ORIGINAL RESEARCH

CURRENT DEVELOPMENTS IN NUTRITION

Bioactive Food Components and Dietary Supplements

Linoleic Acid–Rich Oil Supplementation Increases Total and High-Molecular-Weight Adiponectin and Alters Plasma Oxylipins in Postmenopausal Women with Metabolic Syndrome

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ABSTRACT

Background: The onset of menopause increases the risk of metabolic syndrome (MetS). Adiponectin is an adipokine associated with insulin sensitivity that is lower in people with MetS. Supplementing diets with linoleic acid (LA)-rich oil increased adiponectin concentrations and improved glucose control in women with type 2 diabetes. The effect of LA on adipokines, especially total and the bioactive form of adiponectin, high-molecular-weight (HMW) adiponectin, in women with MetS is unknown.

Objectives: The aim of this study was to explore the effect of supplementation of the diet with an oil rich in LA on adipokines in women with MetS. The effect of the LA-rich oil (LA-oil) on oxylipins, key metabolites that may influence inflammation and metabolism, was also explored.

Methods: In this open-label single-arm pilot study, 18 postmenopausal nondiabetic women with MetS enrolled in a 2-phase study were instructed to consume LA-rich vegetable oil (10 mL/d) as part of their habitual diets. Women consumed an oleic acid–rich oil (OA-oil) for 4 wk followed by an LA-oil for 16 wk. Fasting concentrations of adipokines, fatty acids, oxylipins, and markers of glycemia and inflammation were measured.

Results: After 4 wk of OA-oil consumption, fasting glucose and total adiponectin concentrations decreased whereas fasting C-reactive protein increased. After 16 wk of LA-oil supplementation total and HMW adiponectin and plasma oxylipins increased. Markers of inflammation and glycemia were unchanged after LA-oil consumption.

Conclusions: Supplementation with LA-oil increased total and HMW adiponectin concentrations and altered plasma oxylipin profiles. Larger studies are needed to elucidate the links between these changes and MetS. This trial was registered at clinicaltrials.gov as NCT02063165. *Curr Dev Nutr* 2020;4:nzaa136.

Keywords: linoleic acid, metabolic syndrome, adiponectin, oxylipin, fatty acid composition, adherence

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Abbreviations used: AA, arachidonic acid; ALA, α-linolenic acid; ALM, appendicular lean mass; CRP, C-reactive protein; CUDA, 1-cyclohexyl ureido, 3-dodecanoic acid; DEA, docosatetraenylethanolamide; DiHDoPA, dihydroxydocosapentaenoic acid; DiHOME, dihydroxyoctadecenoic acid; EpO, epoxyoctadecanoic acid; EpODE, epoxyoctadecadienoic acid; EpOME, epoxyoctadecenoic acid; HMW, high molecular weight; HODE, hydroxyoctadecadienoic acid; KODE, keto-octadecadienoic acid; LA, linoleic acid; LA-oil, linoleic acid–rich oil; LMM, lean muscle mass; MetS, metabolic syndrome; OA, oleic acid; OA-oil, oleic acid–rich oil; oxLDL, oxidized low-density lipoprotein; PPARγ, peroxisome proliferator–activated receptor γ; PUHA, 1-phenyl ureido 3-hexanoic acid; SPE, solid phase extraction; TNF-R2, TNF-receptor 2.

Introduction

Metabolic syndrome (MetS) is a group of related conditions including abdominal obesity; elevated triglycerides, glucose, and blood pressure; and low HDL cholesterol (1–3). MetS is associated with higher markers of inflammation and oxidative stress (4) and increases the risk of developing heart disease (5–7) and diabetes (7, 8). The prevalence of MetS increases with age (9–11) and postmenopausal status (11, 12). Indeed, postmenopausal women often experience an increase in adipose mass (13–15), with a shift in adipose to the abdominal

region (13, 15), and a decrease in lean mass (13, 14). Furthermore, compared with premenopausal women, postmenopausal women have higher fasting insulin, fasting glucose, triglycerides, and blood pressure (16).

MetS is associated with reduced concentrations of both total adiponectin (4, 17) and high-molecular-weight (HMW) adiponectin (18). Adiponectin is a peroxisome proliferator–activated receptor-γ (PPARγ) responsive gene product (19) secreted primarily from adipose tissue (20). The HMW form of adiponectin may be the more biologically active form (21). Adiponectin concentrations are negatively associated with insulin resistance (22), waist circumference, and adipose mass (23), and positively associated with lean mass (24) in postmenopausal women. Although adiponectin concentrations often increase in women after menopause (22), postmenopausal women with MetS have reduced concentrations of adiponectin when compared with postmenopausal women without MetS (25), suggesting that adiponectin may be a potential target for the treatment of MetS especially in postmenopausal women.

Linoleic acid (LA) is an essential dietary PUFA that is decreasing in many US crop seed oils (26, 27). Yet, increasing LA in the diet, even without changing other aspects of the diet or dietary fat intake, positively alters adiponectin concentrations, insulin sensitivity (28, 29), and body composition (28, 30, 31). Furthermore, higher blood concentrations of LA are associated with reduced risk of type 2 diabetes (32). LA can be oxygenated either enzymatically or through interactions with reactive oxygen to form oxylipins (33, 34), lipid mediators that are altered in plasma lipoprotein of individuals with MetS (35). In addition, LA (36) and some LA-oxylipins (37–39) are believed to be PPARγ agonists and thus have the potential to influence adiponectin concentrations.

