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RESEARCH ARTICLE

Dietary rapeseed/canola-oil supplementation reduces serum lipids and liver enzymes and alters postprandial inflammatory responses in adipose tissue compared to olive-oil supplementation in obese men

Michael Kruse[1,2], Christian von Loeffelholz[1,2,3,4], Daniela Hoffmann[1], Antje Pohlmann[1], Anne-Cathrin Seltmann[1], Martin Osterhoff[1,2], Silke Hornemann[1,2], Olga Pivovarova[1], Sascha Rohn[5], Gerhard Jahreis[6] and Andreas F. H. Pfeiffer[1,2]

[1] Department of Clinical Nutrition, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany
[2] Department of Endocrinology, Diabetes and Nutrition, Charité University Medicine Berlin, Berlin, Germany
[3] Center for Sepsis Control and Care (CSCC), Integrated Research and Treatment Center, Jena University Hospital, Jena, Germany
[4] Department of Anesthesiology and Intensive Care, Jena University Hospital, Jena, Germany
[5] Institute of Food Chemistry, Hamburg School of Food Science, University of Hamburg, Hamburg, Germany
[6] Department of Nutritional Physiology, Institute of Nutrition, Friedrich Schiller University of Jena, Jena, Germany

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Scope: Obesity is associated with hyperlipidemia, hepatic steatosis, and low-grade inflammation. Studies have shown that MUFA as well as PUFA have beneficial effects on blood lipids and the inflammatory state.

Methods and results: This study investigates the effects of a daily supplementation of either 50 g of rapeseed/canola (RA) or olive (OL) oil over 4 wk on serum lipids, serum liver enzymes, and inflammatory gene expression in subcutaneous (s. c.) adipose tissue in obese men. Consuming RA resulted in increased serum *n*-3 fatty acids and a reduction in total cholesterol, LDL cholesterol, and serum aspartate aminotransferase compared to OL. In s. c. adipose tissue, gene expression of the pro-inflammatory cytokine IL6 was reduced in RA compared to OL. However, after 4 h after a test meal, containing the appropriate oil, white bread, and 400 mL of liquid diet drink (835 kcal in total), gene expression of IL6, IL1B, and EMR1 (egf-like module containing Mucin-like hormone receptor-like 1) was increased in RA and of monocyte chemoattractant protein-1 (CCL2) in both RA and OL.

Conclusion: This demonstrates that consuming RA for 4 wk improves serum lipids, liver enzymes, and basal inflammation in s. c. adipose tissue, but it mediates an acute pro-inflammatory response in adipose tissue upon consuming a meal.

Keywords:
Inflammation / Obesity / Olive oil / Rapeseed oil / Serum lipids

Additional supporting information may be found in the online version of this article at the publisher's web-site

Correspondence: Professor Andreas F. H. Pfeiffer, Department of Clinical Nutrition, German Institute of Human Nutrition Potsdam-Rehbruecke, Arthur-Scheunert-Allee 114–116, 14558 Nuthetal, Germany
E-mail: afhp@dife.de

Abbreviations: **ALA**, alpha-linolenic acid; **ALT**, alanine-aminotransferase; **AST**, aspartate-aminotransferase; **BMI**, body mass index; **CCL2**, chemokine C-C motif ligand 2; **CRP**, C-reactive protein; **DHA**, docosahexaenoic acid; **EMR1**, egf-like module containing Mucin-like hormone receptor-like 1; **EPA**, eicosapentaenoic acid; **FA**, fatty acids; **FFA**, free fatty acids; **γ-GT**, γ-glutamyltranspeptidase; **HOMA IR index**, Homeostatic Model Assessment insulin resistance index; **MCP-1**, monocyte chemoattractant protein-1; **NAFLD**, nonalcoholic fatty liver disease; **OL**, olive oil; **RA**, rapeseed/canola oil; **s. c.**, subcutaneous; **SFA**, saturated fatty acids; **TPC**, total phenolic content

1 Introduction

Obesity, metabolic syndrome, and type 2 diabetes mellitus are global disease burdens leading to major cardiovascular complications and subsequently causing increased medical costs [1–3]. Individuals suffering from obesity present with one or a combination of symptoms such as increased serum levels of LDL cholesterol, triacylglycerols, and free fatty acids (FFA) and reduced serum levels of HDL cholesterol, hypertension, and hepatic steatosis [4, 5]. Furthermore, it is now commonly accepted that obesity is associated with chronic subclinical inflammation of the adipose tissue resulting in increased systemic cytokine release that could accelerate cardiovascular diseases [6]. In case of long-standing and even exacerbated obesity in combination with hypercaloric nutrition and/or sedentary lifestyle, it is very likely that obesity will transform into the metabolic syndrome and overt diabetes mellitus.

In light of the high prevalence of obesity, its associated complications, progression of disease, and medical costs, it seems favorable to find efficient, inexpensive, and easy-to-manage preventive and therapeutic options. So far, initial preventive recommendations address lifestyle changes including exercise and dietary modifications.

As for dietary recommendations, clinical trials have shown that consuming MUFA as well as PUFA can improve serum lipids [7]. A diet rich in MUFA was able to reduce total and LDL cholesterol in moderately obese humans [8]. A diet enriched with *n*-6 PUFA from sunflower oil showed a decrease in postprandial triacylglycerols when compared to an equal amount of butter [9]. Rapeseed oil (RA) contains the *n*-3 fatty acid (FA) alpha-linolenic acid (ALA). Clinical studies using radioisotopes have shown that the elongation of ALA to eicosapentaenoic acid (EPA) and, to a lesser extent, to docosahexaenoic acid (DHA) in the human organism is possible, but it is not very efficient [10]. However, human studies demonstrated that consuming a diet supplemented with ALA-rich vegetable oils increased plasma EPA-concentrations comparable with fish-oil supplementation [11]. Dietary supplementation with ALA as well as with its conversion products EPA and DHA reduced fasting serum triglycerides in normolipidemic humans [12].

It has been shown that the ratio of total to HDL cholesterol is a predictive marker for estimating the risk of coronary artery disease [13]. A meta-analysis performed by Mensink et al. has shown that an equal replacement of dietary saturated FAs (SFA) with *cis* MUFA is able to improve the total/HDL cholesterol ratio [14]. In this analysis, RA was the most powerful oil to reduce total/HDL cholesterol ratio followed by soybean oil and olive oil (OL).

The low-grade inflammation seen in obesity results into elevated serum levels of C-reactive protein (CRP) and cytokines such as TNF-α or IL-6 [6]. Replacing SFA with *n*-6 PUFA was able to reduce postprandial TNF-α and IL-6 levels in obese men [9]. Also, the long-chain *n*-3 FA EPA and DHA are known to have anti-inflammatory effects [15]. A diet supplemented with ALA from walnut and flaxseed oil decreased serum CRP levels in hypercholesterolemic men and women compared to an average American diet [16].

The powerful protective effect of high intake of OL in the context of a Mediterranean diet in Spain was recently demonstrated [17]. However, in northern European countries high intakes of OL are not realistically achievable. Therefore, a replacement of fat with RA, including mayonnaise or bakery products, may be more realistic. We therefore compared OL to RA in this study as a prelude to larger and longer studies.

