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ORIGINAL CONTRIBUTION

Triacylglycerol-rich lipoproteins derived from healthy donors fed different olive oils modulate cytokine secretion and cyclooxygenase-2 expression in macrophages: the potential role of oleanolic acid

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Abstract
Purpose Current evidence suggests that consumption of virgin olive oil (VOO) helps to protect against the development of atherosclerosis and that minor components such as oleanolic acid contribute to this effect. In this study, the effects of triacylglycerol-rich lipoproteins (TRLs) derived from olive oil on inflammatory processes in macrophages and how they are modulated by oleanolic acid was investigated.
Methods TRLs isolated from healthy volunteers 2 and 4 h after a test meal containing VOO, pomace olive oil (POO) (the second pressing of olive oil, enriched in minor components) or POO enriched with oleanolic acid (OPOO) were incubated with macrophages derived from the human monocyte cell line, THP-1.
Results All types of TRLs caused a decrease of about 50% in the secretion of monocyte chemoattractant protein-1 (MCP-1) by the cells. Interleukin (IL)-6 secretion was also significantly decreased by 2 and 4 h VOO TRLs and by 4 h OPOO TRLs. In contrast, increased IL-1β secretion was observed with all 2 h TRL types, and increased tumour necrosis factor-α (TNF-α) production with 2 h VOO and POO, but not OPOO, TRLs. TRLs isolated after 4 h, however, had no significant effects on TNF-α secretion and increased IL-1β secretion only when they were derived from VOO. Cyclooxygenase-2 (COX-2) mRNA expression was strongly down-regulated by all types of TRLs, but protein expression was significantly depressed only by 4 h OPOO TRLs.
Conclusion These findings demonstrate that TRLs derived from olive oil influence inflammatory processes in macrophages and suggest that oleanolic acid may have beneficial effects.

Keywords Triacylglycerol-rich lipoproteins · Olive oil · Oleanolic acid · Cytokine secretion · Cyclooxygenase-2 · Macrophages

Introduction

Triacylglycerol-rich lipoproteins (TRLs) are a heterogeneous mixture of lipoproteins, including chylomicrons, chylomicron remnants (CMR), very low-density lipoproteins (VLDL) and VLDL remnants (intermediate-density lipoproteins) found in the blood following a meal containing fat. Since the density of these lipoproteins overlaps considerably, the isolation of the single classes from postprandial human plasma is impractical, and the TRL fraction has been widely used to study the effects of dietary lipids on vascular disease [1].

Elevating circulating levels of TRLs postprandially have been identified as an independent risk factor for atherosclerosis [1–3]. Atherogenesis begins with endothelial dysfunction and invasion of the artery wall by

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activated leucocytes which then differentiate into macrophages and become lipid-engorged foam cells as they take up lipids from plasma lipoproteins [4]. Recent evidence suggests that TRLs have a direct influence on these events in addition to their indirect effects in increasing in the atherogenicity of the overall lipoprotein profile [5–7]. CMR and TRLs have been shown to enter the artery wall [8, 9], and to be present in human atherosclerotic plaque [10, 11], and to cause leucocyte activation [5, 12], endothelial dysfunction [13–15] and macrophage foam cell formation [13, 16]. However, little information is available about the effects of TRLs on inflammatory processes in macrophages.

The Mediterranean diet is known to protect against atherosclerosis development, and the relatively high virgin olive oil (VOO) content is believed to be a contributory factor [17–19]. The cholesterol-lowering effects of monounsaturated fatty acids (MUFA), a major component of VOO, are well established [20, 21], but recent studies have suggested that minor components of the unsaponifiable fraction of VOO, such as polyphenols, plant sterols, vitamins and carotenoids, may be responsible for some of its protective effects on the vasculature [21], including anti-inflammatory effects on the endothelium [22, 23] and macrophages [24–26]. The triterpenoid, oleanolic acid, is one such component, and previous work has shown that it may modulate the immune response [22, 27–30]. It has been found to inhibit the production of pro-inflammatory cytokines by human peripheral blood mononuclear cells [28] and to induce prostaglandin I_2 release by human coronary smooth muscle cells in a cyclooxygenase-2 (COX-2)-dependent manner [29].

