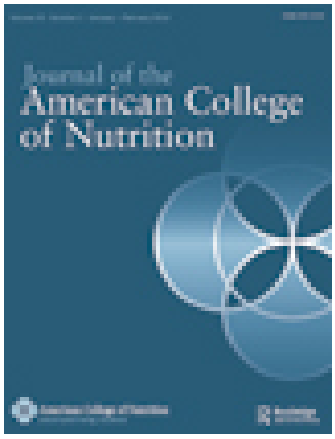

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CLA Does Not Impair Endothelial Function and Decreases Body Weight as Compared with Safflower Oil in Overweight and Obese Male Subjects

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Original Research

CLA Does Not Impair Endothelial Function and Decreases Body Weight as Compared with Safflower Oil in Overweight and Obese Male Subjects

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Key words: endothelial function, metabolic syndrome, cardiovascular disease, PAT index

Objective: Conjugated linoleic acid (CLA) showed a wide range of beneficial biological effects with relevance for cardiovascular health in animal models and humans. Most human studies used olive oil as a reference. This study assessed the effect of CLA as compared with safflower oil on endothelial function and markers of cardiovascular risk in overweight and obese men. Heated safflower oil and olive oil were given for additional descriptive control.

Methods: Eighty-five overweight men (aged 45–68 years, body mass index 25–35 kg/m^2) were randomized to receive 4.5 g/d of the CLA isomeric mixture, safflower oil, heated safflower oil, or olive oil in a 4-week double-blind study. Endothelial function was assessed by peripheral arterial tonometry (PAT) index determination in the fasting and postprandial state (i.e., 4 hours after consumption of a fat- and sucrose-rich meal).

Results: CLA as compared with safflower oil consumption did not impair fasting or postprandial PAT index but decreased body weight. CLA as compared with safflower oil did not change total, low-density lipoprotein (LDL), or high-density lipoprotein (HDL) cholesterol; triglycerides; insulin sensitivity indices; C-reactive protein; soluble adhesion molecules; oxidized LDL; lipoprotein a (Lp[a]); paraoxonase; or platelet-activating factor acetylhydrolase (PAF-AH) activity, but significantly reduced arylesterase activity and increased concentrations of the F_2-isoprostane 8-iso-prostaglandin F $(PGF)_{2\alpha}$.

Conclusion: CLA did not impair endothelial function. Other parameters associated with metabolic syndrome and oxidative stress were not changed or were slightly improved. Results suggest that CLA does not increase cardiovascular risk. Increased F_2-isoprostane concentrations in this context may not indicate increased oxidative stress.

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Scientific congresses where the study was presented: Presented in part at the 44th Scientific Congress of the German Nutrition Society, Halle, March 2007; the 2nd International Congress on CLA, Sardinia, September 2007; "Milchkonferenz 2007" of the Society of Dairy Science, Vienna, September 2007.

INTRODUCTION

Coronary heart disease (CHD) is the most common cause of death in developed countries. The metabolic syndrome is a common predisposing factor in the development of atherosclerotic CHD [1]. Components include central (abdominal) obesity, insulin resistance, arterial hypertension, and dyslipidemia, namely, high triglyceride and low high-density lipoprotein cholesterol (HDL-C) concentrations. All components of the metabolic syndrome can impair endothelial function [2]. The proinflammatory state that accompanies the metabolic syndrome is also associated with endothelial dysfunction [3]. Dysfunction of the coronary or peripheral vascular endothelium constitutes an independent predictor of cardiovascular events [4].

Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of linoleic acid (18:2) with two conjugated double bonds. The predominant isomer of CLA in dairy foods is cis9,trans11-18:2. Industrially produced preparations contain cis9,trans11 and trans10,cis12 isomers in approximately equal concentrations, with minor amounts of other isomers. CLA may have health benefits on markers associated with the metabolic syndrome and cardiovascular risk, and has shown beneficial effects on immune function, but the dramatic effects seen in animal studies mostly have not been confirmed by clinical studies in humans [5]. Some reports have described negative effects in humans. In particular, a 12-week intervention with 4.5 g/d of an isomeric mixture of CLA as compared with olive oil significantly impaired endothelial function, although weight and body mass index (BMI) did not change, and limb skinfold thickness was significantly reduced [6]. Furthermore CLA seemingly exerted negative effects on insulin sensitivity [7–9] and increased oxidative stress [8,10,11].

