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Comparison of dietary conjugated linoleic acid with safflower oil on body composition in obese postmenopausal women with type 2 diabetes mellitus[1–4]

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ABSTRACT

Background: Weight loss may improve glucose control in persons with type 2 diabetes. The effects of fat quality, as opposed to quantity, on weight loss are not well understood.

Objective: We compared the effects of 2 dietary oils, conjugated linoleic acid (CLA) and safflower oil (SAF), on body weight and composition in obese postmenopausal women with type 2 diabetes.

Design: This was a 36-wk randomized, double-masked, crossover study. Fifty-five obese postmenopausal women with type 2 diabetes received SAF or CLA (8 g oil/d) during two 16-wk diet periods separated by a 4-wk washout period. Subjects met monthly with the study coordinator to receive new supplements and for assessment of energy balance, biochemical endpoints, or anthropometric variables.

Results: Thirty-five women completed the 36-wk intervention. Supplementation with CLA reduced body mass index (BMI) (P = 0.0022) and total adipose mass (P = 0.0187) without altering lean mass. The effect of CLA in lowering BMI was detected during the last 8 wk of each 16-wk diet period. In contrast, SAF had no effect on BMI or total adipose mass but reduced trunk adipose mass (P = 0.0422) and increased lean mass (P = 0.0432). SAF also significantly lowered fasting glucose (P = 0.0343) and increased adiponectin (P = 0.0051). No differences were observed in dietary energy intake, total fat intake, and fat quality in either diet period for either intervention.

Conclusions: Supplementation with CLA and SAF exerted different effects on BMI, total and trunk adipose mass, and lean tissue mass in obese postmenopausal women with type 2 diabetes. Supplementation with these dietary oils may be beneficial for weight loss, glycemic control, or both. *Am J Clin Nutr* 2009;90:468–76.

INTRODUCTION

More than 800,000 people each year are newly diagnosed with type 2 diabetes mellitus (T2DM) in the United States (1). Obesity is highly correlated with and a contributing factor for the development of T2DM (2). Android pattern obesity is a dominant criterion leading to insulin resistance and accompanies weight gain, especially in postmenopausal women (3, 4). A fundamental approach to managing T2DM is weight loss, which may improve insulin sensitivity (5). In general, greater weight loss has been associated with a larger magnitude of improvement in glycated hemoglobin (Hb A_{1c}) and fasting glucose concentrations (6–8). These findings suggest that weight loss may enhance the efficacy of a hypoglycemic agent to induce clinically relevant improvements in T2DM management (6–8).

Because weight loss in obese people is difficult to achieve and maintain (9), dietary and pharmaceutical approaches have been the focus of much investigation. The dietary oil, conjugated linoleic acid (CLA), has reduced body weight and adipose in some clinical studies (10, 11) and animal models (12–14) for diet-induced obesity. Therefore, CLA has been promoted as a weight-loss supplement. An effective dose of CLA for loss of weight and adipose in humans may be between 1.4 and 6.4 g CLA mixed isomers (11). The isomer of CLA shown to lower adipose mass in mice is *t*10*c*12-CLA (15). Commercially prepared CLA oil contains approximately 78% total CLA, primarily comprising equal amounts of *c*9*t*11-CLA and *t*10*c*12-CLA. This commercial mixture of CLA oil is used in most clinical studies because naturally occurring CLA from ruminant meats and dairy products is actually quite low in the adipose-lowering *t*10*c*12-CLA isomer (16). In comparison, safflower oil (SAF) is colorless, flavorless, and rich in the essential n–6 (omega-6) fatty acid, linoleic acid (≈78% linoleic acid, *c*9*c*12-linoleate). Linoleic acid has exhibited variable effects on adipogenesis in experimental animal models for obesity (17). SAF is readily available and affordable in US markets for cooking.

The objective of this study was to compare the effects of CLA and SAF on changes in body weight, body composition, and adipose distribution among obese postmenopausal women with

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T2DM. We examined the effects of these dietary oils while subjects continued to use their oral hypoglycemic medications.

SUBJECTS AND METHODS

Subjects

Fifty-five postmenopausal women with T2DM were recruited from the Columbus, Ohio, area. All subjects provided written informed consent and signed authorizations for Health Insurance Portability and Accountability Act before enrolling in the study. The following inclusion criteria were verified at screening: female, ≤70 y of age, postmenopausal (absence of menses ≥1 y), obese [body mass index (BMI; in kg/m^2) >30], Hb A_{1c} ≥6.5% and ≤14%, and normal hepatic enzyme activity. Exclusion criteria ruled out potential subjects with tobacco use, substance abuse, renal or liver disease, malignant tumors, impaired cognitive function, insulin or hormone replacement therapies, or placement of a pacemaker or defibrillator.

Study design

The study was a randomized, double-masked, crossover design conducted at a single site. A product coordinator was responsible for assigning subjects to a treatment order and allocating the dietary oil supplements into coded and numbered containers, ensuring the study would remain double-masked. Study visits were conducted at the Clinical Research Center (CRC) at the Ohio State University. Semiannually, data were summarized by masked study staff and reviewed by the Data Safety Monitoring Committee. The study protocol was approved by the Ohio State University Institutional Review Board and CRC Advisory Board. Recruitment began in April 2004 and ended in July 2007. The study was conducted in agreement with the Declaration of Helsinki and in accordance with International Conference on Harmonization/Good Clinical Practice Guidelines.

