolites Associated with Inflammation and Disease Risk Factors

Compound	Whole chia (fold change)	Milled chia (fold change)	Placebo (fold change)
Glycerol[a]	1.1	1.1	1.1
Glycerate[a]	1.1	−1.1	1.1
Decanoic acid[a]	1.1	1.1	−1.0
5-oxoproline[a]	1.1	1.2	1.2
L-Threonic acid[a]	1.2	1.2	1.3
Laurate[a]	−1.0	−1.4	−1.1
Glycerol, phosphate	1.2	−1.4	1.1
Myristic acid[a]	1.0	1.2	1.1
Palmitoleic acid[a]	1.1	1.3	1.1
Palmitic acid[a]	1.2	−1.4	−1.1
Oleic acid[a]	1.1	−1.2	1.0
trans-9-Octadecenoic acid	1.1	−1.3	1.0
Octadecanoic acid[a]	1.1	1.3	1.1
Nonadecanoic acid	1.0	1.1	1.1
Arachidonic acid[a]	1.0	−1.0	1.1
8,11,14-Eicosatrienoic acid	−1.1	1.1	1.1
Glycerol, myristate	1.2	1.1	1.0
11,14-Eicosadienoic acid[a]	1.3	−1.2	−1.2
11-Eicosenoic acid	1.0	1.0	1.2
Eicosanoic acid	1.0	−1.7	−1.2
Glycerol, pentadecanoate	1.1	1.2	−1.0
2-Monopalmitin	1.2	−1.0	−1.0
Docosahexaenoic acid	1.2	1.1	1.1
1-Palmitoylglycerol	1.2	−1.2	−1.0
Glycerol, heptadecanoate	1.1	1.1	1.0
Monostearin	1.1	1.1	1.1
α-Tocopherol	1.1	1.3	1.1
Cholesterol[a]	1.2	1.1	1.1

[a]Metabolites were verified by internal standard library. Fold change was calculated by the ratio of mean rank between two groups using the Mann-Whitney-Wilcoxon test; a positive value means a higher concentration in poststudy compared to prestudy, and vice versa for negative values.

male and female adults after 12 weeks of ingesting a large quantity (50 g/day) of water-soaked whole chia seed,[20] substantially below the 58% increase measured in the current study after 10 weeks of ingesting 25 g/day milled (but not whole) chia seed. Austria et al.[21] also reported significant increases in plasma ALA when subjects ingested 30 g/day milled but not whole flaxseed over a 3-month period. Thus, milled chia seed, consistent with results from studies using flaxseed, delivers ALA to the human body better than whole chia seed.

After ingestion, ALA is almost completely absorbed and is (1) oxidized to carbon dioxide and water, or (2) incorporated into tissue lipids, or (3) utilized in eicosanoid synthesis. Small amounts of ALA are lost during sloughing of skin and other epithelial cells.[1,2] Approximately 15%–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] A 58% increase in plasma ALA was measured after supplementation with 10 weeks milled chia seed, with a significant 39% increase in plasma EPA. These results are similar to those of others using similar research designs but with ground flaxseed.[21,23]

Despite a corresponding increase in plasma ALA, subjects in the milled chia seed group experienced nonsignificant changes in a variety of risk factor–related outcome measures including lipid profiles, blood pressure, augmentation index, and inflammation. Metabolomics is the measurement of small molecules or metabolites present in biologic samples such as biofluids, tissues, and cellular extracts to elucidate the effect of a particular stimulus on metabolic pathways. In this study, GC-MS methods were utilized to measure metabolic profiles in pre- and post-supplementation serum samples in combination with commercial metabolite libraries and an internal library of approximately 600 internal standards for compound annotation.24 It was hypothesized that global and targeted metabolomics profiling would capture subtle perturbations in metabolites associated with inflammation and disease risk factors from chia seed ALA intake, but the data were nonsupportive. The authors were unable to find any other study using metabolomics to investigate ALA supplementation and potential influences on human health and disease. The use of metabolomics in nutritional sciences is gaining momentum, and should improve understanding regarding the health benefits and underlying molecular pathways related to dietary interventions.24 Most human studies using flaxseed or other ALA supplements have failed to show significant or meaningful changes in traditional measures of inflammation or the blood lipid profile, in concert with the current findings and in contrast to results from studies using fish oil supplements.7,9,27–32 A review of five ALA supplementation studies reported an inconsistent effect of ALA on the blood lipid profile.7 Nelson et al.[28] showed no effect of 8-weeks' 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. Reviews also indicate that ALA supplementation has inconsistent or null effects on vascular function and blood pressure, in concert with the current findings.[17,33]