Obese individuals with low-grade inflammation are at a risk of developing nonalcoholic fatty liver disease (NAFLD) that is associated with elevated serum liver enzymes such as aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT) and hepatic insulin resistance [18]. There appears to be a cross-talk between adipose tissue and liver, since it was shown that increased IL-6 release from obese adipose tissue can induce hepatic insulin resistance [19, 20]. Modulating dietary fat composition may improve NAFLD. Recently, it was shown that *n*-6 PUFA from sunflower oil reduced liver fat content independently of weight loss in obese individuals [21]. A Japanese study showed that EPA and DHA are preventive nutrients for NAFLD in Japanese men [22].

This study aims to analyze the influence of a daily nutritional supplementation of MUFA and PUFA to an isocaloric diet on body composition, serum lipids, serum liver enzymes, and inflammatory gene expression in subcutaneous (s. c.) adipose tissue as primary outcome variables in moderately obese men. Additionally, the postprandial inflammatory response was investigated after a test meal. Most clinical studies compare the nutritional effects of a relatively healthy diet that contains high amounts of MUFA or PUFA with a diet that contains mostly SFA. This study compares a supplementation with either 50 g of OL or RA, both known as favorable nutritional components. Both oils contain a high and almost equal amount of MUFA, whereas RA contains more PUFA, especially ALA and *n*-6 linoleic acid [23]. To circumvent possible confounding effects of micronutrients (e.g., tocopherols, polyphenols), we used cold-pressed extra virgin OL and refined RA with only marginal or even equal concentrations of these compounds.

2 Materials and methods

2.1 Participants

Participants of this study were recruited either from a register of individuals who participated previously in a study performed in our unit or by advertisements in the local newspapers. Only men were included in this study to exclude confounding influences that might result from the hormonal status of estrogens. A total number of 18 moderately obese (body mass index (BMI) 27–35 kg/m^2) men, aged 39–63, were randomly assigned to the OL or the RA arm of the study after a screening examination. Parameters used for randomization

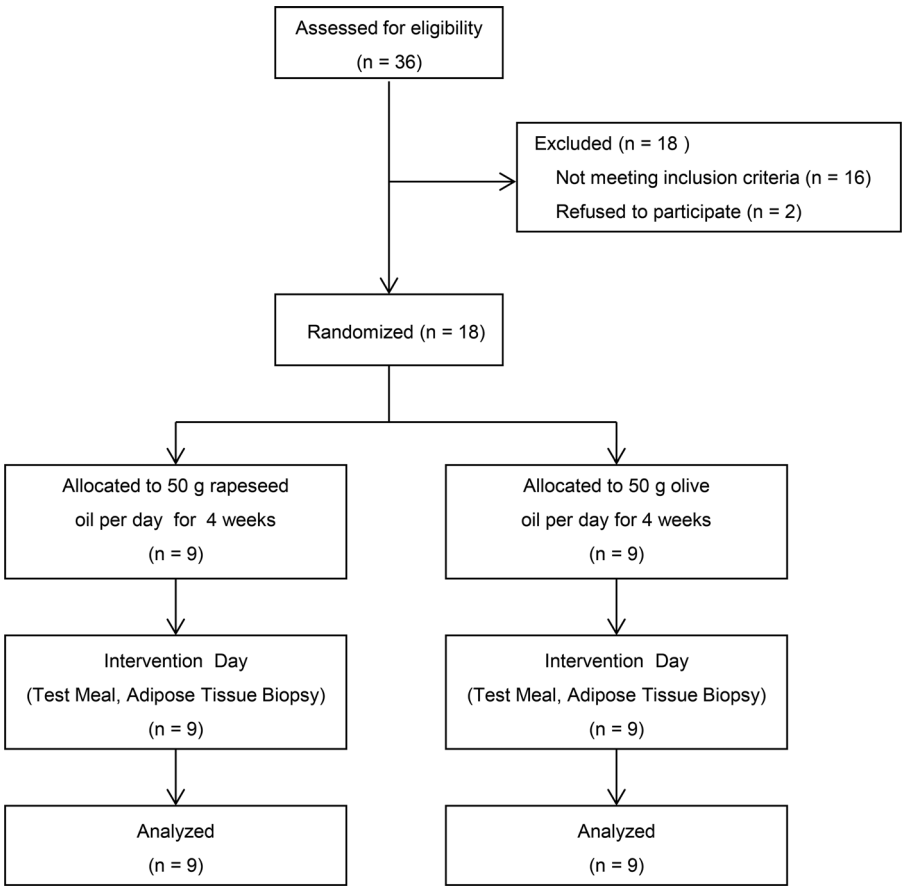

Figure 1. Flow diagram of the study.

were age (years), body weight (kg), height (m), and waist circumference (cm). A computerized program was used to assure that both groups were equally balanced in terms of parameters used for randomization and group size. Individuals diagnosed with diabetes mellitus type 2 or any other illness that would interfere with the study results or were taking medication that would influence the results, especially medication influencing lipid metabolism, liver metabolism, or inflammation, were excluded from the study. All volunteers who were randomized and included completed the study ($n = 18$); there was no dropout individual (Fig. 1). On the screening day, a physical examination including medical history was performed and height (m), body weight (kg), waist and hip circumference (cm) were taken and BMI and waist-to-hip ratio were calculated. Total body fat was determined using an air-displacement plethysmography system [24]. Blood was collected after an overnight fast for analysis of serum lipids, serum glucose, AST, ALT, γ-glutamyl-transpeptidase (γ-GT), monocyte chemoattractant protein-1 (MCP-1), and IL-6. Baseline characteristics of the individuals in the OL and RA groups are shown in Table 1.

All participants gave their written consent after the study design was explained to them in detail. The study protocol was approved by the local ethical committee of the University of Potsdam (1/2010) and was in accordance with the Declaration of Helsinki of 1975, as revised in 1983. For this study, a modification of the CONSORT (Consolidated Standards of Reporting Trials) Statement as listed in the CONSORT 2010 checklist that was published recently [25] was applied. This trial was registered at Current Controlled Trials (http://www.controlled-trials.com), ISRCTN22073289.

2.2 Study design

After randomization, all individuals were asked to fill out a 3-day food record, with 1 day being a weekend day, regarding their dietary habits and send it to our unit. The food record was analyzed by a nutritionist with a specific emphasis on nutrients containing *n*-3 or *n*-6 FA. Individuals were invited for an initial detailed counseling. Energy requirements were calculated based on food records and levels of activity, the appropriate oil was handed out and all individuals were instructed to consume 50 g/day of the appropriate oil for 4 wk. Multiple options regarding how to consume the oils were proposed, e.g. as a salad dressing, as mayonnaise, mixed with curd, as a topping of a toasted slice of bread with herbs and/or spices, or as a pesto or sauce for pasta, but not to use the oils for baking or frying. Individuals were told not to change their dietary habits and to adhere to the recommendations given by the nutritionist. It was the choice of the participants at what time of the day the 50 g of the oil was