The present study examined the effects of CLA compared with safflower oil on endothelial function and other markers related to the metabolic syndrome in overweight or obese men. Native olive oil and heated (thermally oxidized) safflower oil supplements were given for additional descriptive control. First, safflower oil was chosen as the primary control because it is rich in linoleic acid. CLA is an isomer of linoleic acid. CLA and linoleic acid have two double bonds, differ only in the position (and also the configuration) of the double bonds, and have metabolic pathways in common. Second, safflower oil was chosen as the primary control because CLA is frequently produced from safflower oil, as for this study, and thus has a similar background composition. Olive oil was given because most previous studies compared CLA against olive oil. Olive oil contains mainly oleic acid having only one double bond and is a rich source of phenols and terpenes. Isolated phytochemicals or olive oil rich in these compounds may improve postprandial vasodilation [12], increase plasma antioxidant capacity [13], and inhibit adhesion molecule expression [14] as compared with oil poor in phytochemicals. Concentrations of such bioactive compounds vary depending on variety, crop year, and processing. Heated (thermally oxidized) safflower oil was chosen because polyunsaturated oils are frequently heated for food preparation, and because oxidized lipids present in the diet are absorbed and contribute to the pro-oxidant load of a meal [15]. Oils contain tocopherols and tocotrienols as native material or as added during processing. Because α- and γ-tocopherols have both antioxidant and anti-inflammatory properties, the tocopherol content of supplements was standardized in this study. Fat and glucose meals can acutely change endothelial function [16]; therefore, peripheral arterial tonometry (PAT) index and triglyceride, glucose, and insulin concentrations were determined both in the fasting state and following a meal high in fat and sucrose content.

MATERIALS AND METHODS

Subjects

Eighty-five male volunteers aged 45–68 years with a BMI of 25.4–35 kg/m^2 were recruited from the Metabolic Intervention Cohort Kiel (MICK), which was characterized previously [17]. Thirty percent of participants were overweight, and 70% were obese. Accordingly, 76.5% showed a metabolic syndrome according to the criteria of the International Diabetes Federation (2005). Major exclusion criteria were known disorders that affect the digestion and metabolism of food components, use of interfering medication, and established diabetes (fasting glucose levels >125 mg/dl after repeated determination). Nevertheless, impaired fasting glucose concentrations were observed in several instances during the study. Two subjects had CHD, and 4 subjects smoked occasionally. This study was approved by the local Ethics Advisory Committee and was carried out according to the Helsinki Declaration. Participants gave written informed consent before participating in the study.

Protocol and Study Design

This single-center, double-blind, randomized, placebo-controlled study had 4 parallel arms. Subjects were randomly assigned to receive a total of 4.5 g/d of a CLA mixture (containing 3.4 g of 50:50 cis9,trans11-CLA and trans10,cis12-CLA, Tonalin TG 80 [n = 22]), safflower oil (n = 22), heated safflower oil (240°C for 2 hours [n = 20]), or native olive oil (n = 21) for 28 (±2) days. Tocopherol in supplements (609 μg alpha-, 25 μg beta-, 588 μg gamma-, and 255 μg delta-tocopherol, adding up to 793 μg tocopherol equivalents/g oil) was adjusted to that of the CLA mixture. The CLA mixture is a commercial product from Cognis (Monheim, Germany) made from safflower oil. All test materials were encapsulated by Banner, Nl, and were provided by Cognis. This dose of CLA

was chosen because this amount had been given in many previous studies, including that of Taylor et al. [6]. Subjects consumed these test oils as 2 × 4 hard gel capsules daily together with meals. Compliance was considered sufficient if <20% of the capsules were returned (i.e., >80% were indeed consumed). Participants were instructed not to change their eating habits and physical activity routine, and not to use any dietary supplements of vitamins, minerals, or special oil preparations.

Characteristics (including medication, smoking, and alcohol consumption), anthropometric parameters, and fasting blood pressure were assessed, and blood and urine samples were collected at baseline and after the 4-week intervention. Fasting blood samples were collected between 7 AM and 9 AM. A liquid meal (500 ml) was then consumed within 15 minutes that contained 32.5 g protein (casein), 69 g sucrose, 5 g lactose, 55 g butter fat, 10 g ethanol, 644 mg cholesterol, and 9.5 mg retinol. The energy content was 4256 kJ. This meal was prepared by diät+pharma GmbH, Roth, Germany. The fat- and sucrose-rich standardized meal had already been used in previous studies [17]. It equates to a Western-style diet in its extreme form, and both ingredients have been shown to impair acutely endothelial function in the postprandial state [16]. After the meal, blood samples were collected repetitively, after 30 and 60 minutes and then every hour, for measurement of postprandial triglyceride concentrations over a period of 9 hours and of glucose and insulin concentrations over a period of 5 hours. Plasma or serum aliquots were stored at −20°C or −80°C until analysis, depending on the analyte. Twenty-four-hour urine was stabilized with 1 mM ethylenediaminetetraacetic acid (EDTA) and 1 mM hydroxyl-TEMPO (4 hydroxy-2,2,6,6-tetramethyl-piperidin-1-oyl), and aliquots were stored at −20°C.