Pomace olive oil (POO) is produced from the second pressing of the olive skin, flesh and pit fragments (olive pomace), which is left after the extraction of VOO, and as a result, it has an increased concentration of minor components, including oleanolic acid [26, 31]. Like macronutrients such as fatty acids, lipophilic micronutrients such as oleanolic acid are carried from the gut to the liver in CMR, and thus have the opportunity to influence events in the vasculature during this process. The first aim of this study was to investigate the effects of TRLs derived from olive oil on inflammatory processes, including cytokine secretion and COX-2 expression, in macrophages, and the second aim was to establish how these effects may be modulated by the oleanolic acid content of the oil. TRLs were isolated from healthy volunteers after a meal containing VOO, POO or POO enriched with oleanolic acid (OPOO) and their effects on the secretion of the pro-inflammatory cytokines and on the expression of enzymes known to be induced by inflammation were determined in THP-1 macrophages.

Methods

Dietary study

Twelve healthy men (age 23.5 ± 2.4 years, body mass index (BMI) 23.2 ± 1.3 kg/m^2) without a history of digestive or metabolic disorders and with fasting triacylglycerol (TG), cholesterol and glucose concentrations all within normal limits were recruited. All procedures followed were in accordance with Institutional and National ethical standards for human experimentation and with the Helsinki declaration of 1975 as revised in 2000. The volunteers gave written informed consent to a protocol approved by the Institutional Committee on Human Research (Hospital Universitario Virgen del Rocio, Seville, Spain).

At 8 am after an overnight fast, a baseline blood sample was taken from subjects via a catheter in the cubital vein. The test meal consisting of brown bread (71 g) spread with 50 g VOO, POO or OPOO (fatty acid/unsaponifiable fraction composition, Table 1), and skimmed yoghurt (125 g) was then given. Oleicola el Tejar S.A. (El Tejar, Spain) supplied the dietary oils and determined their minor component content. Each subject consumed a test meal containing each of the three oils, with a 1-week wash-out period between each test meal. The subjects were asked to refrain from consuming alcohol or smoking 24 h before

Table 1 Fatty acid and unsaponifiable fraction composition of VOO, POO and OPOO parent oils

	VOO	POO	OPOO
Fatty acid			
16.0	9.75	9.46	9.53
16.1 $n-9$	0.11	0.12	0.12
16.7 $n-7$	0.61	0.65	0.65
18.0	3.11	3.33	3.35
18.1 $n-9$	76.31	74.25	74.39
18.1 $n-7$	2.95	3.14	3.08
18.2 $n-6$	6.00	7.75	7.77
18.3 $n-3$	0.37	0.61	0.42
20.0	0.68	0.61	0.60
20.1 $n-9$	ND	0.08	ND
Unsaponifiable fraction (mg/kg)			
Squalene	2,643	2,543	2,543
Sterols	1,558	2,245	2,245
Tocopherols	172.3	981.2	981.2
Oleanolic acid	ND	32.2	574.9
Fatty alcohols	ND	3,484	3,484

Data are expressed as % total fatty acids or mg/kg (unsaponifiable fraction); *ND* not detected

each experiment. Blood samples were taken 2 and 4 h postprandially. During this time, the subjects were allowed to drink water and/or decaffeinated coffee ad libitum.

TRL isolation and analysis

Serum was obtained from the blood samples by centrifugation (1700*g*, 20 min, 12 °C), and TRLs were isolated by ultracentrifugation as before [33]. Total lipids were extracted from TRL using chloroform: methanol (2:1 v/v) [32] dissolved in chloroform: methanol (2:1 v:v) and stored at −20 °C prior to analysis. The lipid class, TG and phospholipid composition was determined by HPLC as described previously [33, 34].

Culture of THP-1 macrophages

THP-1 monocytes (0.7–1.4 × 10^6 cells/ml) in RPMI 1640 supplemented with foetal bovine serum (10% v/v), penicillin (100 U/ml), streptomycin (100 mg/ml) and 2-mercaptoethanol (50 μM) (culture medium) were differentiated into macrophages by incubation with PMA (200 ng/ml) for 72 h at 37 °C in 5% CO_2: 95% air. Cell viability was >95% in all experiments and was unaffected by incubation with TRLs.

Oil red O staining

Cells (0.7 × 10^6 cells/well) were incubated with TRLs (15 μg cholesterol/ml) for 24 h, washed with PBS and 60% propan-2-ol, then Oil red O (0.5% (w/v) in 40% propan-2-ol/H_2O (v/v)) was added. After 15 min, the stain was removed and cells were washed twice with PBS. Staining density was quantified using Biorad Quantity One software.