Table 1. Baseline characteristics of the participants

	OL	RA	*p*-Value
Participants (*n*)	9	9	
Age (years)	58 ± 2.57	52 ± 2.30	0.080
Body height (m)	1.77 ± 0.01	1.77 ± 0.01	1.000
Body weight (kg)	91.9 ± 3.23	93.5 ± 3.89	0.748
BMI (kg/m^2)	29.2 ± 0.67	29.7 ± 0.87	0.646
Body fat content (%)	27.22 ± 1.37	29.48 ± 1.51	0.214
Waist/Hip ratio	0.98 ± 0.01	0.97 ± 0.01	0.827
Serum total cholesterol (mmol/L)	5.15 ± 0.32	5.24 ± 0.26	0.830
Serum LDL cholesterol (mmol/L)	3.31 ± 0.30	3.38 ± 0.23	0.844
Serum HDL cholesterol (mmol/L)	1.13 ± 0.08	1.16 ± 0.06	0.762
Serum triacylglycerol (mmol/L)	1.57 ± 0.42	1.53 ± 0.27	0.944
Serum FFA (mmol/IL)	0.51 ± 0.05	0.64 ± 0.06	0.152
Serum glucose (mg/dL)	107.50 ± 6.35	103.51 ± 3.42	0.589
Insulin (mU/L)	7.35 ± 1.11	7.70 ± 1.60	0.859
HOMA IR index	1.95 ± 0.29	2.04 ± 0.46	0.785
C-peptide (μg/L)	1.58 ± 0.23	1.29 ± 0.26	0.409
Serum AST (U/L)	33.00 ± 4.20	30.90 ± 2.43	0.667
Serum ALT (U/L)	42.97 ± 9.32	39.64 ± 7.13	0.781
Serum γ-GT (U/L)	43.37 ± 14.14	38.92 ± 6.70	0.780
Serum MCP-1 (pg/mL)	416.0 ± 42.80	421.5 ± 25.47	0.913
Serum IL-6 (pg/mL)	1.70 ± 0.33	1.80 ± 0.34	0.827
CRP (mg/L)	0.90 ± 0.34	1.66 ± 0.74	0.368

Data are given as mean ± SEM. No statistically significant differences were observed between the OL and the RA groups for any of the indicated parameters.

consumed. The oils used in this study were commercially available, cold-pressed extra virgin OL and refined RA. For each individual, the study started on the day after initial nutritional counseling. After 2 wk of oil supplementation, participants were asked again to fill out a 3-day food record and to see our unit for a second in-depth nutritional counseling to ensure that they were still following the initial instructions. A flow diagram of the study is shown in Fig. 1.

2.3 Test meal and adipose tissue biopsy

After 28 days (4 wk) of oil supplementation, participants came to our unit after an overnight fast (fasting started at 22:00 pm on the previous day). At 08:00 am fasting blood samples were taken and anthropometry measurements were performed. At 08:30 am, a biopsy of the s. c. periumbilical adipose tissue was performed and approximately 1 g of tissue was taken, rinsed briefly with 0.9% NaCl saline, immediately snap-frozen in liquid nitrogen, and stored at –80°C for further analyses. At 09:00 am, participants consumed a test meal within 10 min. The test meal contained 25 g of the appropriate oil (OL or RA, 205 kcal), two slices of white bread (each 25 g, total of 130 kcal), 200 mL of Fresubin original DRINK (containing 15% of protein, 55% of carbohydrates, 30% of fat; total of 200 kcal), and 200 mL of ProvideXtra DRINK (containing 11% of protein, 89% of carbohydrates, 0% of fat; total of 300 kcal). The FA composition of the Fresubin original DRINK per 100 mL was <0.01 g of myristic acid (C14:0), 0.34 g of palmitic acid (C16:0), 0.22 g of stearic acid (C18:0), 5.53 g of oleic acid (C18:1), 1.11 g of linoleic acid (C18:2), and 0.27 g of ALA (C18:3). Both test meals contained 19.1 g of protein (80 kcal), 114.9 g of carbohydrates (471 kcal), and 30.5 g of fat (284 kcal). The overall composition of macronutrients of the test meals was 9.6% of protein, 56.4% of carbohydrates, and 34.0% of fat (total of 835 kcal). Blood was collected at 60, 120, and 240 min after the test meal was finished. A second s. c. adipose tissue biopsy was performed 4 h after the test meal at 01:00 pm.

2.4 Determination of TPC and tocopherols of OL and RA

Total phenolic content (TPC) was determined according to Haiyan et al. [26]. Tocopherol analysis was carried out using HPLC on a LC-DAD Smartline series system from Knauer (Berlin, Germany). The separation was carried out on Superspher 4 Si_{60}, 125 × 4 mm column from Merck (Darmstadt, Germany) at a temperature of 21°C, a flow rate of 0.8 mL/min, and fluorescence detection at an emission wavelength of 330 nm (294 nm excitation). The mobile phase was isooctane/ethyl acetate (90:6). The concentrations were calculated from calibration curves prepared for a- and c-tocopherols.

2.5 Blood analysis

Serum parameters (total cholesterol, HDL, triacylglycerols, FFA, AST, ALT, γ-GT, CRP) were measured using standard techniques in a certified laboratory for clinical chemistry at

the German Institute of Human Nutrition. LDL cholesterol was calculated from the above data. Serum insulin and C-peptide were measured using commercially available ELISA (Mercodia, Uppsala, Sweden). Glucose concentrations were measured in venous serum samples (ABX Pentra 400; ABX Diagnostics, Montpellier, France). Serum parameters and serum glucose concentrations were measured at fasting conditions before (screening day) and 4 weeks after the dietary intervention. Homeostatic Model Assessment (HOMA) was used to estimate insulin resistance by calculating the product of fasting serum insulin concentrations (μU/mL) and fasting serum glucose concentration (mg/dL) divided by 405 [27].

Serum MCP-1 was measured using a human chemokine C-C motif ligand 2 (CCL2/MCP-1) Quantikine ELISA (R&D Systems, Wiesbaden, Germany). Serum IL-6 was measured using a human IL-6 Quantikine high-sensitive ELISA (R&D Systems). Blood was collected at the indicated time points and centrifuged immediately. Serum was stored at –80°C for further analysis. MCP-1 and IL-6 were analyzed at fasting conditions on the screening day and after 4 weeks of oil supplementation and at 60, 120, and 240 min after the test meal.

2.6 Lipid extraction and transmethylation

Lipids were extracted from plasma based on the method of Bligh and Dyer as previously detailed [28]. Lipid extracts were methylated by acid- and base-catalyzed procedures using a combination of 0.5 N methanolic sodium hydroxide (Merck, Germany) and 10% (w/w, Supelco, USA) boron trifluoride-methanol (100°C for 5 min each). Subsequently, FA methyl esters were purified by thin-layer chromatography and dissolved in *n*-hexane for analysis. A system of two gas chromatographs/flame ionization detectors (GC/FID) was used to analyze the full FA spectrum from C4 to C26 (GC-17 V3 Shimadzu, Japan; DB-225MS: 60 m, i.d. 0.25 mm, 0.25 μm film thickness; Agilent Technologies, USA) as well as *cis* and *trans* isomers of C18:1, *trans* C18:2 and C18:3 (GC-2010, Shimadzu, Japan; CP-select: 200 m, i. d. 0.25 mm, 0.25 μm film thickness; Varian, The Netherlands) [29]. FA concentrations are expressed as the percentage of the total area of all FA peaks (% of total FA methyl esters).

2.7 Gene expression analysis

mRNA expression analysis was performed in biopsies of s. c. adipose tissue using quantitative real time-PCR as described previously [30]. Gene expression analysis was performed for cytokines (*TNF*, *SERPINE1* (serpin peptidase inhibitor, clade E, nexin, plasminogen activator inhibitor type 1, member 1), *IL1B*, *IL6*, *IL8*, *IL10*) and macrophage markers (*CCL2*, *EMR1* (egf-like module containing Mucin-like hormone receptor-like 1)). The constitutively expressed gene *RPL32* (ribosomal protein L32) was used as a loading control. A list of primer sequences is shown in Supporting Information Table 1.