Endothelial Function

Vascular endothelial function was determined 2 days later by PAT using the Endo-PAT2000 System (Itamar, Caesarea, Israel). PAT index determination was performed twice during each visit, under fasting conditions and 4 hours after intake of the meal described previously. About 20 minutes after arrival or later, pneumatic probes were positioned on the index finger of each hand of subjects in a supine position, with their arms held outstretched on cushions, in a calm room without direct bright light, at 21°C–24°C. After a 10-minute equilibration period (baseline), the blood pressure cuff placed on the dominant upper arm was occluded for 5 minutes and then was released to induce reactive hyperemia, measured for another 5–10 minutes post occlusion. PAT index was calculated as the ratio of the average amplitude of the PAT signal over 1 minute post occlusion divided by the average amplitude of a 3.5-minute baseline interval. This ratio was normalized to the concurrent signal from the contralateral nonoccluded control hand to decrease confounding variables. PAT measurements were analyzed with a computerized, automated algorithm (Itamar Medical Ltd, Caesarea, Israel). Therefore, no intraobserver or interobserver variability was noted. PAT measurement is a relatively new technique, but it has been approved by the U.S. Food and Drug Administration (FDA). PAT results correlate with flow-mediated dilatation (FMD) readings [18]. In a Framingham cohort study, BMI, diabetes mellitus, smoking, and cholesterol in total plasma (T-C)/HDL-C all were inversely related to the PAT ratio [19].

Biochemical Analyses

Safety parameters were analyzed by an accredited routine laboratory (University Hospital Kiel). Glucose, T-C, HDL-C, low-density lipoprotein cholesterol (LDL-C), and triglycerides were analyzed in plasma by enzymatic methods (Thermo Fischer Scientific, Dreieich, Germany) using a Konelab 20i Clinical Analyzer (Thermo Fischer Scientific), and insulin by radioimmunoassay (RIA) (Adaltis, Bologna, Italy). Homeostasis model assessment-insulin resistance (HOMA-IR) was calculated as ([fasting insulin mU/L × fasting glucose mmol/L]/22.5). For the postprandial course of triglyceride, glucose, and insulin concentrations, the area under the curve (AUC) was calculated according to the trapezoidal rule. Soluble adhesion molecules sVCAM, sICAM, and sE-selectin were determined by enzyme-linked immunosorbent assay (ELISA) (Bender MedSystems, Vienna, Austria). Detection limits were 0.59, 2.17, and 0.33 ng/ml, and intra-assay coefficients of variation (CVs) were 3.1%, 4.1%, and 5.4%, respectively. High-sensitivity C-reactive protein (hs-CRP) was determined by enzyme immunoassay (EIA) (DRG, Marburg, Germany) with a detection limit of 0.1 ng/ml and an intra-assay CV of 4.2%. Lipoprotein (a) (Lp[a]) was determined by a turbidimetric assay (Thermo Fischer Scientific, detection limit 30 ng/ml, intra-assay CV 2.9%) using a Konelab 20i clinical analyzer and oxidized LDL (oxLDL) by ELISA (Mercodia, Uppsala, Sweden, detection limit <1 mU/L, intra-assay CV 6.4%). The oxLDL antibody used here recognizes aldehyde-modified lysine groups on LDL. The urinary isoprostane 8-iso-prostaglandin F(2alpha) (8-iso-$PGF_{2\alpha}$), an F_2-isoprostane, was determined as described previously [20]. Urinary 8-iso-$PGF_{2\alpha}$ was adjusted for urinary creatinine excretion. Platelet-activating factor acetylhydrolase (PAF-AH) activity [21], paraoxonase (PON) arylesterase activity [22], and activity of PON toward paraoxon [23] were assessed as described previously.