The objectives of this pilot study were to determine the impacts of LA-oil supplementation on total and HMW adiponectin and explore the impact of LA-oil supplementation on oxylipin profiles in postmenopausal women with MetS.

Methods

Study design and participants

This pilot and feasibility study (NCT02063165) was a single-arm, 2-phase, open-label intervention study approved by the Ohio State University Institutional Biomedical Review Board (protocol number 2012H0259). All participants provided written informed consent before study participation. This study was conducted according to the Declaration of Helsinki. Partial descriptions of the study design, methods, participant inclusion and exclusion criteria, and participant characteristics have been previously reported (24).

Overweight or obese postmenopausal women were recruited from the greater Columbus Ohio area over a 1-y period (2014–2015). Inclusion and exclusion criteria were verified at prescreening and during the screening visit. Inclusion criteria were female, postmenopausal ($\geq$1 y without menses), age $\geq$50 and $\leq$69 y, BMI (in kg/m^2) $\geq$25 and $\leq$55, waist circumference $\geq$88 cm, stable medical therapy and weight for $\geq$3 mo, and $\geq$1 of the following: triglyceride >150 mg/dL or triglyceride-lowering medication, HDL cholesterol <50 mg/dL or lipid-lowering medication, blood pressure >130 mm Hg systolic or >85 mm Hg diastolic or blood pressure-lowering medication, and blood glucose >100 and <126 mg/dL. Exclusion criteria included self-reported substance abuse; impaired cognitive function; current or previous diagnosis of heart, renal, or circulatory diseases or type 2 diabetes; current use of supplements or medications known to affect body composition; current or previous use of oral hypoglycemic agents or exogenous insulin; and gastrointestinal diseases or disorders.

Study visits began between 06:00 and 09:30 after an overnight fast for $\geq$10 h and abstaining from consuming the study oils on the morning of each study visit. Participants were asked not to alter their dietary intake or physical activity throughout the study. Participants received the placebo oil [oleic acid (OA)-rich oil; OA-oil] during a first 4-wk run-in period (weeks −4, 0) and then received the LA-oil for the 16-wk dietary intervention period (weeks 0, 4, 8, 12, and 16). Participants were asked to return unused oils and report any adverse events at each study visit.

Dietary oils

Participants were instructed to consume 1 teaspoon (5 mL) of the study oil twice per day during the entire study. Participants were given examples of ways to incorporate the oils into their diet without using the oils for cooking or baking. Participants consumed high-OA safflower oil (LouAna Oils) during the run-in period (7.3 g OA per 9.3 g fat) and high-LA safflower oil (Arista Industries, Inc.) during the intervention period (6.9 g LA per 9.3 g fat). Fatty acid composition of the oils was verified every 3 mo. The OA-oil for the run-in has a similar fatty acid composition to the LA-oil used during the intervention period, with the substitution of OA for LA (**Supplemental Table 1**). Owing to inability to acquire LA-rich safflower oil toward the end of the study, 2 participants were given grapeseed oil, 1 from week 12 to week 16 and 1 from week 8 to week 16, during the intervention. The grapeseed oil (Pompeian Inc.) contained the same amount of LA (6.9 g LA per 9.3 g fat) and an almost identical fatty acid composition to the high-LA safflower oil.

Adherence to protocol

To assess adherence, participants received 1 bottle of preweighed oil at each study visit and a 1-teaspoon measuring spoon at the beginning of the study to measure the oils. Each bottle of returned oil was weighed to calculate the oil consumed during each 4-wk interval between study visits. In addition, participants were instructed to self-record daily consumption. Expected oil consumption each day was 9.3 g or 10 mL (2 teaspoons) during both the run-in and intervention periods.

Fatty acids

Fasting blood samples collected at each study visit were prepared for plasma and erythrocyte samples and used for fatty acid analysis. A subset of 12 women consented to receive adipose biopsies performed by a medical doctor at weeks 0 and 16 as previously described (40). Total lipids were extracted from plasma and adipose samples using chloroform:methanol (2:1, vol:vol) and 0.88% potassium chloride wash (41). FAMEs from plasma, adipose, and study oils were prepared using 5% hydrochloric acid in methanol (42) at 76°C. Erythrocyte fatty acids were extracted and methylated using boron trifluoride at 100°C (43–45). Analysis of all FAMEs was completed as previously described (46). Retention times of sample FAMEs were compared to standards (Matreya, LLC, and Nu-Check Prep Inc.). The percentage of each fatty acid from the total identified is reported (47).