2.8 Statistical analysis

All data are given as mean ± SEM. Statistical analysis was performed using SPSS 20.0 (SPSS, Chicago, IL, USA). Normal distribution was tested using the Kolmogorov–Smirnov test. Paired Student's *t*-test was used to test for statistical significance within groups and unpaired Student's *t*-test was used to test for statistical significance between groups. A two-sided *p*-value <0.05 was considered significant.

3 Results

3.1 Energy consumption, dietary intake, and body composition

For OL, energy intake from fat increased from 37.4% at baseline to 47.9% after 4 weeks of oil consumption and for RA from 38.9 to 44.3%, respectively (Supporting Information Table 2). However, the relative increase in fat consumption did not affect body weight or body composition. As shown in Table 2, there were no significant changes for OL or RA in body weight, BMI, body fat content or waist/hip ratio over the time of intervention. RA led to a 1.59-fold increase ($p = 0.016$) in ALA and to a 1.26-fold increase ($p = 0.003$) in EPA plasma-concentration, respectively (Fig. 2). There was no increase in DHA observed ($p = 0.341$). For participants in the RA group, we saw a significant increase in plasma *n*-3 PUFA as shown in Table 3. Since plasma *n*-6 PUFA remained unchanged, this resulted in a significant decrease in the plasma *n*-6/*n*-3 ratio in this group (Table 3). We did not observe any changes in plasma *n*-6 or *n*-3 PUFA or in the plasma *n*-6/*n*-3 ratio in the OL group. An analysis of FA, tocopherols, and TPC of the OL and RA used in this study is shown in Supporting Information Table 3. OL and RA did not show any notable differences in alpha-tocopherol and TPC. As expected, gamma-tocopherol was not present in RA.

3.2 Serum lipids, glucose, insulin, HOMA IR, C-peptide, and CRP

After 4 wk of RA-oil supplementation, we observed a reduction of 11.6% in total cholesterol ($p = 0.004$) and of 15.2% in LDL cholesterol ($p = 0.003$), respectively, in fasting serum (Fig. 3). These changes were not seen for OL. A mean reduction of 0.55 ± 0.14 mmol/L in total cholesterol and of 0.45 ± 0.11 mmol/L in LDL cholesterol was achieved for RA. For OL, a mean reduction of 0.17 ± 0.19 mmol/L in total cholesterol and of 0.13 ± 0.17 mmol/L in LDL cholesterol was seen. However, these reductions did not reach significance when compared between groups (Table 4). The total/HDL cholesterol ratio significantly decreased from 4.65 ± 0.32 to 4.36 ± 0.33 ($p = 0.001$) in the OL group. In the RA group, it dropped from 4.65 ± 0.41 to 4.43 ± 0.35; however, this did not reach significance ($p = 0.229$, Fig. 3F). Fasting glucose levels tended

Table 2. Body weight, BMI, and body composition before and after dietary intervention

	OL			RA		
	Baseline	4 weeks	*p*-Value	Baseline	4 weeks	*p*-Value
Body weight (kg)	91.9 ± 3.23	92.1 ± 3.27	0.601	93.5 ± 3.89	93.1 ± 3.76	0.182
BMI (kg/m²)	29.2 ± 0.67	29.3 ± 0.66	0.825	29.7 ± 0.87	29.5 ± 0.85	0.105
Body fat content (%)	27.22 ± 1.37	26.73 ± 1.41	0.432	29.48 ± 1.07	28.28 ± 0.82	0.114
Waist/hip ratio	0.98 ± 0.01	0.97 ± 0.02	0.391	0.97 ± 0.01	0.98 ± 0.01	0.194

Data are given as mean ± SEM. *p*-Values are given for the comparison between baseline and 4 wk of oil consumption for the OL and RA groups.

to be lower after dietary intervention in both groups, however not significantly (Tables 3 and 4). We observed a significant decrease in fasting serum insulin levels in the RA group (Table 3). The mean reduction in insulin levels in the RA group was 2.19 ± 0.75 mU/L and almost reached significance ($p = 0.058$) when compared to the OL group that showed a slight mean increase in insulin levels of 0.26 ± 0.77 mU/L (Table 4). For RA, the calculated HOMA insulin resistance (HOMA IR) index significantly dropped from 2.04 at baseline to 1.40 after 4 wk of oil consumption ($p < 0.018$). The HOMA IR index also tended to be lower after OL consumption; however this was not significant. There were no differences between baseline levels and 4 wk of oil supplementation in either the OL or RA group for HDL cholesterol, triacylglycerols, FFA, C-peptide, or CRP (Fig. 3, Tables 3 and 4).

3.3 Liver enzymes

Serum levels of AST were significantly reduced by 18.0% after 4 wk of RA supplementation compared to baseline ($p = 0.037$), whereas no differences were observed for OL

