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Daily flaxseed consumption improves glycemic control in obese men and women with pre-diabetes: a randomized study

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ABSTRACT

The study hypothesis was that fasting glucose, insulin, fructosamine, C-reactive protein, and interleukin-6 decrease and adiponectin increases with daily flaxseed consumption in overweight or obese individuals with pre-diabetes. In this randomized, cross-over study overweight or obese men and postmenopausal women (n = 25) with pre-diabetes consumed 0, 13, or 26 g ground flaxseed for 12 weeks. Glucose, insulin, homeostatic model assessment (HOMA-IR), and normalized percent of α-linolenic fatty acid (ALA) were significantly different by treatment (multiple analysis of variance, $P = .036$, $P = .013$, $P = .008$, $P = .024$ respectively). Paired *t* tests showed glucose decreased on the 13 g intervention compared to the 0 g period [13g = –2.10 ± 1.66 mg/L (mean ± SEM), 0 g = 9.22 ± 4.44 mg/L, $P = .036$]. Insulin decreased on the 13 g intervention but not the 26 g ($P = .021$) and 0 g ($P = .013$) periods (13 g = –2.12 ± 1.00 mU/L, 26 g = 0.67 ± 0.84 mU/L, 0g = 1.20 ± 1.16 mU/L). HOMA-IR decreased on the 13 g period but not on the 26 g ($P = .012$) and 0 g ($P = .008$) periods (13g = –0.71 ± 0.31, 26g = 0.27 ± 0.24, 0g = 0.51 ± 0.35). The α-linolenic fatty acid decrease for the 0 g period was different than the 13 g ($P = .024$) and 26 g ($P = .000$) periods (13 g = 0.20 ± 0.04, 26g = 0.35 ± 0.07, 0g = –0.01 ± 0.07). Fructosamine, high sensitivity C-reactive protein, adiponectin, and high-sensitivity interleukin-6 had no significant differences. Flaxseed intake decreased glucose and insulin and improved insulin sensitivity as part of a habitual diet in overweight or obese individuals with pre-diabetes.

1. Introduction

Seventy-nine million Americans have pre-diabetes [1], a condition characterized by high blood glucose concentrations (fasting plasma glucose ≥100–125 mg/dL or hemoglobin A_{1c} [HbA_{1c}] 5.7%-6.4%) due to ineffective insulin or 'insulin resistance' [1,2]. Most people with pre-diabetes are overweight or obese (eg, body mass index [BMI] >25 kg/m^2) and are at 3

Abbreviations: ALA, α-linolenic fatty acid; ANOVA, analysis of variance; BMI, body mass index; CRP, C-reactive protein; ED, enterodiol; EL, enterolactone; g, gram(s); HbA_{1c}, hemoglobin A_{1c}; hs-CRP, high sensitivity C-reactive protein; hs-IL-6, high-sensitivity interleukin-6; HOMA-IR, homeostatic model assessment; IL-6, interleukin-6; MANOVA, multiple analysis of variance; mg, milligram(s); RIA, radioimmunoassay; SDG, secoislariciresinol diglucoside; SD, standard deviation; SEM, standard error of the means.

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times higher risk for developing type 2 diabetes than normal weight (BMI 18.5-24.9 kg/m^2) individuals [3]. Obesity increases insulin resistance [4] and low-grade inflammation that are associated with the development of diabetes [5]. Although the long-term complications associated with type 2 diabetes are well-recognized, some long-term damage to the body, especially the heart and circulatory system, may already be occurring during pre-diabetes.

Modest changes in dietary intake and exercise can delay, if not prevent, the onset of diabetes in people with pre-diabetes. Inclusion of omega-3 fatty acids in the diet is associated with improvements in insulin sensitivity and glycemic control [6]. Epidemiological studies have reported a lower prevalence of impaired glucose tolerance (pre-diabetes) in populations consuming 1–2 g/d of long chain omega-3 polyunsaturated fatty acids [6]. Increased soluble, viscous fiber in the diet lowers the glucose response to carbohydrate-containing foods, by delaying gastric emptying and absorption of glucose [7]. Flaxseed contains soluble fiber, α-linolenic acid (ALA), and linoleic acid [8]. It is also the richest source of the lignan secoisolariciresinol diglucoside, which, after ingestion, is further metabolized to enterodiol and enterolactone [9]. A growing body of evidence suggests that secoisolariciresinol diglucoside metabolites and flaxseed consumption may protect against the metabolic syndrome and risk of progressing from pre-diabetes to type 2 diabetes by reducing lipid and glucose concentrations, delaying postprandial glucose absorption and decreasing oxidative stress and inflammation [10–13].