Assay of cytokine secretion

THP-1 macrophages (0.7 × 10^6 cells/well) were incubated with TRLs (15 μg cholesterol/ml) for 16 h. The concentration of IL-1β, IL-6, TNF-α and MCP-1 in the medium was determined by ELISA according to the manufacturers' instructions.

mRNA analysis

THP-1 macrophages (0.7 × 10^6 cells/well) were incubated with TRLs (15 μg cholesterol/ml) for 16 h, and total RNA was then extracted (RNAeasy Plus MiniKit, Qiagen, Crawley, UK) with DNAase I treatment according to the manufacturers' instructions. Omniscript reverse transcriptase and oligo-(dT) (Qiagen) were used for reverse transcription, and mRNA abundance for COX-1, COX-2, iNOS and the housekeeping gene β-2-microglobulin were determined by real-time polymerase chain reaction (qPCR) using SYBR green quantitative fluorescence and an Opticon 2 LightCycler system (MJ Research, Waltham, Massachusetts, USA). Four candidate housekeeping genes, β-2-microglobulin glyceraldehyde phosphate dehydrogenase, β-actin and ribosomal protein L13a were tested under the experimental conditions used, and β-2-microglobulin was selected as it showed no changes. The forward and reverse primers and the PCR conditions employed are shown in Supplementary (S) Table 1. Ct values were determined by automated threshold analysis (Opticon Monitor 2 software). Data were normalised using values obtained for β-2-microglobulin, and the fold change in mRNA expression in TRL treated when compared to control macrophages was determined by the method of Pfaffl [35].

Immunoblotting

THP-1 macrophages (1.4 × 10^6 cells/well) were incubated with TRLs (15 μg cholesterol/ml, 30 min) prior to incubation with LPS (1 μg/ml) for 16 h. Cells were then lysed by the addition of 100–250 μl lysis buffer (76.5 mM Tris (pH 6.8), 10% glycerol, 2% sodium dodecyl sulphate (SDS), 200 mM sodium orthovanadate, 10 mg/ml protease inhibitor) for 5 min at 4 °C. Lysates were heated to 100 °C (5 min), centrifuged at 9000*g* (10 min),mixed with sample buffer (240 mM Tris (pH 6.8), 40% glycerol, 8% SDS and 0.04% bromophenol blue (2:1, v:v)), and β-mercaptoethanol (1% final concentration) was then added. After heating to 100 °C (5 min), treated lysates were electrophoresed in running buffer (25 mM Tris, 0.192 M glycine and 0.1% (w/v) SDS) (80 mA, 1 h). Proteins were wet transferred onto polyvinylidene fluoride membranes (Bio-Rad (Hemel Hempstead, UK), blocked with 5% milk in Tris buffered saline (0.1% Tween) (TBST) (1 h), washed in 0.1% TBST (6 × 10 min) and incubated with COX-1 or COX-2 primary antibodies (Cell Signalling Technology, Hitchin, Herts, UK) (1:1,000 v/v) in 0.1% TBST containing 10% BSA (4 °C overnight). After washing with 0.1% TBST (6 × 10 min), membranes were incubated with anti-rabbit antibodies conjugated with horseradish protein 1:10,000 (v:v) in 0.1% TBST containing 0.2% BSA for 1 h. Immunoreactive proteins were detected using enhanced chemiluminescence (GE Health Care, Bucks, UK).

Statistical analysis

Data were analysed by one-way or two-way ANOVA followed by Dunnett's or Bonferroni's multiple comparison test as appropriate.

Results

Lipid and fatty acid composition of VOO, POO and OPOO oils and TRLs

The fatty acid and unsaponifiable composition of VOO, POO and OPOO is shown in Table 1. There were no significant differences between the fatty acid composition of the three test oils, however, there were important differences in the minor component composition. Oleanolic acid was undetectable in VOO, but present in POO (32.2 mg/kg) and in OPOO at a higher level (574.9 mg/kg) by supplementation. VOO also lacked fatty alcohols, while POO and OPOO contained almost 3,500 mg/kg. In addition, the sterol and tocopherol content was considerably higher in POO and OPOO when compared to VOO.