Oxylipins and other lipid mediators

Lipids were first isolated using 10:8:11 (vol:vol:vol) cylcohexane:isopropanol:ammonium acetate and treated with a mineral base to release free fatty acids from esterified lipid pools. Alkaline stable products were then purified by solid-phase extraction and quantified by electrospray ionization LC–tandem MS as previously described (48, 49). Briefly, 100 µL of plasma was enriched with butylated hydroxytoluene, EDTA, and deuterated surrogates in methanol, diluted with isopropanol, homogenized on a mechanical bead beater, and extracted with cyclohexane. The extract was split into organic and aqueous phases with 0.1 M ammonium acetate and centrifugation at 4°C for 5 min at 10,000 × *g*. The aqueous phase was re-extracted with cyclohexane, then the organic phases were combined, dried under vacuum, and reconstituted in 40 µL 1:1 methanol:toluene (vol:vol). To release esterified lipids, the 40 µL total lipid extract was mixed with 100 µL 0.5 M methanolic sodium methoxide, topped with nitrogen, mixed, and incubated for 1 h at 60°C. A 100-µL aliquot of water was then added, the vial was flushed with nitrogen, and then was returned to 60°C for another hour. This 2-step procedure promotes efficient transformation of lipophilic esters including cholesterol esters and triglycerides. Oxylipin free acids were then isolated from cooled samples with 1-cc 10-mg Oasis HLB solid-phase extraction (SPE) 96-well plates (Waters Corp Inc.). The SPE wells were washed with ethyl acetate and methanol and conditioned with 2 mL 0.1% arachidonic acid (AA; 20:4n–6) / 5% MeOH. Hydrolysates were diluted with 0.5 mL 0.1% AA / 5% MeOH and neutralized with 10 µL 20% glacial acetic acid (∼0.17 M), transferred to the SPE wells, and further diluted with 0.5 mL wash solution, which was used to rinse the hydrolysate vial to yield a final solution with 16% organics. The sample was loaded onto the column and extracted by gravity, air dried for 20 min with a light vacuum, and mediators were eluted with 0.5 mL 1.0% acetic acid in methanol, followed by 1.0 mL ethyl acetate, and collected in deep-well polypropylene 96-well plates containing 10 µL 20% glycerol in methanol. Solvents were removed under a vacuum, then residues were reconstituted in 100 µL 100 nM cyclohexylureidododecanoic acid (Sigma-Aldrich) internal standard solution, chilled, and filtered by centrifugation for ∼1 min through a 0.2-µm polyvinylidene difluoride membrane 400-µL 96-well microfilter plate (Agilent Technologies), with eluents collected in 0.45-mL polypropylene microplates. Filtered samples were mat-capped and analyzed directly against authentic calibration standards on an API 6500 QTRAP (Sciex) after residue separation on a 2.1 × 150 mm 0.17-µm BEH column (Waters Corp). Analytes were generally quantified against 6- to 9-point calibration curves. Signals for the parent fatty acids, acylethanolamide of EPA (20:5n–3) and palmitoleic acid (16:1n–7), were assessed based on published mass transitions and retention times relative to quantified compounds, and are reported here as relative abundance across the experimental data sets. Calibrants and internal standards were either synthesized [(1-cyclohexyl ureido, 3-dodecanoic acid (CUDA), 1-phenyl ureido 3-hexanoic acid (PUHA)] or purchased from Cayman Chemical, Medical Isotopes, Avanti Polar Lipids Inc., or Larodan Fine Lipids. Data were processed with AB Sciex MultiQuant version 3.0.

Biochemical analysis

Fasting concentrations of triglyceride, HDL cholesterol, LDL cholesterol, and total cholesterol were measured from whole blood obtained from a finger stick (Cholestech LDX analyzer, Cholestech Corporation). Fasting serum glucose, insulin, and TNF-receptor 2 (TNF-R2) concentrations were analyzed by the Clinical Research Core laboratories for all study visits (at the Ohio State Wexner Medical Center). Serum glucose concentrations were analyzed with an adaptation of the hexokinase-glucose-6-phosphate dehydrogenase method (50) using the Dimension Xpand Clinical Chemistry System (Siemens Medical Diagnostics). Insulin was measured using a solid-phase enzyme-labeled chemiluminescent immunometric assay (Siemens Immulite 1000; Siemens Healthcare Diagnostics Product Ltd.). Insulin intra-assay and interassay CVs were 5.7% and 6.7%, respectively. The HOMA-IR (51) was used to estimate insulin resistance. TNF-R2 concentrations were measured with the Ultra-Sensitive kit from Meso Scale Discovery using a MESO QuickPlex SQ 120. Plasma concentrations of leptin, oxidized LDL (oxLDL), and total adiponectin and high-molecular-weight (HMW) adiponectin were measured using ELISA kits from R&D Systems Inc., Mercodia AB, and Alpco, respectively. Serum concentrations of IL-6 were analyzed using an ELISA kit from R&D Systems Inc. and serum C-reactive protein (CRP) concentrations were measured by ELISA from Immundiagnostik AG.

Clinical assessment and body composition

Blood pressure, temperature, and pulse were measured at each study visit after the participant had been seated and rested for ≥5 min. Weight was measured to the nearest 0.1 kg using a digital scale at each study visit. Height was measured to the nearest 0.1 cm at week −4 and week 16 using a wall-mounted stadiometer.

DXA equipped with Corescan (Lunar iDXA; GE Healthcare) was used to determine whole-body composition and visceral adipose tissue at weeks 0 and 16. Data are reported as mass (kg) and mass normalized to BMI. To assess the validity of this instrument in the study population, 16 participants consented to have the scan twice at the week 0 visit. The CV was 0.37% for lean muscle mass (LMM), 1.09% for appendicular lean mass (ALM), 0.88% for trunk adipose, 0.42% for total adipose, and 5.27% for Corescan visceral fat.

Diet assessment

Dietary intake was assessed in all participants between week −4 and week 0. Participants were instructed to keep one 3-d food record (1 weekend day and 2 weekdays). A registered dietitian reviewed and completed the food records with the participants. The data were entered into Nutrition Data Systems for Research (NDSR, version 2014, University of Minnesota). Dietary data, except kilocalories (kcal), are expressed per 1000 kcal consumed.