Serum interleukin-6 (IL-6), an inflammatory cytokine produced by adipocytes and immune cells, may play a role in the insulin resistance of obesity and type 2 diabetes [14]. IL-6 increases hepatic C-reactive protein (CRP) synthesis, another biomarker of inflammation [15]. Human studies report high CRP concentrations are positively associated with the presence of cardiovascular disease, obesity, and insulin resistance in population-based and clinical studies [16–19]. Adiponectin is an anti-inflammatory adipocyte secretory protein that may have a protective role in diabetes development [20,21]. Several epidemiologic studies report a lower incidence of diabetes for those with higher adiponectin concentrations [22,23].

The current study was conducted to determine the effect of flaxseed consumption on glycemic control, cytokines, and adipokines in overweight and obese individuals with pre-diabetes. We hypothesized that fasting glucose, insulin, fructosamine, CRP, and IL-6 would decrease and adiponectin would increase with daily flaxseed consumption in overweight or obese individuals with pre-diabetes. To test this hypothesis, we performed primary analysis of a randomized crossover design to determine the impact of consuming 13 or 26 g (2 or 4 tablespoons) of ground flaxseed, compared to a control period for 12 weeks on fasting glucose, insulin, fructosamine, CRP, IL-6, and adiponectin in 11 overweight or obese men and 14 overweight or obese postmenopausal women with pre-diabetes. Flaxseed is safe, inexpensive, and widely available for consumption. A few studies show it can improve insulin sensitivity and not adversely affect inflammatory responses [11,24–26]. Thus, flaxseed supplementation may benefit people with pre-diabetes who are also overweight or obese.

2. Methods and materials

2.1. *Subjects and dietary protocol*

Potential participants who were overweight or obese but had no diagnosis of type 1 or type 2 diabetes were screened for inclusion/exclusion criteria by the study personnel and for pre-diabetes (impaired fasting glucose between 100 and 125 mg/dL) by a fasted blood draw. Participants were classified as overweight if their BMI was ≥25.0 and ≤29.9 and obese if their BMI was ≥30.0. Individuals excluded from the study included: people who (1) were on prescribed oral hypoglycemic medication or insulin injection; (2) had any diagnosed acute illness or chronic illness other than impaired glucose tolerance that would impact glucose metabolism or was not controlled; (3) had an allergy or food intolerance to flaxseed; (4) smoked at the time of study enrollment; (5) had intensive exercise or unusual dietary habits (e.g., more than 150 minutes of moderate-intensity aerobic activity per week or 75 minutes of vigorous-intensive aerobic activity per week or elimination of one or more food groups from their diet); (6) used anti-inflammatory medications on a regular basis; (7) had regular intake of flaxseed, flaxseed oil or fish oil supplementation (eg, consumed one or more times a week on a consistent basis), fish (<340 g of fish/week) or soy (eg, consumed one or more times a week on a consistent basis); or (8) women who had a menstrual cycle within the last 6 months. One hundred thirty-seven potential participants from the communities surrounding the University of Colorado Colorado Springs and the University of Montana were screened for the study. Of these, 73 (53%) did not meet the inclusion criteria for the following reasons: 5% were smokers; 10% had too high of a supplement, soy, or flax intake; 15% did not meet the BMI criteria; 22% were on an oral hypoglycemic medication; and 48% did not meet the inclusion criteria for other reasons which included still having regular menstrual cycles, falling outside of the age or glucose range, or rigorous goals for weight loss and exercise in the next 10 months (Fig. 1). Participants who met the eligibility criteria were enrolled by study personnel and assigned to an intervention sequence by the primary investigators. The protocol was approved by the University of Colorado Colorado Springs and the University of Montana Institutional Review Boards, and participants provided informed written consent before entering the study. The study is registered with ClinicalTrials.gov, NCT01698112.

Three treatment periods during the study included the control period (no flaxseed supplementation, 0 g), 13 g (2 tablespoons) per day flaxseed treatment, or 26 g (4 tablespoons) per day flaxseed treatment. The ground flaxseed was provided by Bob's Red Mill Natural Foods, Inc., Milwaukie, OR, in one lot, and was frozen at 0°C until use. The flaxseed was weighed by trained study personnel using an electronic food scale and provided to the participants in pre-weighed doses every other week during intervention periods. The study followed a randomized crossover design. Zero g, 13 g or 26 g ground flaxseed was consumed for 12 weeks, followed by a 2-week washout period before initiation of the next treatment period. Participants consumed the ground flaxseed