The lipid composition of TRLs was not affected by the type of oil consumed, with TG being the predominant lipid at both 2 and 4 h (Fig. 1). The fatty acid composition of the TG fraction of the TRLs isolated 2 and 4 h after consumption of the three test meals was generally similar to that of the parent oils. Oleic acid (18:1 $n-9$ cis) was the main fatty acid in all TRL types (42–49%) (STable 2), with no significant differences between those derived from VOO, POO or OPOO or between the two time points. Other major fatty acids present were palmitic acid (16:0) (17–19%), stearic acid (7–12%) and linoleic acid (18:2 n-6) (15-18%), and their proportions were not significantly different between the diet groups or the 2 and 4 h time points. MUFA (>95% oleic acid) accounted for 48–54% of the total TG fatty acid content in all TRL types, while 26–32% was saturated fatty acids (SFA) and 21–25% polyunsaturated fatty acids (PUFA) (STable 3).

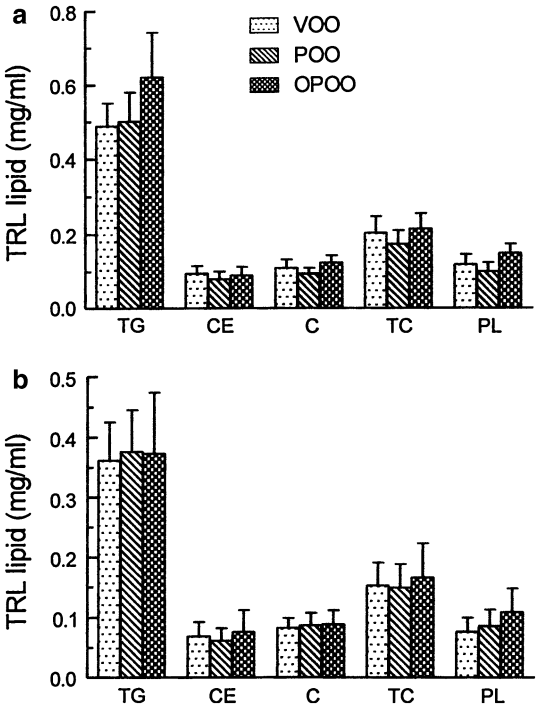

Fig. 1 Lipid classes composition of TRL obtained from blood samples taken from volunteers 2 h (**a**) or 4 h (**b**) after the intake of a test meal containing VOO, POO or OPOO. *CE* cholesterol esters, *FC* free cholesterol, *TC* total cholesterol. Data shown are the mean from 9 (VOO, 2 h), 10 (4 h all oils) or 11 (POO and OPOO, 2 h) subjects and *error bars* show the SEM

As expected, the fatty acid profile of the PL fraction of the TRLs did not resemble that of the parent oil as closely as the TG fraction. In addition to oleic acid (15–21%), the other main fatty acids present were palmitic acid (22–25%), stearic acid (20–27%) and linoleic acid (14–18%), and there were also significant amounts of 20:3 ($n-6$) (5–7%), arachidonic (20:4, $n-6$) (2.4–3.6%)and docosahexaenoic (22:6, $n-3$) (2.2–2.5%) acids present. The proportions of all fatty acids were similar in TRLs derived from all the test oils and isolated at 2 and 4 h (data not shown). Thus, SFA (46–55% total fatty acids) predominated in the PL fraction of all TRLs, followed by PUFA (29–35%), while MUFA accounted for 18–25% (STable 3).

Induction of lipid accumulation in macrophages by TRLs

TRLs isolated 2 h after consumption of the test meal had no significant effect on the amount of lipid accumulation observed by Oil Red O staining. In contrast, TRLs isolated after 4 h caused an increase intracellular lipid levels of approximately 2-fold ($P < 0.001$), regardless of the oil from which they were derived.

The effect of TRLs on chemokine/cytokine secretion by macrophages

Secretion of MCP-1 was markedly decreased by about 50% by all TRL types, and there were no differences between the effects of those derived from different oils or isolated at 2 or 4 h (VOO, POO both time points, $P < 0.001$; OPOO, 2 h $P < 0.001$, 4 h $P < 0.01$) (Fig. 3a). IL-6 secretion was also decreased by 2 h TRLs, but this change only reached significance in the case of VOO (−54%, $P < 0.05$) (Fig. 3b). Similar decreases were observed with TRLs isolated after 4 h, with those derived from VOO (−62.5%, $P < 0.05$) and OPOO (−54%, $P < 0.05$) causing significant changes. In contrast, TNF-α secretion was significantly increased by VOO (+63%, $P < 0.05$) and POO (+83%, $P < 0.01$), but not OPOO, TRLs isolated after 2 h, but in this case there was a clear difference between the effects of particles isolated at the two different time points, since 4 h TRLs had no significant effects regardless of the