Statistical analysis

All statistical analysis was performed with a significance level of 0.05. Analysis of oxylipins and other lipid mediators was performed using JMP Pro 14.1 (JMP, SAS Institute). Before analysis, outliers in the metabolite values were removed using the robust Huber M test. Next, data were transformed to obtain normal distributions using Johnson's transformation. Differences in metabolite mean concentrations were assessed using ANCOVA, with the time of visit as fixed effect and subject as covariate. This approach emphasized the treatment effect (difference between week 0 and week 16) and reduced interindividual variance. The *P*-value threshold was adjusted using Benjamini–

TABLE 1 Participant characteristics[1]

Characteristic	$n = 16$
Age, y	59.8 ± 5.1
Cessation of menses, y	9.1 ± 8.8
Race	
Caucasian	81 (13)
Black or African American	19 (3)
Education	
High school diploma/some college	50 (8)
4-Y degree/some graduate school	19 (3)
Graduate degree	31 (5)
Use of lipid-lowering medications	6 (1)
Use of blood pressure–lowering medications	13 (2)
Dietary intake	
Energy, kcal/d	1663 ± 486
Protein, g/1000 kcal	46 ± 11
Carbohydrate, g/1000 kcal	115 ± 16
Total fat, g/1000 kcal	41 ± 5.1
Saturated fat, g/1000 kcal	14 ± 3.0
Monounsaturated fat, g/1000 kcal	14 ± 2.3
Polyunsaturated fat, g/1000 kcal	9.4 ± 2.7
Linoleic acid, g/1000 kcal	8.1 ± 2.3

[1]Values are mean ± SD or % (*n*) of participants.

Hochberg false discovery rate correction with a $q = 0.2$ (52). The fold difference used in the oxylipin/endocannabinoid network visualizations was calculated by dividing the week-16 time point mean by the week-0 time point mean. Group means were calculated using normally distributed data.

For the remaining analyses, paired *t* tests were used to test for differences in measures from the prebaseline visit (week −4) to the baseline visit (week 0). For analysis of measurements during the intervention period (weeks 0–16), linear mixed models with random intercepts to account for within-subject correlation over time were fitted for each measure. All 16 participants who completed the baseline visit (week 0) were included in these models. If there were significant changes in an outcome over time (based on the test of the week effect), linear contrasts in the mixed models were used to test for differences from baseline (week 0) to each of weeks 4, 8, 12, and 16. All tables show means ± SDs. The ratio of adiponectin to leptin was log transformed for modeling to better approximate normality of residuals. Hypothesis tests were conducted at a 5% type 1 error rate. Adjustments for multiple comparisons were not made, because this is a hypothesis-generating pilot study with a limited sample size. However, only testing for differences from baseline in models with a significant week effect helped reduce the chance of a false finding (i.e., reduced type 1 error). Statistical analyses were conducted using Stata version 15 (53).

Results

Participants' characteristics and adverse events

Eighteen participants enrolled and 15 completed the study. Two withdrew before the week 0 visit and 1 before the week 8 visit. Reasons for withdrawal included adverse events (related or unrelated) and scheduling conflicts. There were 11 reported adverse events related to the consumption of the study oils (5 with OA-oil and 6 with LA-oil). All adverse events were gastrointestinal complaints with 72% of them described as mild and 64% resolving before being reported.

Table 1 reports characteristics of the participants who completed at least the week 0 study visit ($n = 16$). The current DRI for linoleic acid is the Adequate Intake of 11 g/d for women aged ≥51 y (54). Dietary assessment revealed that the mean habitual intake of LA was 13.7 ± 6.2 g/d and with the addition of the LA-oil the mean intake increased to 20.6 ± 6.2 g/d.

Adherence and changes in measurements during the run-in period

The mean consumption of the OA-oil during the 4-wk run-in period was 92% ($n = 16$) of that expected from the oil weight and 96% ($n = 5$) of that expected from self-report (Table 2). The OA-oil did not alter plasma or erythrocyte LA or OA concentrations (**Supplemental Table 2**). Fasting serum glucose ($P = 0.0001$) and fasting plasma total adiponectin concentrations ($P < 0.0001$) decreased whereas CRP ($P = 0.046$) increased from week −4 to week 0. There were no other changes in any of the study measurements measured after the 4-wk run-in period.

Adherence and changes in measurements during the intervention period

Participants were instructed to consume 10 mL (2 teaspoons) of the LA-oil per day during the 16-wk intervention period. Adherence measured by the oil weight was similar throughout the intervention period with a mean of 81% (weeks 0–4; $n = 16$), 79% (weeks 4–8; $n = 14$), 78% (weeks 8–12; $n = 14$) and 79% (weeks 12–16; $n = 13$). During the intervention period fewer participants completed the self-reported oil consumption at each visit than returned the unused oil. Self-reported adherence was on average 96% (weeks 0–4; $n = 9$), 92% (weeks 4–8; $n = 8$), 93% (weeks 8–12; $n = 6$), and 90% (weeks 12–16; $n = 7$).

Table 3 presents plasma, erythrocyte, and adipose tissue fatty acid composition results. LA in plasma, erythrocytes, and adipose tissue did not change significantly between week 0 and week 16. However, in both plasma and erythrocytes LA significantly increased ($P = 0.05$) at weeks 8 and 12 compared with week 0. AA was unchanged in erythrocytes ($P = 0.86$) and adipose tissue ($P = 0.30$), but decreased in plasma

TABLE 2 Adherence to OA-oil and LA-oil consumption[1]

	Week −4 to week 0	Week 0 to week 4	Week 4 to week 8	Week 8 to week 12	Week 12 to week 16
Weight, %	92 ± 38 (16)	81 ± 32 (16)	79 ± 33 (14)	78 ± 28 (14)	79 ± 23 (13)
Self-record, %	96 ± 5 (5)	96 ± 4 (9)	92 ± 8 (8)	93 ± 10 (6)	90 ± 11 (7)

[1]Values are mean ± SD (*n*); specifically, mean ± SD percentage of adherence (expected oil consumption) from either weight of the oil or self-record of daily teaspoon consumption. OA-oil consumption week −4 to week 0; LA-oil consumption week 0 to week 16. Participants were instructed to consume 9.3 g or 10 mL/d (2 teaspoons) of oil. LA-oil, linoleic acid–rich oil; OA-oil, oleic acid–rich oil.