Recruitment occurred over a 2.9-y period. After providing written informed consent, subjects reported to the CRC for a screening visit to document and assess demographic information, medical history, Hb A_{1c}, hepatic enzyme concentrations, height and weight to determine BMI, cognitive function with the use of an Orientation-Memory-Concentration test (18), and willingness to comply with the study protocol. Eligible subjects were randomly assigned in block fashion to 2 groups based on their BMI and Hb A_{1c} concentrations at screening. All subjects received CLA and SAF treatments in the crossover design. Subjects reported to the CRC in the early morning (before 1000) to control for diurnal variations in hormone concentrations. Subjects fasted overnight for a minimum of 10 h before each study visit and were asked to abstain from taking their prescribed diabetes medications and treatment capsules the morning of each study visit. The initial diet period (diet period 1) was 16 wk in duration, followed by a 4-wk washout period and a second 16-wk diet period (diet period 2). A 4-wk washout period is typical of feeding trials with fatty acid supplementation (19). Adverse events were reported at each monthly visit.

Subjects consumed 8 dietary oil capsules daily, with instructions to take 2 capsules with each meal and 2 capsules at night for a total of 8.0 g dietary oil daily. Each CLA capsule contained 1.0 g CLA-80 oil. The CLA treatment capsules (Cognis Corporation, Cincinnati, OH) provided 6.4 g CLA isomers and 1.6 g oil composed primarily of oleic and palmitic acids per day. Each SAF capsule contained 1.0 g SAF. The fatty acid composition of oils is shown in **Table 1** (20). Oils were periodically analyzed for composition throughout the duration of the study (approximately every 6 mo), and composition did not change.

Anthropometry

Dual-energy X-ray absorptiometry (DXA; Lunar Radiation Corp, Madison, WI) with LUNAR PRODIGY software (version 5.6; Lunar Radiation Corp) was used to determine body composition. Data for lean mass and adipose mass for total body and for the trunk compartment were expressed as absolute mass (in g). To assess validity of this instrument on our population for trunk adiposity, a subset of 12 subjects consented to have the technician scan them twice at 1 visit. The CV for measurement of trunk adiposity was 1.83%.

Height was measured to the nearest 0.1 cm with the use of a calibrated wall-mounted stadiometer (Healthometer Professional Products, Bridgeview, IL) at the screening visit. Weight was assessed with the use of a calibrated digital scale (Healthometer Professional Products) at each study visit and recorded to the nearest 0.1 kg. Waist and hip circumferences were recorded to the nearest 0.1 cm at the beginning and end of each diet period with the use of an anthropometric tape measure with the subject standing upright. Triceps and subscapular skinfold thicknesses were assessed by traditional methods with the use of a Lange caliper (Quick Medical Supply, Snoqualmie, WA) (21).

Sagittal abdominal diameter (SAD) was measured as a surrogate marker of visceral adiposity (22). The measurement was taken with a sliding abdominal caliper (Holtain-Kahn Abdominal Caliper; Holtain Ltd, Crymych, United Kingdom) with the subject lying in the recumbent position with hips flexed. The measurement was recorded to the nearest 0.1 cm at the level of the iliac crest.

Biochemical analyses

Fasting blood samples were analyzed by the General CRC Laboratory Core for glucose, insulin, leptin, and adiponectin. Glucose was measured by enzymatic assay (YSI, Yellow Springs, Ohio), and insulin, leptin, and adiponectin were measured by radioimmunoassay (insulin kit: Siemens Medical, Los Angeles, CA; leptin and adiponectin kits: Linco Research Inc, St Charles, MO). Serum concentrations of alanine transaminase (ALT) and aspartate transaminase (AST) were measured with the use of

TABLE 1
Fatty acid composition of the dietary supplements[1]

	SAF	CLA
	% fatty acid	*% fatty acid*
16:0 (palmitic acid)	5.8	1.5
18:0 (stearic acid)	2.0	1.6
18:1n−9 (oleic acid)	12.0	13.1
18:2n−6 (linoleic acid)	78.4	0
*c*9*t*11-CLA	0	41.6
*t*10*c*12-CLA	0	40.4

[1] The percentage of fatty acid was measured by gas chromatography (20). SAF, safflower oil; CLA, conjugated linoleic acid.

enzyme activity assays by the Ohio State University Medical Center clinical laboratory.

Quantitative glycemic measures

The homeostasis model assessment for insulin resistance (HOMA-IR), which correlates well with the "gold standard" euglycemic-hyperinsulinemic clamp (23), was used as a proxy measurement of insulin sensitivity.

Assessment of adherence

Adherence for inclusion was established as ≥70% of supplements consumed and was assessed monthly by self-report, counts of returned supplements, and accumulation of fatty acids in the blood. To maintain the double-masked nature of the study, a product coordinator was responsible for administering the capsules. Serum fatty acids were measured in each diet period. The accumulation of linoleic acid and *t*10*c*12-CLA were used as biomarkers for adherence to consuming SAF and CLA supplements, respectively, using methods we have previously published for fish-oil supplementation (24).

Assessment of diet and activity

To account for the potential effect of behavioral changes on study endpoints, we analyzed measures of energy balance. On 4 occasions during the study, subjects kept diet and activity records for 3 consecutive days (2 weekdays and 1 weekend day). For records of dietary intake, subjects recorded the date, time, type, preparation, and amount of each food and beverage consumed. These records were checked for accuracy before analysis with the Nutrition Data Systems for Research (University of Minnesota, Minneapolis, MN). Data were analyzed for energy (in kcal), distribution of macronutrients, and fatty acids. For records of physical activity, subjects recorded all occupational and leisure activity in 15-min increments with the use of a grid and numerical coding system. Physical activity was calculated according to established approximate energy expenditure (in kcal · kg^{-1} · 15 min^{-1}) to estimate daily average energy cost of physical activity and metabolic equivalents per day (Met-h/d) (25).