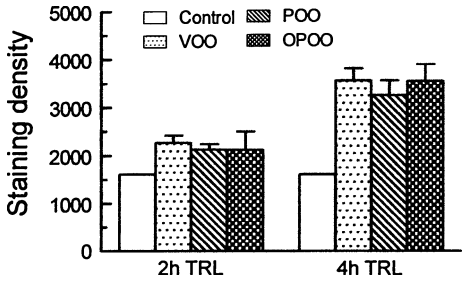

Fig. 2 TRLs (15 μg cholesterol/ml) obtained from blood samples taken from volunteers 2 or 4 h after a test meal containing VOO, POO or OPOO or an equal volume of saline (Control) were incubated with THP-1 macrophages for 24 h. Cells were then stained with Oil red O, and staining was quantified by density analysis. Data shown are the mean from 3 subjects and *error bars* show the SEM. ***$P < 0.001$ versus Control

test oil consumed (Fig. 3c). All three types of TRLs isolated after 2 h also increased the secretion of IL-1β by the cells (+46–55%; VOO, OPOO, $P < 0.05$; POO $P < 0.01$), but with 4 h TRLs, this effect was significant only when the test meal included VOO (+47%, $P < 0.05$) (Fig. 3d). Production of the anti-inflammatory cytokine, TGF-β, was not affected by any of the three TRL types from either time point of isolation (Fig. 3e).

The effects of TRLs on the expression of mRNA for COX-1, COX-2 and iNOS by macrophages

The abundance of COX-2 mRNA was decreased after incubation with all three TRL types, and this effect was particularly marked with 4 h TRLs derived from OPOO (2 h TRLs, −63–70%; 4 h TRLs, VOO, −63%, POO, −71%, OPOO, −84%; $P < 0.001$ in all cases) (Fig. 4a). In contrast, COX-1 mRNA levels were not significantly changed in any conditions (Fig. 4b). In addition, TRLs caused no significant changes in iNOS mRNA levels regardless of the test oil used or the time of isolation (Fig. 4c).

The effects of TRLs on the expression of protein for COX-1 and COX-2 by macrophages

COX-2 protein was undetectable by immunoblotting in untreated macrophages, but became apparent after pre-incubation with LPS (1 μg/ml). Experiments with TRLs, therefore, were carried out following pre-incubation of the cells with LPS, and COX-1 and COX-2 protein expression was assessed by immunoblotting (Fig. 5). As expected, COX-1 protein levels were not significantly changed by treatment with TRLs (sample: control ratio; 2 h, VOO