TABLE 3 Fatty acids composition during the LA-rich oil intervention period[1]

	Week 0	Week 4	Week 8	Week 12	Week 16	*P* value[2]
Plasma						
OA	19 ± 1.7	18 ± 2.7	18 ± 2.3	17 ± 1.9	18 ± 2.10	0.07
ALA	0.65 ± 0.21	0.64 ± 0.19	0.60 ± 0.17	0.55 ± 0.16	0.62 ± 0.17	0.13
LA	31 ± 4.8	32 ± 4.5	33 ± 4.1*	33 ± 4.3*	32 ± 4.3	0.05
AA	7.6 ± 2.5	7.3 ± 2.5	7.1 ± 2.1*	7.4 ± 2.1*	7.2 ± 2.4*	0.01
EPA	0.47 ± 0.13	0.53 ± 0.17	0.47 ± 0.14	0.49 ± 0.12	0.45 ± 0.09	0.32
DHA	2.3 ± 0.45	2.2 ± 0.67	2.0 ± 0.41	2.0 ± 0.40	2.1 ± 0.33	0.22
Erythrocyte						
OA	14 ± 1.1	13 ± 0.78	14 ± 1.0	13 ± 0.95	13 ± 1.2	0.49
ALA	0.17 ± 0.06	0.16 ± 0.07	0.14 ± 0.06	0.13 ± 0.06*	0.18 ± 0.06	0.01
LA	13 ± 2.1	14 ± 2.0	14 ± 1.9*	14 ± 2.1*	14 ± 1.6	0.05
AA	15 ± 2.1	16 ± 2.0	16 ± 2.0	16 ± 1.8	16 ± 1.6	0.86
EPA	2.1 ± 0.38	2.1 ± 0.36	2.1 ± 0.31	2.0 ± 0.33	2.0 ± 0.32	0.39
DHA	3.4 ± 0.89	3.3 ± 0.86	3.3 ± 0.77	3.1 ± 0.55	3.2 ± 0.68	0.32
Adipose tissue						
OA	44 ± 2.4	_	_	_	45 ± 2.9	0.16
ALA	1.0 ± 0.26	_	_	_	1.0 ± 0.25	0.60
LA	20 ± 2.8	_	_	_	20 ± 3.3	0.14
AA	0.59 ± 0.25	_	_	_	0.54 ± 0.22	0.30
EPA	0.21 ± 0.058	_	_	_	0.22 ± 0.084	0.62
DHA	0.16 ± 0.079	_	_	_	0.13 ± 0.066	0.07

[1]Values are mean ± SD percentages of total fatty acids. *Significantly different from week 0 at $P < 0.05$ level. Plasma and erythrocyte tests: week 0, $n = 16$; week 4, $n = 16$; week 8, $n = 15$; week 12, $n = 14$; week 16, $n = 15$. Adipose tests: week 0, $n = 12$; week 16, $n = 10$. AA, arachidonic acid; ALA, α-linolenic acid; LA, linoleic acid; OA, oleic acid.
[2]*P* value from visit effect in linear mixed model.

($P = 0.01$) starting at week 8 and remained lower than week 0 at both weeks 12 and 16. α-Linolenic acid (ALA; 18:3n–3) decreased at week 8 in erythrocytes but did not change in plasma ($P = 0.13$) or adipose tissue ($P = 0.60$). OA, EPA, and DHA (22:6n–3) were all unaltered in plasma, erythrocytes, and adipose tissue during LA-oil supplementation.

Total adiponectin increased at week 4 during the intervention period and remained higher at week 16 than at week 0 (**Table 4**). HMW adiponectin was also significantly increased from week 0 to week 16 of the intervention period ($P < 0.0001$). No changes were seen in leptin, TNF-R2, and CRP, but IL-6 concentrations were altered during the intervention period ($P = 0.0007$), increasing from week 0 to week 8 and then decreasing from week 8 to week 16. As a result, only the week 8 concentration was significantly different from week 0. Glucose ($P < 0.001$), insulin ($P = 0.03$), and HOMA-IR values ($P = 0.004$) transiently increased over the intervention period but were unchanged by week 16 compared with week 0. Starting at week 4, HOMA-IR and glucose were elevated (compared with week 0) and remained higher at weeks 8 and 12, whereas insulin concentrations rose at weeks 8 and 12. HDL, LDL,

TABLE 4 Biochemical measurements during the LA-oil intervention period[1]