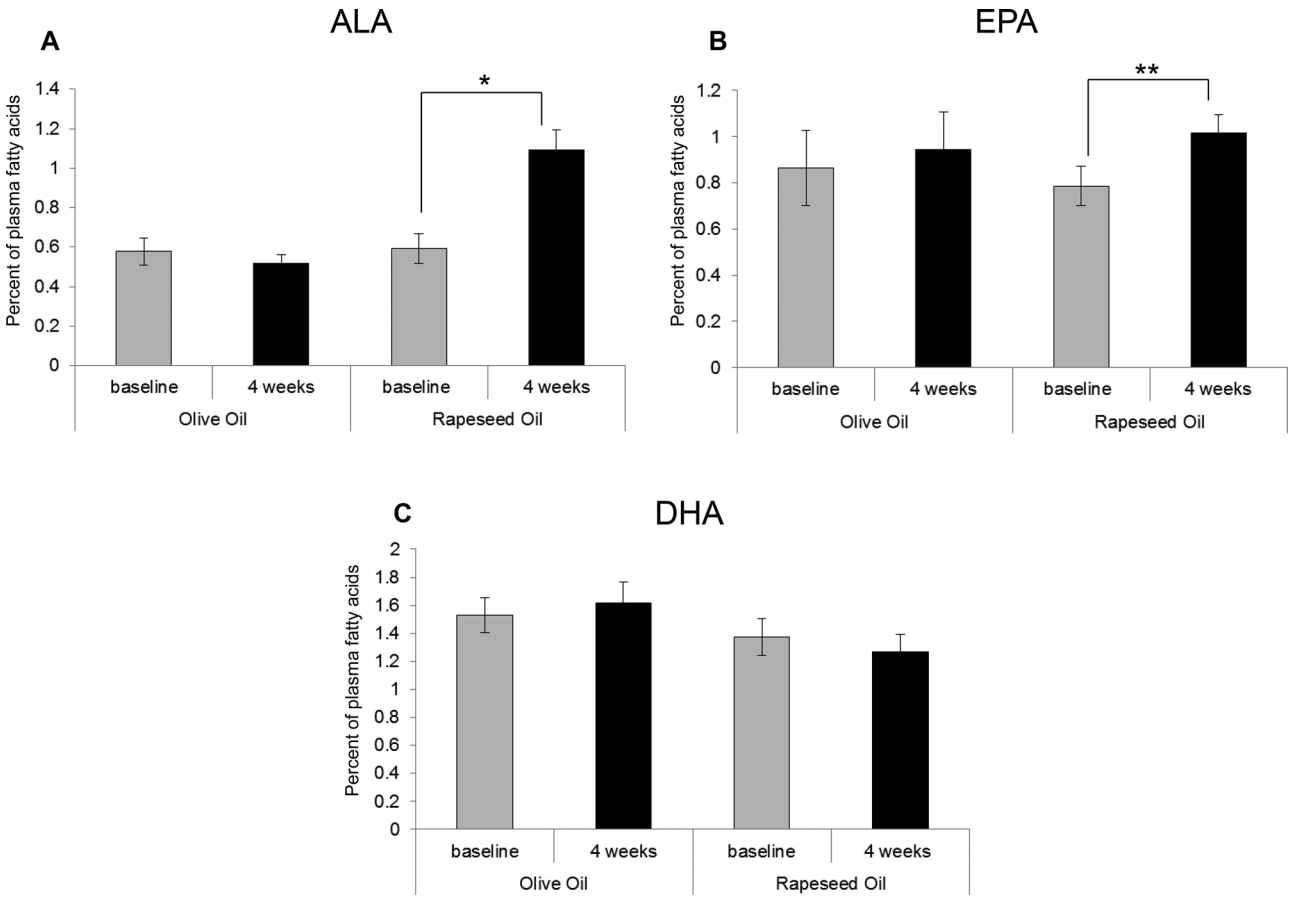

Figure 2. Percentage of ALA (A), EPA (B), and DHA (C) of plasma FA of fasting individuals before (baseline) and after (4 wk) daily supplementation with OL or RA. Data represent mean ± SEM; *$p = 0.016$, **$p = 0.003$.

Table 3. Blood glucose, insulin, C-peptide, CRP, plasma *n*-6 and *n*-3 PUFA content, and plasma *n*-6/*n*-3 ratio before and after dietary intervention

	OL			RA		
	Baseline	4 weeks	*p*-Value	Baseline	4 weeks	*p*-Value
Glucose (mg/dL)	107.50 ± 6.35	91.47 ± 4.63	0.068	103.51 ± 3.42	98.77 ± 3.34	0.089
Insulin (mU/L)	7.35 ± 1.11	9.15 ± 2.41	0.438	7.71 ± 1.61	5.52 ± 1.10	0.019
HOMA IR index	1.95 ± 0.29	1.53 ± 0.19	0.157	2.04 ± 0.46	1.40 ± 0.31	0.018
C-peptide (μg/L)	1.58 ± 0.23	2.05 ± 0.35	0.348	1.29 ± 0.26	1.12 ± 0.28	0.068
CRP (mg/L)	0.90 ± 0.34	0.71 ± 0.22	0.616	1.66 ± 0.74	1.97 ± 0.95	0.593
Total *n*-6 PUFA (%)	34.42 ± 2.53	33.58 ± 1.68	0.644	34.22 ± 1.39	34.47 ± 0.99	0.726
Total *n*-3 PUFA (%)	3.67 ± 0.30	3.68 ± 0.34	0.982	3.59 ± 0.26	4.17 ± 0.21	0.002
n-6/*n*-3 ratio	9.76 ± 0.97	9.48 ± 0.61	0.749	9.95 ± 0.84	8.43 ± 0.46	0.021

Data are given as mean ± stSEM. *p*-Values are given for the comparison between baseline and 4 wk of oil consumption for the OL and RA groups.

supplementation (Fig. 4). For serum levels of ALT we saw a reduction of 28.8% in the RA group; however, this did not reach significance (Fig. 4). No changes in the OL group were observed for serum levels of ALT. Neither RA nor OL supplementation changed serum levels of γ-GT (data not shown).

3.4 IL-6 and MCP-1 serum concentrations

After 4 wk of oil supplementation, we did not observe any significant changes in fasting IL-6 or MCP-1 serum levels (Supporting Information Fig. 1A and C, respectively) in both RA and OL groups. IL-6 serum levels increased within 4 h after the test meal in both groups and were slightly more elevated in RA compared to OL, but this difference did not reach significance. There was no increase in serum MCP-1 after the test meal in both groups. When postprandial serum levels of IL-6 and MCP-1 were individualized for each participant to fasting levels prior to the test meal, participants who consumed RA had increased IL-6 levels compared to OL, but that did not reach significance (Supporting Information Fig. 1B). There were no changes in individual

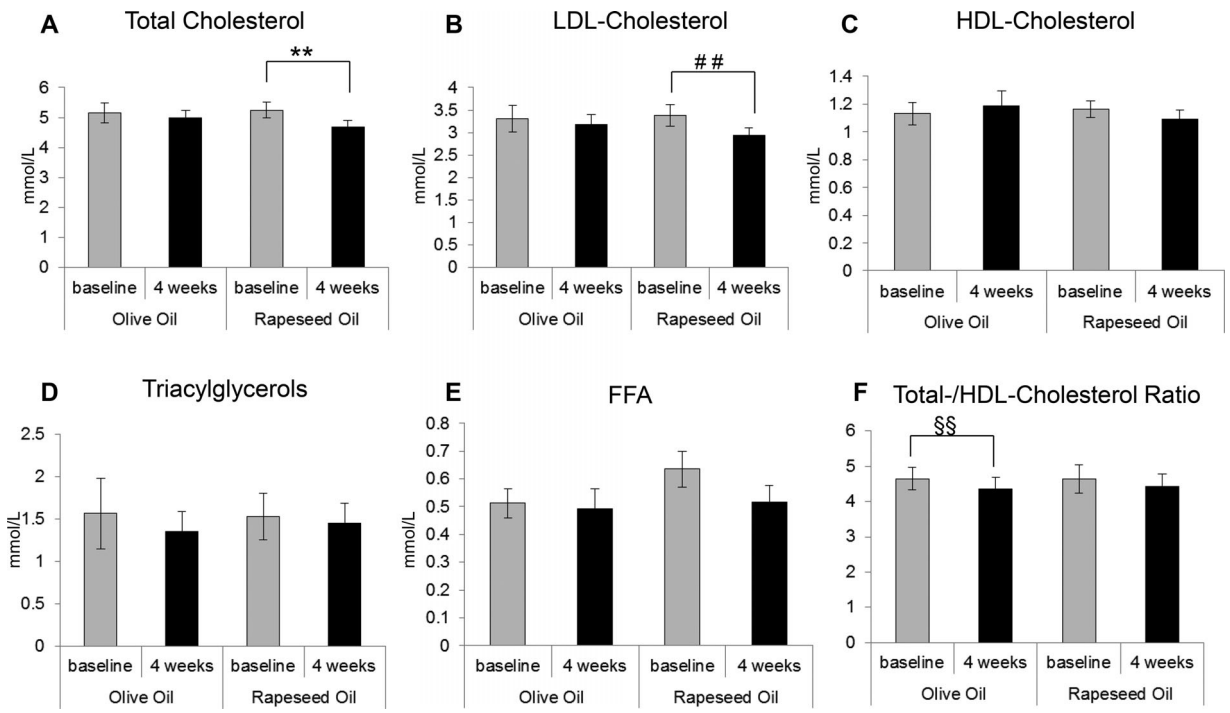

Figure 3. Serum levels of total (A), LDL (B), and HDL (C) cholesterol; triacylglycerols (D); and FFA (E) of fasting individuals before (baseline) and after (4 wk) daily supplementation with OL or RA. (F) Ratio of total/HDL cholesterol. Data represent mean ± SEM; $^{**}p = 0.004$, $^{\#\#}p = 0.003$, $^{\S\S}p = 0.001$.