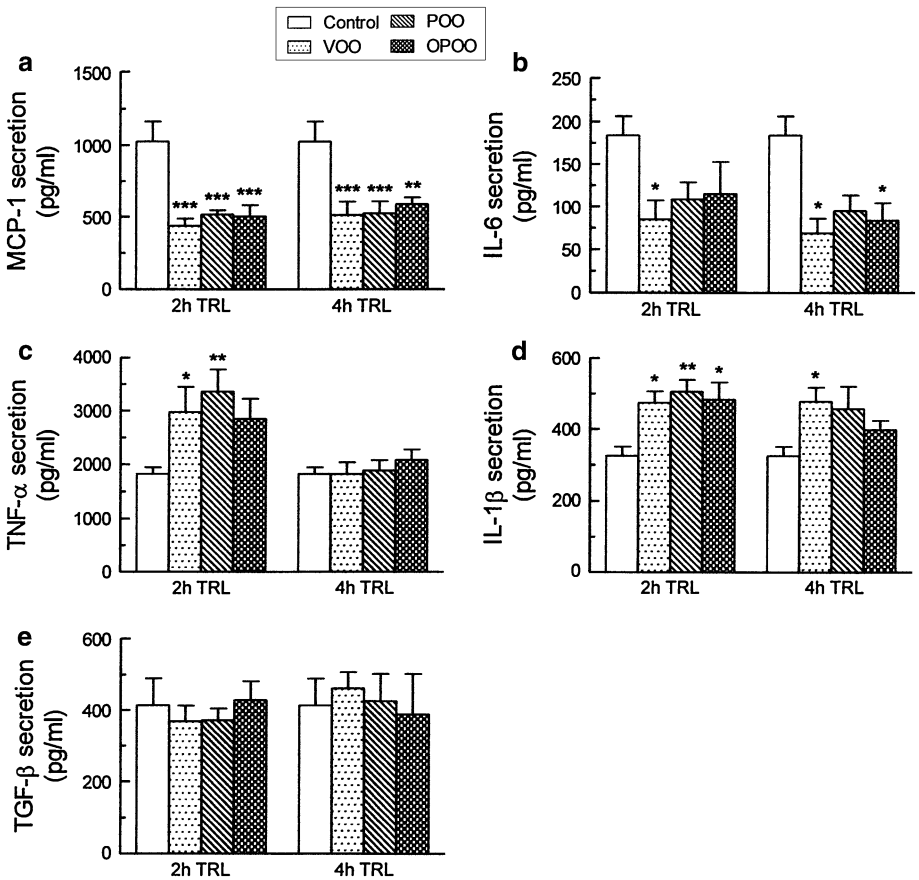

Fig. 3 TRLs (15 μg cholesterol/ml) obtained from blood samples taken from volunteers 2 or 4 h after a test meal containing VOO, POO or OPOO or an equal volume of saline (Control) were incubated with THP-1 macrophages for 16 h. Cell culture media was analysed for MCP-1 (**a**), IL-6 (**b**), TNF-α (**c**), IL-1β (**d**) and TGF-β (**e**) by ELISA. Data shown are the mean from 5 to 12 separate experiments each using TRLs from different subjects, except for TGF-β and 4 h VOO TRLs ($n = 4$) and OPOO TRLs ($n = 3$). *Error bars* show the SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus Control

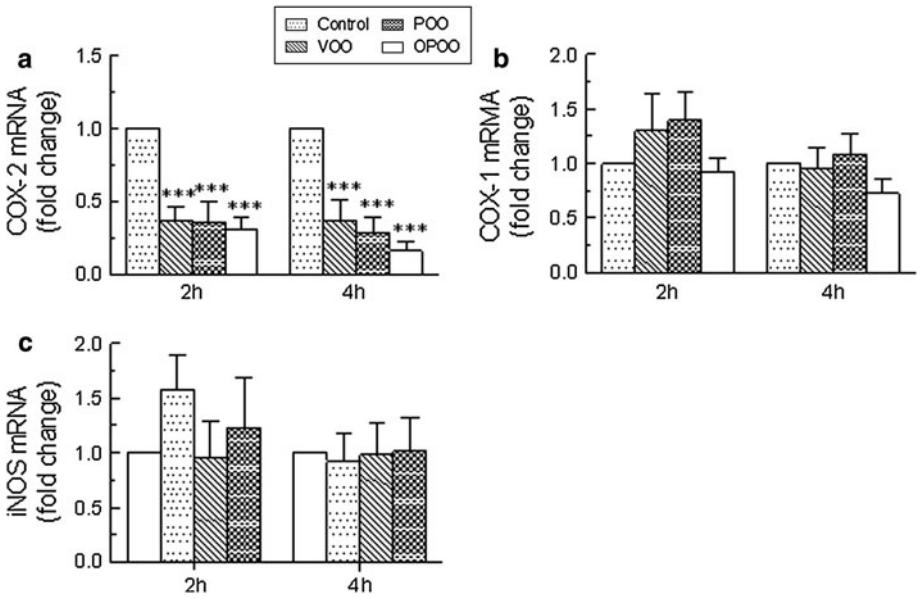

Fig. 4 TRLs (15 μg cholesterol/ml) obtained from blood samples taken from volunteers 2 or 4 h after a test meal containing VOO, POO or OPOO or an equal volume of saline (Control) was incubated with THP-1 macrophages for 16 h and the abundance of mRNA transcripts for **a** COX-2; **b** COX-1 and **c** iNOS was determined by qPCR. Data shown are the mean from 8 (COX-2, Control; iNOS, Control; COX-1, OPOO), 9 (iNOS OPOO), 10 (COX-2, all TRL types; COX-1, VOO TRLs) or 11 (COX-1, POO TRLs; iNOS, VOO, POO) separate experiments each using TRLs from different subjects. *** $P < 0.001$ versus Control