Statistical analyses

Block randomization was based on Hb A_{1c} and BMI at screening. Random sequence was generated by a statistician. On the basis of the study design and exploratory data analysis, a mixed model, including the effects of week and treatment and interactions between week and treatment, was used to fit the data from week 0 to week 36. PROC MIXED procedure in SAS 9.1 (SAS Institute, Cary, NC) was used to conduct the data analysis. This mixed-effects model treats the measurements from each subject as repeated measures, taking into account the fact that measurements from the same subject are correlated. Compound symmetry variance-covariance structure was used to estimate

TABLE 2
Analysis of subjects according to treatment assigned at baseline, as a single cohort, and according to completion of 36-wk intervention[1]

	Subjects by dietary treatments assigned at baseline		Average for full cohort at baseline (*n* = 55)	Subjects who completed intervention compared with those who dropped out	
	SAF to CLA (*n* = 33)	CLA to SAF (*n* = 22)		Completed (*n* = 35)	Dropouts (*n* = 20)
Age (y)	60.1 ± 7.3[2]	59.4 ± 7.3	59.7 ± 7.3	60.1 ± 7.9	58.8 ± 6.0
BMI (kg/ m^2)	36.3 ± 6.1	37.1 ± 7.2	36.6 ± 6.5	35.7 ± 6.2	38.2 ± 6.9
Race (*n*)					
African American	9	5	14	7	7
White	21	15	36	26	10
American Indian	1	0	1	1	0
Asian	1	0	1	1	0
Other	1	0	1	0	1
Education (*n*)					
High school or equivalent	3	6	9	4	5
2 y college	2	4	6	4	2
4 y college	7	2	9	6	3
Master's degree	1	2	3	2	1
Time since diagnosis of diabetes (y)	8.59 ± 4.87	11.86 ± 7.01	9.89 ± 5.97	9.71 ± 6.24	10.22 ± 5.57
Medication users (*n*)					
Sulfonylureas	8	5	32	23	9
Biguanides	7	6	31	19	12
Thiazolidinediones	4	1	19	13	6
Incretin mimetic	1	0	1	0	1
α-Glucosidase inhibitor	0	1	1	1	0
Combination therapy	13	9	8	5	3

[1] SAF, safflower oil; CLA, conjugated linoleic acid. There were no significant differences between subjects who completed the study and subjects who dropped out of the study for all demographic variables.
[2] Mean ± SD (all such values).

the error variance and covariance among weeks for each subject to account for correlations within subjects. Orthogonal contrasts are used in the mixed model to evaluate the change of BMI over time. Data in Tables 3–5 are expressed as means ± SEMs. All data are reported at a 5% level of significance. Data were not subject to intent-to-treat analysis.

RESULTS

Of the 55 randomly assigned subjects, 35 subjects completed both diet periods of this crossover study. The number of subjects retained at each clinical visit were as follows: diet period 1: CLA, $n = 22$ in week 0, $n = 17$ in week 4, $n = 17$ in week 8, $n = 17$ in week 12, $n = 16$ in week 16; SAF, $n = 33$ in week 0, $n = 31$ in week 4, $n = 28$ in week 8, $n = 27$ in week 12, $n = 27$ in week 16; diet period 2: CLA, $n = 27$ in week 20, $n = 27$ in week 24, $n = 24$ in week 28, $n = 24$ in week 32, $n = 22$ in week 36; SAF, $n = 16$ in week 20, $n = 14$ in week 24, $n = 14$ in week 28, $n = 13$ in week 32, $n = 13$ in week 36. Reasons for withdrawal appeared largely unrelated to the study intervention because occurrences did not differ between treatment groups and included time commitment ($n = 3$), gastrointestinal complaints ($n = 3$), unrelated health concerns ($n = 6$), worsened glycemia ($n = 2$), inability to obtain venous access ($n = 3$), and loss of contact ($n = 3$). Various adverse events occurred throughout the duration of the study, but differences between treatments were not detected. Baseline characteristics of the study population are presented in **Table 2**. When comparing the diet groups, no differences were observed for age, ethnicity, BMI, duration of diabetes, and Hb A_{1c} between the groups at baseline. No effect of the 4-wk washout period was observed on endpoints of this study.

Anthropometric variables

A significant decrease in BMI was observed over the course of both diet periods with CLA supplementation ($P = 0.0022$). BMI also decreased significantly with CLA supplementation during the last half of each diet period (between weeks 8 and 16 for diet period 1 and between weeks 28 and 36 for diet period 2) (**Figure 1**). Supplementation with SAF did not alter BMI. When the dietary oils were compared with each other, effects of CLA on body weight and BMI (**Table 3**) were significantly different from SAF.

Total adipose mass measured by DXA was significantly decreased by CLA supplementation. SAF had no effect on total adipose mass, but it significantly reduced trunk adipose mass and increased lean tissue mass. Neither SAF nor CLA supplementation significantly altered waist circumference, waist-hip ratio, SAD, or skinfold thickness measurements during the study (Table 3).

Biochemical measurements

CLA had no significant effect on fasting glucose or insulin (**Table 4**). In contrast, SAF significantly decreased fasting glucose. Presumably because of the lower fasting glucose concentrations, HOMA-IR analyses showed a significant improvement of insulin sensitivity with SAF supplementation. When comparing the 2 dietary oil treatments, there was a significant difference between treatments observed in fasting glucose and HOMA-IR.