Table 4. Mean changes from baseline to 4 wk of oil consumption

	OL	RA	*p*-Value
Body weight (kg)	0.27 ± 0.49	−0.38 ± 0.33	0.225
BMI (kg/m²)	0.04 ± 0.19	−0.22 ± 0.12	0.265
Body fat content (%)	−0.49 ± 0.59	−1.78 ± 0.93	0.267
Waist/hip ratio	−0.01 ± 0.01	0.01 ± 0.01	0.118
Serum total cholesterol (mmol/L)	−0.17 ± 0.19	−0.55 ± 0.14	0.128
Serum LDL cholesterol (mmol/L)	−0.13 ± 0.17	−0.45 ± 0.11	0.132
Serum HDL cholesterol (mmol/L)	0.06 ± 0.05	−0.07 ± 0.04	0.061
Total/HDL cholesterol ratio	−0.28 ± 0.06	−0.21 ± 0.16	0.695
Serum triacylglycerol (mmol/L)	−0.22 ± 0.21	−0.07 ± 0.18	0.612
Serum FFA (mmol/L)	−0.02 ± 0.09	−0.12 ± 0.08	0.415
Serum glucose (mg/dL)	−15.78 ± 6.82	−4.75 ± 2.45	0.153
Insulin (mU/L)	0.26 ± 0.77	−2.19 ± 0.75	0.058
HOMA IR index	0.31 ± 0.49	−0.48 ± 0.20	0.154
C-peptide (μg/L)	0.63 ± 0.47	−0.16 ± 0.08	0.118
Serum AST (U/L)	−0.60 ± 2.89	−5.57 ± 2.23	0.192
Serum ALT (U/L)	−1.53 ± 2.93	−11.41 ± 5.85	0.150
CRP (mg/L)	−0.20 ± 0.38	0.31 ± 0.56	0.462

Data are given as mean ± SEM. *p*-Values are given for the comparison of mean changes from baseline to 4 wk of oil consumption between the two groups.

postprandial serum MCP-1 levels (Supporting Information Fig. 1D).

3.5 Postprandial response of inflammatory gene expression in s. c. adipose tissue after 4 wk of OL or RA supplementation

Four hours after the test meal, there was a significant 1.61-fold increase ($p = 0.030$) in gene expression of *IL1B* in abdominal s. c. adipose tissue in the RA group compared to the fasting state (Fig. 5A). However, in the OL group no change in postprandial *IL1B* gene expression was observed ($p = 0.944$). After 4 wk of oil supplementation, we saw a strong reduction of 73.5% in *IL6* gene expression levels in the RA group compared to OL ($p = 0.001$) in the fasting state (Fig. 5B). Interestingly, *IL6* gene expression was 2.11-fold increased ($p = 0.032$) 4 h after the test meal in the RA group, whereas a slight but nonsignificant decrease was observed in the OL group ($p = 0.226$, Fig. 5B). After the test meal, gene expression levels of *CCL2* were increased 1.53-fold ($p = 0.009$) and 1.52-fold ($p = 0.043$) compared to fasting levels in the RA and OL groups, respectively (Fig. 5C). For *EMR1* a 1.35-fold increase ($p = 0.049$) in gene expression was observed postprandially in the RA group, whereas no significant difference was observed in the OL group ($p = 0.682$, Fig. 5D). We did not see any significant differences in postprandial gene expression levels of *IL8*, *IL10*, *SERPINE1* (serpin peptidase inhibitor, clade E, nexin, plasminogen activator inhibitor type 1, member 1) or *TNF* in neither OL nor RA group (Fig. 6).

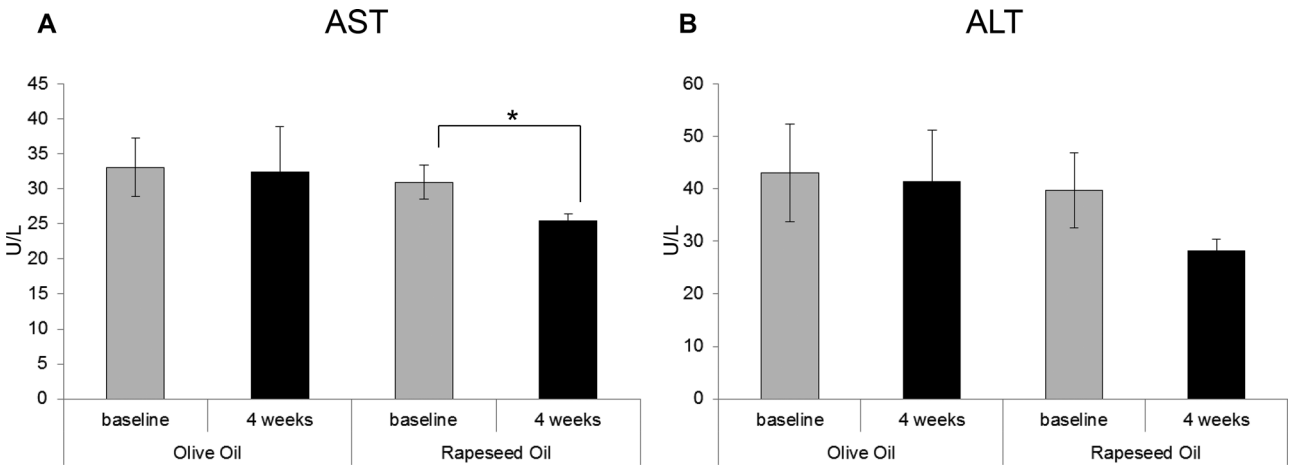

Figure 4. Serum levels of AST (A) and ALT (B) of fasting individuals before (baseline) and after (4 wk) daily supplementation with OL or RA. Data represent mean ± SEM; *$p = 0.037$.