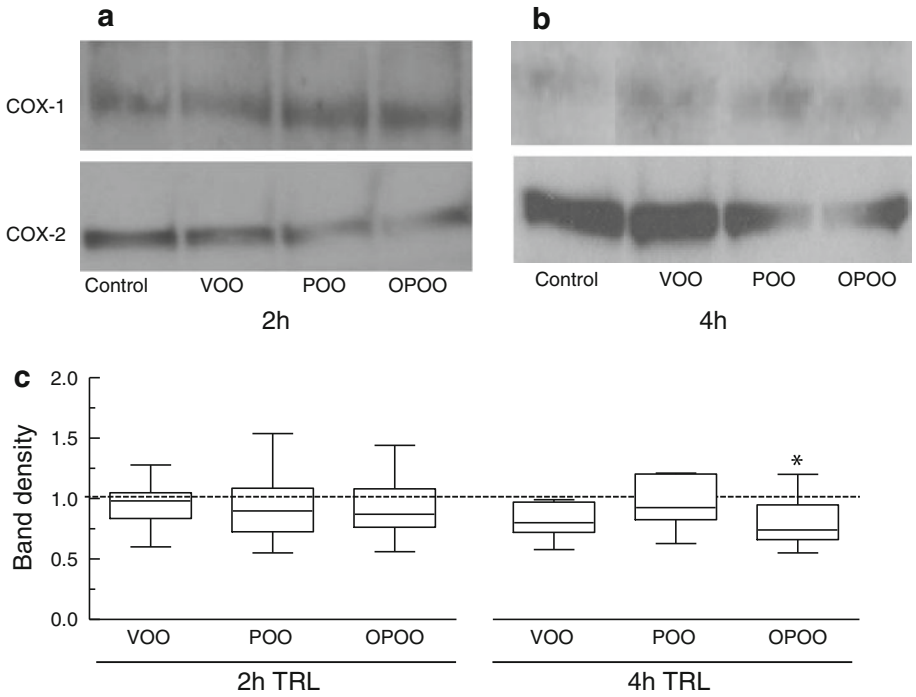

Fig. 5 TRLs (15 μg cholesterol/ml) obtained from blood samples taken from volunteers 2 or 4 h after a test meal containing VOO, POO or OPOO or an equal volume of saline (Control) were incubated with THP-1 macrophages for 16 h after prior incubation with LPS (1 μg/ml) for 30 min. Cells were lysed, and COX-1 and COX-2 protein concentrations were assessed by immunoblotting. **a**, **b** representative blots for 2 and 4 h samples, respectively. **c** Band density was quantified using Quantity One software, data for COX-2 were normalised using values for COX-1 and are expressed as fold change compared with the Control values. Data shown are the mean from 9 (except 4 h POO TRLs, where $n = 10$) separate experiments each using TRLs from a different subject. *Error bars* show the SEM. *$P < 0.05$ versus Control

0.84 ± 0.04, POO 1.02 ± 0.24, OPOO 0.99 ± 0.13; 4 h, VOO 1.38 ± 0.54, POO 1.39 ± 0.45, OPOO 0.85 ± 0.09), allowing it to be used as a housekeeping gene for normalisation of COX-2 protein levels. In contrast to the marked decrease observed in COX-2 mRNA abundance, protein concentrations (Fig. 5a, b, c) showed a significant decrease only with 4 h TRLs derived from OPOO (Fig. 5c).

Discussion

Current evidence suggests that TRLs promote inflammatory processes by increasing pro-inflammatory cytokines, adhesion molecules, and ROS production and expression within the vessel wall [36–38], but that those derived from an olive oil–rich diet have a less inflammatory effect compared with TRLs derived from butter, vegetable oil or fish oil [39–41]. Little is known, however, about the effects of TRLs on inflammatory processes in macrophages.

To investigate the effects of TRLs derived from olive oil on macrophage inflammatory processes and how they may be modified by changes in the content of the lipophilic minor component oleanolic acid, we used THP-1 macrophages and lipoproteins isolated 2 or 4 h after consumption of a test meal containing VOO, POO or OPOO, three oils with a similar fatty acid composition (Table 1), but differing in their content of minor components, and in particular, oleanolic acid. Analysis of the lipid classes present in the TRLs and their fatty acid composition demonstrated that there were no significant differences between those derived from VOO, POO or OPOO (Fig. 1, STables 2, 3). Thus, any differences in the effects of TRLs derived from the three different oils are likely to be due to differences in their content of minor components.

Previous studies have demonstrated that TRLs isolated postprandially [42] or obtained from hypertriglyceridemic subjects [43] induce macrophage foam cell formation. In the present study, TRLs isolated 4 h following consumption of the test meals were found to cause a 2-fold increase in lipid accumulation in THP-1 macrophages, while TRLs isolated after 2 h had no significant effect (Fig. 2). These results are consistent with the findings of Palmer et al. [42], who used TRLs isolated 5 h postprandially, and indicate that the induction of foam cell formation by TRLs is dependent on the time at which they are recovered. TRLs isolated 2 h after consumption of a fat meal are likely to contain a higher proportion of chylomicrons, which are not taken up well by macrophages, while those recovered after 4 h would be expected to contain a higher proportion of CMR [1, 7] which are taken up more easily, resulting in foam cell formation.