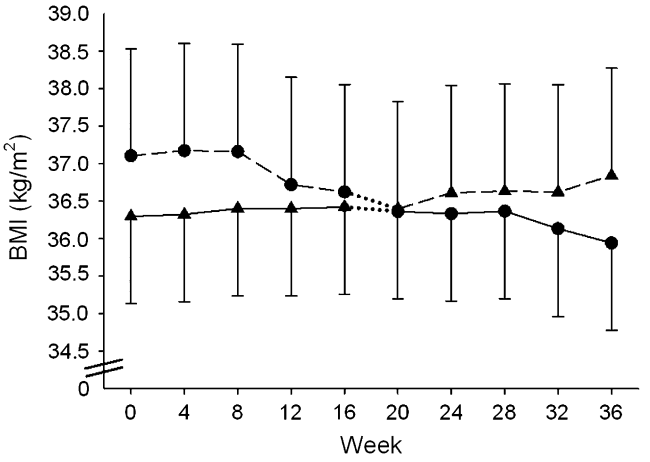

FIGURE 1. Mean (±SEM) time-dependent effect of conjugated linoleic acid (CLA) in reducing BMI in obese postmenopausal women with type 2 diabetes. (●) Subjects receiving CLA supplementation; (▲) subjects receiving safflower oil (SAF) supplementation. Subjects indicated with the dashed line received CLA from weeks 0 to 16 then crossed over to receive SAF from weeks 20 to 36. Subjects indicated with the solid line received SAF from weeks 0 to 16 then CLA from weeks 20 to 36. Dotted line indicates the washout period (weeks 16–20). Diet period 1: CLA, $n = 22$ in week 0, $n = 17$ in week 4, $n = 17$ in week 8, $n = 17$ in week 12, $n = 16$ in week 16; SAF, $n = 33$ in week 0, $n = 31$ in week 4, $n = 28$ in week 8, $n = 27$ in week 12, $n = 27$ in week 16; diet period 2: CLA, $n = 27$ in week 20, $n = 27$ in week 24, $n = 24$ in week 28, $n = 24$ in week 32, $n = 22$ in week 36; SAF, $n = 16$ in week 20, $n = 14$ in week 24, $n = 14$ in week 28, $n = 13$ in week 32, $n = 13$ in week 36.

Supplementation with CLA had no effect on change of the adipokines adiponectin or leptin. Supplementation with SAF significantly increased adiponectin but did not alter leptin concentrations (Table 4). When comparing the 2 dietary treatments, there was not a significant difference between treatments on adipokines in our study. Two markers of hepatic function, alanine transaminase and AST, were unchanged by dietary CLA. SAF decreased AST significantly.

Assessment of adherence

Self-reports of compliance indicated >90% of supplements were consumed. Furthermore, <10% of distributed supplements were returned from subjects who completed the 36-wk intervention. As a biomarker of adherence for supplements, we measured serum accumulation of fatty acids that are prominent in CLA and SAF supplements. Linoleic acid comprises 78.4% of SAF and was significantly increased in plasma by 11%. The isomer *t*10*c*12-CLA comprises 40.4% of CLA supplements and was increased by 235% in plasma (data not shown).

Assessment of energy balance

No significant differences were observed in reported dietary energy intake measured in calories between the 2 groups (**Table 5**) (26). No significant differences were observed between the groups for food sources of energy or for intakes of total fat, carbohydrate, and protein; saturated fat; polyunsaturated fatty acids; linoleic acid; or monounsaturated fatty acids. Physical activity was unchanged throughout the course of the study.

TABLE 3
Differential effects of conjugated linoleic acid (CLA) and safflower oil (SAF) on total and central adipose mass and lean mass[1]

	Diet period 1		Diet period 2			
	Baseline (week 0)	ΔWeeks 0–16	Baseline (week 20)	ΔWeeks 20–36	*P* for trend[2]	*P* for comparison of treatments[3]
Body weight (kg)						0.032
SAF	99.16 ± 3.29	−0.11 ± 0.55	97.18 ± 4.14	0.90 ± 0.79	0.415	
CLA	98.86 ± 4.13	−1.25 ± 0.71	98.78 ± 3.30	−0.86 ± 0.59	0.024	
BMI (kg/m^2)						0.000
SAF	36.3 ± 1.2	0.1 ± 0.2	36.4 ± 1.4	0.5 ± 0.2	0.054	
CLA	37.1 ± 1.4	−0.5 ± 0.2	36.4 ± 1.2	−0.4 ± 0.2	0.002	
Adipose tissue (g)						0.074
SAF	42,994 ± 2272	80 ± 667	43,203 ± 2877	135 ± 906	0.849	
CLA	44,656 ± 2872	−1076 ± 849	42,150 ± 2281	−1591 ± 721	0.019	
Trunk adipose tissue (g)						0.039
SAF	24,391 ± 1227	−1203 ± 852	25,680 ± 1674	−1943 ± 1267	0.042	
CLA	25,506 ± 1655	1075 ± 1184	23,587 ± 1267	314 ± 942	0.361	
Lean tissue (g)						0.193
SAF	45,149 ± 1615	1402 ± 594	46,390 ± 2046	654 ± 808	0.043	
CLA	46,489 ± 2040	−412 ± 756	46,513 ± 1625	599 ± 642	0.851	
Waist circumference (cm)						0.904
SAF	111.3 ± 2.3	−1.0 ± 0.7	110.8 ± 2.9	1.0 ± 1.0	0.949	
CLA	112.0 ± 2.9	−0.7 ± 0.9	110.1 ± 2.3	0.6 ± 0.7	0.915	
Waist-hip ratio						0.190
SAF	0.91 ± 0.01	0.00 ± 0.00	0.92 ± 0.02	−0.01 ± 0.01	0.874	
CLA	0.90 ± 0.02	0.01 ± 0.01	0.91 ± 0.01	0.02 ± 0.01	0.084	
Sagittal abdominal diameter (cm)						0.449
SAF	27.3 ± 0.6	0.1 ± 0.4	27.6 ± 0.9	0.7 ± 0.5	0.239	
CLA	27.7 ± 0.8	0.3 ± 0.5	27.0 ± 0.7	−0.2 ± 0.4	0.900	
Triceps skinfold thickness (mm)						0.629
SAF	41.7 ± 1.8	0.2 ± 1.0	40.8 ± 2.3	0.8 ± 1.4	0.550	
CLA	41.3 ± 2.2	−0.6 ± 1.2	41.7 ± 1.8	0.5 ± 1.0	0.940	
Subscapular skinfold thickness (mm)						0.184
SAF	45.9 ± 1.7	−3.5 ± 1.2	43.2 ± 2.3	1.5 ± 1.8	0.366	
CLA	43.1 ± 2.2	0.7 ± 1.6	42.1 ± 1.8	1.4 ± 1.4	0.328	

[1] All values are means ± SEMs, pooled across SAF and CLA groups. In this crossover study, 55 women began supplementation with either SAF or CLA for 16 wk for diet period 1 (weeks 0–16) and diet period 2 (weeks 20–36). Thirty-five women completed all 36 wk. The results were derived from tests for orthogonal contrasts used in the mixed-effects model.
[2] Within-diet treatment of both diet periods (final – initial value).
[3] Difference of effects of diet treatments (SAF compared with CLA).