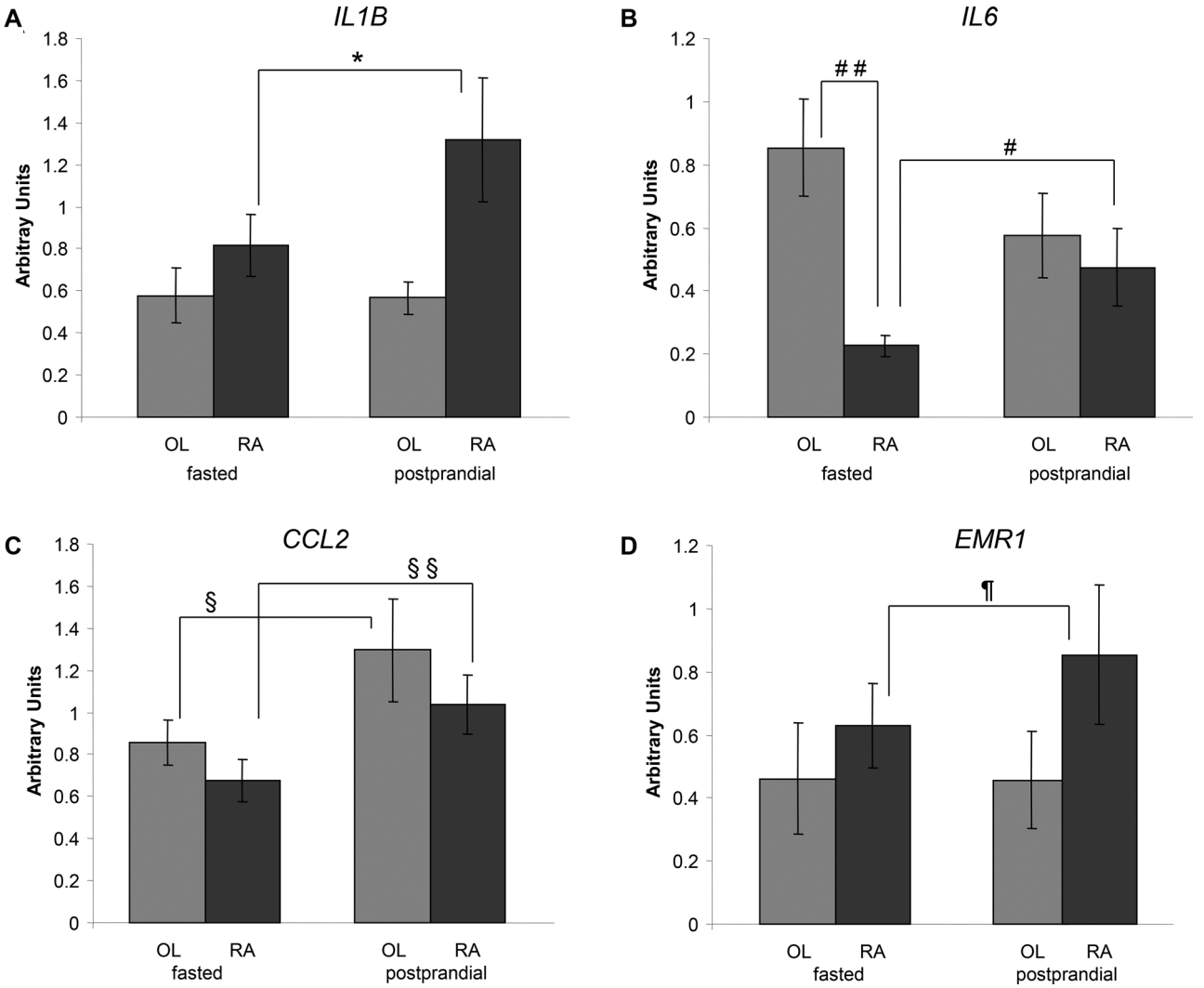

Figure 5. Gene expression levels of *IL1B* (A), *IL6* (B), *CCL2* (C), and *EMR1* (D) in s. c. adipose tissue. Gene expression was analyzed in a biopsy of adipose tissue 4 wk after daily supplementation with OL or RA after an overnight fast (fasting) and 4 h after a test meal (postprandial). Data represent mean ± SEM; $^{*}p = 0.030$, $p = 0.032$, $^{\#\#}p = 0.001$, $^{\S}p = 0.043$, $^{\S\S}p = 0.009$, $^{\P}p = 0.049$.

4 Discussion

In this study, we compared the effects of a daily dietary supplementation with either 50 g of OL or 50 g of RA over 4 wk on serum lipids, serum liver enzymes, and inflammatory markers in serum and s. c. adipose tissue. Both oils are known to be favorable nutritional components and are recommended for a healthy diet [31, 32]. However, studies comparing the effects of RA and OL on these parameters simultaneously are lacking. Our main findings are decreased levels of total and LDL cholesterol and of the liver enzyme AST in serum of individuals who consumed RA. Unexpectedly, participants in the RA group showed a postprandial pro-inflammatory response in s. c. adipose tissue after 4 wk of oil consumption.

The intention of this study was to investigate the effects of oil supplementation without the confounding effects of weight change. Therefore, the daily diet consumed by the participants (including the oil supplementation) was isocaloric. As shown in Table 2, body weight, BMI, body fat content, and waist/hip ratio were not significantly different between participants in the RA and OL groups before the study and remained stable after the study was completed, indicating no relevant changes in adiposity over the duration of the study. The addition of RA or OL to the diet caused an increase in the relative amount of fat consumed in both groups (Supporting Information Table 2). However, since the overall daily diet was isocaloric, this indicates that the relative increase in fat consumption did not affect body weight or body fat content of the participants of both groups. To ensure compliance of food consumption, we measured serum concentrations of ALA that is present in RA but almost absent in OL as shown by an analysis of the FA content of both oils used in this study

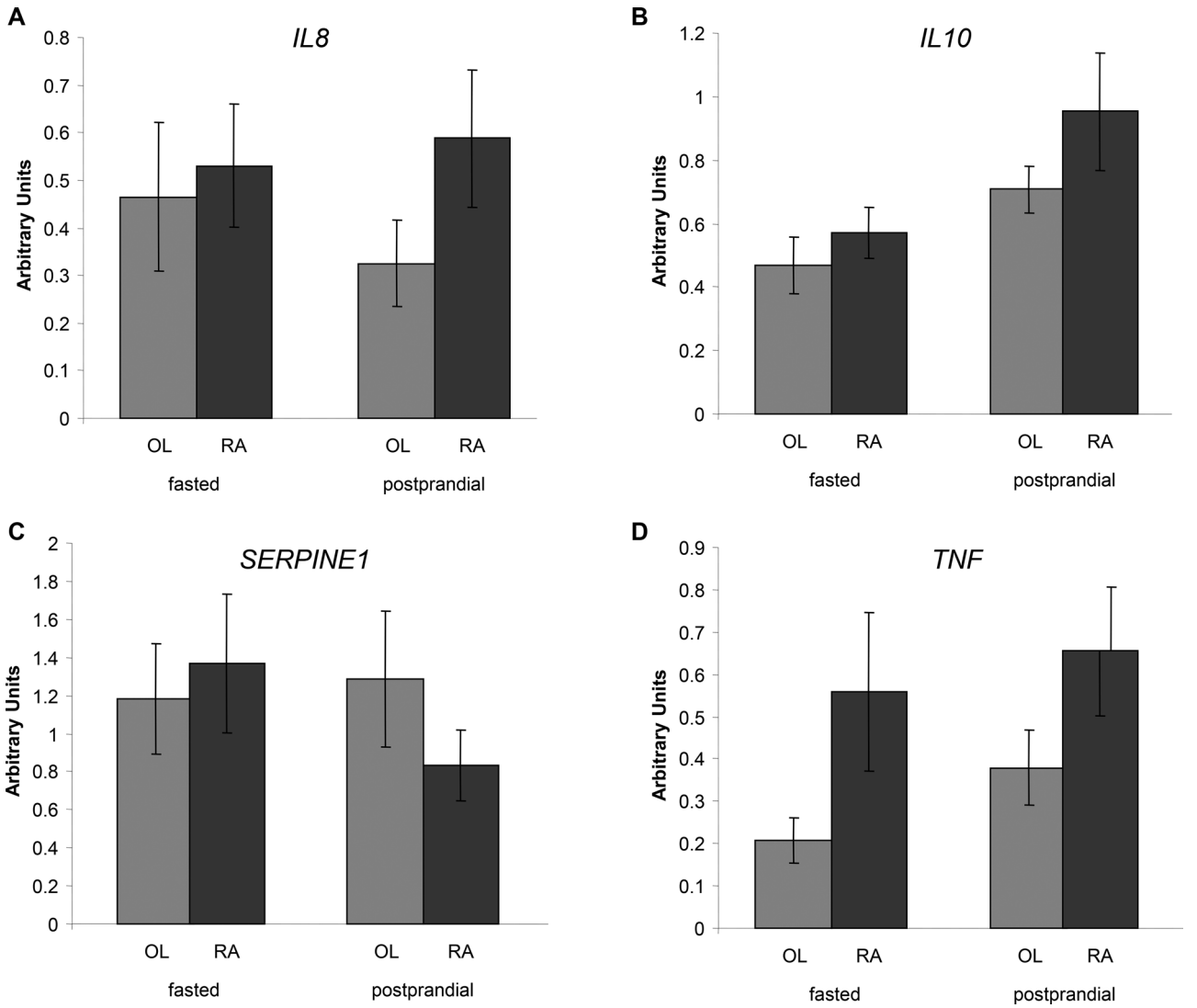

Figure 6. Gene expression levels of *IL8* (A), *IL10* (B), *SERPINE1* (serpin peptidase inhibitor, clade E, nexin, plasminogen activator inhibitor type 1, member 1; C), and *TNF* (D) in s. c. adipose tissue. Gene expression was analyzed in a biopsy of adipose tissue 4 wk after daily supplementation with OL or RA oil after an overnight fast (fasting) and 4 h after a test meal (postprandial). Data represent mean ± SEM.