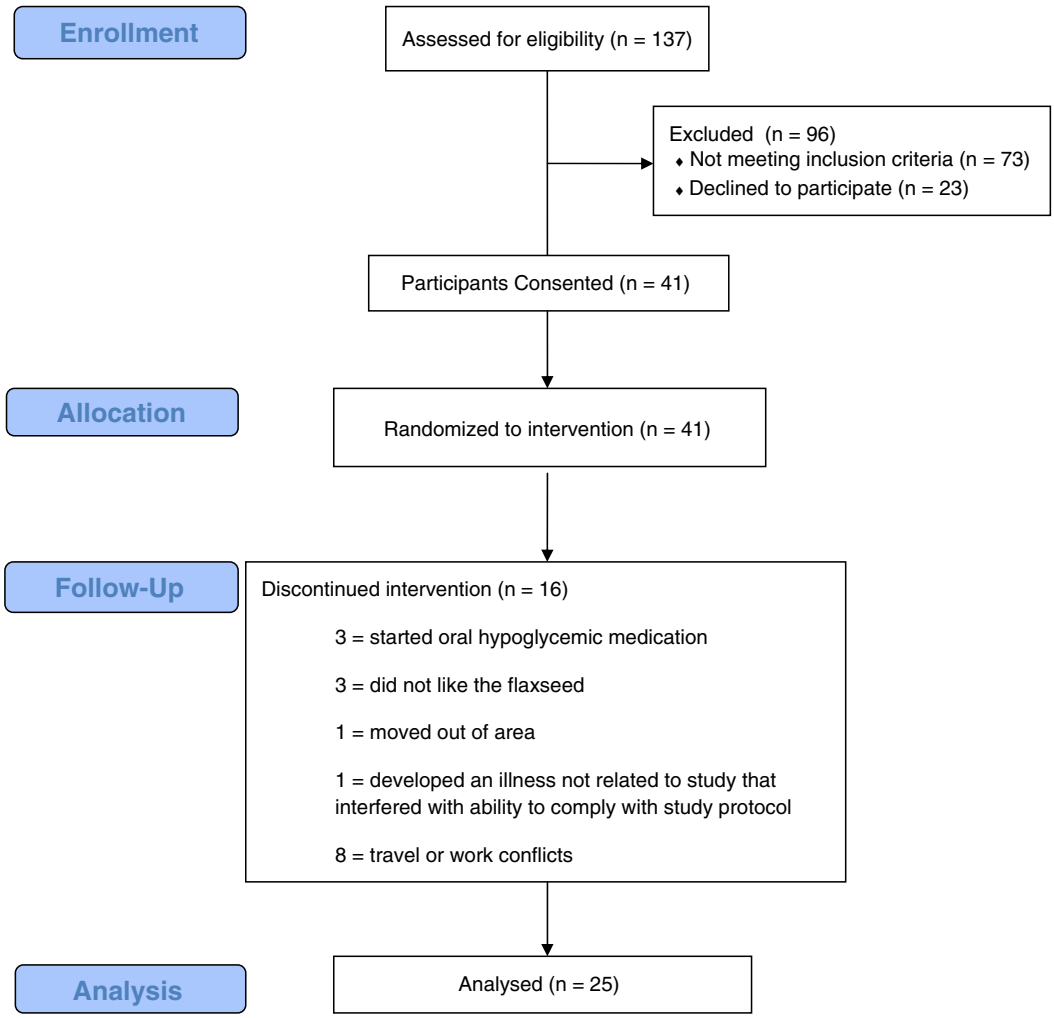

Fig. 1 – Consolidated Standards of Reporting Trials (CONSORT) flow diagram.

as part of their normal meal or snack and were instructed to not to bake, cook, or microwave the supplement but rather to add it to prepared foods. Participants also completed a 2-week wash-in period to allow for weight stabilization, baseline collection of diet information, and familiarization with diet recording protocols prior to beginning the first of the three treatment periods.

2.2. *Sampling and analysis of anthropometrics and dietary intake*

Participants completed one 24-hour diet record each week during the treatment periods plus four nonconsecutive 24-hour diet records at baseline (40 diet records total). These records were analyzed to determine changes in macro- and micronutrient intake during the study (ESHA Food Processor SQL version 10.2.2; ESHA Research, Salem, OR, USA). Weight was measured every other week during the treatment periods, and percent body fat was measured at week 0 and week 12 of each treatment period on an electronic scale (Tanita TBF-300A). Height was measured at the beginning of the study with a stadiometer. Body mass index was calculated using the standard formula, weight (kg)/height $(m)^2$.