Statistical Analysis

The primary parameter defined before the study was the intervention effect on fasting endothelial function (i.e., the change from baseline to 4 weeks). Besides CLA, 3 reference oils were administered. Safflower oil was chosen as a primary control for the reasons outlined before. The other 2 were given

Table 1. Endothelial Function and Physical Characteristics*

	CLA (n = 21)	Safflower Oil (n = 21)	Olive Oil (n = 19)	Safflower Oil, Heated (n = 20)	*p* All Groups	*p* CLA vs Safflower
PAT index fasting						
Baseline	1.89 ± 0.43	2.04 ± 0.38	2.18 ± 0.63	2.19 ± 0.65	0.391	0.209
Change	−0.03 ± 0.45	−0.08 ± 0.62	−0.18 ± 0.52	−0.15 ± 0.65	0.830	0.421
PAT index 4 h pp						
Baseline	1.72 ± 0.25	1.92 ± 0.39 †	2.00 ± 0.37†	2.28 ± 0.68†	0.009	0.046
Change	0.20 ± 0.48	0.06 ± 0.47	−0.14 ± 0.31	−0.14 ± 0.67	0.171	0.291
Body weight (kg)						
Baseline	88.6 ± 9.1	87.1 ± 9.1	88.0 ± 10.0	93.5 ± 12.6	0.395	0.497
Change	−1.13 ± 1.65	0.04 ± 1.29 †	0.14 ± 0.96†	−0.04 ± 1.69†	0.051	0.031
BMI (kg/m^2)						
Baseline	28.3 ± 2.3	28.2 ± 2.0	27.8 ± 1.3	28.9 ± 2.6	0.763	0.801
Change	−0.35 ± 0.53	0.02 ± 0.42	0.04 ± 0.29	−0.02 ± 0.52	0.067	0.042
Systolic BP (mmHg)						
Baseline	141.4 ± 16.9	142.1 ± 17.0	136.8 ± 19.7	136.5 ± 15.4	0.482	0.801
Change	−10.8 ± 17.4	−9.1 ± 11.2	−4.7 ± 17.5	−2.3 ± 13.6	0.323	0.990
Diastolic BP (mmHg)						
Baseline	90.4 ± 8.7	86.4 ± 12.5	84.0 ± 9.7	84.4 ± 8.4	0.075	0.128
Change	−6.9 ± 10.9	−0.7 ± 8.7	−1.1 ± 9.5	0.04 ± 9.0	0.112	0.057
Pulse (min^{-1})						
Baseline	68.8 ± 9.7	69.1 ± 10.0	66.7 ± 9.6	67.2 ± 10.6	0.752	0.900
Change	−4.4 ± 6.7	−5.8 ± 8.4	−3.6 ± 9.5	−3.2 ± 12.3	0.680	0.687

* Means ± SD.
† Significantly different from CLA (post hoc Mann-Whitney).

for additional descriptive control. Because most parameters showed no normal distribution, baseline values and intervention-dependent changes between all 4 groups were compared using Kruskal-Wallis (analysis of variance [ANOVA] on Ranks) followed by post hoc multiple Mann-Whitney analysis. Because the primary comparison was between CLA and safflower oil treatment, *p* values for this individual analysis are presented separately. The software system was Statgraphics-Plus 4.1 (Manugistics Inc, Rockville, MD). Level of significance was set at $p \leq 0.05$. Means ± standard deviation (SD) are given.

RESULTS

Baseline body weight and BMI (Table 1), waist circumference (WC, 106.9 ± 8.0 cm), and waist/hip ratio (WHR, 1.00 ± 0.05) were not different between the 4 groups, nor were age, alcohol use, tobacco use (12.9% smokers), and permitted medication. All subjects were compliant. The supplements caused no adverse side effects. Three subjects from 3 different treatment groups were excluded from statistical analysis because they took interfering medication during the intervention period. One subject in the olive oil group should not have been included because of the inability to perform endothelial peripheral arterial tonometry (EndoPAT). Safety parameters (blood cell count; the electrolytes sodium, potassium, and calcium; enzyme activities of glutamic-oxaloacetic transaminase [GOT = AST], glutamic-pyruvic transaminase [GPT = ALT], cholinesterase, alkaline phosphatase, glutamyl transpeptidase, and creatinine) were not different between groups before and after intervention (data not shown). Erythrocytes, hemoglobin, hematocrit, and glutamic-pyruvic transaminase decreased during intervention in all groups, but concentrations remained within the common range.

Our study determined endothelial function in the fasting state and 4 hours after consumption of a fat- and sucrose-rich meal. Fasting PAT indices at baseline were not different between groups (Table 1), but the postprandial PAT index at baseline was lower in the CLA group as compared with all other groups ($p < 0.05$) (Table 1). Postprandial PAT indices were not lower than fasting indices. The 4-week interventions did not change fasting PAT indices, although CLA administration improved the postprandial PAT index not significantly by around 6.9% as compared with safflower oil.