DISCUSSION

In the present study, we observed a significant reduction of BMI with 6.4 g CLA supplementation/d. Because BMI had not yet reached a plateau, it is possible that further reductions in BMI are achievable with a longer length of supplementation. The reduced BMI found in our study supports other studies (27–30), which have shown weight loss by CLA. However, at least one comparative-controlled study has reported no effect of CLA on weight loss (31). We suspect these differences in study outcomes could be attributed to differences in dosages of CLA or durations of treatment. In our study, weight loss was not detected until after 8 wk of CLA supplementation in either diet period, suggesting that an extended period of supplementation may be necessary to see weight-reducing effects of CLA.

The CLA-induced weight loss in our study may be attributed to the reduction of adipose tissue mass (1.08 and 1.60 kg in diet periods 1 and 2, respectively). This magnitude of adipose loss averaged 3.20% of starting adipose mass. Importantly, the adipose-lowering effect of CLA occurred without a change in lean tissue mass, which is particularly significant because postmenopausal women are at risk of losing lean tissue mass (32). Unlike some studies that reported a regional specific reduction of adipose tissue by CLA (22, 28), we did not find a pattern of depot-specific adipose reduction. Although the adipose-lowering effect of CLA has been observed in several cohorts of men with varying degrees of body mass (11), our study is the first to show the adipose-lowering effects in obese postmenopausal women. Weight gain with an increase in body fat percentage and a concomitant redistribution of fat from peripheral to intraabdominal depots are common after menopause (4). These alterations in body composition increase the risk of developing metabolic syndrome (3).

This study is the first to show that such a modest amount (≈1-2/3 teaspoon or 8 mL) of a linoleic acid–rich oil may have a profound effect on body composition in women. Although CLA reduced total body adiposity, SAF reduced trunk adipose mass in both diet periods. The loss observed in our study (1.20 and 1.90 kg) translates to an average loss of 6.3% of starting adipose mass of the trunk region. To our knowledge, this magnitude of reduction has not been reported in an intervention that used a linoleic acid–rich oil. Furthermore, SAF increased total body lean tissue mass (gains averaging 1.4 and 0.6 kg, an ≈1.6% increase from starting lean mass). Importantly, the effect

TABLE 4
Effect of dietary oils on serum metabolites and adipokines[1]

	Diet period 1		Diet period 2			
	Baseline (week 0)	ΔWeeks 0–16	Baseline (week 20)	ΔWeeks 20–36	*P* for trend[2]	*P* for comparison of treatments[3]
Fasting glucose (mg/dL)						0.011
SAF	148 ± 8	−19 ± 8	159 ± 11	−11 ± 11	0.034	
CLA	145 ± 10	5 ± 10	138 ± 9	11 ± 9	0.137	
Fasting insulin (μU/mL)						0.462
SAF	19.9 ± 2.1	−1.7 ± 1.8	13.3 ± 2.8	−1.1 ± 2.6	0.186	
CLA	15.4 ± 2.6	−1.1 ± 2.3	17.8 ± 2.2	1.4 ± 2.0	0.763	
HOMA-IR						0.050
SAF	7.1 ± 0.9	−1.3 ± 0.8	5.3 ± 1.2	−0.8 ± 1.2	0.027	
CLA	5.4 ± 1.1	0.1 ± 1.0	6.2 ± 1.0	1.3 ± 0.9	0.592	
Leptin (ng/mL)						0.053
SAF	30 ± 4	1 ± 1	27 ± 5	3 ± 2	0.134	
CLA	29 ± 5	0 ± 2	31 ± 4	−2 ± 1	0.215	
Adiponectin (μg/mL)						0.059
SAF	7.3 ± 1.1	0.8 ± 0.6	7.9 ± 1.3	2.4 ± 0.8	0.005	
CLA	9.5 ± 1.3	−0.3 ± 0.8	8.3 ± 1.1	0.8 ± 0.6	0.878	
Alanine aminotransferase (U/L)						0.059
SAF	28.6 ± 1.6	−2.2 ± 1.4	25.1 ± 2.1	−2.4 ± 2.0	0.063	
CLA	25.2 ± 1.9	−1.1 ± 1.8	24.8 ± 1.7	3.0 ± 1.6	0.430	
Aspartate aminotransferase (U/L)						0.034
SAF	31.4 ± 2.0	−4.5 ± 2.0	27.2 ± 2.7	−2.5 ± 2.8	0.043	
CLA	28.2 ± 2.4	−1.7 ± 2.5	26.8 ± 2.1	4.8 ± 2.1	0.347	

[1] All values are means ± SEMs, pooled across safflower oil (SAF) and conjugated linoleic acid (CLA) groups. In this crossover intervention study, 55 women began supplementation with either SAF or CLA oil for 16 wk for diet period 1 (weeks 0–16) and diet period 2 (weeks 20–36). Thirty-five women completed all 36 wk of the intervention. The results were from tests for orthogonal contrasts used in the mixed-effects model. HOMA-IR, homeostasis model assessment for insulin resistance.
[2] Within-diet treatment of both diet periods (final – initial value).
[3] Difference of effects of diet treatments (SAF compared with CLA).

of SAF was independent of diet or activity changes (Table 5). Recently, a diet containing corn oil exhibited a dual effect on adipogenesis in mice: In a high-carbohydrate diet, corn oil enhanced adipogenesis, but in a high-protein diet corn oil was antiadipogenic in mice (17). Although there was no difference in dietary carbohydrate or protein intake between diet groups in either diet period, the interaction between SAF and specific macronutrients was not tested in our study.