2.3. *Sampling and analysis of blood*

Three 15-mL venous forearm blood samples were taken 2 to 3 days before the participants began each feeding period and immediately after they completed each feeding period. Plasma was extracted and frozen at –45°C until analyzed for glucose, insulin, fructosamine, adiponectin, and fatty acids. Serum was separated by refrigerated centrifugation at 1000*g* for 10 min and stored at –20°C until analyzed for high-sensitivity IL-6 (hs-IL-6) and high sensitivity C-reactive protein (hs-CRP).

Plasma glucose, insulin, fructosamine, adiponectin, and serum hs-CRP were measured by a commercial laboratory (Centura Laboratory, Centura Health, Colorado Springs, CO, USA) using standard commercial methodologies. Serum hs-IL-6 was measured by high-sensitivity Quantikine Human IL-6 Immunoassay (R&D Systems Inc, Minneapolis, MN, USA) that employed the quantitative sandwich enzyme immunoassay technique. Standards and samples were measured at 450 nm (microplate reader, BioTek ELIU808IU). If samples were diluted, the concentrations read from the standard curve were multiplied by the dilution factor. Data was reduced using software (BioTek KC ver 3.0) and a 4-parameter logistic curve-

fit analysis was used to create standard curves. This commercially available kit has been analyzed for specificity and cross-reactivity. Inter- and intra-assay coefficients of variation for the hs-IL-6 analyses were 8.12% and 7.76%, respectively. Plasma fatty acids were measured using a modified Folch procedure [27], and the fatty acids were methylated with BF_3 in methanol. Fatty acid methyl esters were determined by gas chromatography (Hewlett Packard, Palo Alto, CA, USA; GC model 6890) equipped with a fused capillary column (30 m × 0.25 mm × 0.25 μm DB-23; J&W Scientific, Folsom, CA, USA)[27]. The identity of individual fatty acids was determined by comparing retention times with standard mixtures of fatty acids (NuChek 68A, NuChek 96; NuChek Prep Inc, Elysian, MN, USA, and Supelco PUFA2, Sigma-Aldrich Canada Ltd, Mississauga, ON, Canada). Individual fatty acids are reported and analyzed as % of total fat. Inter- and intra-assay coefficients of variation for the fatty acid analyses for pooled human plasma were less than 5% for linoleic acid.

2.4. Statistical analyses

Data are expressed as means (±SD) change (end value – beginning value) for each end point during each diet treatment period. Data were tested for normality via the Kolmogorov-Smirnov and Shapiro-Wilk tests and no transformations were necessary for analysis. Data were analyzed for statistical significance by analysis of variance (ANOVA) with repeated measures, with time and treatment as factors. Once a significant change was identified by ANOVA, paired *t* tests were performed to determine differences by treatment. Dietary intake data was analyzed using multivariate analysis with the intervention and time as independent variables. $P \leq .05$ was considered statistically significant. Sample size was determined using continuous data sample size determinations. SPSS version 19 (SPSS, Chicago, IL, USA) was used for all analyses.

This study used a crossover, repeated measures design in which each participant served as his or her own control. Using continuous data sample size determinations, a sample size of ~28 participants was necessary to provide a power of 80% to detect a decrease fasting glucose of at least 27 ± 65 mg/dL [28], fasting insulin of at least 2.8 ± 8.9 mU/L [29], fructosamine by 86 ± 96 μmol/L [30], and adiponectin of at least 1.32 ± 3.32 μg/mL [31] at an α of .05. Therefore, an initial sample size of 40 participants was recruited to provide sufficient numbers to detect differences by repeated measures of analysis of variance and allow for a ~35% dropout rate over the course of the study.

3. Results

3.1. Participant recruitment and retention, anthropometry and dietary intake

Forty-one participants began the study, with 25 participants completing all 3 study treatment arms (Fig. 1). The reasons for participants dropping out included: starting an oral hypoglycemic medication (n = 3), did not like the flaxseed (n = 3), moved out of the area (n = 1), developed an illness not related to the study that interfered with ability to comply to study protocol (n = 1) and travel or work conflicts (n = 8).