	Week 0	Week 4	Week 8	Week 12	Week 16	*P* value[2]
Total adiponectin, μg/mL	6.8 ± 2.7	9.0 ± 3.2*	8.4 ± 3.0*	9.1 ± 3.5*	8.3 ± 3.2*	<0.0001
HMW adiponectin, μg/mL	3.7 ± 2.0	_	_	_	4.9 ± 2.1*	<0.0001
Leptin, ng/mL	30 ± 19	_	27 ± 21	_	29 ± 23	0.61
Adiponectin to leptin ratio[3]	0.3 ± 0.3	_	0.4 ± 0.2*	_	0.5 ± 0.6*	0.0058
IL-6, pg/mL	2.3 ± 1.6	2.7 ± 1.6	3.5 ± 2.3*	2.9 ± 1.3	1.9 ± 0.89	0.0007
oxLDL, U/L	72 ± 21	66 ± 17	64 ± 16	62 ± 16	64 ± 19	0.09
CRP, ng/mL	5445 ± 4676	3658 ± 3428	_	_	4079 ± 3653	0.09
TNF-R2, pg/mL	5074 ± 1346	5266 ± 1138	5313 ± 1209	5089 ± 1212	5055 ± 1162	0.24
Glucose, mg/dL	92 ± 9.5	101 ± 10*	100 ± 7.6*	100 ± 10*	95 ± 11	<0.0001
Insulin, µU/mL	12 ± 5.7	12 ± 4.8	13 ± 6.9*	13 ± 6.4*	11 ± 4.9	0.03
HOMA-IR	2.6 ± 1.3	3.2 ± 1.4*	3.1 ± 1.8*	3.3 ± 1.7*	2.6 ± 1.3	0.004
Triglycerides, mg/dL	146 ± 50	153 ± 81	152 ± 60	136 ± 46	150 ± 57	0.82
HDL cholesterol, mg/dL	54 ± 15	54 ± 15	55 ± 14	54 ± 15	55 ± 16	0.87
LDL cholesterol, mg/dL	112 ± 29	111 ± 36	107 ± 28	111 ± 27	109 ± 24	0.93
Total cholesterol, mg/dL	196 ± 35	196 ± 43	192 ± 35	193 ± 31	195 ± 29	0.92

[1]Values are means ± SDs. *Significantly different from week 0 at $P < 0.05$ level; n varies from 14 to 16. CRP, C-reactive protein; HMW, high molecular weight; LA-oil, linoleic acid–rich oil; oxLDL, oxidized low-density lipoprotein; TNF-R2, TNF-receptor 2.
[2]*P* value from visit effect in linear mixed model.
[3]Log transformed for analysis but shown as untransformed means ± SDs at each time point.