CLA decreased body weight as compared with safflower oil and other oils ($p < 0.05$). Reduction in BMI was not significant. WC and WHR did not change (data not shown). CLA did not affect systolic blood pressure (BP); it decreased

Table 2. Plasma and Urinary Analytes*

		CLA (n = 21)	Safflower Oil (n = 21)	Olive Oil (n = 19)	Safflower Oil, Heated (n = 20)	*p* All Groups	*p* CLA vs Safflower
Fasting							
Glucose (mmol/L)	Baseline	6.20 ± 0.98	6.10 ± 0.74	6.19 ± 0.70	6.14 ± 1.26	0.834	0.744
	Change	−0.09 ± 0.37	0.01 ± 0.39	0.01 ± 0.94	0.08 ± 0.99	0.286	0.435
Insulin (mU/L)	Baseline	13.3 ± 4.7	13.9 ± 6.7	15.2 ± 5.5	19.9 ± 9.8	0.069	0.900
	Change	−2.2 ± 4.3	−1.6 ± 6.7	−2.0 ± 6.7	−4.0 ± 9.9	0.791	0.435
HOMA-IR	Baseline	3.81 ± 1.44	3.91 ± 1.84	4.35 ± 1.66	6.03 ± 4.41	0.191	0.960
	Change	−0.66 ± 1.23	−0.44 ± 1.81	−0.49 ± 2.05	−1.43 ± 4.41	0.826	0.406
Total cholesterol (mmol/L)	Baseline	5.87 ± 0.94	5.75 ± 1.01	5.56 ± 0.87	5.62 ± 0.91	0.741	0.821
	Change	−0.27 ± 0.59	−0.01 ± 0.39	0.18 ± 0.72	0.07 ± 0.61	0.173	0.083
HDL cholesterol (mmol/L)	Baseline	1.46 ± 0.29	1.56 ± 0.30	1.51 ± 0.47	1.30 ± 0.33†‡	0.029	0.379
	Change	−0.02 ± 0.23	−0.03 ± 0.21	0.00 ± 0.31	0.06 ± 0.14	0.145	0.725
LDL cholesterol (mmol/L)	Baseline	3.90 ± 0.81	3.81 ± 0.84	3.51 ± 0.75	3.77 ± 0.74	0.405	0.706
	Change	−0.26 ± 0.49	−0.07 ± 0.31	−0.06 ± 0.54	−0.07 ± 0.57	0.388	0.070
Triglycerides (mmol/L)	Baseline	1.59 ± 0.64	1.53 ± 0.93	2.26 ± 2.68	1.96 ± 2.00	0.635	0.268
	Change	0.03 ± 0.62	−0.09 ± 0.53	−0.38 ± 1.55	−0.06 ± 1.33	0.801	0.554
Lp(a) (μmol/L)	Baseline	1.22 ± 1.22	1.90 ± 1.34	1.40 ± 1.07	0.92 ± 0.79	0.146	0.119
	Change	−0.06 ± 0.32	0.21 ± 0.48	0.03 ± 0.70	0.00 ± 0.31	0.489	0.163
hs-CRP (mg/L)	Baseline	1.8 ± 1.0	2.5 ± 2.5	3.5 ± 2.9	2.6 ± 2.2	0.664	0.880
	Change	0.31 ± 1.4	−0.60 ± 1.9	−0.12 ± 2.9	−0.24 ± 1.7	0.563	0.191
s-VCAM (ng/mL)	Baseline	755 ± 223	710 ± 184	819 ± 282	952 ± 328	0.087	0.615
	Change	−21.5 ± 104	50.0 ± 134	−69.0 ± 201	−27.3 ± 122	0.077	0.119
s-ICAM (ng/mL)	Baseline	277.6 ± 50.1	291.2 ± 69.5	308.6 ± 66.4	289.2 ± 75.1	0.537	0.725
	Change	1.9 ± 41.5	7.3 ± 31.4	5.3 ± 18.0	2.3 ± 22.3	0.878	0.615
s-E-Selectin (ng/mL)	Baseline	46.9 ± 23.3	48.0 ± 20.2	52.4 ± 18.5	48.8 ± 15.3	0.745	0.744
	Change	−0.6 ± 7.7	0.4 ± 5.8	−0.3 ± 5.0	2.4 ± 5.6	0.666	0.880
PON paraoxonase (U/L)	Baseline	153.2 ± 70.0	154.1 ± 72.0	129.7 ± 72.5	150.5 ± 68.0	0.613	0.938
	Change	0.3 ± 19.7	11.0 ± 17.6	−1.0 ± 11.3	5.0 ± 20.7	0.078	0.124
PON arylesterase (U/mL)	Baseline	179.4 ± 42.6	174.1 ± 24.0	205.0 ± 49.3	165.5 ± 35.1	0.060	0.774
	Change	−3.2 ± 23.4	13.4 ± 20.0†	−6.5 ± 24.4‡	9.7 ± 18.4†§	0.026	0.039
PAF-AH (U/L)	Baseline	34.4 ± 10.6	34.0 ± 9.2	33.7 ± 8.9	36.4 ± 9.9	0.812	0.990
	Change	0.89 ± 5.25	0.58 ± 3.31	0.52 ± 3.98	0.56 ± 5.43	0.876	0.521
8-iso-$PGF_{2\alpha}$ (pg/mgCrea)	Baseline	62.8 ± 18.0	56.9 ± 20.2	63.4 ± 27.6	54.9 ± 15.4	0.263	0.107
	Change	147.0 ± 89.3	5.2 ± 12.6†	4.5 ± 11.0†	7.3 ± 10.3†	<0.001	<0.001
OxLDL (U/L)	Baseline	80.9 ± 21.6	74.8 ± 20.0	71.1 ± 17.6	69.1 ± 22.1	0.350	0.435
	Change	−4.44 ± 12.6	−0.69 ± 11.3	−0.04 ± 8.7	2.89 ± 10.2	0.410	0.481
Postprandial area under curve							
Glucose (mmol × L^{-1} × h)	Baseline	28.5 ± 3.6	28.0 ± 3.2	27.7 ± 2.5	28.4 ± 4.3	0.975	0.898
	Change	−0.17 ± 1.69	−0.66 ± 2.33	0.48 ± 2.54	−0.58 ± 3.06	0.915	0.821
Insulin (mU × L^{-1} × h)	Baseline	172 ± 101	187 ± 140	180 ± 80	225 ± 125	0.312	0.265
	Change	2.7 ± 71.2	21.2 ± 61.2	36.6 ± 88.2	−19.1 ± 104.2	0.251	0.694
Triglycerides (mmol × L^{-1} × h)	Baseline	20.7 ± 7.8	18.6 ± 11.1	23.1 ± 20.4	22.7 ± 18.1	0.590	0.151
	Change	0.68 ± 6.65	0.37 ± 6.46	−0.60 ± 6.43	−0.03 ± 9.66	0.987	0.979