Participant characteristics are shown in Table 1. All participants were nonsmokers and reported moderate-intensity or vigorous-intensity exercise for 30 minutes or less per day, 3 to 5 days per week. The average BMI at baseline was 30.4 ± 1.1 kg/m^2

Table 1 – Participant characteristics *

Characteristic	Men	Women	All participants
N †	11	14	25
Age (y) †	56.9 ± 8.3	60.0 ± 4.1	58.6 ± 6.3
Weight (kg) †	93.8 ± 10.4	82.5 ± 11.2	87.5 ± 12.0
BMI (kg/m^2) †	29.2 ± 2.9	31.3 ± 6.5	30.4 ± 5.3
BMI category			
Overweight (n) †	6	8	14
Obese (n) †	5	6	11
			Normal range
Plasma			
Glucose (mg/dl) †	109 ± 11	104 ± 4	106 ± 8	<100
Insulin (mU/L) ‡	12.0 ± 5.1	9.6 ± 4.6	10.7 ± 4.9	3–25
Fructosamine (μmol/L) ‡	232 ± 18	229 ± 20	221 ± 50	190–270
Adiponectin (μg/mL) ‡	5.8 ± 2.6	11.0 ± 5.6	8.9 ± 5.3	
BMI 25–30 (kg/m^2)	7.0 ± 2.7			Men 4–20
		11.4 ± 7.1		Women 5–28
BMI > 30 (kg/m^2)	4.3 ± 1.7			Men 2–20
		10.3 ± 3.4		Women 4–22
Serum			
hs-IL6 (pg/mL) ‡	0.78 ± 0.44	0.72 ± 0.40	0.75 ± 0.41	0.447–9.96
hs-CRP (mg/dL) ‡	3.6 ± 4.2	2.7 ± 2.0	3.1 ± 3.1	1.0–3.0 mg/L

* Values are the means ± SD (n=25 for age, weight, BMI, BMI category, glucose, insulin, hs-IL6, hs-CRP; n = 24 for fructosamine; n = 23 for adiponectin).
† Participant characteristics at baseline.
‡ Participant characteristics at entry to first intervention.

(Table 1). Body weight, BMI, or percent fat mass were not significantly different compared to the baseline, nor between treatment periods (data not presented). Fourteen participants were classified as overweight, and 11 participants were classified as obese. No significant relationships between gender, body weight, BMI, or percent fat mass and the changes, or lack of changes, in glucose, insulin, HOMA-IR, inflammatory or anti-inflammatory biomarkers were found; therefore, all further analyses were conducted based on the intervention (control, low-dose flaxseed, high-dose flaxseed). The biomarker values at the beginning of each intervention were analyzed to determine if there was any effect of order or carryover effect from the previous period. No statistically significant differences or correlations were noted; therefore, the 2-week washout between intervention periods was sufficient.

Table 2 shows the nutrient composition of the ground flaxseed supplement. Participants reported the ground flaxseed was palatable when added to foods such as applesauce, pudding, stews, or fruit smoothies and return of the tubes that contained the flaxseed (empty or containing flaxseed that was not consumed) indicated compliance with the intervention protocol (>98% of flaxseed consumed during interventions). Based on self-administered 24-hour dietary recalls, no significant differences were observed in mean daily energy, protein, carbohydrate, fat, saturated fat, polyunsaturated fat, monounsaturated fat, trans-fat, total fiber, cholesterol, omega-3 fatty acids or omega-6 fatty acids between each treatment period of the study (Table 3). Soluble fiber and Vitamin E intake were significantly different between treatment periods (Table 3).

Table 3 – Dietary intake across treatment periods*

	Control	Low dose	High dose
Total kcal	2145 ± 851	2279 ± 696	2417 ± 929
Protein (g)	90 ± 24	98 ± 19	102 ± 36
Carbohydrate (g)	248 ± 124	263 ± 101	262 ± 98
Total fat (g)	88 ± 40	90 ± 34	106 ± 59
Saturated fat (g)	27 ± 10	28 ± 9	30 ± 13
Polyunsaturated fat (g)	10 ± 6	9 ± 6	13 ± 7
Monounsaturated fat (g)	19 ± 12	19 ± 12	22 ± 12
Cholesterol (mg)	290 ± 117	314 ± 133	318 ± 177
Omega-3 fatty acids (g)	0.86 ± 0.6	0.70 ± 0.4	1.90 ± 3
Omega-6 fatty acids (g)	8 ± 6	7 ± 5	9 ± 6
Trans-fatty acids (g)	0.8 ± 1.0	0.8 ± 0.8	1.0 ± 1.9
Total fiber (g)	22 ± 10	24 ± 9	30 ± 18
Soluble fiber (g)†	1.9 ± 1.5	1.9 ± 1.7	2.4 ± 1.3
Vitamin E (mg)†	5 ± 3	6 ± 7	7 ± 3.5
Selenium (μg)	78 ± 64	71 ± 30	83 ± 56

* Data presented as means ± SD. n = 25 for all dietary intake values presented.
† Significantly different, as determined by ANOVA, between control and high dose (26 g) treatment periods ($P < .05$).