As previously discussed, postmenopausal women are at increased risk of developing central obesity (3, 4). Anthropometric measurements, including waist circumference and SAD, did not show a significant reduction in abdominal adipose in our study. However, with the use of DXA, we found decreased trunk adipose during SAF supplementation. Regional differences in patterns of obesity have been linked with changes of adipokine production (33). Adiponectin may be associated with subcutaneous adipose depots. In the present study, SAF significantly increased adiponectin concentrations by an average of 20.3%, whereas no significant changes in adipokines were observed with CLA supplementation. Although an increase in adiponectin concentrations was observed, we were not able to detect changes of subcutaneous adipose (eg, adipose mass of hips, thighs, etc), as measured by DXA. One explanation is that the increase of serum adiponectin by SAF is due to an altered ratio of visceral adipose: subcutaneous adipose (34); however, DXA technology cannot distinguish between visceral and subcutaneous adipose depots. In addition, linoleic acid is a modest ligand for peroxisome proliferator-activated receptor γ (PPARγ) (35). Transcription of adiponectin is responsive to the PPARγ ligands (eg, thiazolidinediones). The promoter region of the adiponectin gene appears to have a functional responsive element for PPARγ (36, 37). The induction of adiponectin could be due to changes in transcription of PPARγ mRNA by transactivation of PPARγ by linoleic acid. Future investigations into adipokine production with dietary-oil supplementation may be a key determinant in linking dietary fat quality with reducing obesity and attenuating metabolic complications.

Postmenopausal women are at substantially increased risk of developing insulin resistance (4). Dietary saturated fat has been associated with decreased peripheral insulin sensitivity (38). However, it is unknown how polyunsaturated fats contribute to improved insulin sensitivity. Our study has shown that SAF lowered glycemia and reduced HOMA-IR, which may be attributed to lower trunk adipose mass, increased adiponectin, or both. In support of the interactive effect of trunk adipose mass with glycemic control, visceral fat gained over a 7-y period was associated with significant deterioration of glucose-insulin homeostasis after correction for total fat gain (39). The effect of increasing visceral fatness with a decline of glycemic control may increase lipid supply to the liver and β cells, also known as a lipotoxic effect (40). Lipotoxicity can lead to increased hepatic glucose production and β cell failure, both of which lead to poor glycemic control.

TABLE 5
Diet and physical activity[1]

	Diet period 1		Diet period 2			
	Baseline (week 0)	ΔWeeks 0–16	Baseline (week 20)	ΔWeeks 20–36	*P* for trend[2]	*P* for comparison of treatments[3]
Energy (kcal)[4]						0.500
SAF	1746 ± 75	−154 ± 92	1547 ± 14	141 ± 144	0.938	
CLA	1925 ± 96	−395 ± 126	1527 ± 86	222 ± 102	0.287	
Carbohydrate (g)						0.612
SAF	197 ± 10	−20 ± 12	180 ± 14	4 ± 17	0.453	
CLA	200 ± 12	−28 ± 15	184 ± 10	−2 ± 13	0.129	
Protein (g)						0.267
SAF	78 ± 4	−2 ± 4	71 ± 5	7 ± 6	0.541	
CLA	85 ± 5	−10 ± 6	74 ± 4	3 ± 5	0.333	
Fat (g)						0.578
SAF	77 ± 5	−10 ± 6	63 ± 7	15 ± 9	0.606	
CLA	85 ± 6	−19 ± 8	62 ± 5	16 ± 6	0.791	
PUFA (g)						0.448
SAF	15 ± 1	−2 ± 2	12 ± 2	4 ± 2	0.594	
CLA	18 ± 2	−5 ± 2	12 ± 1	4 ± 2	0.587	
Linoleic acid (g)[5]						0.619
SAF	13 ± 1	−2 ± 1	10 ± 2	4 ± 2	0.315	
CLA	15 ± 1	−3 ± 2	11 ± 1	4 ± 2	0.739	
MUFA (g)						0.658
SAF	30 ± 2	−4 ± 2	25 ± 3	4 ± 4	0.923	
CLA	33 ± 3	−9 ± 3	24 ± 2	6 ± 3	0.589	
SFA (g)						0.891
SAF	26 ± 2	−3 ± 2	21 ± 3	7 ± 3	0.386	
CLA	26 ± 2	−3 ± 3	20 ± 2	5 ± 2	0.565	
Activity (Met eq)[6]						0.475
SAF	158 ± 5	9 ± 8	166 ± 7	−3 ± 13	0.706	
CLA	161 ± 7	−2 ± 10	162 ± 6	−7 ± 9	0.516	

[1] All values are means ± SEMs, pooled across safflower oil (SAF) and conjugated linoleic acid (CLA) groups. Values are from diet only. PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid; Met eq, metabolic equivalents.

[2] Within-diet treatment of both diet periods (final – initial value).

[3] Difference of effects of diet treatments (SAF compared with CLA).

[4] Dietary intake of women was measured by 3-d diet records administered at weeks 0, 16, 20, and 36. Forty-five women returned diet records from visit at week 0. Thirty-five women completed diet records for all 36 wk of the intervention.

[5] From diet only; therefore, this value does not include linoleic acid from the SAF supplement.

[6] Activity was measured by using the method of Bouchard et al (26). Data from activity records were interpreted by a blinded reviewer, and all records were interpreted by the same individual. Data are reported as the average of 3-d recordings for each record. The results were from tests for orthogonal contrasts used in the mixed-effects model.