* Means ± SD.
† Significantly different from CLA.
‡ Significantly different from safflower oil.
§ Significantly different from olive oil (post hoc Mann-Whitney).

diastolic BP slightly but not significantly more than safflower oil.

CLA as compared with safflower oil consumption changed neither fasting indices of insulin sensitivity and glycemic control, namely, fasting glucose, insulin, and HOMA, nor postprandial glucose and insulin response (Table 2). CLA decreased total and LDL-C concentrations somewhat more than safflower oil, by 4.2% and 3.9% ($p = 0.08$ and 0.07), and decreased the T-C/HDL-C ratio by 5.3% (not significant). No changes in HDL-C, fasting triglycerides, and postprandial triglyceride response were noted (Table 2).

OxLDL concentration at baseline correlated positively with T-C (r = 0.455), LDL-C (r = 0.488), and fasting glucose (r = 0.305), and negatively with fasting (r = −0.282) and

postprandial PAT index (r = −0.342) (n = 81). No significant treatment-dependent changes in oxLDL were noted, nor were significant treatment-dependent changes in other inflammatory markers, namely, Lp(a), hs-CRP, and the soluble adhesion molecules sVCAM, sICAM, and sE-selectin (Table 2). CLA as compared with safflower oil consumption did not affect PAF-AH activity but decreased PON activity by 7.1% (not significant) and PON arylesterase activity by 8% ($p < 0.05$). CLA intervention significantly increased urinary F_2-isoprostanes compared with safflower oil and other treatments ($p < 0.001$).