3.2. *Glycemic control and inflammatory markers*

Table 4 shows differences by treatment for fasting glucose, insulin, HOMA-IR, fructosamine, hs-CRP, IL-6, adiponectin, and ALA. Plasma glucose and insulin, HOMA-IR, and normalized percent of plasma ALA showed a significant difference by treatment (multiple analysis of variance, $P = .036$, $P = .013$, $P = .008$, and $P = .024$, respectively). Paired t tests showed change in plasma glucose for the 13 g treatment period was significantly different than the 0 g period ($P = .036$). The change in plasma insulin for the 13 g treatment period was significantly different than the 26 g ($P = .021$) and 0 g ($P = .013$) periods. The change in HOMA-IR for the 13 g period was significantly different than the 26 g ($P = .012$) and 0 g ($P = .008$). The change in normalized percent of plasma ALA for the 0 g period was significantly different than the 13 g ($P = .026$) and the 26 g ($P = .018$) periods and the 13 g period was significantly different than the 26 g ($P = .000$) period. Raised plasma ALA demonstrated compliance to the study was good. The mean changes in plasma fructosamine, serum hs-CRP, adiponectin, and IL-6 were not significantly different between treatment periods.

4. Discussion

Pre-diabetes is characterized by hyperglycemia due to ineffective insulin or "insulin resistance." Over time, people with

Table 2 – Macro- and micronutrient analysis of the ground flaxseed

	13 g ground flaxseed	26 g ground flaxseed
Total kilocalories	70 without dietary fiber 82 with dietary fiber	140 without dietary fiber 164 with dietary fiber
Carbohydrate (g)	4	8
Carbohydrate (kcal)	16	32
Protein (g)	3	6
Protein (kcal)	12	24
Fat (g)	6	12
Fat (kcal)	54	108
α-linolenic acid (omega-3 ALA; g)	2.9	5.8
Total fiber (g)	3	6
Soluble fiber (g)	1	2
Insoluble fiber (g)	2	4
Lignans (mg)		
Secoisolariciresinol diglucoside	56.99	113.98
Matairesinol	0.68	1.37
Lariciresinol	0.69	1.38
Pinoresinol	0.69	1.38

Table 4 – Biological values and change measured from the control, low dose flaxseed and high dose flaxseed treatment periods [*,†]

	Control			Low dose			High dose		
	0 wk	12 wk	Δ	0 wk	12 wk	Δ	0 wk	12 wk	Δ
Plasma									
Glucose (mg/dL)	105 ± 22	113 ± 19	+8 ± 21[a]	112 ± 17	110 ± 17	−2 ± 8[b]	112 ± 18	113 ± 18	+1 ± 12[a,b]
Insulin (mU/L)	9.4 ± 4.3	11.4 ± 8.1	+2.0 ± 6.8[a]	11.5 ± 6.6	9.6 ± 5.2	−2.0 ± 4.7[b]	10.5 ± 4.7	11.5 ± 7.1	+1.0 ± 4.3[a]
Fructosamine (μmol/L)	226 ± 17	231 ± 16	+5 ± 12	228 ± 18	230 ± 17	+2 ± 18	230 ± 20	229 ± 17	−1 ± 13
HOMA-IR	2.5 ± 1.2	3.1 ± 2.0	+0.7 ± 1.8[a]	3.2 ± 1.9	2.6 ± 1.4	−0.7 ± 1.5[b]	2.9 ± 1.4	3.3 ± 2.0	+0.4 ± 1.2[a]
Adiponectin (μg/mL)	9.4 ± 5.1	9.1 ± 5.6	−0.0 ± 1.4	8.4 ± 4.7	8.9 ± 5.5	+0.4 ± 1.7	9.3 ± 6.2	9.2 ± 5.2	−0.1 ± 2.4
Serum									
hs-IL6 (pg/mL)	0.67 ± 0.23	0.64 ± 0.26	−0.04 ± 0.21	0.68 ± 0.30	0.71 ± 0.33	+0.03 ± 0.24	0.75 ± 0.39	0.78 ± 0.38	+0.03 ± 0.39
hs-CRP (mg/L)	2.9 ± 3.0	3.8 ± 3.8	+0.9 ± 2.1	3.0 ± 3.2	3.4 ± 3.2	+0.4 ± 2.4	3.2 ± 2.8	2.8 ± 2.7	−0.4 ± 1.2
Linoleic acid (%)	30.7 ± 2.9	30.3 ± 4.7	−0.4 ± 3.2	30.1 ± 4.6	29.4 ± 4.4	−0.8 ± 3.0	31.5 ± 4.0	30.5 ± 4.1	−1.0 ± 2.6
ω-6 fatty acid (%)	39.6 ± 3.3	39.7 ± 5.3	+0.1 ± 3.6	38.8 ± 4.6	38.3 ± 4.8	−0.5 ± 3.6	38.6 ± 7.9	39.4 ± 3.8	+0.8 ± 8.6
ω-3 fatty acid (%)	2.3 ± 0.9	2.3 ± 0.7	+0.0 ± 0.9	2.1 ± 0.8	2.5 ± 0.6	+0.4 ± 0.8	2.3 ± 0.8	2.7 ± 0.9	+0.5 ± 0.7
ALA (%)	0.5 ± 0.3	0.5 ± 0.2	−0.0 ± 0.3[a]	0.5 ± 0.2	0.7 ± 0.2	+0.2 ± 0.2[b]	0.5 ± 0.3	0.8 ± 0.3	+0.3 ± 0.3[c]
EPA (%)	0.5 ± 0.4	0.4 ± 0.3	−0.0 ± 0.4	0.4 ± 0.3	0.5 ± 0.3	+0.1 ± 0.3	0.5 ± 0.4	0.6 ± 0.4	+0.1 ± 0.4
DHA (%)	1.0 ± 0.3	1.0 ± 0.4	+0.0 ± 0.3	1.0 ± 0.	1.0 ± 0.3	+0.1 ± 0.4	1.1 ± 0.3	1.0 ± 0.3	−0.1 ± 0.4