Our data are in partial disagreement with Vuksan et al.[34] This study utilized a single-blind crossover design with 20 adults with type 2 diabetes who were given 37 g/day chia seed or wheat bran for 12 weeks, with a 4–6-week washout period. Chia seed supplementation was related to a 6.3 mm Hg decrease in systolic blood pressure, a 40% reduction in CRP, and a 21% drop in vonWillebrand factor (vWF), with no changes in body mass or blood total cholesterol, LDL cholesterol, HDL cholesterol, or triglycerides. Much of the change in CRP, vWF, and systolic blood pressure in this study, however, was due to contrasting increases in these measures during the wheat bran supplement period, and are inconsistent with most other well-controlled ALA-supplementation studies. Crossover designs in studies of this type pose several challenges, including carryover effects from the washout period (which often cannot be specified), and compliance issues due to the prolonged total study period.

Conclusions

Milled compared to whole chia seed supplementation for 10 weeks resulted in higher plasma ALA and EPA levels. Nonetheless, these data combined with those from the authors' previous publication[20] do not support the short-term strategy of having postmenopausal, overweight women use whole or milled chia seed supplements (25–50 g/day) high in

ALA to help lower chronic inflammation or improve blood pressure, vascular function, and blood lipid profiles over a 10–12-week period. This conclusion is strengthened by the lack of group differences over time in both traditional disease-related biomarkers and global and targeted metabolomics procedures. Whether or not these findings can be applied to younger adults remains to be determined in subsequent investigations. The typical serving size for chia seed is 4–12 g, and we tested intake levels at the upper limits of human consumption, but without measurable influences on health. Future research is warranted to test higher ALA intake levels using chia seed oil. These findings, however, should not discourage individuals from using chia seed and other ALA-rich foods. These foods are nutrient dense, and high, chronic ALA intake has been associated in epidemiologic investigations with multiple health benefits.[8,13–15]

Disclosure Statement

No competing financial interests exist.