Earlier studies with CLA in Zucker diabetic fatty rats have shown that CLA could attenuate the development of impaired glucose tolerance and hyperinsulinemia (14, 41). However, further studies have shown that *t*10*c*12-CLA could also lead to impaired insulin sensitivity in human subjects when adipose-lowering effects were observed (42). Subsequent studies have shown mixed results: One other study showed CLA worsens glucose tolerance (43), whereas another showed no such effect (44). In our cohort, we did not observe an effect of CLA on surrogate markers of insulin sensitivity (Table 4). Furthermore, CLA did not alter nonesterified free fatty acids or triglyceride concentrations (data not shown). Although weight loss >7% is shown to improve management of glycemia in people with T2DM (45), it appears that the weight and adipose loss by CLA in our study was not of sufficient magnitude to improve markers of glycemia.

There were some limitations to our study design. First, our study population was obese postmenopausal women, impairing the possibility to generalize our results to nonobese men and women or to premenopausal nondiabetic women. In addition, we assessed energy and nutrient intakes with the use of repeated 3-d diet records that may not accurately reflect subtle changes in calories that may have occurred because of the daily addition of 72 kcal from oil. Another limitation is the choice of 16-wk crossover interventions that may not provide adequate time to show maximum results on changes in outcomes measured. Finally, we recognize that there are limitations that occur in a free-living population that could be better measured in a controlled environment.

The current dietary recommendations from the American Heart Association and the American Diabetes Association suggest that polyunsaturated fatty acids (PUFAs) should account for

≤10% of calories (45, 46). Until recently, there was an absence of recommendations to intentionally include n−6 PUFA-rich oils as part of a healthy and calorically balanced diet. In January 2009, an advisory paper was published that emphasized the importance of including the n−6 fatty acid linoleic acid as well as other PUFAs, such as n−3 PUFAs, for heart health (47). Our findings suggest that dietary supplementation of oils rich in the n−6 linoleic acid decreases trunk adipose, increases lean tissue mass, and improves glycemic control which may reduce the risk of heart disease as well as other comorbidities from poorly controlled diabetes. Our data also suggest that at a dose of 6.4 g/d, CLA has a significant effect on lowering body weight and total adipose mass without altering lean tissue mass in obese postmenopausal women who are not also on a weight-loss diet or exercise plan. The use of lower doses of CLA over longer durations of intervention may prove to be an effective weight-loss aid.