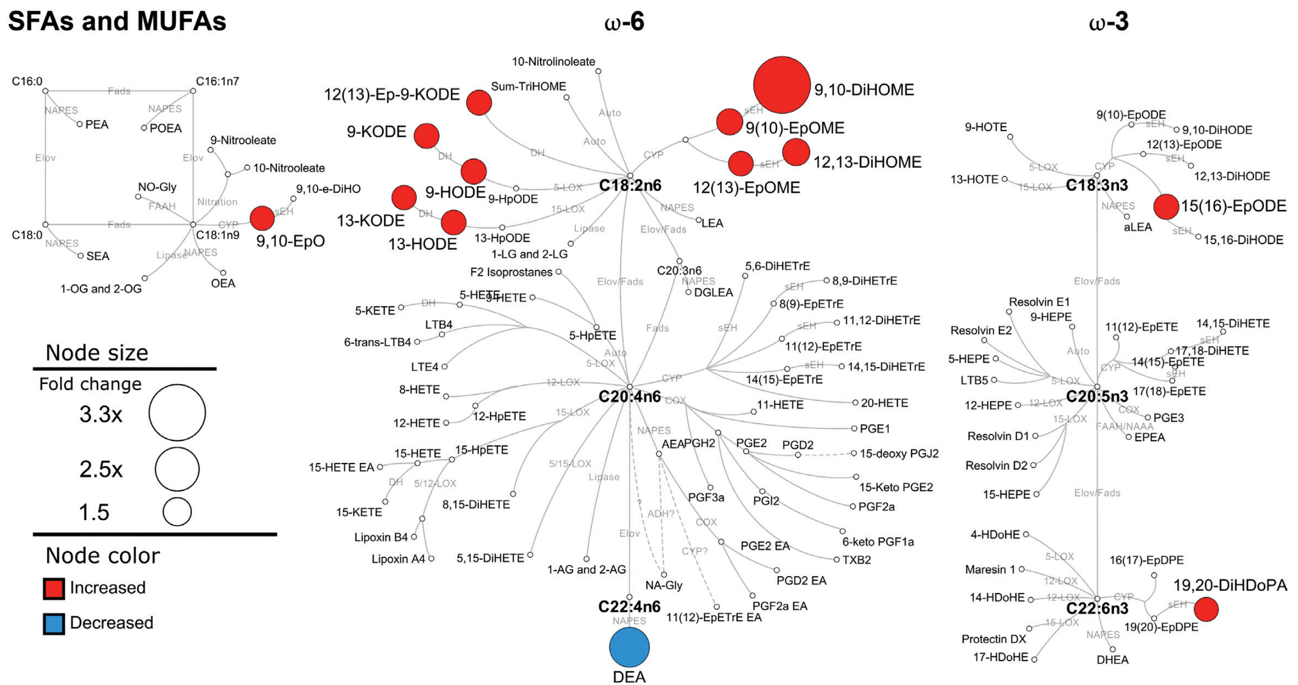

FIGURE 1 Changes in oxylipins and acylethanolamides from week 0 to week 16 of LA-oil supplementation. Network presents fatty acid metabolic pathways, including SFAs and MUFAs and ω-3 and ω-6 fatty acids with oxylipins and endocannabinoid synthesis pathways. Node size represents the fold change and the color represents the directionality of the change: red, increased after 16 wk; blue, decreased after 16 wk. ADH, alcohol dehydrogenase; AEA, arachidonoylethanolamine; AG, arachidonoylglycerol; COX, cyclooxygenase; CYP, cytochrome P450; DEA, docosatetraenylethanolamide; DGLEA, dihomo-gamma-linolenoyl ethanolamide; DH, dehydrogenase; DHEA, docosahexaenoyl ethanolamide; DiHDoPA, dihydroxydocosapentaenoic acid; DiHETrE, dihydroxyeicosatrienoic acid; DiHO, dihydroxyoctadecanoic acid; DiHODE, dihydroxyoctadecadienoic acid; DiHOME, dihydroxyoctadecenoic acid; EA, ethanolamide; Elov, fatty acids elongase; EpETrE, epoxyeicosatrienoic acid; EpO, epoxyoctadecanoic acid; EpODE, epoxyoctadecadienoic acid; EpOME, epoxyoctadecenoic acid; FAAH, fatty acid amide hydrolase; Fads, fatty acid desaturase; HDoHE, hydroxydocosahexaenoic acid; HEPE, hydroxyeicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; HOTE, hydroxyoctadecatrienoic acid; HpETE, hydroperoxyl-eicosatetraenoic acid; HpODE, hydroperoxyoctadecadienoic acid; KETE, keto-eicosatetraenoic; KODE, keto-octadecadienoic acid; LG, linoleoylglycerol; LOX, lipoxygenase; LTB, leukotriene B; LTE, leukotriene E; NAPES, N-acylphosphatidylethanolamine-specific; OEA, oleoylethanolamine; OG, oleoylglycerol; PEA, palmitoylethanolamine; PGD, prostaglandin D; PGE, prostaglandin E; PGF, prostaglandin F; PGI, prostaglandin I; PGJ, prostaglandin J; POEA, palmitoleoyl ethanolamide; SEA, stearoyl ethanolamide; sEH, soluble epoxide hydrolase; TriHOME, trihydroxyoctadecaenoic acid.

and total cholesterol, oxLDL, and triglyceride concentrations were unaltered with LA-oil supplementation.

Concentrations of esterified oxylipins and acylethanolamides in the plasma were measured at week 0 and week 16 (**Figure 1**). Nine LA-oxylipins significantly increased from week 0 to week 16 (**Supplemental Table 3**). 9,10-dihydroxyoctadecenoic acid (DiHOME) had the largest increase followed by 12,13-DiHOME, 9(10)-epoxyoctadecenoic acid (EpOME), 12(13)-EpOME, 13-hydroxyoctadecadienoic acid (HODE), 13-keto-octadecadienoic acid (KODE), 9-HODE, 9-KODE, and 12(13)-Ep-9-KODE, all with a 1.5-fold increase. One OA-oxylipin, 9,10-epoxyoctadecanoic acid (EpO), 1 ALA-oxylipin, 15(16)-epoxyoctadecadienoic acid (EpODE), and 1 DHA-oxylipin, 12,20-dihydroxydocosapentaenoic acid (DiHDoPA), also increased from week 0 to week 16. The only acylethanolamide altered during the intervention period was the AA-derived docosatetraenylethanolamide (DEA), which decreased.

Body composition analyzed by DXA at week 0 and week 16 revealed no changes in total fat, visceral fat, trunk fat, or LMM/BMI in the women (**Table 5**). ALM/BMI increased by 3%, but it was not statistically significant ($P = 0.06$). BMI, blood pressure, temperature, and pulse were unaltered during the intervention period (**Table 6**).

TABLE 5 Body composition measurements during the LA-oil intervention period[1]

	Week 0	Week 16	*P* value[2]
Total fat, kg	34 ± 8.4	34 ± 9.7	0.30
Visceral fat, kg	1.0 ± 0.39	1.0 ± 0.42	0.64
Trunk fat, kg	19 ± 4.5	18 ± 5.2	0.28
ALM/BMI	0.65 ± 0.064	0.67 ± 0.056	0.06
LMM/BMI	1.4 ± 0.15	1.4 ± 0.15	0.16

[1]Values are means ± SDs. Week 0, $n = 14$; week 16, $n = 13$. ALM, appendicular lean mass; LA-oil, linoleic acid–rich oil; LMM, lean muscle mass.
[2]*P* value from visit effect in linear mixed model.

TABLE 6 Vital signs and BMI during the LA-oil intervention period[1]

	Week 0	Week 4	Week 8	Week 12	Week 16	*P* value[2]
Pulse, bpm	70 ± 9.3	71 ± 9.0	75 ± 11	74 ± 9.4	71 ± 11	0.10
Temperature, °C	36 ± 0.43	36 ± 0.39	36 ± 0.43	36 ± 0.41	36 ± 0.30	0.16
Systolic BP, mm Hg	125 ± 16	124 ± 17	130 ± 15	129 ± 13	122 ± 15	0.14
Diastolic BP, mm Hg	79 ± 7.4	78 ± 7.8	80 ± 7.4	78 ± 7.4	78 ± 9.2	0.62
BMI	31 ± 4.3	31 ± 4.7	31 ± 4.8	31 ± 4.9	31 ± 5.1	0.94

[1]Values are means ± SDs. *Significantly different from week 0 at $P < 0.05$ level. Week 0, $n = 16$; week 4, $n = 16$; week 8, $n = 15$; week 12, $n = 15$; week 16, $n = 15$. BP, blood pressure; LA-oil, linoleic acid–rich oil.
[2]*P* value from visit effect in linear mixed model.