DISCUSSION

Taylor et al. [6] were the first to determine the effects of CLA on endothelial function. Based on the wrist cuff variant of the brachial artery FMD technique, consuming CLA as compared with olive oil impaired endothelial function dramatically, by more than 50%. However, other symptoms of the metabolic syndrome and inflammatory markers were not impaired, except for increased F_2-isoprostane concentrations. The authors themselves speculated that reduced limb skinfold thickness, caused by CLA, may have interfered with FMD measurement. The present study reexamined the effect on endothelial function. In this study, the 4-week CLA mixture supplementation did not change the fasting PAT index as compared with safflower oil and all other oils. The postprandial PAT index was slightly but not significantly improved by 6.9% as compared with safflower oil, and even more when compared with olive and heated safflower oil. Our findings are consistent with those of other groups that used somewhat different ways to assess endothelial elasticity following consumption of cis9,trans11-CLA enriched milk [24] or a cis9,trans11-CLA supplement [25]. Contrary to expectations, the postprandial PAT index in the present study was hardly lower than the fasting value. It might be that the high protein content of the meal prevented fat-induced impairment [26], or that endothelial function had already returned to near-baseline values at this time point [16]. Alternatively, because tocopherol can prevent a postprandial inflammatory response [27], a supply of tocopherol with supplements may have attenuated or prevented postprandial impairment of endothelial function. Nevertheless, postprandial PAT readings substantiate results in the fasting state.

The favorable trend in endothelial function was accompanied by a slightly (not significantly) reduced diastolic blood pressure following intake of CLA as compared with safflower and other oils. An effect on blood pressure so far has not been observed in other human studies [6,24,25], but the combination of CLA and calcium reduced pregnancy-associated hypertension [28], and CLA supplementation reduced blood pressure in spontaneously hypertensive rats [29].

Here, CLA as compared with safflower oil consumption reduced body weight ($p < 0.05$) and BMI (not significantly). In several human studies, CLA reduced body weight or decreased fat mass, increased lean body mass [30], increased basal energy expenditure [31], prevented weight gain during the holiday season [32], or limited weight gain in obese children [33]. It might be that subtle metabolic changes lead to weight reduction only if CLA consumption is combined with physical exercise [34], as confirmed in experimental animals [35]. Our study was carried out during the summer season, when increased physical activity is likely.

CLA reduced total and LDL-C concentrations not significantly more than safflower oil consumption. A significant reduction was observed by Moloney et al. [7], although most other human studies observed no changes in lipid levels [5,6]. CLA did not change fasting glucose, insulin, and HOMA or the postprandial response of glucose and insulin to the fat- and sucrose-rich meal. Previous observations of the effects of CLA on insulin sensitivity indices were somewhat conflicting. This may be due to the different CLA preparations used, as both the cis9,trans11-isomer [8] and the trans10,cis12-isomer [9] decreased insulin sensitivity as compared with olive oil, while the CLA mixture did not [9]. Moloney et al. [7] found adverse effects of CLA only on some fasting insulin sensitivity indices, but not on postprandial glucose, insulin, and C-peptide response to a glucose load. Other long-lasting studies found no adverse effects of CLA [6,36] but beneficial effects on serum insulin and free fatty acids in women exercising regularly [37]. In most studies, CLA did not affect glucose and insulin concentrations [5].

Anti-inflammatory properties are attributed to CLA on the basis of decreased cyclooxygenase activity and decreased concentrations of cytokines and prostaglandin E_2 (PGE_2) in in vitro and animal studies [5]. In humans, however, CLA did not change serum concentrations of tumor necrosis factor-alpha (TNF-α) [6,10,11,36] or interleukin (IL)-6 and IL-8 [36]. However, when peripheral blood mononuclear cells (PBMCs) were challenged with lipopolysaccharide, cells of subjects consuming CLA released less proinflammatory TNF-α and IL-6 and more anti-inflammatory IL-10 than control cells of healthy subjects consuming high-oleic sunflower oil [38]. Similar effects were observed in pigs [39]. CLA supplementation also decreased the in vitro production of TNF-α, interferon-γ, and IL-5 in stimulated PBMCs of allergic subjects [40]. Adding CLA to a typical Western diet produced no physiologic change in blood coagulation or platelet function in healthy adults [41]. In some instances, CLA consumption increased CRP concentration. But at the same time, CLA did not change other inflammatory markers, namely, sVCAM, TNF-α, and sTNF-α receptors [11], nor did it increase γ-tocopherol, while it left serum α-tocopherol concentration unchanged [42]. In our study, CLA showed no adverse effects on concentrations of CRP, sICAM, sE-selectin, and sVCAM.

These findings are thus in line with those of most previous studies. CLA did not induce expression of proinflammatory cytokines in adipose tissue of mice when compared with sunflower oil [43], and it decreased expression of several anti-inflammatory genes in adipose tissue of humans when compared with linoleic acid [44]. The study of Herrmann et al. [44] shows that changes in gene expression depend on the CLA isomers consumed, and also on the peroxisome proliferator-activated receptor (PPAR)gamma2 polymorphism. It thus provides several clues regarding why different studies may have different outcomes.