TRLs have been found to influence inflammatory processes in the vasculature by increasing leucocyte activation [5, 12, 36], modulating endothelial cell function [14], increasing vascular sensitivity [36, 44] and decreasing the expression of genes involved in smooth muscle cell proliferation and inflammation [39]. Previous work has demonstrated that the secretion of the pro-inflammatory chemokine/cytokines, MCP-1, TNF-α, IL-6 and IL-1β by macrophages is down-regulated by CMR, while secretion of the anti-inflammatory cytokine, TGF-β, is unaffected [45, 46]. The current study indicates that TRLs also down-regulate MCP-1 and IL-6 production by the cells, while having no effect on TGF-β secretion (Fig. 3a, b, e). In contrast to the findings with CMR, however, TRLs were shown to increase macrophage TNF-α and IL-1β secretion when they were isolated 2 h postprandially, although the effect on TNFα was abolished when they were obtained after 4 h (Fig. 3c). Thus, these findings support the idea that CMRs have anti-inflammatory effects on macrophages, but suggest that in vivo other TRLs present postprandially such as chylomicrons and VLDL may modulate the cellular response.

COX-2 and iNOS are inducible enzymes that have been implicated in inflammatory pathways and diseases, including atherosclerosis. Induction of the expression of iNOS during inflammation contributes to endothelial dysfunction [47], but we found no evidence for a role for TRLs in the regulation of iNOS expression (Fig. 4c). COX enzymes catalyse the formation of prostaglandins. COX-1 is expressed constitutively in most cells, including macrophages, but the expression of COX-2 is induced during inflammation by many mediators including cytokines such as IL-1β and TNF-α [48]. COX-2 levels have been found to be up-regulated in atherosclerotic lesions [48]. The present study shows that all types of TRLs markedly decreased macrophage COX-2 mRNA expression (Fig. 4a), while, as expected, that of the constitutively expressed COX-1 was unaffected (Fig. 4b). COX-2 protein levels also tended to decrease, although in this case, significance was reached only in the case of OPOO TRLs isolated 4 h postprandially (Fig. 5c). These findings are consistent with our previous work which demonstrated that CMR down-regulate COX-2 mRNA expression and that this effect may be mediated by the suppression of the activity of nuclear factor-κB [45]. Down-regulation of COX-2 expression is usually considered to be atheroprotective, since it reduces inflammatory prostaglandin production. However, Chan et al. [49] have reported that COX-2 inhibition in macrophages caused enhanced foam cell formation, and other studies have suggested that it may adversely affect platelet function by causing an imbalance between thromboxanes and prostaglandins [50]. The suppression of COX-2 induction by TRLs, therefore, is likely to be anti-inflammatory, but may have other less beneficial effects.

Although, in general, the effects of TRLs isolated following the three test diets had quantitatively similar effects, unlike VOO and POO TRLs, OPOO TRLs did not cause a significant rise in TNF-α secretion (Fig. 3c) by macrophages when isolated after 2 h, or in IL-1β secretion when isolated after 4 h (Fig. 3d). Furthermore, COX-2 protein expression was significantly decreased in cells treated with 4 h TRLs derived from OPOO, but not VOO or POO, when compared untreated controls (Fig. 5c), which corresponded to a stronger down-regulation of

COX-2 mRNA expression (Fig. 4a). Thus, these results provide only limited support for previous findings suggesting that oleanolic acid may have an anti-inflammatory effect on vascular cells, and further work is needed before definitive conclusions can be drawn [23–28].

In summary, the work presented here demonstrates that TRLs derived from olive oil cause foam cell formation and modulate some of the inflammatory processes in macrophages that are involved in the pathogenesis of atherosclerosis. The particles had both inflammatory (increased TNF-α and IL-1β secretion) and anti-inflammatory (decreased MCP-1 and IL-6 secretion, decreased COX-2 expression) effects. However, TRLs isolated 4 h postprandially, and therefore likely to contain a higher proportion of remnant particles, were less inflammatory than those isolated after 2 h, although they caused greater lipid accumulation in the cells.

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Conflict of interest The authors declare that they have no conflict of interest.

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