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REFERENCES

1. American Diabetes Association. Report on incidence of type 2 diabetes. Alexandria, VA: American Diabetes Association, 2005.
2. Leahy JL. Pathogenesis of type 2 diabetes mellitus. Arch Med Res 2005; 36:197–201.
3. Carr MC. The emergence of the metabolic syndrome with menopause. J Clin Endocrinol Metab 2003;88:2404–11.
4. Ley CJ, Lees B, Stevenson JC. Sex- and menopause-associated changes in body-fat distribution. Am J Clin Nutr 1992;55:950–4.
5. Ahmad F, Considine RV, Bauer TL, Ohannesian JP, Marco CC, Goldstein BJ. Improved sensitivity to insulin in obese subjects following weight loss is accompanied by reduced protein-tyrosine phosphatases in adipose tissue. Metabolism 1997;46:1140–5.
6. Berne C. The Orlistat Swedish type 2 diabetes study group: a randomized study of orlistat in combination with a weight management programme in obese patients with type 2 diabetes treated with metformin. Diabet Med 2005;22:612–8.
7. Vettor R, Serra R, Fabris R, Pagano C, Federspil G. Effect of sibutramine on weight management and metabolic control in type 2 diabetes: a meta-analysis of clinical studies. Diabetes Care 2005;28:942–9.
8. Finer N, Ryan DH, Renz CL, Hewki AC. Prediction of response to sibutramine therapy in obese non-diabetic and diabetic patients. Diabetes Obes Metab 2006;8:206–13.
9. Mauro M, Taylor V, Wharton S, Sharma AM. Barriers to obesity treatment. Eur J Intern Med 2008;19:173–80.
10. Belury MA. Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action. Annu Rev Nutr 2002;22:505–31.
11. Whigham LD, Watras AC, Schoeller DA. Efficacy of conjugated linoleic acid for reducing fat mass: a meta-analysis in humans. Am J Clin Nutr 2007;85:1203–11.
12. Park Y, Albright KJ, Storkson JM, Liu W, Cook ME, Pariza MW. Changes in body composition in mice during feeding and withdrawal of conjugated linoleic acid. Lipids 1999;34:243–8.
13. Liu LF, Purushotham A, Wendel AA, Belury MA. Combined effects of rosiglitazone and conjugated linoleic acid on adiposity, insulin sensitivity, and hepatic steatosis in high-fat-fed mice. Am J Physiol Gastrointest Liver Physiol 2007;292:G1671–82.
14. Houseknecht KL, Vanden Heuvel JP, Moya-Camarena SY, et al. Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker diabetic fatty *fa/fa* rat. Biochem Biophys Res Commun 1998; 244:678–82.
15. Park Y, Storkson JM, Albright KJ, Liu W, Pariza MW. Evidence that the *trans*-10,*cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice. Lipids 1999;34:235–41.
16. Martins SV, Lopes PA, Alfaia CM, et al. Contents of conjugated linoleic acid isomers in ruminant-derived foods and estimation of their contribution to daily intake in Portugal. Br J Nutr 2007;98:1206–13.
17. Madsen L, Pedersen LM, Liaset B, et al. cAMP-dependent signaling regulates the adipogenic effect of n−6 polyunsaturated fatty acids. J Biol Chem 2008;283:7196–205.
18. Katzman R, Brown T, Fuld P, Peck A, Schechter R, Schimmel H. Validation of a short Orientation-Memory-Concentration Test of cognitive impairment. Am J Psychiatry 1983;140:734–9.
19. Ruiz-Gutierrez V, Perona JS, Pacheco YM, Muriana FJ, Villar J. Incorporation of dietary triacylglycerols from olive oil and high-oleic sunflower oil into VLDL triacylglycerols of hypertensive patients. Eur J Clin Nutr 1999;53:687–93.
20. Belury MA, Kempa-Steczko A. Conjugated linoleic acid modulates hepatic lipid composition in mice. Lipids 1997;32:199–204.
21. Stolarczyk LM, Heyward VH. Applied Body Composition Assessment. Champaign, IL: Human Kinetics, 2004:33–7.
22. Riserus U, Berglund L, Vessby B. Conjugated linoleic acid (CLA) reduced abdominal adipose tissue in obese middle-aged men with signs of the metabolic syndrome: a randomised controlled trail. Int J Obes Metab Disord 2001;25:1129–35.
23. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
24. Begum R, Belury M, Burgess JR, Peck LW. Effect of n−3 and n−6 PUFA supplementation on maintenance of hematocrit in hemodialysis patients. J Ren Nutr 2004;14:233–41.
25. Pereira MA, FitzerGerald SJ, Gregg EW, et al. A collection of Physical Activity Questionnaires for health-related research. Med Sci Sports Exerc 1997;29(suppl):S1–205.
26. Bouchard C, Tremblay A, Leblanc C, Lortie G, Savard R, Thériault G. A method to assess energy expenditure in children and adults. Am J Clin Nutr 1983;37:461–7.
27. Gaullier JM, Halse J, Hoye K, et al. Supplementation with conjugated linoleic acid for 24 months is well tolerated by and reduces body fat mass in healthy, overweight humans. J Nutr 2005;135:778–84.
28. Gaullier JM, Halse J, Høivik HO, et al. Six months supplementation with conjugated linoleic acid induces regional-specific fat mass decreases in overweight and obese. Br J Nutr 2007;97:550–60.
29. Sneddon AA, Tsofliou F, Fyfe CL, et al. Effect of a conjugated linoleic acid and omega-3 fatty acid mixture on body composition and adiponectin. Obesity (Silver Spring) 2008;16:1019–24.
30. Belury MA, Mahon A, Banni S. The conjugated linoleic acid (CLA) isomer, t10c12-CLA, is inversely associated with changes in body weight and serum leptin in subjects with type 2 diabetes mellitus. J Nutr 2003;133(suppl):257S–60S.
31. Zambell KL, Keim NL, Van Loan MD, et al. Conjugated linoleic acid supplementation in humans: effects on body composition and energy expenditure. Lipids 2000;35:777–82.
32. Jacobsen DE, Samson MM, Kezic S, Verhaar HJ. Postmenopausal HRT and tibolone in relation to muscle strength and body composition. Maturitas 2007;58:7–18.
33. Cnop M, Havel PJ, Utschneider KM, et al. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. Diabetologia 2003;46:459–69.
34. Vardhana PA, Dicken C, Tortoriello DV, Chu M, Carmina E, Lobo RA. Increasing adiposity in normal ovulatory women affects adipocytokine expression in subcutaneous and visceral abdominal fat. Int J Gynaecol Obstet 2009;104:121–4.
35. Kliewer SA, Sundseth SS, Jones SA, et al. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptor α and γ. Proc Natl Acad Sci USA 1997; 94:4318–23.
36. Iwaki M, Matsuda M, Maeda N, et al. Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. Diabetes 2003;52:1655–63.

37. Pfützner A, Marx N, Walcher D, Löbig M, Seidel D, Forst T. Impact of rosiglitazone on visfatin and adiponectin plasma concentrations in patients with type 2 diabetes and coronary artery disease. Clin Lab 2008; 54:237–41.
38. Vessby B, Unsitupa M, Hermansen K, et al. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: the KANWU study. Diabetologia 2001;44:312–9.
39. Lemieux S, Prud'homme D, Nadeau A, Tremblay A, Bouchard C, Després JP. Seven-year changes in body fat and visceral adipose tissue in women. Association with indexes of plasma glucose-insulin homeostasis. Diabetes Care 1996;19:983–91.
40. Lee Y, Hirose H, Ohneda M, Johnson JH, McGarry JD, Unger RH. β-Cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-β-cell relationships. Proc Natl Acad Sci U S A 1994;91:10878–82.
41. Ryder JW, Portocarrero CP, Song XM, et al. Isomer-specific antidiabetic properties of conjugated lioleic acid. Imporved glucose tolerance, skeletal muscle insulin action, and UCP-2 gene expression. Diabetes 2001;50:1149–57.
42. Riserus U, Arner P, Brismar K, Vessby B. Treatment with dietary *trans*10*cis*12 conjugated linoleic acid causes isomer-specific insulin resistance in obese men with the metabolic syndrome. Diabetes Care 2002;25:1516–21.
43. Moloney F, Yeow TP, Mullen A, Nolan JJ, Roche HM. Conjugated linoleic acid supplementation, insulin sensitivity, and lipoprotein metabolism in patients with type 2 diabetes mellitus. Am J Clin Nutr 2004;80: 887–95.
44. Eyjolfson V, Spriet LL, Dyck DJ. Conjugated linoleic acid improves insulin sensitivity in young, sedentary humans. Med Sci Sports Exerc 2004;36:814–20.
45. American Diabetes Association. Nutrition Recommendations and Interventions for Diabetes: a position statement of the American Diabetes Association. Diabetes Care 2007;30(suppl):S48–65.
46. American Heart Association. American Heart Association dietary recommendations for lowering risk of cardiovascular disease. Available from: http://www.americanheart.org (cited 2008).
47. Harris WS, Mozaffarian D, Rimm E, et al. Omega-6 fatty acids and risk for cardiovascular disease: a science advisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. Circulation 2009;119: 902–7.