We identified additional biomarkers of inflammatory and oxidative stress response. PON and PAF-AH are enzymes with anti-inflammatory function [45]. They protect LDL against lipid peroxidation by hydrolyzing oxidized phospholipids. It is not completely clear whether PON or arylesterase [46], or rather lactonase activity [45,47], is responsible for this protective PON function. In previous studies, CLA protected PON-1 against oxidative inactivation [48], and cis9,trans11-CLA increased PON activity in plasma from hamsters [49]. In this study, CLA reduced arylesterase activity significantly by 8% and PON activity not significantly by 7.1% as compared with safflower oil. PAF-AH activity was not changed. One may wonder whether decreased enzyme activity indicates increased CLA-induced oxidative stress. Other reports have described unexpected reductions in PON activity, namely, with caloric restriction in rats [50] and with increased vegetable consumption in humans [51]. We interpret the findings to indicate that the metabolic condition following CLA consumption became less pro-oxidant, and that therefore less enzyme activity was required. Furthermore, even though increased oxidative stress reduces PON-1 and PON-3 activity in serum, it may increase PON-2 activity in tissues [45].

Lp(a) is a specific atherogenic LDL particle that carries oxidized phospholipids [52]. As in a previous study [36], CLA did not change Lp(a). OxLDL is another atherogenic particle carrying oxidized phospholipids. The present study is the first to examine the effects of CLA on plasma oxLDL concentration. Plasma oxLDL correlates with endothelial dysfunction [52] and with symptoms of the metabolic syndrome [53,54], but most oxLDL is found in vessel walls [52]. The fact that oxLDL concentrations of our participants at study entry correlated positively with T-C, LDL-C, and glucose concentrations, and negatively with endothelial function, fits with previous observations. The fact that CLA reduced oxLDL concentration modestly but not significantly and did not change Lp(a) concentration speaks against increased CLA-induced production of oxidized phospholipids.

The F_2-isoprostane 8-iso-$PGF_{2\alpha}$ is an established index for nonenzymatic lipid peroxidation [55]. Elevated concentrations in plasma or urine are frequently associated with traits of the metabolic syndrome such as hypercholesterolemia, diabetes, and obesity [56], although this is not always the case [53,54]. Among numerous studies performed, CLA consumption consistently increased concentrations of urinary 8-iso-$PGF_{2\alpha}$ [6,8–10,40,42] and did so in this study. In a recent study, CLA consumption did not increase malondialdehyde—another index of lipid peroxidation [42]. Of note, conditions of oxidative stress frequently result in an increase in both markers of oxidative stress (i.e., malondialdehyde and 8-iso-$PGF_{2\alpha}$ alike [55]).

Oxidative stress generates numerous D_2-, E_2-, and F_2-isoprostane species [55,56], both free and esterified to phosphatidylcholine, in tissues and in plasma [55]. Esterified isoprostanes disturb cell membrane integrity [57], and an esterified E_2-isoprostane is among proinflammatory oxidized phospholipids in lipoproteins [58]. The release of isoprostanes from phospholipids is catalyzed by PON, PAF-AH, and other soluble phospholipases A_2 [58,59]. CLA did not amplify PON or PAF-AH activity, rendering increased release of free F_2-isoprostanes by these enzymes unlikely. However, we cannot exclude a role for other soluble phospholipase A_2 activities, which were not tested. According to recent data, CLA competes with 8-iso-$PGF_{2\alpha}$ for peroxisomal β-oxidation and by this mechanism blocks its degradation [60]. In line with this observation, we previously reported that children with peroxisomal β-oxidation deficiency exhibit impaired metabolic clearance of 8-iso-$PGF_{2\alpha}$ [61]. Thus further investigation has to provide evidence of whether CLA interacts with 8-iso-$PGF_{2\alpha}$ exclusively or with several D_2-, E_2-, and F_2-isoprostanes.

CONCLUSION

Four-week supplementation with a CLA-mixture (Tonalin 80 TG) as compared with safflower oil in overweight and obese men did not impair endothelial function as measured by the PAT index; it decreased weight and moderately but not significantly improved diastolic blood pressure, some serum lipids, and some markers of inflammation and oxidative stress. The way in which increased 8-iso-$PGF_{2\alpha}$ concentrations fit into this context needs to be clarified in additional studies, although delayed degradation may be an explanation.

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