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

[*] Values are the means ± SD (n = 25 for Control, Low Dose and High Dose glucose, fructosamine, HOMA-IR, hs-IL6, hs-CRP, linoleic acid, ω-6 fatty acid, ω-3 fatty acid, ALA, EPA, and DHA; n = 24 for control, low-dose and high-dose insulin; n = 23 for control, low-dose and high-dose adiponectin); Δ's in the same row with different superscripts are significantly different ($P < .05$) as determined by multiple analysis of variance.

[†] Values for serum fatty acids presented as normalized percentages (%) of total fat.

pre-diabetes who do not control their blood glucose concentrations may progress to type 2 diabetes and experience complications including cardiovascular disease, peripheral vascular disease, hyperlipidemia, retinopathy, neuropathy, and/or microalbuminuria and nephropathy. Optimal (and conventional) interventions for pre-diabetes are glucose control through diet, exercise, and, if necessary, medications. An efficient but still uncommon intervention is flaxseed supplementation as an adjunct therapy to conventional treatment(s). Very few randomized clinical studies have examined the influence of flaxseed or defatted flaxseed supplementation on glycemic control or inflammation in people who are healthy [12,25,32] or have type 2 diabetes [33,34] or dyslipidemia [35–38]. Only one study prior to ours reported flaxseed's influence on glycemic control and inflammatory biomarkers in obese glucose intolerant people [26]. Thus, we examined the effects of varying doses of ground flaxseed supplementation on biomarkers associated with pre-diabetes, specifically, glycemic control and response (eg, glucose, insulin, and fructosamine), inflammation (IL-6 and CRP), and obesity/adipokines (adiponectin).

The current study found that a low dose of daily flaxseed supplementation decreased insulin resistance in overweight and obese, glucose-intolerant people. Fasting insulin values significantly decreased with daily consumption of 13 g of ground flaxseed compared to 0 g (control) or 26 g ground flaxseed. There were, however, no significant differences for change in plasma insulin between 26 g ground flaxseed and the control intervention. Similarly, 13 g ground flaxseed significantly decreased plasma glucose and the HOMA-IR index compared to control (0 g flaxseed) but there were no differences for change in glucose or HOMA-IR index between 13 g and 26 g ground flaxseed or between 26 g flaxseed and control. These results differ from others reporting decreased HOMA-IR following flaxseed supplementation without significant changes in insulin [26,33,35]. Studies administering much higher doses of flaxseed than ours have shown no significant changes in plasma glucose or insulin concentrations [26,33,34]. The outcome difference might be related to differences in sample size (9 vs. 25 participants) [26], methods of delivering flaxseed supplementation (eg, baked in bread, flaxseed-derived lignans) [26,33,35], or participant disease states (eg, type 2 diabetes, hypercholesterolemia) [33,35].

Fasting fructosamine values did not change with daily consumption of 13 or 26 g ground flaxseed. Previous studies report flaxseed supplementation either decreases [33] or does not affect [34] HbA_{1c} in people with type 2 diabetes. To our knowledge, this is the first study to determine the effect of flaxseed on fructosamine in people who are overweight or obese and glucose intolerant.