(Supporting Information Table 3). We saw a significant increase of ALA and also of EPA, which probably was a result of ALA elongation [33], in serum of participants in the RA group, but not in the OL group, indicating a high adherence to oil consumption (Fig. 2). DHA, which is also a conversion product of ALA, however was not changed in both groups.

The decreased serum levels of total and LDL cholesterol of participants in the RA group, who consumed a higher amount of PUFA, are in line with similar studies performed previously. A study in healthy adults showed that consumption of a PUFA-rich diet over 20 days resulted in a reduction of plasma LDL cholesterol levels, but had no effects on HDL cholesterol and triacylglycerol concentrations [34]. A meta-analysis performed by Mensink et al. revealed that an isocaloric replacement of SFA with *cis* unsaturated FA had a positive effect on the reduction of the total/HDL cholesterol ratio, which is a risk marker for developing cardiovascular diseases [14]. Moreover, these data showed that PUFA are more favorable than MUFA to achieve this goal and in terms of dietary oils RA is superior to OL. We observed a decrease of the total/HDL cholesterol ratio in both groups that interestingly reached significance in the OL group.

Clinical studies have shown that RA fortified with micronutrients has beneficial effects on plasma cholesterol [35]. Since the concentrations of micronutrients of both oils used in this study are only slightly different, the effects observed here are very unlikely to be caused by these compounds.

Vessby et al. reported improved insulin sensitivity in healthy human individuals consuming an isocaloric MUFA-rich diet for 3 months compared to individuals consuming

an isocaloric diet rich in saturated fats [36]. This effect was only seen when the total fat content was below 37 energy%. In our study, we could see improved insulin sensitivity determined by the HOMA IR index despite an increase in energy from fat above 40% after oil consumption for RA and OL that, however, was statistically significant for RA only (Table 3).

Clinical studies in patients suffering from NAFLD have shown that dietary supplementation with *n*-3 PUFA is sufficient to reduce serum liver enzymes and to improve hepatic steatosis. A study where 1 g/day of *n*-3 PUFA, containing EPA and DHA, was administered for 12 months to patients with NAFLD showed a decrease in the liver enzymes ALT and AST and in hepatic fat content [37]. Moreover, this study showed a reduced *n*-6/n-3 FA ratio in serum of patients who received the *n*-3 PUFA treatment compared to controls. In obese individuals suffering from NAFLD overnutrition induces liver oxidative stress with concomitant depletion of hepatic *n*-3 long-chain PUFA resulting in an increased *n*-6/*n*-3 long-chain PUFA ratio [38]. In our study, we could achieve a significant increase in plasma *n*-3 PUFA and a subsequent reduction in plasma *n*-6/*n*-3 ratio (Table 3) with an easy-to-manage daily supplementation of 50 g of RA. Consequently, we also observed a reduction in liver enzymes in serum of our moderately obese individuals in the RA group.

n-3 PUFA have beneficial effects on lipid metabolism by regulating the expression of key genes resulting in increased lipolysis and FA oxidation and decreased lipogenesis [39]. Interestingly, for pro-inflammatory cytokines such as TNF-α and IL-6, it was shown that these mediators induce similar effects like *n*-3 PUFA in terms of adipocyte lipid metabolism. For instance, TNF-α reduces the activity and expression of LPL that in turn decreases lipid accumulation in adipocytes [40]. It was reported recently that *n*-3 PUFA are able to mediate anti- and pro-inflammatory responses [41]. These responses seem to be species- and tissue-specific and both anti- and pro-inflammatory responses could occur at the same time. There is also some controversy of the effects among different studies. As for anti-inflammatory responses, a reduction in TNF-α, IL-1β, and IL-6 in human mononuclear cells [42, 43] and an increase in IL-10 in murine adipocytes [44] were described. However, as for pro-inflammatory responses, an increase in TNF-α and IL-6 production was observed after EPA and DHA treatment in a rat peritoneal macrophage model [45]. Since RA contains a higher amount of PUFA compared to OL, it was of great interest to investigate inflammatory responses in our dietary interventional study. A novel finding of this study is that a postprandial increase in gene expression of *IL1B*, *IL6*, *CCL2*, and *EMR1* in the RA group and of *CCL2* in the OL group was observed in s. c. adipose tissue. Elevated *CCL2* and *EMR1* gene expression are indicators for increased macrophage invasion in adipose tissue [46, 47], whereas IL-1β and IL-6 are viewed as pro-inflammatory cytokines [48]. All four mediators are known to be upregulated in the chronic status of inflammation in obesity [6, 48]. In light of the observation that pro-inflammatory cytokines use the same mechanisms as *n*-3 PUFA for the induction of positive effects on adipose tissue metabolism, we propose that the increase in gene expression of cytokines in our study could be interpreted as a favorable result. We thus propose a hormetic response to the brief inflammatory stimulus resulting in a long-term protective response. Such a hormetic response has been recently discussed for IL-6 [49].

Interestingly, we observed a strong decrease in *IL6* gene expression after 4 wk of oil consumption in the fasting state in the RA group compared to the OL group, indicating reduced long-term *IL6* gene expression after RA consumption. However, upon a meal stimulus *IL6* gene expression increased acutely in the postprandial state in the RA group. This increase was not observed in the OL group. Chronically elevated IL-6 serum levels are known to induce peripheral and hepatic insulin resistance [19, 20, 50], whereas acute, short-term elevated IL-6 serum levels improve insulin sensitivity [49]. It was shown that muscle contraction during exercise is able to acutely increase serum IL-6 levels up to 100-fold [51]. It could therefore be speculated that RA consumption can mimic short-erm, "exercise-like" IL-6 induction in adipose tissue. Any modulating effects of micronutrients could be ruled out, as we did not observe any notable differences in these compounds between the oils. However, further work is needed to prove this hypothesis. For serum IL-6 levels, we could observe a strong postprandial increase in both groups after the test meal with a slightly higher, nonsignificant increase in the RA group compared to the OL group.

Limitations of our study are the limited number of participants and the male gender, therefore, restricting its generalizability. Also, parametric tests may not always be robust with such small sample sizes. Moreover, longer time frames are needed to show the durability of the responses. Our study provides data on postprandial inflammatory responses after the oil intervention. Future studies should address comparisons between test meals before and after the dietary intervention.

In conclusion we have shown that a daily supplementation of 50 g of RA might be considered as a potential therapeutic tool and a favorable, easy-to-manage daily nutritional supplementation for the treatment of liver steatosis, since a reduction of serum liver enzymes was achieved in obese individuals. Moreover, we demonstrated that RA induced a postprandial inflammatory response that could be seen as a beneficial positive hormetic effect. Further clinical studies should address these aspects as well as the underlying mechanisms of the induction of cytokines in adipose tissue.

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