References

1. Burdge GC. Metabolism of alpha-linolenic acid in humans. Prostaglandins Leukot Essent Fatty Acids 2006;75:161–168.
2. Arterburn LM, Hall EB, Oken H. Distribution, interconversion, and dose response of n-3 fatty acids in humans. Am J Clin Nutr 2006;83(6 suppl):1467S–1476S.
3. U.S. Department of Agriculture, Agricultural Research Service. 2010. Nutrient Intakes from Food: Mean Amounts Consumed per Individual, by Gender and Age. What We Eat in America. NHANES 2007–2008. Online document at: www.ars.usda.gov/ba/bhnrc/fsrg Accessed November 2, 2011.
4. Food and Nutrition Board, Institute of Medicine. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). A report of the Panel on Macronutrients, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Washington, DC: National Academy Press, 2002.
5. Wang C, Harris WS, Chung M, et al. n-3 Fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: A systematic review. Am J Clin Nutr 2006;84:5–17.
6. Sun Q, Ma J, Campos H, et al. Blood concentrations of individual long-chain n-3 fatty acids and risk of nonfatal myocardial infarction. Am J Clin Nutr 2008;88:216–223.
7. Balk EM, Lichtenstein AH, Chung M, et al. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: A systematic review. Atherosclerosis 2006;189:19–30.
8. American Heart Association Nutrition Committee, Lichtenstein AH, Appel LJ, Brands M, et al. Diet and lifestyle recommendations revision 2006: A scientific statement from the American Heart Association Nutrition Committee. Circulation 2006;114:82–96.
9. Prasad K. Flaxseed and cardiovascular health. J Cardiovasc Pharmacol 2009;54:369–377.
10. Whelan J, Rust C. Innovative dietary sources of n-3 fatty acids. Annu Rev Nutr 2006;26:75–103.
11. Stark AH, Crawford MA, Reifen R. Update on alpha-linolenic acid. Nutr Rev 2008;66:326–332.
12. Blasbaig TL, Hibbeln JR, Ramsden CE, et al. Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century. Am J Clin Nutr 2011; 93:950–962.
13. Djoussé L, Biggs ML, Lemaitre RN, et al. Plasma omega-3 fatty acids and incident diabetes in older adults. Am J Clin Nutr 2011;94:527–533.
14. Farina EK, Kiel DP, Roubenoff R, et al. Dietary intakes of arachidonic acid and alpha-linolenic acid are associated with reduced risk of hip fracture in older adults. J Nutr 2011; 141:1148–1153.
15. Lucas M, Mirzaei F, O'Reilly EJ, et al. Dietary intake of n-3 and n-6 fatty acids and the risk of clinical depression in women: A 10-y prospective follow-up study. Am J Clin Nutr 2011;93:1337–1343.
16. de Goede J, Verschuren WM, Boer JM, et al. Alpha-linolenic acid intake and 10-year incidence of coronary heart disease and stroke in 20,000 middle-aged men and women in the Netherlands. PLoS One 2011;6:e17967.
17. Egert S, Stehle P. Impact of n-3 fatty acids on endothelial function: Results from human interventions studies. Curr Opin Clin Nutr Metab Care 2011;14:121–131.
18. Geleijnse JM, de Goede J, Brouwer IA. Alpha-linolenic acid: Is it essential to cardiovascular health? Curr Atheroscler Rep 2010;12:359–367.
19. Poudyal H, Panchal SK, Waanders J, et al. Lipid redistribution by *a*-linolenic acid-rich chia seed inhibits stearoyl-CoA desaturase-1 and induces cardiac and hepatic protection in diet-induced obese rats. J Nutr Biochem 2012;23:153–162.
20. Nieman DC, Cayea EJ, Austin MD, et al. Chia seed does not promote weight loss or alter disease risk factors in overweight adults. Nutr Res 2009;29:414–418.
21. Austria JA, Richard MN, Chahine MN, et al. Bioavailability of alpha-linolenic acid in subjects after ingestion of three different forms of flaxseed. J Am Coll Nutr 2008;27:214–221.
22. Bloedon LT, Balikai S, Chittams J, et al. Flaxseed and cardiovascular risk factors: Results from a double blind, randomized, controlled clinical trial. J Am Coll Nutr 2008;27: 65–74.
23. Taylor CG, Noto AD, Stringer DM, et al. Dietary milled flaxseed and flaxseed oil improve N-3 fatty acid status and do not affect glycemic control in individuals with well-controlled type 2 diabetes. J Am Coll Nutr 2010;29:72–80.
24. Zulyniak MA, Mutch DM. Harnessing metabolomics for nutrition research. Curr Pharm Biotechnol 2011;12:1005–1015.
25. Chicco AG, D'Alessandro ME, Hein GJ, et al. Dietary chia seed (*Salvia hispanica* L.) rich in alpha-linolenic acid improves adiposity and normalises hypertriacylglycerolaemia and insulin resistance in dyslipaemic rats. Br J Nutr 2009; 101:41–50.
26. Ayerza R Jr, Coates W. Effect of dietary alpha-linolenic fatty acid derived from chia when fed as ground seed, whole seed and oil on lipid content and fatty acid composition of rat plasma. Ann Nutr Metab 2007;51:27–34.
27. Wilkinson P, Leach C, Ah-Sing EE, et al. Influence of alpha-linolenic acid and fish-oil on markers of cardiovascular risk in subjects with an atherogenic lipoprotein phenotype. Atherosclerosis 2005;181:115–124.
28. Nelson TL, Stevens JR, Hickey MS. Inflammatory markers are not altered by an eight week dietary alpha-linolenic acid intervention in healthy abdominally obese adult males and females. Cytokine 2007;38:101–106.

29. Kaul N, Kreml R, Austria JA, et al. A comparison of fish oil, flaxseed oil and hempseed oil supplementation on selected parameters of cardiovascular health in healthy volunteers. J Am Coll Nutr 2008;27:51–58.
30. Harper CR, Edwards MC, Jacobson TA. Flaxseed oil supplementation does not affect plasma lipoprotein concentration or particle size in human subjects. J Nutr 2006;136:2844–2848.
31. Vedin I, Cederholm T, Freund Levi Y, et al. Effects of docosahexaenoic acid-rich n-3 fatty acid supplementation on cytokine release from blood mononuclear leukocytes: The OmegAD study. Am J Clin Nutr 2008;87:1616–1622.
32. Calder PC. Polyunsaturated fatty acids, inflammatory processes and inflammatory bowel diseases. Mol Nutr Food Res 2008;52:885–897.
33. Wendland E, Farmer A, Glasziou P, Neil A. Effect of alpha linolenic acid on cardiovascular risk markers: A systematic review. Heart 2006;92:166–169.
34. Vuksan V, Whitham D, Sievenpiper JL, et al. Supplementation of conventional therapy with the novel grain Salba (*Salvia hispanica* L.) improves major and emerging cardiovascular risk factors in type 2 diabetes: Results of a randomized controlled trial. Diabetes Care 2007;30:2804–2810.