IL-6 and CRP are sensitive physiological biomarkers of subclinical systemic inflammation and are proposed to play key roles in the relationship among obesity, inflammation, insulin resistance, hyperglycemia, and type 2 diabetes [39–42]. Our study found no significant differences in IL-6 or CRP between the 3 treatment periods. These findings agree with other studies that show no significant changes in IL-6 following flaxseed supplementation when administered in capsule form or incorporated into baked goods [25,26,34,35]. Our CRP results mostly agree with other investigations that report CRP concentration remained the same following flaxseed or flaxseed lignan supplementation, while CRP concentration in the control supplementation group increased [24–26,35,43]. Our study participants were similar to those in the recent study conducted by Rhee and Brunt [26]. And, like the Rhee and Brunt study, our IL-6 and CRP concentrations remained in normal or near normal ranges across the three treatment periods. As has been proposed by others, overweight or obese participants may not have had low grade systemic inflammation even though they were a high cardiovascular disease risk group [26,44].

Adiponectin is an anti-inflammatory adipocyte secretory protein that may have a protective role in diabetes development [45,46]. We did not see a significant change in adiponectin values with either dose of flaxseed. To our knowledge, only 2 studies in the literature have reported the effect of either flaxseed oil or milled flaxseed on adiponectin concentrations [34,37]. Of these, one was conducted in non-diabetic, dyslipidemic men [37] while the other was conducted in normal weight individuals with well-controlled type 2 diabetes [34]. No significant changes were found for adiponectin following flaxseed supplementation in either of these previous two studies [34,37], which is in agreement with our results.

The mechanism(s) by which flaxseed exerts its effect on glycemic control has yet to be fully identified and a plausible explanation for the observed changes with the low-dose intervention but not the high-dose intervention has yet to be documented. Flaxseed contains two components that have been reported to influence the incidence of pre-diabetes as well as type 2 diabetes: soluble fiber and lignans. In this study, we were unable to assess the specific roles of fiber and lignans on the biomarkers measured due to the use of whole ground flaxseed. Flaxseed also contains the n-3 fatty acid α-linolenic acid (ALA) which has been shown to have positive effects on inflammation and cardiovascular disease even though its role in the development and treatment of pre-diabetes and type 2 diabetes is still controversial [24–26,35–38,47]. In this study, the flaxseed and its ALA component did not change CRP or hs-IL-6 values that were measured as markers of inflammation. Alpha-linolenic acid concentrations were used as a marker of compliance with the interventions and ALA increased with increased consumption of ground flaxseed. This increase was statistically significant with the high dose of the flaxseed (26 g/day) compared to the control period and there was a non-significant increase with the low dose of flaxseed (13 g/day; $P = .067$) compared to the control period. Based on the changes in ALA concentrations, we believe that the participants were compliant with both interventions.

There are limitations that will be acknowledged and addressed regarding this study. A control period was utilized rather than a placebo since an inert placebo for flaxseed was not identified. Although it is unlikely, not taking a supplement during the control period may have inadvertently had an impact on the biomarkers measured even though participants completed all other aspects of the study (diet records, every other week study visits, weight checks, blood draws, etc.) during that period. Participants were asked to store flaxseed at 0°C until use but verification of participant compliance with these instructions was not assessed. Prolonged storage of the flaxseed at temperatures greater than 0°C may have resulted in instability of the active components and influenced the biomarkers assessed during the study.

Participants in this study did not have elevated inflammatory markers which may have impaired our ability to assess improvement in those biomarkers associated with flaxseed consumption. Control for consumption of anti-inflammatory medications was self-reported by participants. Participants may not have provided accurate information about their use of anti-inflammatory medications during the study period, confounding our ability to observe improvement in those values associated with flaxseed consumption. Future studies that explore the relationship between flaxseed intake and reductions in inflammation should include individuals that have elevated inflammatory markers at baseline.

In conclusion, we accept the hypothesis that regular low dose (13 g) flaxseed consumption improves biomarkers for pre-diabetes, including insulin, glucose, HOMA-IR and fructosamine values. We found insufficient evidence to accept the hypothesis that that regular low dose or high dose (26 g) flaxseed consumption improves CRP or IL-6 values. We also found insufficient evidence to explain why the low dose flaxseed improved biomarkers for pre-diabetes but this effect was not observed for the high dose intervention. This study was designed as a short-term trial and, based on the results obtained, provides enough preliminary evidence to support a longer-term multi-center trial exploring this relationship. A longer-term trial should include measurement of HbA_{1c} in addition to fructosamine as markers of long-term glycemic control. In addition, we recommend that participants track their fasting and post-prandial glucose values daily using a glucometer to better assess the day-to-day impact of flaxseed consumption on glucose concentrations.

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