Discussion

The risk of MetS increases in women after menopause (12) and MetS increases the likelihood of developing heart disease (5–7) and type 2 diabetes (7, 8). Despite studies showing LA can reduce inflammation and favorably influence glycemia and body composition in women with diabetes (28, 29) and healthy men and women (31), little is known about the effect of LA in MetS. Alarmingly, the supply of LA in the US diet is decreasing as more plant seed oil crops are of the high-OA variety (26, 27). The objectives of this single-arm open-labeled pilot study were to investigate the effects of consuming an oil rich in LA on adiponectin concentrations, fatty acid and oxylipin profiles, and parameters of MetS and to evaluate oil consumption adherence in postmenopausal women with MetS.

There was a transient increase of LA concentrations at weeks 8 and 12; however, the 3% and 4% increase in plasma and erythrocyte LA, respectively, was not significantly different between week 0 and week 16. This is in contrast to our previous study, where a similar amount of LA supplementation, provided to participants in capsule form, increased serum LA concentrations by 11% after 16 wk in a larger cohort of postmenopausal women with type 2 diabetes (28). However, unlike our previous study, the participants in this study were asked to measure out 10 mL/d of the provided study oil using a teaspoon (also provided). The delivery of loose oil may have increased the variability of the actual dose consumed.

Lower concentrations of adiponectin are associated with MetS (18), insulin resistance (55), and risk of type 2 diabetes (56). Both total adiponectin and HMW adiponectin were increased at week 16 compared with week 0, by 22% and 32%, respectively. Whereas the elevation of adiponectin was sustained during the 16-wk intervention period, there was a counterintuitive transient increase in glucose, insulin, and HOMA-IR, which returned to baseline levels at week 16. This is in contrast to our previous study where LA-oil supplementation increased adiponectin by 30% but decreased glucose and HOMA-IR in postmenopausal women with diabetes (28, 29). This lack of a consistent change in glycemic measurements may be linked to the less severe hyperglycemia in this cohort of women with MetS. Furthermore, it is possible that the adiponectin and the markers of glycemia during the intervention period were influenced by the initial decrease of adiponectin and glucose during the run-in period (week −4 to week 0). It is not clear if the increase in adiponectin and glucose during the intervention period would have occurred had there not also been a decrease from week −4 to week 0 when the women consumed the OA-oil. Further examination into effects of both LA and OA on adiponectin and glucose is needed. A study of longer duration, adequate power, or more standardized dosing, e.g., delivery of oil by providing mixed foods or supplements, is needed to fully understand the effects of LA on these markers of glycemia in women with MetS.

Oxylipins have a variety of physiological functions (33, 34) and LA-oxylipins in particular may play a role in energy metabolism (57–59) and inflammation (60). 9,10-DiHOME and 12,13-DiHOME are inversely associated with BMI (57, 61) and waist circumference (61) in healthy individuals. 12,13-DiHOME is also negatively associated with HOMA-IR (57, 59), triglyceride concentrations (57), and adipose mass (59). In mice, 12,13-DiHOME can amplify fatty acid uptake in brown adipose tissue (57) and skeletal muscle (58). 13-HODE and 9-HODE can reduce adipocyte lipid storage and adipokine production in adipocytes (62) and may also play a role in atherosclerosis (63). Although concentrations of 9(10)-EpOME and 12(13)-EpOME are positively associated with microvascular function (64), women with type 2 diabetes have higher concentrations of these epoxides than women without diabetes (48). To our knowledge only 2 other studies have assessed the impact of increasing LA in the diet for 4 wk on oxylipin concentrations in humans (64–66). In the current study an analysis of changes in total esterified oxylipins after 16 wk of LA-oil supplementation was conducted. Not only were LA-oxylipins increased at week 16, but OA-, ALA-, and DHA-oxylipins were as well. The relative change in the 9 LA-oxylipins that increased was larger than the relative change of either plasma or erythrocyte LA. This is consistent with a previous study in men that saw larger relative increases in many LA-oxylipins than in plasma LA after 4 wk of safflower oil supplementation (66). In addition, this increase in LA-oxylipins, in spite of a lack of change in LA, was previously seen in the serum of rats fed a diet with safflower oil for 6 wk (67). In this study, 13-HODE and 9-HODE, both believed to be PPARγ agonists (37–39), demonstrated a 28% and 30% increase, respectively, which is similar to the percentage increases in total adiponectin and HMW adiponectin, suggesting a potential role of these oxylipins in altering adiponectin concentrations. However, more research is needed to identify the physiological effects of LA-oil supplementation, LA-oxylipins, and MetS.

There are several limitations to this study. First, this is a small, open-labeled pilot study in women only, with each individual acting as her own control, as opposed to an independent and double-blinded control group. Second, because there was no control group without LA-oil and there was no washout period between the run-in period using OA-oil and the commencement of using LA-oil at week 0, the results of the 16-wk intervention period may have been influenced by some carryover effects from the run-in period. Third, the daily oil dose was not premea-

sured for the study participants, increasing the likelihood of variation in the oil consumed per person each day. Fourth, 2 of the participants were given grapeseed oil for 4 or 8 wk, which, although it has a nearly identical fatty acid profile to the high-LA safflower oil, is not identical in terms of other compounds including polyphenols and antioxidants. Lastly, the generalizability of our findings to a broader group of people with MetS, e.g., men, youth, and elderly, is not realistic given the limited size of the cohort.

In conclusion, the increases of total and HMW adiponectin and LA-oxylipins after 16 wk of LA-oil supplementation merit further investigation. A future study should evaluate the effects of supplementation of LA in men and women with MetS to improve insulin sensitivity and affect cellular pathways downstream from adiponectin in a large, randomized, placebo-controlled clinical trial.

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